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Emily Winokur March 21, 2017

Vesicular monoamine transporter 2 (VMAT2) as a potential mediator of social and repetitive behaviors in mice

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Abstract

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Vesicular monoamine transporter 2 (VMAT2) is a synaptic vesicle protein that packages monoamines (dopamine, serotonin, norepinephrine, and histamine) into synaptic vesicles. Aberrant VMAT2 functioning has been associated with disorders that often include symptoms such as depression, changes in fear responsiveness, social dysfunction, social anxiety, and an increased incidence of repetitive behaviors. It is likely that VMAT2 expression has an effect on social and repetitive behaviors as VMAT2 also has a role in the mesolimbic dopamine pathway, a pathway involved in social behavior, and VMAT2 is found in serotonergic neurons, also implicated in social and repetitive behaviors. Transgenic mice expressing 5% of the normal levels of VMAT2 and mice over-expressing VMAT2 provide a unique tool to study the contribution of VMAT2 to these behaviors. While previous research indicates changes in depressive- and anxiety-like behavior and changes in fear-like behavior in these transgenic mouse lines, little research has explored the effect of over- or under-expression of VMAT2 on social functioning and repetitive behaviors. This study uses a standard battery of mouse social tests to provide a basis for understanding how VMAT2 mediates social behavior and repetitive behaviors thus contributing to the development of pharmacological interventions as well as mouse models to study other disorders. Here, I show that the over- or under-expression of VMAT2 has no *consistent* effect on social behavior in mice. However, VMAT2 under-expression has an effect on the total time engaged in social behavior and the total time spent following a novel stimulus mouse. Additionally, I show that VMAT2 expression has an effect on repetitive behaviors such as self-grooming, digging, and jumping.

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Abstract

Vesicular monoamine transporter 2 (VMAT2) is a synaptic vesicle protein that packages monoamines (dopamine, serotonin, norepinephrine, and histamine) into synaptic vesicles. Aberrant VMAT2 functioning has been associated with disorders that often include symptoms such as depression, changes in fear responsiveness, social dysfunction, social anxiety, and an increased incidence of repetitive behaviors. It is likely that VMAT2 expression has an effect on social and repetitive behaviors as VMAT2 also has a role in the mesolimbic dopamine pathway, a pathway involved in social behavior, and VMAT2 is found in serotonergic neurons, also implicated in social and repetitive behaviors. Transgenic mice expressing 5% of the normal levels of VMAT2 and mice over-expressing VMAT2 provide a unique tool to study the contribution of VMAT2 to these behaviors. While previous research indicates changes in depressive- and anxiety-like behavior and changes in fear-like behavior in these transgenic mouse lines, little research has explored the effect of over- or under-expression of VMAT2 on social functioning and repetitive behaviors. This study uses a standard battery of mouse social tests to provide a basis for understanding how VMAT2 mediates social behavior and repetitive behaviors thus contributing to the development of pharmacological interventions as well as mouse models to study other disorders. Here, I show that the over- or under-expression of VMAT2 has no *consistent* effect on social behavior in mice. However, VMAT2 under-expression has an effect on the total time engaged in social behavior and the total time spent following a novel stimulus mouse. Additionally, I show that VMAT2 expression has an effect on repetitive behaviors such as self-grooming, digging, and jumping.

Introduction

Synaptic vesicles mediate the release of neurotransmitters at synaptic terminals throughout the brain. Vesicular monoamine transporter 2 (VMAT2; *SLC18A2*) is a synaptic vesicle transport protein found primarily in the central nervous system with two primary purposes related to neurotransmission and neuronal cell health (Guillot and Miller, 2009; Caudle et al., 2008; Cliburn et al., 2016). First, VMAT2 packages monoamines (dopamine, serotonin, norepinephrine, epinephrine, and histamine) into vesicles for the release from synaptic terminals. VMAT2 transports these neurotransmitter molecules by coupling with an H^+-ATPase antiporter to maintain an electrochemical gradient and exchanging two protons for one monoamine molecule (Guillot and Miller, 2009; Parsons, 2000). VMAT2 also sequesters dopamine into synaptic vesicles, thereby reducing cytosolic dopamine concentrations, oxidative stress, and the subsequent destruction of dopamine neurons (Lohr et al., 2014; Goldstein et al., 2013; Alter et al., 2013; Liu and Edwards, 1997). In the brain, VMAT2 is present in both small vesicles and dense core vesicles in neural regions with high monoaminergic activity (Nirenberg et al., 1995; Caudle et al., 2008; Cliburn et al., 2016)

Normal monoaminergic neurotransmission is critically dependent on VMAT2 for vesicular uptake of monoamine molecules. Faulty monoaminergic neurotransmission has been implicated in a variety of disorders including schizophrenia, attention deficit hyperactivity disorder, dystonia, Huntington's disease, addiction, depression, post-traumatic stress disorder (PTSD), and Parkinson's Disease (PD) (Creese et al., 1988; Eisenberg et al., 1988; Song et al., 2012; Klawans et al., 1972; Ritz et al., 1988; Coppen, 1967; Davidson, 1997; Pifl et al., 2014; Caudle et al., 2007; Rilstone et al., 2013, Hornykiewicz, 1998). Specifically, aberrations in VMAT2 functioning have been associated with a variety of diseases and disorders. Previous

research indicates reduced vesicular uptake in patients with PD, changes to VMAT2 binding patterns in patients with schizophrenia and bipolar disorder type I, and decreased VMAT2 expression in the ventral tagmental area (VTA) and nucleus accumbens (NAC) in a rat model of depression (Pifl et al., 2014; Zubieta et al., 2001; Schwartz et al., 2003). Additionally, in a study with trauma-exposed European-American women of 3742 single nucleotide polymorphisms (SNPs) in more than 300 genes, a significant association was found between the haplotype for the gene which encodes VMAT2, and PTSD (Solovieff et al., 2014). Moreover, in a post-mortem analysis of PTSD-affected brains and control brains, the brains of patients diagnosed with PTSD had significantly reduced mRNA for the VMAT2 protein in multiple brain areas (Bharadwaj et al., 2016).

The implication that VMAT2 has a role in PTSD indicates that VMAT2 could have an effect on various complex behaviors. While PTSD is characterized by aberrant fear responses, other symptoms include depression, social anxiety, social withdrawal, and cognitive changes (Berton et al., 2006; Hofmann et al., 2003; Etkin and Wager, 2007; DSMV, 2013). Furthermore, the implication of VMAT2 in other disorders such as bipolar disorder and schizophrenia that often contain an aspect of social anxiety and/or dysfunction indicates that VMAT2 gene dose could mediate social behavior in mice (Zubieta et al., 2001; Schwartz et al., 2003; DSMV, 2013). Collectively, these studies show that VMAT2 could be a promising pharmacological target for a variety of diseases; thus, more research is needed to understand the contribution of VMAT2 to complex behaviors, specifically, social behaviors.

The neural circuitry underlying social behavior is another indicator that VMAT2 could have an effect on social behavior in mice. In the mesolimbic dopamine system, dopamine cells originating in the ventral tagmental area (VTA) project to the nucleus accumbens (NAc), medial

prefrontal cortex (mPFC), and amygdala (Young et al., 2011). The mesolimbic dopamine system is critical for social behavior, maternal behavior, and appetitive drugs of abuse (Young et al., 2011; O'Connell and Hofmann, 2011). The dopaminergic projections from VTA, a neural region known to express VMAT2, to various neural regions implicated in appetitive behaviors, pleasure, and social behavior further indicates that changes in VMAT2 expression could be associated with changes in social behavior (Cliburn et al., 2016; O'Connell and Hofmann, 2011; Swanson, 1982). Furthermore, serotonin, another monoamine packaged by VMAT2, has been implicated in social deficits and repetitive behaviors (Kane et al., 2012). Mice lacking serotonin display social deficits, communication deficits, and an increased incidence of repetitive behaviors, thus recapitulating many behaviors observed in Autism Spectrum Disorder (ASD) (Kane et al., 2012; Veenstra-Vanderweele et al., 2012). Given the changes in social and repetitive behavior seen in patients with ASD, it is likely that VMAT2 gene dose also has an effect on repetitive behaviors. Additionally, patients with schizophrenia, another disorder associated with aberrant VMAT2 function often also exhibit repetitive behaviors, thus supporting the idea that VMAT2-transgenic mice likely have alterations in repetitive behaviors (Luchins et al., 1992). As VMAT2 can affect neurotransmission in the mesolimbic dopamine system as well as serotonergic neurotransmission, more research is needed to understand how varying levels of VMAT2 expression specifically affect social and repetitive behaviors.

