Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Joyce Liu April 10, 2023

Norepinephrine and Dopamine Contribute to Distinct Repetitive Behaviors Induced by Predator Odor Stress

by

Joyce Liu

David Weinshenker, Ph.D.

Adviser

Neuroscience and Behavioral Biology

David Weinshenker, Ph.D.

Adviser

Paul Lennard, Ph.D.

Committee Member

LaTonia Taliaferro-Smith, Ph.D.

Committee Member

2023

Norepinephrine and Dopamine Contribute to Distinct Repetitive Behaviors Induced by Predator Odor Stress

by

Joyce Liu

David Weinshenker, Ph.D.

Adviser

An abstract of

a thesis submitted to the Faculty of Emory College of Arts and Sciences

of Emory University in partial fulfillment

of the requirements of the degree of

Bachelor of Sciences with Honors

Neuroscience and Behavioral Biology

2023

Abstract

Abstract

Norepinephrine and Dopamine Contribute to Distinct Repetitive Behaviors Induced by Predator Odor Stress

By Joyce Liu

Exposure to stressful stimuli, such as novel environments or shock, elicits repetitive and defensive behaviors in mice, many of which are mediated by the catecholamine neurotransmitters norepinephrine (NE) and dopamine (DA). *Dbh* -/- mice lack the enzyme dopamine-beta-hydroxylase (DBH), which converts DA to NE. Thus, these mice lack NE and have elevated levels of DA as compared to NE-competent controls. We investigated the repetitive behavioral responses of *Dbh* -/- mice and their NE-competent littermates (*Dbh* +/-) to predator odor exposure. We found that while *Dbh* +/- mice engage in vigorous defensive burying in the presence of predator odor but not water, *Dbh* -/- show higher levels of grooming, regardless of the environment, and very little defensive burying in the presence of predator odor. Pharmacological blockade of NE neurotransmission through alpha-1, alpha-2, and beta adrenoreceptors decreased defensive burying in *Dbh* +/- mice, while blockade of DA neurotransmission through D1 receptors decreased grooming in *Dbh* -/- mice. Together, these results suggest that NE transmission is required for predator odor stress-induced defensive burying, while DA transmission through D1 receptors facilitates grooming. These results shed light on the neurochemistry that contributes to innate responses to psychological stress and may help identify neurotransmitters and circuits that underlie repetitive behaviors that are exacerbated by stress, as seen in neuropsychiatric disorders like Tourette's syndrome, obsessive-compulsive disorder, and autism spectrum disorder.

Norepinephrine and Dopamine Contribute to Distinct Repetitive Behaviors Induced by Predator Odor Stress

by

Joyce Liu

David Weinshenker, PhD.

Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences

of Emory University in partial fulfillment

of the requirements of the degree of

Bachelor of Sciences with Honors

Neuroscience and Behavioral Biology

2023

Acknowledgements

I would like to thank Dr. David Weinshenker for his invaluable support and mentorship throughout my time in his laboratory. I would like to thank Dr. LaTonia Taliaferro-Smith for her continued guidance and helping me start on my research journey. I would like to thank Dr. Paul Lennard for encouraging me to think critically throughout this project. I would like to thank Dr. Daniel Lustberg for his mentorship, emotional support, and development of this project. I would like to thank Dr. Kate McCann and the rest of the Weinshenker Lab at Emory University for their encouragement and support throughout my time in the lab.

Table of Contents

1. Introduction

Psychological stress from noxious or unfamiliar stimuli, such as novel environment, tail pressure, or foot shock, alters norepinephrine (NE) and dopamine (DA) signaling in mice (Finlay et. al., 1995; Shanks et. al., 1991; Miura et. al., 2002; Berridge et. al., 1999). Alterations in the transmission of these catecholamines play a role in the mouse's behavioral response to such stressors. For example, inhibition of the noradrenergic system in mice that were repeatedly exposed to foot shock reduced aggressive behavior (Olson et. al., 2011). Additionally, attenuating NE release reduced exploratory behavior in mice after restraint stress (Berridge and Dunn, 1989). Foot shock and restraint stress have also been shown to alter DA neuron firing rate, which was correlated with higher locomotor activity after amphetamine administration (Valenti et. al., 2011).

Psychological stressors also elicit repetitive, defensive, and risk assessment behaviors in mice that include defensive burying, immobility, and flat-back approach (Garbe et. al., 1993; Kemble and Bolwahnn, 1997). Certain defensive behaviors such as defensive burying in the marble burying test are facilitated by NE transmission (den Hartog et. al., 2020; Lustberg et. al., 2020a). Mice also engage in passive, non-defensive behavior such as grooming, which is facilitated by DA transmission (Blackburn et. al., 1992; Murray and Waddington, 1989). While these innate, repetitive stress responses can be adaptive for self-soothing or survival in mice (De Boer et. al., 1990; Korte et. al., 1992), they can also reflect a model of pathological stress-induced or stress-exacerbated repetitive behaviors and tics seen in neuropsychiatric disorders, including obsessive-compulsive disorder (OCD), Tourette's syndrome, and autism spectrum disorder (ASD). For example, the *Sapap 3* -/- mouse model of OCD, which displays excessive grooming, is associated with abnormalities in DA receptor expression (Welch et. al. 2007, Wood

et. al., 2018). Additionally, administration of prazosin, an alpha-1 adrenoreceptor, has been shown to reduce tics in a mouse model of Tourette's syndrome (Nordstrom et. al., 2015). While a mouse model certainly cannot reflect the full complexity of these neuropsychological disorders, understanding the neurochemistry underlying stress-induced repetitive behaviors in mice can shed light on the contributions of NE and DA signaling to similar symptoms in these disorders.

Dopamine-beta-hydroxylase (DBH) converts DA to NE in noradrenergic neurons, and DBH knockout (*Dbh -/-*) mice lack NE and have elevated levels of DA (Bourdélat-Parks et. al., 2005; Thomas et al., 1998; Thomas et al., 1995). Therefore, they are a useful tool in helping us understand the relative contributions of NE and DA to stress-induced behaviors. These mice lack species-typical behavioral responses to innate stressors such as novel environments and cage change (Lustberg et. al., 2020a, Lustberg et. al., 2020b), and they show higher levels of grooming than NE-competent littermates in the presence of novelty stress (Lustberg et. al., 2022).

In this study, we sought to expand our understanding of the role of NE and DA signaling in stress response to predator odor. While previously tested stressors such as novelty represent moderate stressors, predator odor is a severe, ethologically relevant stressor. Given previously demonstrated behavioral indifferences of *Dbh -/-* mice to species-typical stressors (Lustberg et. al., 2020b; Lustberg et. al., 2022, Tillage et. al., 2021), we hypothesized that predator odor stress would elicit different behavioral responses in *Dbh -/-* and *Dbh +/-* mice. In this study, we investigated repetitive exploratory (light digging), defensive (defensive burying), and non-defensive consummatory (grooming) behaviors in *Dbh -/-* mice and NE-competent *Dbh +/-* controls while exposed to predator odor. We then sought to parse the contributions of NE and DA signaling to these repetitive behaviors. We used pharmacological manipulation to investigate

NE transmission through alpha-1, alpha-2, and beta adrenoceptors as well as DA transmission through D1 and D2 receptors in *Dbh* +/- and *Dbh* -/- mice. Alpha-1 adrenoceptors are coupled to G_q proteins, while beta adrenoceptors are coupled to G_s proteins, and upon binding of NE, both receptors will elicit downstream excitatory functions (Wu et. al., 1992; Arriza et. al., 1992). Alpha-2 receptors are coupled to G_i proteins, leading to inhibitory functions upon binding of NE (Chabre et. al., 1994). D1 receptors are coupled to excitatory G_s proteins, and D2 receptors are coupled to inhibitory G_i proteins (Neve, 2013). Because these receptors are all so widely expressed throughout the central nervous system as well as in the body periphery (Kuhar et. al., 1999; Neve, 2013), we hope to more clearly delineate precise functions of each receptor and understand the neurocircuitry underlying specific stress-induced repetitive behaviors.