Transgenic mice under- or over-expressing VMAT2 provide a unique tool to study the effect of VMAT2 on mouse behavior and neurological functioning. A complete knockout of VMAT2 results in pups dying within a few days of birth. However, mice expressing 5% of the normal levels of VMAT2 survive into adulthood (Caudle et al., 2007, Wang et al, 1997). These VMAT2-deficient mice (VMAT2-LO) have reduced vesicular uptake of dopamine, an age-

dependent reduction in striatal dopamine, increased dopamine-related oxidative toxicity as indicated by an increased DOPAC/DA ratio, increased tyrosine hydroxylase activity, progressive decline in DAT immunoreactivity, and a progressive loss of dopamine terminals and cell bodies in the substantia nigra pars compacta (Caudle et al., 2007). VMAT2-LO mice also exhibit a significant reduction in dopamine, norepinephrine, and serotonin in the striatum, cortex, and hippocampus (Taylor et al., 2009; Caudle et al., 2007; Mooslehner et al., 2001). VMAT2-LO mice serve as a mouse model of Parkinson's Disease (PD) and recapitulate both motor and non-motor symptoms of PD including decreased locomotor activity, age-dependent deficits in social and non-social olfactory acuity, sleep disturbances, gastrointestinal dysfunction, and depression (Caudle et al., 2007; Taylor et al., 2009; Braak et al., 2003; Langston, 2006; Cummings, 1992).

In contrast, VMAT2-overexpressing mice, known as VMAT2-HI mice, have approximately a three-fold increase in VMAT2 mRNA, a three-fold increase in VMAT2 in striatal homogenate, a three-fold increase in VMAT2 in vesicle fractions, increased vesicular volume, increased dopamine uptake, increased stimulated dopamine release in the dorsal striatum, increased extracellular dopamine, and an increased protection against the detrimental of effects dopaminergic neurotoxins on dopamine neurons compared to their wild-type (VMAT2-WT) littermates (Lohr et al., 2014). Additionally, VMAT2-HI mice display increased locomotor activity and reduced anxiety- and depressive-like behavior as indicated by fewer marble buried in a marble burying assay and reduced immobility time in a forced swim test (Lohr et al., 2014). These improved outcomes on depressive- and anxiety-like behavior add to the growing weight of evidence that VMAT2 mediates a variety of behaviors in a mouse model.

As VMAT2 affects depressive-like behavior, an aspect of PTSD, unpublished data using VMAT2 transgenic mice further suggests that VMAT2 affects other PTSD-like behaviors.

Consistent with PTSD symptoms, VMAT2-LO mice display altered freezing behavior in response to contextual shock stimuli and display maladaptive fear learning in a fear tone-shock conditioning paradigm (Cliburn et al., *in preparation*, personal communication). In contrast, VMAT2-HI mice show normal freezing to cued and contextual fear but have an increased rate of extinction to the fear cue (Cliburn et al., *in preparation*, personal communication). These results indicate that VMAT2 mediates fear responsiveness in mice. Because PTSD often also includes social anxiety and/or dysfunction, VMAT2-gene dose could mediate social behaviors in mice, thus VMAT2-transgenic mice could represent a novel mouse model to study risk and resilience to PTSD-like phenotypes and associated behaviors.

Despite converging lines of evidence indicating that VMAT2 gene dose could mediate social behavior, little research has explored the effect of over- and under-expression of VMAT2 on social behavior in mice. Furthermore, the implication of the serotonergic system in Autism spectrum disorder (ASD) which is associated with changes in both social and repetitive behaviors as well as the presence of repetitive behaviors in other disorders associated with VMAT2 collectively indicates that VMAT2 gene dose could also affect repetitive behaviors (Silverman et al., 2012; Veenstra-Vanderweele et al., 2012; Luchins et al., 1992). Additionally, given the implication of VMAT2 gene dose in resilience to PTSD-like behavior, VMAT2-HI mice likely exhibit other behaviors indicative of stress-resilience when in the stress-inducing novel environment. To address this, VMAT2-transgenic mice will also be assessed for repetitive behaviors that can also be signs of stress (i.e. self-grooming, digging, jumping, and circling) during a ten-minute habituation to a novel cage.

Based on the results of limited data indicating that older VMAT2-LO mice show no preferential exploration of foreign animal's bedding scent as well as the implication of VMAT2

in other disorders with changes in social behavior, I hypothesize that VMAT2 gene dose mediates social behavior in mice. To test this hypothesis, I characterize social behavior in VMAT2 transgenic mouse lines using tests of non-social and social olfactory habituation and dishabituation, social approach, social memory, and social interaction. I predict that VMAT2-LO mice will display decreased social behavior and VMAT2-HI mice will show no difference from the wild-type. Consistent with previous research on stress resilience in VMAT2-transgenic mouse lines, I predict that, during the scored ten-minute habituation phase, due to the stress-inducing novel environment, VMAT2-LO mice will have increased self-grooming compared to the VMAT2-HI mice. Additionally, previous research indicates that VMAT2-HI mice bury fewer marbles in a marble assay, therefore, I predict that VMAT2-HI mice will also have decreased digging compared to the VMAT2-WT mice (Lohr et al., 2014). Finally, unpublished data indicates that the VMAT2-LO mice display altered escape behavior during a forced swim test, thus I predict that VMAT2-LO mice will have increased jumping behavior compared to VMAT2-WT mice (Lohr et al., 2014). Overall, these studies will provide a basis for understanding the contribution of VMAT2 to the changes in social and repetitive behaviors seen in a variety monoamine-related disorders.

Methods

Mice:

VMAT2-LO mice were generated as previously described (Cliburn et al., 2016; Lohr et al., 2016). VMAT2-overexpressing mice (VMAT2-HI) were created using a bacterial artificial chromosome transgene to add an extra three copies of the VMAT2 gene with its promotor and regulatory regions (Lohr et al., 2014; Cliburn et al., 2016). All genotypes used in this study were of the same genetic background (Charles River C57BL/6), and VMAT2-WT littermates were used as the control group (Lohr et al., 2016). Animals were group-housed for the duration of the study. Mice received food and water *ad libitum* on a 12:12 light cycle. All procedures were conducted in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Emory University.

Olfactory Habituation/Dishabituation

An olfactory habituation/dishabituation assay was used to test the ability of mice to detect and discriminate between both non-social and social odors (Yang and Crawley, 2009). Each mouse was individually placed in a clean cage and allowed 5 minutes to habituate to an unscented cotton-tipped applicator placed as it would be for the duration of the test (Fig. 1). During testing, mice were presented with three cotton-tipped applicators per scent of peppermint (McCormick peppermint extract, 1:10 dilution), lemon (McCormick lemon extract, 1:10 dilution), and vanilla (Casa Papantla, 1:10 dilution). The cotton tip of the applicator was dipped in the appropriate solution for two seconds between presentations. Mice were also presented three times each with two different odors from foreign bedding. These scents were collected by running the cotton tip of the cotton-tipped applicator back and forth on the bottom of the cage of a novel mouse ten

times. To preserve the scent, cotton-tipped applicators that had swabbed the cages were sealed and stored in a freezer set at -80° C. Each of the 15 total (9 non-social, 6 social) scent iterations was presented for 2 minutes with an inter-stimulus interval of 30 seconds. Total time spent exploring the cotton tip, defined as sniffing within 2cm of the tip, was recorded for each presentation in real time by the experimenter. The experimenter was kept blind to the experimental groups.

Social Approach

A social approach assay was used to quantify social approach behavior and social memory in mice (Yang et al., 2011). Mice were individually placed in the center chamber of a 3-chambered apparatus (Fig. 2A) and allowed 10 minutes to habituate (Fig. 2B). The sides were then opened and the mouse was allowed 10 minutes to habituate to the whole apparatus (Fig. 2B). Time spent in each chamber was later analyzed through video recordings. A stimulus mouse was then placed under a mesh wire cup (3.8-cm bottom diameter, rust-proof/rust-resistant, noncorrosive, steel wire with space between bars to allow for interaction) on one side of the chamber, and an identical empty upside-down cup was placed on the opposite side (Fig. 2B). To prevent the subject mouse from climbing on top of the cup, a right-side up Solo cup was placed on top of the inverted cup and a weight was placed in the Solo cup. The subject mouse was given 10 minutes to freely explore the apparatus. Time spent near the stimulus mouse under the cup (novel mouse) and time spent near the inverted cup without the mouse (novel object) was later analyzed through video recordings. The side of the novel mouse was counterbalanced across all subjects. In the final stage, the original stimulus mouse was moved to the opposite side of the chamber, and a new subject mouse was placed under a cup on the original side. The subject mouse was given 10 minutes to explore the apparatus, and time near the novel and familiar stimulus mouse was

observed through video recordings (Fig. 2B). The apparatus was cleaned between subjects. All videos were scored by an experimenter blind to the experimental groups.