2. Methods

2.1 Mice

Dbh −/− mice were maintained on a mixed 129/SvEv and C57BL/6 J background, as previously described (Thomas et al., 1998; Thomas et al., 1995). *Dbh* -/- males were bred to *Dbh* +/- females. Norepinephrine is required for embryonic development, so pregnant *Dbh* +/− dams were administered drinking water containing the β-adrenergic receptor (AR) agonist isoproterenol and the α1AR agonist phenylephrine (20 μg/ml each; Sigma-Aldrich) with vitamin C (2 mg/ml) from E9.5–E14.5, and the synthetic NE precursor L-3,4-dihydroxyphenylserine (DOPS; 2 mg/ml; Lundbeck, Deerfield, IL) + vitamin C (2 mg/ml) from E14.5-parturition to prevent embryonic lethality resulting from complete *Dbh* deficiency (Mitchell et al., 2008; Thomas et al., 1995). *Dbh* −/− mice are easily distinguished from their NE-competent littermates by their visible delayed growth and bilateral ptosis phenotypes.

A total of 35 mice (4-10 months of age) was used for all experiments. The groups consisted of 19 *Dbh* +/- mice (n = 8 male, n = 11 female) and 16 *Dbh* -/- mice (n = 10 male, n = 6 female). *Dbh* +/− littermates were used as controls because their behavior and catecholamine levels are indistinguishable from wild-type (*Dbh*+/+) mice (Bourdélat-Parks et al., 2005; Szot et al., 1999; Thomas et al., 1998).

Because no sex differences in stress-induced digging or grooming have been reported in past literature (Londei et. al., 1998; Smolinsky et. al., 2009; Dixit et. al., 2020) or were observed in pilot experiments from this study, male and female mice from the same *Dbh* genotype were evenly distributed across drug treatment groups, and data were pooled between sexes. All animal procedures and protocols were approved by the Emory University Animal Care and Use Committee in accordance with the National Institutes of Health guidelines for the care and use of

laboratory animals. Mice were maintained on a 12 h/12 h light/dark cycle (7:00/19:00 h) with access to food and water ad libitum except during behavioral testing. Behavioral testing was conducted under standard lighting conditions during the light cycle (ZT5-ZT8) in the same room where the mice were housed to minimize the stress of cage transport on test days.

2.2 Behavioral Analysis

Mice were removed from their home cages and placed individually into a clean standard mouse cage with a cotton nestlet square pre-soaked with either 1 mL of deionized water (odorless control) or a predator odor (bobcat urine; Maine Outdoor Solutions, LLC, Hermon, Maine). A clear plexiglass cover was placed on top of the cages to prevent odor dispersal and mouse escape during the experiment. The first 10 min of each mouse's exposure to either predator odor or water were filmed using a front-facing digital camera in order to assess active and passive coping behaviors. Mice were returned to their home cages after the exposure task. Time spent in defensive burying, light digging, and grooming was manually scored by a trained observer blind to genotype and treatment using digital stopwatches. Defensive burying was defined as vigorous and directed displacement of bedding material, involving both front and back paws, often towards the nestlet with the odor stimulus. Light digging behavior was defined as casual, non-directed displacement of the bedding material, involving only the front paws and/or nose. Grooming behavior was defined as repetitive licking or scratching of the paws, tail, or body.

2.3 Drugs

The following drugs were administered i.p. to dissect the relative contributions of NE and DA receptor signaling to behavior: prazosin (0.5 mg/kg, alpha-1 adrenergic receptor antagonist) (Sigma-Aldrich, St. Louis, MO); propranolol (5 mg/kg, beta-adrenergic receptor antagonist) (Sigma-Aldrich); atipamezole (1 mg/kg, alpha-2 adrenergic receptor antagonist) (Sigma-Aldrich), flupentixol (0.25 mg/kg, nonspecific DA receptor antagonist) (Sigma-Aldrich); SCH-23390 (0.003 mg/kg, D1 receptor antagonist) (Sigma-Aldrich); L-741,626 (10 mg/kg, D2 receptor antagonist) (Sigma-Aldrich). All drugs were dissolved in bacteriostatic saline except prazosin and L-741,626. Prazosin was first dissolved in 1.5% DMSO and 1.5% Cremophor EL before being added to saline, and L-741,626 was first dissolved in 10% ethanol and 1.5% Cremophor EL before being added to saline. All drugs were administered 30 min before behavioral testing. Bacteriostatic saline was used for all mice as a vehicle and control for injection stress.