Social Interaction

A social interaction test was used to measure repetitive behaviors during a habituation phase and various social behaviors towards a novel mouse of the same sex and similar size (Silverman et al., 2010; McFarlane et al., 2008). Mice were individually placed in a clean cage with the nestlet removed and allowed 10 minutes to habituate to the novel cage (Fig. 3B). A clear piece of plexi glass was placed over the top of the cage with a slight opening for airflow, and a video camera was placed directly on top such that the whole cage was visible (Fig 3A). Mice were video recorded for the duration of the habituation phase. Videos were subsequently scored by the experimenter who was blind to experimental groups for self-grooming, circling, jumping, and digging behavior (Fig. 3B). In the social interaction phase, a novel same-sex stimulus mouse weighing within 3g of the subject mouse was then placed in the cage, and their behavior was video recorded for 10 minutes with the aforementioned video camera set-up (Fig. 3A-B). Videos were later scored by the experimenter for following, sniffing, huddling, and wrestling behavior. The experimenter was blind to the experimental groups.

Videos

All videos were recorded using a GoPro Hero 5 Session. Videos were played via QuickTime move player and duration of behaviors was timed via hand-help stopwatch (Marathon Adanac 3000 Digital Stopwatch Timer).

Statistical Analyses

Statistics were performed using Graphpad Prism 5 (La Jolla, CA). Significant outliers were removed prior to data analysis using Grubbs outlier test ($p<0.05$). Data was tested for a

parametric distribution using the Kolmogorov-Smirnov test ($p<0.05$). If parametric, data was analyzed by repeated measures of analysis of variance when comparing more than two groups or t-tests when only comparing only two groups. When data was non-parametric, the data was analyzed by the Mann-Whitney U-test or rank-transformed then analyzed via one- or two-way ANOVA. With two-way ANOVAs comparing the effect and interaction of two different independent variables, when there was a significant main effect or a trend towards a main effect of a variable of interest, post hoc ANOVA tests were used to compare groups with the one variable. If a one-way ANOVA indicated a difference in behavior across all genotypes, Tukey's multiple comparisons tests were used to determine differences between only two genotypes on the same measure. For parametric data, a two-sample t-test was used to determine differences between two genotypes according to *a priori* hypotheses, as explained below. For non-parametric data, a Chi-squared table test or Mann-Whitney test was used to assess differences between only two groups when an *a priori* hypothesis regarding a difference between two genotypes existed.

Results

Olfactory habituation/dishabituation

Non-social odors

Olfactory habituation and dishabituation was conducted as previously described in Yang and Crawley (2009). A two-way ANOVA using stimulus familiarity (presentation-1,2, or 3) for non-social scents and genotype as factors indicated no interaction between stimulus familiarity and genotype ($F_{4,70}$=1.15, p=0.34) (Fig. 4B). There was also no main effect of genotype on sniffing of non-social odors ($F_{2.35}$=1.75, p=0.19) (Fig. 4B). However, there was a significant main effect of familiarity on the amount of time the mice sniffed the cotton tip ($F_{2,70}$=25.40, p<0.0001) indicating a difference in exploratory time with each successive presentation of the same odor (Fig. 4B). These results indicate that, across genotypes, there was no difference in exploration of non-social odors, but overall, mice differentially explored successive presentations of non-social scents.

Exploratory analysis to determine olfactory habituation patterns of each genotype individually revealed a significant main effect of familiarity of non-social odors in VMAT2-WT mice (F_2=5.361, p=0.0084) and VMAT2-LO mice (F_2=7.951, p=0.0013) (Fig. 4B). These results indicate that both genotypes had a significant difference in time spent exploring the cotton tip across presentations. VMAT2-WT and VMAT2-LO mice showed a decrease in exploration time between the first and second presentations and first and third presentations of the non-social odors (Tukey's test, p<0.05) but not the second and third presentation (Fig. 4B). Familiarity had no effect on the amount of time that VMAT2-HI mice explored the non-social odors (F_2=1.122, p=0.3421) indicating that VMAT2-HI mice did not habituate to the non-social odors.

Social odors

For social odors, a two-way ANOVA of stimulus familiarity and genotype indicated no significant interaction between the two variables ($F_{4,70}$=0.58, p=0.68) (Fig. 4C). There was a significant main effect of familiarity ($F_{1,70}$=38.32, p<0.0001) indicating that the mice had differential exploration of successive presentations of the same social odor (Fig. 4C). However, there was no effect of genotype on investigation of social odors ($F_{2,35}$=0, p=1.0).

Exploratory analysis of the habituation to social odors for each genotype via one-way ANOVA revealed a significant effect familiarity on the exploration of social odors in VMAT2-LO mice (F_2=7.893, p=0.0013) and VMAT2-WT mice (F_2=6.071, p=0.0048) (Fig. 4C). Both the VMAT2-LO and VMAT2-WT mice spent significantly more time exploring the cotton tip during the first presentation of a social odor than the second or third presentation (Tukey's test, p<0.05). There was no effect of familiarity on the exploration of social odors for VMAT2-HI mice (F_2=2.254, p=0.13), thus, VMAT2-HI mice did not habituate to social odors.

Social Approach

Social Exploration

The purpose of this experiment was to test sociability as more time exploring the novel mouse than the novel object indicates a social phenotype (Yang et al., 2011). A two-way ANOVA analyzing genotype and exploration of the two stimuli overall showed no interaction between genotype and exploration ($F_{2,36}$=1.52, p=0.23) and no significant main effect of genotype on overall exploration ($F_{2,36}$=2.68, p=0.082) (Fig. 5A). However, there was a significant main effect of the stimulus (mouse or object) ($F_{1,36}$=48.09, p<0.0001) indicating that the mice overall spent significantly more time exploring the mouse than the object (Fig. 5A). Post-hoc comparisons of the time exploring the mouse and the time exploring the object revealed

that each genotype spent significantly more time exploring the mouse ($p<0.05$) (Fig. 5A). A one-way ANOVA to assess the effect of genotype on the extent of preference for the mouse revealed no effect of genotype ($F_2=1.518$, $p=0.23$) (Fig. 5B). These results indicate that genotype had no effect the strength of preference for the mouse (Fig. 5B).

Number of entries to the chamber containing the novel mouse was significantly different across genotypes (one-way ANOVA, $F_2=13.34$, $p<0.0001$) (Fig. 5C). Post-hoc analysis via Tukey's multiple comparisons test indicated that VMAT2-WT mice had significantly more entries to the chamber with the mouse than the VMAT2-LO mice ($p<0.05$), but there was no difference in the number of entries to the chamber with the mouse between the VMAT2-WT and VMAT2-HI mice. Similarly, a one-way ANOVA to analyze the effect of genotype on the number of entries into the chamber containing the object indicated a significant difference across genotypes ($F_2=12.19$, $p<0.0001$) (Fig. 5C). Post-hoc comparisons using Tukey's multiple comparisons test revealed than VMAT2-WT mice had significantly more entries to the chamber with the object than the VMAT2-LO mice ($p<0.05$), but there was no difference between the VMAT2-WT and VMAT2-HI mice (Fig. 5C). These results indicate that VMAT2-LO mice had significantly fewer entries to both the chamber containing the novel mouse and the chamber containing the novel object.

Social Memory

The purpose of the final stage of the social approach assay was to measure social memory and preference for social novelty (Yang et al., 2011). A two-way ANOVA analyzing the effect of genotype and exploration of the two stimulus mice showed no interaction between genotype and exploration ($F_{2,36}=0.22$, $p=0.81$) (Fig. 6A). Genotype did not affect preference for either mouse ($F_{2,36}=2.22$, $p=0.12$). There was no main effect of familiarity on the time that the subject mouse

spent in proximity to the novel or familiar mouse ($F_{1,36}$=1.87, p=0.18) (Fig. 3A). A one-way ANOVA to assess the effect of genotype on difference in preference for the novel mouse compared to the familiar mouse revealed that there was no effect of genotype, and none of the genotypes showed a significant preference for either mouse (Fig. 6B). These results indicate that none of the genotypes had a preference for the novel mouse, and there was no difference in social memory or preference for social novelty across the genotypes.