2.4 Statistical Analysis

The effects of the water vs predator odor on repetitive behaviors in *Dbh* +/- and *Dbh* -/- mice were compared using 2-way ANOVA (genotype x odorant), with post hoc Tukey tests for multiple comparisons where appropriate. The effects of drugs vs vehicle on time spent engaged in repetitive behaviors were assessed using paired sample t-tests, where each mouse was tested in both control and drug conditions. Although we were not sufficiently powered to detect sex differences, unpaired sample t-tests were used for preliminary analyses. No significant sex differences were detected (data not shown), and male and female data were pooled within each genotype and treatment group.

3. Results

3.1 *Dbh -/-* mice exhibit decreased digging and increased grooming in response to predator odor.

We first assessed the responses of *Dbh -/-* and NE-competent *Dbh +/-* mice to predator odor and water by quantifying 3 behaviors: light digging that reflects exploratory behavior (Fig. 1A), aggressive digging that signifies defensive burying/tunneling (Fig. 1B), total digging that combines the times spent in light digging and defensive burying behavior (Fig. 1C) and auto-grooming (Fig. 1D) (Lustberg et. al., 2022). Control animals displayed modest levels of light digging and grooming following water exposure, and no defensive burying. Predator odor dramatically increased defensive burying and total digging but had no impact on light digging or grooming. By contrast, *Dbh -/-* mice displayed high levels of grooming in response to water, and were indifferent to predator odor (no emergence of defensive burying and no change in grooming or total digging). For light digging, there was a trend for a main effect of genotype that did not reach statistical significance [$F(1,24) = 4.06$, $p = 0.056$], and no main effect of odorant [$F(1,24) = 2.61$, $p = 0.12$] or an odorant x genotype interaction [$F(1,24) = 0.89$, $p = 0.36$]. This indicated that light digging behavior did not differ between genotypes or the odorant presented. For defensive burying, there were main effects of genotype [$F(1,24) = 15.96$, $p = 0.0005$], odorant [$F(1,24) = 15.96$, $p = 0.0005$], and odorant x genotype interaction [$F(1,24) = 15.96$, $p = 0.0005$]. Post hoc analysis revealed that *Dbh +/-* mice showed significantly increased defensive burying ($p < 0.0001$) in the predator odor condition as compared to water, and they also engaged in significantly more defensive burying ($p < 0.0001$) than the *Dbh -/-* mice in the predator odor condition (Fig. 1B). Specifically, neither genotype of mice engaged in defensive burying in the water condition, but control mice engaged in defensive burying in the presence of bobcat odor while *Dbh -/-* mice did not. For total digging, there was a main effect of genotype [$F(1,24) =$

35.46, p <0.0001], odorant [F(1,24) = 7.98, p = 0.0094], and an odorant x genotype interaction [F(1,24) = 25.34, p <0.0001]. Post hoc analysis revealed that *Dbh* +/- mice showed significantly increased total digging (p < 0.0001) in the predator odor condition as compared to water, and they also engaged in significantly more total digging (p < 0.0001) than the *Dbh* -/- mice in the predator odor condition (Fig. 1C). For grooming, there was a main effect of genotype [F(1,24) =18.82, p = 0.002], but no main effect of odorant [F(1,24) = 0.7293, p = 0.40] or odorant x genotype interaction [F(1,24) = 0.57, p = 0.46].