Number of entries to the novel mouse was significantly different across genotypes (one-way ANOVA, F_2=14.30, p<0.0001) (Fig. 6C). Post-hoc analysis via Tukey's multiple comparison test indicated that VMAT2-WT mice had significantly more entries to the chamber with the novel mouse than the VMAT2-LO mice (p<0.05), and the VMAT2-HI mice had significantly more entries to the chamber with the novel mouse than the VMAT2-WT mice (Fig. 6C). Similarly, a one-way ANOVA to analyze the effect of genotype on the number of entries into the chamber containing the familiar mouse showed a significant difference across genotypes (F_2=13.49, p<0.0001) (Fig. 6C). Post-hoc comparisons using Tukey's multiple comparisons test indicated that VMAT2-WT mice had significantly more entries to the chamber with the familiar mouse than the VMAT2-LO mice (p<0.05), and the VMAT2-HI mice had significantly more entries to the chamber with the familiar mouse than the VMAT2-WT mice (Fig. 6C).

Social Interaction

Individual Behavior

Mice were scored for self-grooming, digging, and jumping behavior as defined by Silverman et al. (2016). Non-parametric analysis of time engaged in self-grooming behavior (KS=0.2952, p=0.0029) revealed that VMAT2-LO spent significantly more time self-grooming than the VMAT2-HI mice (U= 16, Z=3.00, p=0.0026) (Fig. 7A). VMAT2-WT mice spent

significantly more time digging than the VMAT2-LO mice (t_{27}=2.331, p=0.028) (Fig. 7B). There was no difference in the duration of digging between the VMAT2-WT and VMAT2-HI mice (t_{24}=0.06553, p=0.95). A significantly greater proportion of VMAT2-HI mice, compared to VMAT2-WT mice, showed any jumping behavior (χ^2=4.8571, p=0.028) (Fig. 7C).

Social Behavior

A ten-minute social interaction was scored for face sniffing, body/genital sniffing, following, huddling, allogrooming, wrestling, mounting, and total interaction time. There was no difference between genotypes in time spent engaged in sniffing (face or body/genitals), allogrooming behavior, huddling behavior, or wrestling behavior. For overall interaction time, non-parametric analysis (KS=0.2618, p=0.0045) revealed that the VMAT2-LO mice spent significantly more time overall engaged in social behavior than the VMAT2-WT mice (U=60, Z=2.14, p=0.032) (Fig. 8B). Non-parametric (KS=0.3614, p<0.0001) analysis of time the subject mouse spent following the stimulus mouse indicated that the VMAT2-LO mice spent significantly more time following the stimulus mouse than VMAT2-WT mice (U=61, Z=2.28, p=0.023) (Fig. 8A).

Discussion

The present study tested VMAT2-transgenic mice on a battery of social assays to examine the extent to which VMAT2 gene dose mediates social behavior. Additionally, the present study examined the effect of under- or over-expression of VMAT2 on the incidence and duration of repetitive behaviors. Overall, there is not enough evidence to conclude that VMAT2 gene dose *consistently* has an effect on social or repetitive behaviors in mice, but compared to their wild-type littermates, both VMAT2-LO and VMAT2-HI mice show alterations in some aspects of social and repetitive behaviors.

Olfaction is a critical component of social behavior. Differences in non-social olfactory acuity could confound overall social behavior in mice. Olfaction was assessed via an olfactory habituation and dishabituation assay with both non-social and social odors. Genotype did not affect overall exploration of the non-social odors of peppermint, lemon, and vanilla or social odors taken from the dirty cages of single-housed male mice (Fig. 4A). Both VMAT2-LO and VMAT2-WT mice showed characteristic dishabituation to the non-social odors, but the VMAT2-HI mice did not exhibit this pattern (Fig. 4B) (Yang and Crawley, 2011). With social odors, VMAT2-transgenic mice showed no significant differences across genotypes in habituation (Fig. 1C). The VMAT2-LO and VMAT2-WT mice showed a significant decrease in sniffing time between the first and second presentation of the social odor, but the VMAT2-HI mice did not show this decrease (Fig. 4C).

The similarity in non-social olfactory habituation and dishabituation between VMAT2-LO and VMAT2-WT mice is complementary to previous data indicating that younger VMAT2-LO mice exhibit no olfactory deficits while older VMAT2-LO mice have a deficit in odor discrimination (Taylor et al., 2009). The data collected in this paper used mice 4-9 months old.

Given previous research indicating a progressive decline in olfactory acuity for the VMAT2-LO mice and the olfactory deficits seen in PD, it is likely that deficits in non-social and social olfactory acuity are not evident in the present protocol with younger VMAT2-LO mice, but differences would emerge in older mice (Caudle et al., 2007; Taylor et al., 2009; Braak et al., 2003; Langston, 2006). Because there was no difference in the behavior of the VMAT2-LO mice towards the social odors, these data do not support the initial hypothesis that VMAT2-LO mice would exhibit social deficits. However, future studies wherein the mice have a choice between a social and non-social scent would better indicate social olfactory acuity and the preference for social odors in transgenic mice.

While there was no difference across genotypes in exploration of non-social and social odors, exploratory analysis within each genotype on habituation to the non-social and social scents revealed that VMAT2-HI mice did not exhibit the characteristic olfactory dishabituation as described by Yang and Crawley (2009). There are multiple potential explanations for these results. A one-way ANOVA to assess the effect of genotype on exploration of just the first presentation of the social odor has a trend towards a significant effect of genotype (F_2=2.725, p=0.080). The VMAT2-HI mice may not exhibit the characteristic dishabituation to non-social odors because they had a trend towards a reduction in exploration of the first non-social odor presentation (Fig. 4B). Additionally, the altered dishabituation pattern seen in the VMAT2-HI mice could be due to motor movement. The VMAT2-HI mice have more ambulations in the active period (dark cycle), and no difference during the inactive period (light cycle) (Lohr et al., 2014). While the olfactory assay was performed during the light cycle, it is possible that the novel environment induced an increase in locomotor activity analogous to that seen in the dark cycle, such that the VMAT2-HI mice spent more time moving around the cage (Lohr et al.,

2014). The VMAT2-HI mice might have little interest in the non-social scent, thus, the increased locomotor activity could have caused the the mice to only sniff the cotton tipped applicator when in proximity. Likely, the mice exhibited no habituation as movement and the consequent chance exploration due to the proximity of the scent did not change over the duration to the assay.

VMAT2-immunoreactivity has been observed in cells that project from the subventricular zone to the olfactory bulb, and VMAT2 is expressed in the monoaminergic olfactory tubercle (Xu, 1996; Cliburn et al., 2016). The olfactory tubercle is implicated in the reward system and sensory integration (Ikemoto, 2007; Wesson and Wilson, 2011). In rodents, odors are used for communication and to convey information about sex, species, social dominance, health status, and reproductive status (Arakawa et al., 2008; Harvey et al., 1989; Lin et al., 2005). Rodents prefer to be in the presence of a social odor than no odor indicating that social odors could be inherently rewarding (Nelson and Panksepp, 1996; Trezza et al., 2011). The aforementioned VMAT2 distribution indicates that VMAT2-overexpression could alter sensory integration of rewarding stimuli, and the observed differences in VMAT2-HI habituation to social scents could be due to motivational differences. This explanation is complemented by unpublished behavioral comparisons of the VMAT2-WT and VMAT2-HI mice that indicate that VMAT2-HI mice have increased latency to uncover a buried piece of food following an 18-hour fast ($t=2.661$, $p=0.020$) (Supplemental 1). The increased time to find the buried food could be due to olfactory deficits (the VMAT2-HI mice cannot smell the palatable food), motivational differences (the VMAT2-HI mice have reduced motivation to find the food), or a combination of both factors. As VMAT2-HI mice may have reduced olfactory acuity, reduced motivation, and increased locomotion, an olfactory preference assay as described in Taylor et al. (2009) would better control for confounding behavioral differences.

A three-chambered apparatus was used to test both social exploration and social memory in VMAT2-transgenic mice. When the mice were given a choice between a novel mouse and a novel object, all genotypes spent significantly more time exploring the mouse than the object (Fig. 5A). These results indicate that overall, VMAT2-transgenic mice exhibit sociability, defined as significantly more time spent exploring the mouse than the object (Yang et al., 2011). While all genotypes spent more time exploring the novel mouse, there was a trend ($p<0.10$) towards a significant main effect of genotype on the amount of time spent exploring the stimuli overall (mouse and object) (Fig. 5A). However, genotype had no effect on the extent of preference for the mouse (Fig. 5B). These results could be confounded by the side preference of the mice. During the habituation phase, mice were scored for the time spent in the left and right chambers. A t-test combining the time on the left and time on the right side across all genotypes revealed a difference in the time the mice spent on either side ($t=2.175$, $p=0.033$). There was no effect of genotype on side preference, but comparisons of side preference within each genotype revealed that the VMAT2-WT mice showed a significant preference for the right side, while the VMAT2-LO and VMAT2-HI mice showed a trend towards a preference for the right side (Supplemental 2). This preference could be due to the light conditions or a smell in the room that consistently drew the mice to the right side of the apparatus. In attempt to nullify the effect of side preference, the side of the novel mouse was counterbalanced across all mice.