3.2 Adrenergic antagonists suppress predator odor-induced digging behavior in control animals.

The lack of defensive burying in the *Dbh* -/- mice suggested that NE is required for defensive burying in response to predator odor. To determine whether this is true in normal mice and which receptors are involved, we next assessed the effects of adrenergic receptor (AR) antagonists on the predator odor responses of *Dbh* +/- mice. The compounds tested included prazosin (0.5 mg/kg, α1AR antagonist), propranolol (5 mg/kg, βAR antagonist), a mix of prazosin (0.5 mg/kg) and propranolol (5 mg/kg), and atipamezole (1 mg/kg, α2AR antagonist). Comparing the drug-treated mice to those treated with saline vehicle, propranolol [t(7) = 3.13, p = 0.017] (Fig. 2B) and the prazosin + propranolol cocktail [t(6) = 2.80, p = 0.031] (Fig. 2C) significantly reduced light digging, while prazosin [t(7) = 1.43, p = 0.20] (Fig. 2A) and atipamezole [t(6) = 1.68, p = 0.15] (Fig. 2D) had no effect. These results indicate that βARs, but not αARs, promote this exploratory behavior. All compounds tested significantly reduced defensive burying (prazosin: t(7) = 2.46, p = 0.044; propranolol: t(7) = 2.85, p = 0.025; prazosin + propranolol: t(6) = 4.00, p = 0.0072; atipamezole: t(6) = 3.38, p = 0.015) (Fig. 3) and total digging (Fig. 4) with the exception of prazosin, which showed a strong trend (prazosin: t(7) =

2.35, p = 0.051; propranolol: t(7) = 3.41, p = 0.011; prazosin + propranolol: t(6) = 7.89, p = 0.0002; atipamezole: t(6) = 2.85, p = 0.029]. These results suggest that α1AR, α2AR, and βAR activation all contribute to predator odor-induced defensive burying. Prazosin was the only compound that attenuated grooming in control mice (Fig. 5) (propranolol: t(7) = 0.85, p = 0.43; prazosin: t(7) = 3.03, p = 0.019; prazosin + propranolol: t(6) = 0.17, p = 0.87; atipamezole: t(6) = 0.74, p = 0.49). Overall, these results indicated that defensive burying and total digging were the predator odor-induced behaviors most sensitive to blocking NE transmission.

3.3 D1, but not D2 antagonists attenuate excessive grooming in *Dbh -/-* mice in the presence of predator odor.

Because the AR antagonists recapitulated the decrease in predator odor-induced burying but not the increase in grooming observed in the *Dbh -/-* mice, we suspected that the grooming was the result of excessive DA transmission from noradrenergic neurons in the knockouts, and assessed the effects of DA receptor antagonists on predator odor response in *Dbh -/-* mice. The drugs tested included flupentixol (0.25 mg/kg, nonselective DA receptor antagonist), SCH-23390 (0.003 mg/kg, D1 receptor antagonist), L-741,626 (10 mg/kg, D2 receptor antagonist), and a mixture of SCH-23390 (0.003 mg/kg) and L-741,626 (10 mg/kg). Flupentixol [t(12) = 2.76, p = 0.0037] (Fig. 9A), SCH-23390 [t(6) = 5.89, p = 0.0011] (Fig. 9B), and the SCH-23390 + L741,626 cocktail 626 [t(6) = 7.50, p = 0.0003] (Fig. 9D) all significantly reduced grooming in *Dbh -/-* mice in the presence of predator odor, while L-741,626 alone had no effect [t(7) = 0.75, p = 0.48] (Fig. 9C). There were no significant effects of any drug on light digging (Fig. 6), defensive burying (Fig. 7), or total digging (Fig. 8), with the exception that L-741-626 increased total digging in the *Dbh -/-* mice by ~10 sec over the course of the 10 min test [t(7) = 2.81, p =

0.026] (Fig. 8C). It is worth noting that mice administered SCH-23390 either alone or in combination with L-741,626 ceased nearly all movement but were responsive to gentle prodding and regained normal mobility upon being returned to their home cages. These results suggest that excessive grooming in *Dbh -/-* mice is mediated by D1, but not D2 transmission.

3.4 α1AR blockade has no effect on behavioral response to predator odor in *Dbh -/-* mice.