The VMAT2-WT and VMAT2-HI mice had significantly more entries to the chamber containing the mouse and the chamber containing the object than the VMAT2-LO mice (Fig. 5C), but there was no difference in time spent exploring the two stimuli. The number of entries to the chambers is indicative of general motor movement, and these data complement unpublished data on distance traveled in an open field test (Supplemental 2). Both the VMAT2-

WT and VMAT2-HI mice traveled a significantly greater distance than the VMAT2-LO mice (Supplemental 2). Despite decreased movement, the VMAT2-LO mice still spent more time in the chamber with the mouse. The presence of a live conspecific in one of the chambers was likely salient enough for the VMAT2-LO mice to overcome their decreased movement and still exhibit and a comparable level of sociability. Additionally, with increased motor movement, the VMAT2-HI mice could have spent a greater amount of time moving around the center chamber. Instead, they moved about the whole apparatus and had no difference in the time they spent interacting with the novel mouse. These data do not support the initial hypothesis that the VMAT2-LO mice would display reduced social behavior. The VMAT2-LO mice exhibited no difference in sociability from the VMAT2-WT or VMAT2-HI mice. Consistent with the original hypothesis, the VMAT2-HI mice did not show any difference in sociability from the VMAT2-WT mice.

During the final phase, the subject mouse could investigate either a novel mouse on one side of the apparatus or a familiar mouse on the other side of the apparatus. Previous research indicates that wild-type C57BL/6J mice show a significant preference for the novel mouse (Nadler et al., 2004). However, in this study, none of the genotypes had a significant preference for the novel mouse (Fig. 6A). Because even the VMAT2-WT mice did not preferentially explore the novel mouse, these data indicate that the test did not work appropriately. These results could be confounded by the aforementioned side preference as the the side of the novel mouse was counterbalanced across all subjects but not even within genotypes. Even with the confounding effect of side preference, genotype had no effect on the amount of time that the mice spent interacting with the novel or familiar mouse. Similar to the social exploration phase, the VMAT2-HI and VMAT2-WT mice had significantly more entries to both the chamber

containing the novel mouse and the chamber containing the familiar mouse (Fig. 6C). Again, these results are consistent with the total distance covered during an open-field test (Supplemental 2). Despite reduced movement, the VMAT2-LO mice show no difference from the VMAT2-WT mice in a social memory task. Similarly, despite increased movement, the VMAT2-HI mice show no difference in social behavior in this assay. Overall, behavior on the three-chambered social approach assay indicates that there is no difference in social approach or social memory of VMAT2-transgenic mice, but these results could be confounded by the overall side preference of the mice.

VMAT2 has been implicated in a variety of diseases including schizophrenia, bipolar disorder, depression, and PTSD (Zubieta et al., 2001; Schwartz et al., 2003; Solovieff et al., 2014; Bharadwaj et al., 2016). Additionally, the serotonergic system, a monoaminergic neurotransmitter system, has been implicated in ASD (Silverman et al., 2012; Veenstra-Vanderweele et al., 2012). Because repetitive behaviors are implicated in both schizophrenia and ASD, the mice were also scored for repetitive behaviors during a ten-minute habituation to a novel cage (Tracy et al., 1996; Silverman et al., 2012; Veenstra-Vanderweele et al., 2012). The prevalence of excessive repetitive behaviors and social defects in the VMAT2-LO mice could be indicative of an Autism-like phenotype. Additionally, a difference between genotypes in self-grooming, circling, digging, or jumping behaviors could be indicative of other affective states.

Altered self-grooming behavior can be symptomatic of a state of disorder (Kalueff et al., 2015). Multiple studies indicate that self-grooming behavior is altered by acute stress, and it can also be a sign of both discomfort and comfort in the environment (Denmark et al., 2010; Kalueff and Murphy, 2007; Minuer et al., 2003; Kalueff, 2004). Given unpublished data indicating increased susceptibility to fear cues in VMAT2-LO mice and resiliency to fear cues in VMAT2-

HI mice in a fear conditioning paradigm, I hypothesized that the VMAT2-LO mice model susceptibility to stress while the VMAT2-HI mice model resilience to stress. In the habituation phase of the present study, self-grooming is likely indicative of stress; thus, in order to best capture the spectrum of stress susceptibility and resilience, I performed the Mann-Whitney U-test (non-parametric distribution) on the duration of self-grooming between the VMAT2-LO and VMAT2-HI mice. The VMAT2-LO mice exhibited significantly more self-grooming behavior than the VMAT2-HI mice (Fig. 4A). A greater proportion of interrupted grooming bouts, determined via analysis of motor movement sequence, can also be indicative of increased stress (Kalueff and Tuohimma, 2014). While motor sequences were not analyzed in the current study, the incidence of self-grooming was used as a proxy for number of transitions and interruptions in self-grooming behavior. The VMAT2-LO mice also had a greater incidence of self-grooming than the VMAT2-HI mice (t_{21}=2.462, p=0.023). The increased self-grooming in the VMAT2-LO mice compared to the VMAT2-HI mice is in contrast to the motor phenotypes of the transgenic mice. As VMAT2-LO mice display decreased movement and the VMAT2-HI mice show increased motor movement the difference in the duration of self-grooming behavior is more noteworthy (Caudle et al., 2007; Taylor et al., 2009; Lohr et al., 2014; Kalueff et al., 2015). Together, these results augment previous research indicating stress resilience in the VMAT2-HI mice, but future studies need to analyze the specific motor movement sequences of the transgenic mice.

Previously, VMAT2-HI mice have been shown to have decreased anxiety-like behavior as indicated by fewer marbles buried during a marble burying assay (Lohr et al., 2014). Because these comparisons were made between the VMAT2-HI and the VMAT2-WT mice, digging behavior was assessed between the two genotypes with the *a priori* hypothesis that the VMAT2-

HI mice would have reduced digging. This hypothesis was not supported as the VMAT2-HI mice exhibited no significant difference in digging behavior compared to the VMAT2-WT mice (Fig. 3B). The absence of a difference in baseline digging behavior indicates that the VMAT2-HI mice likely do have decreased anxiety-like behavior. In contrast, the VMAT2-LO mice exhibited significantly reduced digging behavior compared to the VMAT2-WT mice (Fig. 7B). Unpublished data suggests that VMAT2-LO and VMAT2-WT mice have no significant difference in the number of marbles buried in a marble burying test. While these results do not show an increase in anxiety-like behavior for the VMAT2-LO mice, the the VMAT2-LO mice may have an increased anxiety-like phenotype. They have reduced digging and locomotor activity, but they bury comparable numbers of marbles in a marble burying test. If the VMAT2-LO mice had the same baseline digging and locomotor activity as the VMAT2-WT mice, they would likely bury more marbles in a marble burying test thus indicating increased anxiety-like behavior.

During the habituation phase, very few mice overall showed any jumping behavior, however, a significantly greater proportion of VMAT2-HI mice exhibited *any* jumping behavior when compared to the proportion of VMAT2-WT mice (Fig. 7C). Because there was a clear piece of plexi-glass over the cage during this phase, the jumping behavior could have been indicative of an escape-like behavior. However, jumping behavior can also be an adaptive behavior and indicative of increased fitness, particularly in younger mice (Henderson, 1981). Additionally, mice exhibit more jumping behavior following an amphetamine injection, thus, the increased dopamine release in the VMAT2-HI mice may be analogous to the increased neurotransmission due to amphetamine (Lal et al., 1976; Lohr et al., 2014). The jumping

behavior in this assay is likely not a repetitive behavior and is more likely indicative of increased movement as a result of VMAT2-overexpression.