Our data indicated that NE transmission was responsible for digging behaviors, while DA promoted grooming. However, because we saw a significant reduction in grooming in the *Dbh +/-* mice treated with prazosin, and DA can signal through α1ARs (Paladini et. al., 2001; Cilz et. al., 2014; Özkan et. al., 2017), we speculated that some of the excessive grooming observed in *Dbh -/-* mice might be driven by DA-mediated α1AR transmission. To test this hypothesis, we administered prazosin (0.5 mg/kg) to *Dbh -/-* mice in the presence of predator odor but failed to observe a drug effect on any behavior including grooming (Fig. 10). Combined with the outcomes from the DA receptor antagonist experiments, these results suggest the while α1ARs modestly contribute to predator odor-induced grooming in normal mice, they are not required for the excessive grooming response of *Dbh -/-* mice, which appears to be mediated by D1 receptors.

3.5 D1 receptor blockade induces behavioral arrest in *Dbh +/-* control mice.

To determine whether SCH-23390-induced suppression of all behavior was unique to *Dbh -/-* mice or was a general effect of the drug in the presence of predator odor, we administered SCH-23390 (0.003 mg/kg) to predator odor-exposed *Dbh +/-* mice and found a significant decrease in light digging [$t(6) = 3.85$, $p = 0.0085$] (Fig. 11A), defensive burying [$t(6) = 4.64$, $p = 0.0035$] (Fig. 11B), total digging [$t(6) = 4.89$, $p = 0.0027$] (Fig. 11C), and grooming [$t(6) = 3.47$, $p =$

0.013] (Fig. 11D). While *Dbh* +/- mice treated with SCH-23390 were qualitatively more mobile than the *Dbh* -/- mice treated with SCH-23390, they remained mostly still until returned to their home cage. These results indicate that an interaction between predator odor and D1 blockade induces behavioral arrest in both normal and *Dbh* -/- mice.

4. **Discussion**

In this study, we investigated the effects of manipulating catecholamine transmission on innate, repetitive behavioral responses to predator odor-induced stress in mice by comparing *Dbh* -/- mice, which lack NE and instead produce DA in their "noradrenergic" neurons, to their *Dbh* +/- littermates that have normal catecholamine content. We also used pharmacological manipulation to target NE and DA signaling, allowing us to assess the effects of both chronic and acute catecholamine manipulation.

Predator odors such as bobcat urine are ethologically relevant psychological stressors that elicit intense, innate behavioral responses in mice (Ferrero et. al., 2011, Janitzky et. al., 2015). Stress results in modulation of catecholamine levels (Irwin et. al., 1986), which in turn alters behavioral reactivity to stress (Olson et. al., 2011). In our study, we assessed light digging, defensive burying, and grooming behaviors in the presence of bobcat urine.

In behavioral experiments where mice have substrate to dig through, light digging is interpreted as an exploratory behavior but not necessarily an active stress coping mechanism because it is not stress-specific (Londei et. al., 1998). In support of this idea, light digging was similar in control mice exposed to either water or bobcat urine, with a reduction in *Dbh -/-* mice regardless of exposure type. In our pharmacology experiments, we found that blockade of NE signaling through beta, but not alpha-1 or alpha-2 ARs, decreased light digging. This is consistent with our previous findings that *Dbh -/-* mice show reduced exploratory activity in novel environments that are not inherently dangerous (Lustberg et. al., 2020b).

By contrast, defensive burying is considered an active coping strategy in response to stress, in which the mouse attempts to remove or avoid aversive stimuli. In the shock probe defensive burying test, mice will use bedding to bury electrified prods and remove the source of shock pain

(Tillage et. al., 2020). Rats who engage in this defensive burying behavior in response to an electrified shock prod have been shown to have lower hypothalamic-pituitary-adrenal (HPA) axis activation as compared to rats who are forced to freeze in the presence of the shock prod, indicating that defensive burying is an active stress-coping strategy by lowering circulating corticosterone levels (De Boer et. al., 1990; Korte et. al., 1992). The marble burying test has also demonstrated that defensive burying is stress-sensitive, with stressed mice burying more marbles than unstressed mice (Kedia and Chattarji, 2014), and we have shown that *Dbh -/-* mice bury fewer marbles than controls (Lustberg et. al., 2020a).