During the social interaction test, there was no difference across genotypes in face sniffing, body or genital sniffing, huddling, allogrooming, wrestling, or mounting behavior. VMAT2-LO mice had an overall increase in cumulative time engaged in social behavior (Fig. 8A). VMAT2-LO subject mice spent significantly more time than the VMAT2-WT subject mice following the novel stimulus mouse around the cage (Fig. 8B). Collectively, these results could indicate that VMAT2-LO mice are hyper-social in the presence of a novel mouse or are more interested in the novel conspecific. The increase in social behavior in the VMAT2-LO mice could be due to a compensatory change in oxytocin neurotransmission as oxytocin and serotonergic circuits are both critical in the social reward circuitry (Dolen et al., 2013). However, this was not the case in the social approach assay wherein the VMAT2-LO mice exhibited no greater preference for the novel mouse than the VMAT2-WT or VMAT2-HI mice. The presence of a freely-moving novel mouse could lead to differential social behavior than when the novel mouse is confined to the region under a wire cup, thus future research should examine behavioral differences in the subject mice depending on the condition of the stimulus mice.

VMAT2 gene dose had an effect on various repetitive behaviors as VMAT2-HI mice had reduced self-grooming compared to VMAT2-LO mice, VMAT2-WT mice had increased digging behavior compared to the VMAT2-LO mice, and the VMAT2-HI mice had an increased incidence of jumping behavior. However, in a standard battery of social tests, there was no consistent effect of VMAT2-deficiency or overexpression on social behavior. These social tests involved interactions with a novel mouse, however, interactions with a novel mouse do not encapsulate the range of social deficits or social anxiety phenotypes exhibited in other disorders

(PTSD, bipolar disorder, schizophrenia, depression) in which VMAT2 is implicated (Zubieta et al., 2001; Schwartz et al., 2003; DSMV, 2013). These disorders often include aspects of social anxiety, social withdrawal, social dysfunction, and altered empathic responses (Zubieta et al., 2001; Schwartz et al., 2003; DSMV, 2013). In humans, the social issues associated with these disorders is not limited to interactions with novel individuals.

In order to better address the role of VMAT2 in the social components of these disorders, mice should be tested in assays that involve their behavior towards familiar conspecifics, thus more closely mirroring the social phenotype seen in humans. This could include studies evaluating the subject's response to the distress of a familiar cage- or littermate compared to the distress of a novel mouse, studies of prosocial behavior, studies of social recognition, and studies examining ultrasonic vocalizations (Langford et al., 2006). Differences in these assays could be indicative of a social deficit analogous to aberrant social behavior in humans. Evidence suggests that VMAT2-HI mice are resilient to many behavioral stressors, thus, studies of social defeat in VMAT2-transgenic mice would help understand both stress resilience and social behavior. Because VMAT2 has been implicated in the neural reward circuitry, we also need more tests of reward (Panksepp and Lahvis, 2006; Panksepp et al., 2007).

The present study provides a foundation for future research to further examine the effect of VMAT2 on aspects of social behavior and repetitive behaviors. Because VMAT2 is a promising pharmacological target for a variety of disorders, understanding how VMAT2 mediates social behavior across a range of studies could aid the development of pharmacological interventions as well as mouse models to study other diseases and disorders.

Figures

Figure 1. Experimental set-up for olfactory habituation/dishabituation assay. Cotton-tipped applicator dipped in solution was hung from metal cage cover using Elmer's All Purpose Tack. Cotton tip was about 1.5" from the bedding across all subjects and scent iterations.

A. Three-chamber apparatus used for social approach assay

B. Phases of social approach assay

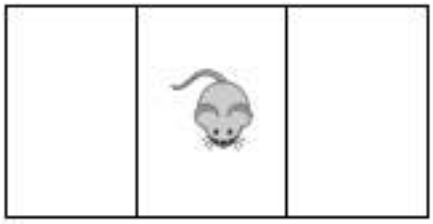
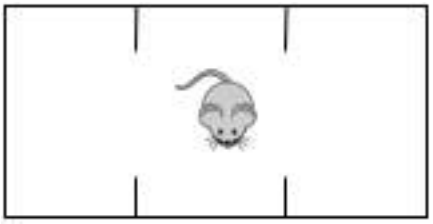
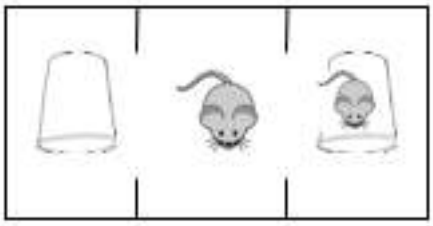
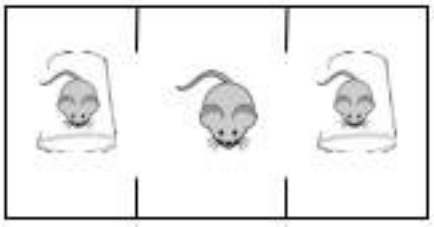
Phase 1	**Phase 2**
	
Phase 3	**Phase 4**
	

Figure 2. Social approach apparatus and experimental design. A. Three-chamber apparatus used for all subjects in the study. **B.** Phases of the social approach assay. Phase 1: subject mouse habituates to the center chamber. Phase 2: subject mouse habituates to the whole apparatus. Phase 3: novel mouse under a cup on one side of the chamber and nothing under identical cup on the other side. Phase 4: novel mouse under a cup on one side and mouse from phase 3 under a cup on the opposite side.

A. Equipment set-up for social interaction assay

B. Phases of social interaction assay

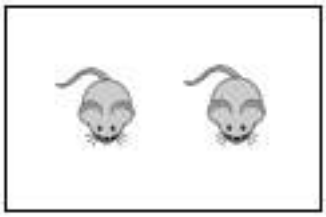
Individual Behavior	Social Interaction
	
Videos scored for: Self-grooming behavior Circling behavior Jumping behavior Digging behavior	**Videos scored for:** Face sniffing behavior Genital/body sniffing behavior Following behavior Allogrooming behavior Huddling behavior Wresting behavior Mounting behavior Total interaction time

Figure 3. Social interaction equipment set-up and experimental design. A. A piece of clear plexi-glass was placed on top of a clean cage with room for air flow. The Go Pro Hero 5 Session was placed on top of the plexi-glass. **B.** Phase 1: subject mouse alone in the cage and allowed 10 minutes to habituate. Phase 2: novel same-sex and similar size stimulus mouse placed in the cage with the subject mouse for 10 minutes.

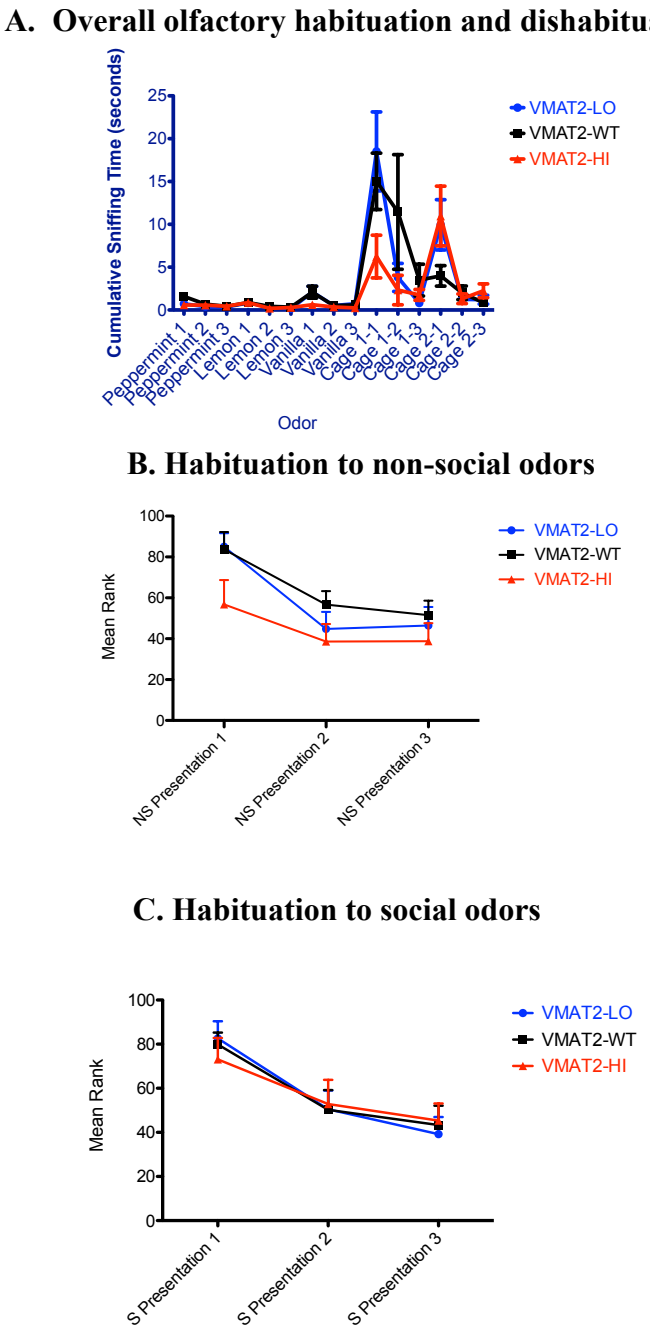
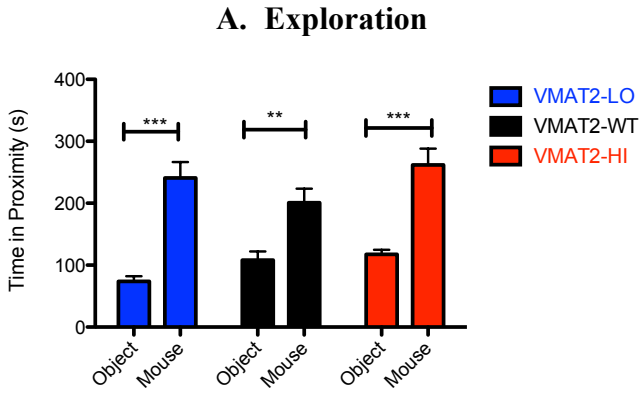
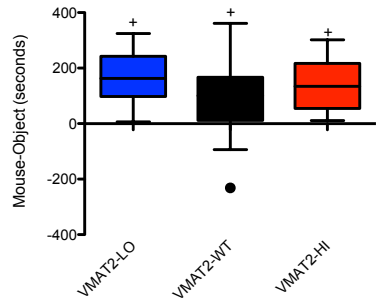


Figure 4. Olfactory habituation and dishabituation to non-social and social scents in VMAT2 transgenic mice. A. Mice habituated to successive presentations of the same odor and dishabituated when a novel odor was presented. The first presentation of each scent elicited the greatest amount of sniffing, and sniffing time declined in subsequent presentations of the same scent. Results represent mean cumulative sniffing time for each two-minute presentation ± SEM. **B.** Overall, mice habituated to successive presentations of non-social odors. Dots represent mean rank ± SEM. **C.** Overall, mice habituated to successive presentations of social odors. Dots represent mean rank ± SEM. *N*=14 VMAT2-LO mice, 15 VMAT2-WT mice, and 9 VMAT2-HI mice.





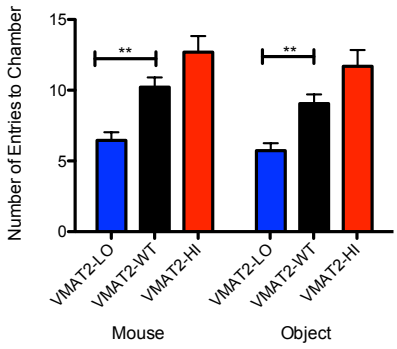
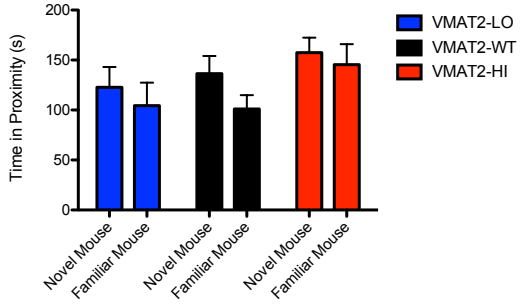
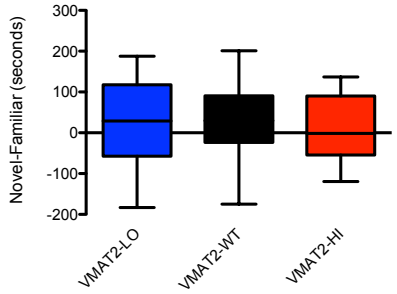


Figure 5. Social exploration for VMAT2 transgenic mice. **A.** All three genotypes spent significantly more time in proximity to the novel mouse than the novel object. Bars indicate cumulative time ± SEM. **B.** There was no significant difference between genotypes and preference for the novel mouse. Box-and-whisker plot represents cumulative exploration time of the mouse-cumulative exploration time of the object ± SEM. (+) indicates that difference was significantly greater than zero. **C.** The VMAT2-WT and VMAT2-HI mice had significantly more entries to the chamber containing the mouse and the chamber containing the novel object. Bars represent total number of entries to the chamber ± SEM. *N*=11 VMAT2-LO mice, 18 VMAT2-WT mice, and 10 VMAT2-HI mice.

A. Exploration



B. Time exploring novel mouse – time exploring familiar mouse



C. Entries

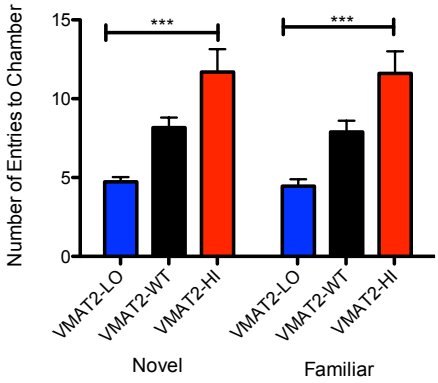


Figure 6. Social memory for VMAT2 transgenic mice. A. All three genotypes showed no preference for the novel mouse over the familiar mouse. Bars indicate cumulative time ± SEM. **B.** There was no significant difference between genotypes and preference for the novel mouse compared to the familiar mouse. Box-and-whisker plot represents cumulative exploration time of the novel mouse-cumulative exploration time of the familiar mouse ± SEM. **C.** The VMAT2-HI mice had significantly more entries to the chamber containing the novel mouse and the chamber containing the familiar mouse than the VMAT2-WT mice. The VMAT2-WT mice had significantly more entries than the VMAT2-LO mice. Bars represent total number of entries to the chamber ± SEM. *N*= 11 VMAT2-LO mice, 18 VMAT2-WT mice, and 10 VMAT2-HI mice.

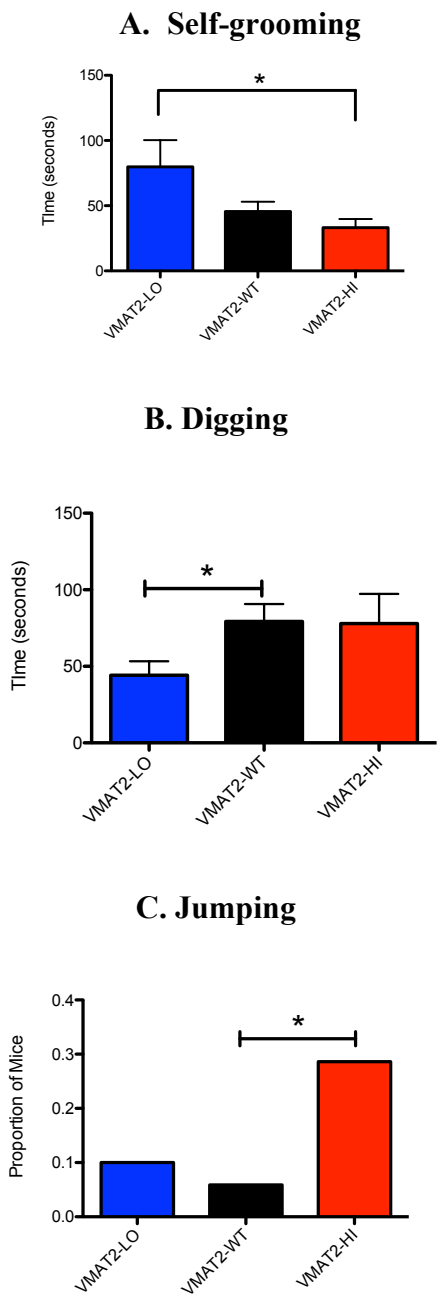


Figure 7. Individual behavior of VMAT2 transgenic mice in a novel cage for ten minutes. **A.** VMAT2-LO mice spend significantly more time engaged in self-grooming behavior than VMAT2-HI mice. Bars represent average cumulative time self-grooming ± SEM. **B.** VMAT2-WT mice spend significantly more time digging than VMAT2-LO mice. Bars represent average cumulative time digging ± SEM. **C.** A significantly greater proportion of VMAT2-HI mice show jumping behavior than VMAT2-WT mice. Bars represent proportion of mice that showed any jumping behavior. *N*=14 VMAT2-LO mice, 17 VMAT2-WT mice, and 10 VMAT2-HI mice.