We found that both *Dbh -/-* and *Dbh +/-* mice engage in similarly low levels of defensive burying when exposed to a novel cage with a water-soaked cotton nestlet, indicating that this environment is not particularly stressful. *Dbh +/-* mice, but not *Dbh -/-* mice, show increased defensive burying when exposed to predator odor as compared to water. Our pharmacology experiments demonstrated that blocking NE signaling in *Dbh +/-* mice through alpha-1 and beta ARs decreased defensive burying and total digging. These results confirm that predator odor elicits behavioral stress responses that are dependent on NE, and are consistent with our past work that shows NE signaling through these receptors is required for novel odorant stress-induced repetitive digging (Lustberg et. al., 2022). Blockade of NE signaling through alpha-1 ARs has been previously shown to decrease defensive behavior in the presence of a traumatic cue in rats (Ketenci et. al., 2020). Additionally, antagonism of beta ARs was reported to block cocaine withdrawal-induced defensive burying in rats (Harris and Aston-Jones, 1993). Based on these data, we expected that increasing NE transmission via blockade of autoinhibitory alpha-2 ARs on the locus coeruleus (Aghajanian and VanderMaelen, 1982) would facilitate stress responses, but instead atipamezole also attenuated defensive burying and total digging. Alpha-2

ARs are also expressed by neurons targeted by noradrenergic innervation (Timmermans and van Zwietan, 1982), so it is possible that the effects of atipamezole on digging behavior were mediated by these post-synaptic receptors. Another possible explanation is that the increase in NE release from noradrenergic neurons after atipamezole administration led to an increase in other stress responses that we did not measure, such as freezing, which could have occluded digging. For example, atipamezole has been shown to cause higher levels of freezing in mice after footshock stress (Murchison et. al., 2004). Altogether, the results from our genetic and pharmacological experiments suggest that noradrenergic dysregulation of any kind through alpha-1, alpha-2, and beta ARs is detrimental to active stress coping response through defensive burying in mice. Future pharmacological experiments, such as administering norepinephrine to *Dbh -/-* mice, can help confirm the role of norepinephrine in defensive burying in response to predator odor exposure.

Tying our work into neurocircuitry, past work by other members in our lab has shown that several regions innervated by the locus coeruleus (LC), the major noradrenergic nucleus in the brain, show higher neuronal activity (as measured by c-fos protein induction) following predator odor exposure in *Dbh +/-* mice than *Dbh -/-* mice. These include the anterior cingulate cortex (ACC), the dorsal bed nucleus of the stria terminalis (dBNST), the periaqueductal gray (PAG), and the lateral septum (LS). These regions receive noradrenergic innervation through alpha-1, alpha-2, and beta ARs and have been implicated in predator response (Daniel and Rainnie, 2015; Endres and Fendt, 2008; Janitzky et. al., 2015; Jhang et. al., 2018. They represent possible regions where NE signaling mediates digging behavior and are candidates for site-specific infusions of drugs that manipulate adrenergic signaling.

Grooming, unlike digging, has not been considered a stress coping response, as mice will groom in situations of both comfort and stress (Smolinsky et. al., 2009). Repetitive, purposeless self-grooming is often observed in animal models of obsessive-compulsive disorder (OCD), Tourette's syndrome, and autism spectrum disorder (Nordstrom and Burton, 2002; Welch et. al., 2007; Lewis, 2011). Pharmacological stimulation of D1 receptors has been shown to induce stereotyped self-grooming in rats, whereas stimulation of D2 receptors did not (Berridge and Aldridge, 2000). Another study also found that D1, but not D2 receptor signaling is necessary for novelty-induced grooming in mice (Drago et. al., 1999). We found that *Dbh -/-* mice groom more than *Dbh +/-* mice regardless of the odorant presented. Because of these and other studies implicating the role of increased DA signaling in excessive grooming behavior, we suspected that it was mediated by the ectopic DA rather than the lack of NE in *Dbh -/-* mice. Indeed, our pharmacology experiments revealed that DA signaling through D1, but not D2, receptors is necessary for excessive grooming behavior in *Dbh -/-* mice in the presence of predator odor. We also previously showed that *Dbh -/-* mice groom more than *Dbh +/-* mice in the presence of a novel odorant, and this excessive grooming was reduced by administration of the non-selective DA receptor antagonist flupentixol (Lustberg et. al., 2022). Therefore, the results from our genetic and pharmacological experiments suggest that the neurochemistry and circuity that drive grooming in response to predator odor is similar to the circuitry for grooming in other situations, including novel odors and/or environments.