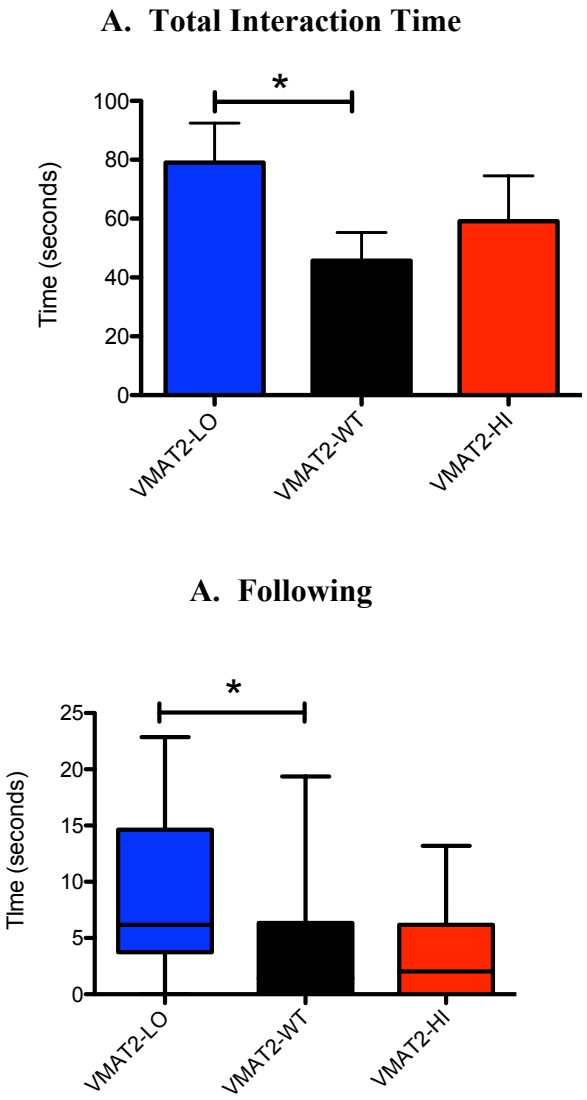


Figure 8. Following and total interaction time for VMAT2 transgenic mice. A. VMAT2-LO subject mice spent significantly more time interacting overall with the stimulus mouse. Bars represent average cumulative interaction time ± SEM. **B.** VMAT2-LO subject mice spent significantly more time following the stimulus mouse than the VMAT2-WT mice. Box-and-whisker plot represents distribution of time spent following. *N*=14 VMAT2-LO mice, 17 VMAT2-WT mice, and 10 VMAT2-HI mice.

Supplemental Methods

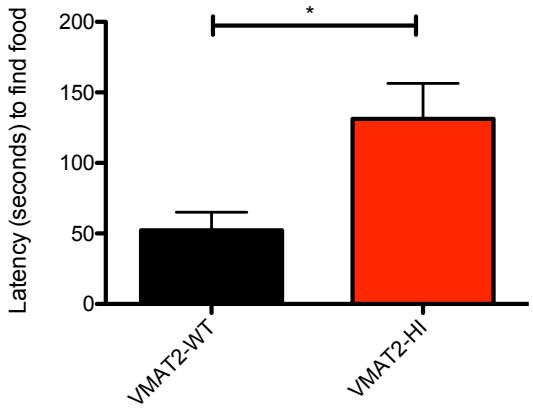
Buried food test:

The protocol was adapted from Yang et al. (2009). All food was removed from each mouse cage sixteen hours prior to testing. Precautions were taken to ensure that no pellets were in the bedding. To ensure that the food was palatable, each mouse was given one Fruit Loop. In all cages, the Fruit Loop was consumed during the overnight period prior to formal testing. Each mouse was placed in a clean cage and allowed 5 minutes of habituation. The mouse was placed back in original cage while the experimenter buried one Fruit Loop in the bottom right corner of the cage approximately 1 cm below the surface of the bedding. The mouse was placed in the top left of the cage and total time to uncover the cereal was recorded by a live experimenter blind to the experimental groups.

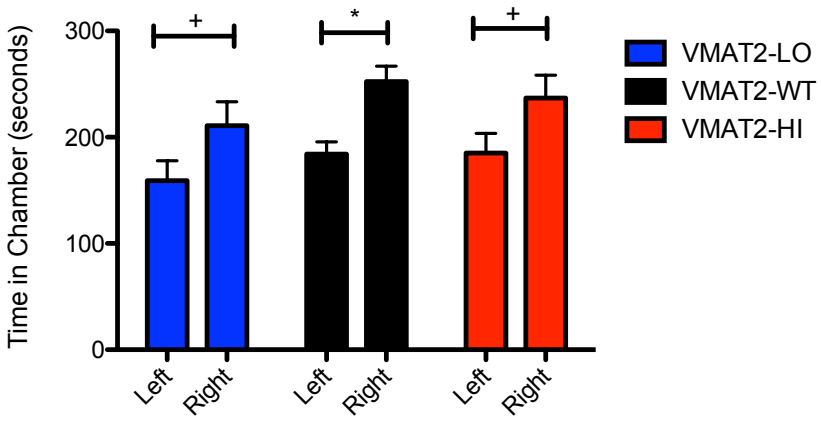
Open field test:

Mice were individually placed in a large circular chamber and allowed ten minutes to freely explore. Time spent in the center of the apparatus, time spent in the side of the apparatus, and total movement was recorded by TopScan 2.0. CleverSysm Inc (Reston, VA).

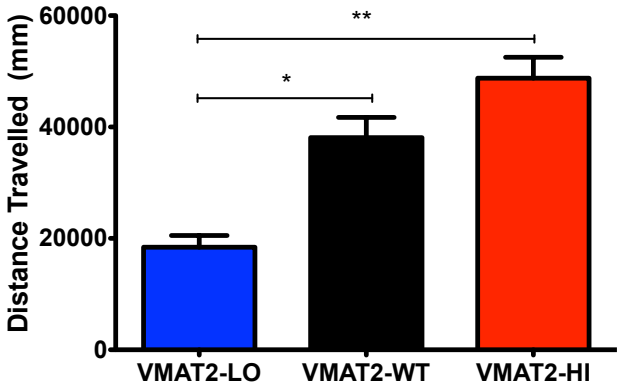
Supplemental Figures



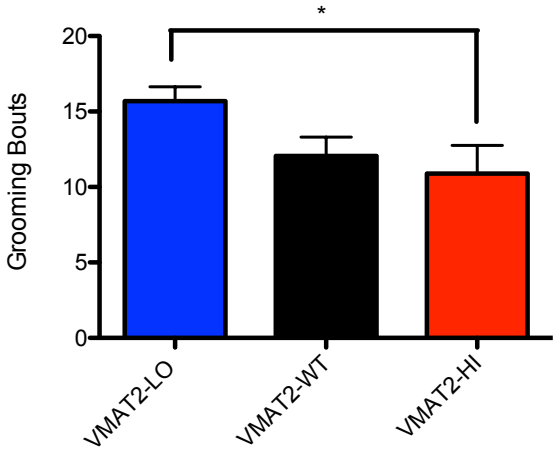
Supplemental 1. Latency to find buried food following an 18-hour fast for VMAT2-WT and VMAT2-HI mice. VMAT2-HI mice took significantly more time to find buried food following an 18-hour fast (t=2.661, p=0.20). Bars represent mean latency to find the food ± SEM. *N*= 7 VMAT2-WT females and 8 VMAT2-HI females.



Supplemental 2. Side preference in a three-chambered apparatus for VMAT2-transgenic mice. The VMAT2-WT mice showed a significant preference for the right side (t=3.676, p=0.0008). The VMAT2-LO and VMAT2-HI mice showed an insignificant trend towards a preference for the right side (p<0.10). (+) indicates trend (p<0.10). Bars represent mean time on that side ± SEM. *N*= 11 VMAT2-LO mice, 18 VMAT2-WT mice, and 10 VMAT2-HI mice.



Supplemental 3. Distance traveled during an open field test for VMAT2-transgenic mice. VMAT2-WT and VMAT2-HI mice traveled a significantly greater distance during an open field test than the VMAT2-LO mice. Bars represent mean distance traveled ± SEM. *N*=2 VMAT2-LO mice, 10 VMAT2-WT mice, and 12 VMAT2-HI mice.



Supplemental 4. Number of bouts of self-grooming for VMAT2-transgenic mice. The VMAT2-LO mice had significantly more bouts of self-grooming. Bars represent mean number of bouts of self grooming ± SEM. *N*= 13 VMAT2-LO mice, 16 VMAT2-WT mice, and 10 VMAT2-HI mice.

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