Interestingly, specifically blocking D1 receptors with SCH-23390 administration resulted in a universal suppression of movement in both the *Dbh +/-* mice and *Dbh -/-* mice, indicating that D1 signaling contributes to a variety of predator odor-induced behaviors in mice, including general locomotion. Another study using SCH-23390 also showed that it can induce catalepsy in

mice, which is defined as cessation of movement regardless of external stimuli (Chinen and Frusa-Filho, 1999), although at a much higher dose (0.1 mg/kg) than the one we used (0.003 mg/kg). Nevertheless, other studies have indicated that D1 receptor signaling is required for many aspects of locomotor activity, including cocaine-induced hyperactivity in mice (Cabib et. al., 1991) and rearing in rats placed in a novel cage (Dreher and Jackson, 1989). Importantly, suppression of activity caused by SCH-23390 was specific to the novel cage change with predator odor, as the mice returned to mostly normal levels of locomotor activity immediately upon return to the home cage, indicating an interaction between predator odor presence and/or the novel environment with the drug action. Although this result alone confounds the interpretation that D1 receptors are specifically important for grooming in the *Dbh -/-* mice, when combined with our data that flupentixol (D1 + D2 antagonist) suppresses grooming but not general locomotion, while L741-626 (D2 antagonist) has no effect, it strongly implicates the D1 receptor. In the future, site-specific infusions of D1-acting drugs into various target areas that mediate responses to predator odor stress can likely untangle the circuits for general locomotor activity and grooming. Past work by other members in our lab has shown that the medial amygdala (MeA) shows higher neuronal activity (as measured by c-fos protein induction) following predator odor exposure in *Dbh -/-* mice than *Dbh +/-* mice. Therefore, the MeA is a possible region where DA signaling mediates grooming behavior, particularly due to its connections to the olfactory bulb (Winans and Scalia, 1970; Scalia and Winans, 1975).

Additionally, we found that blockade of alpha-1 AR signaling in *Dbh +/-* mice decreased grooming. Given that DA is important for grooming behavior, and previous reports that DA can activate alpha-1 ARs under some conditions (Paladini et. al., 2001; Cilz et. al., 2014; Özkan et. al., 2017), we hypothesized that prazosin might be suppressing grooming by blocking DA

signaling through the alpha-1 AR. However, we found that prazosin had no effect on grooming or any other behavioral responses in *Dbh* -/- mice, refuting this idea. This is consistent with our previous finding that prazosin had no effect on the increased locomotor response to amphetamine in *Dbh* -/- mice (Weinshenker et. al., 2002). A previous study showed that centrally acting terazosin, which is also an alpha-1 antagonist, inhibited motor activity in mice in a novel cage (Stone et. al., 1999). It is possible that prazosin acted similarly to suppress overall motor activity, resulting in the decrease in grooming and digging in the *Dbh* +/- mice. Site-specific infusion of prazosin or terazosin into discrete brain regions may be required to determine the specific contribution of alpha-1 ARs to grooming vs digging in response to predator odor.

Our results indicate that NE signaling governs both exploratory and defensive burying/digging behaviors. Specifically, NE signaling through beta ARs contributes to light digging, while NE signaling through alpha-1, alpha-2, and beta ARs contributes to defensive burying. Our results also suggest that DA signaling through D1, but not D2, receptors contributes to excessive grooming.

Results from our study may have clinical implications, such as for *Toxoplasma gondii* infection. As a parasite that reproduces in cats as part of its life cycle, *T. gondii* impairs rodents' innate fear of cat odor, increasing the chance that the parasite reaches its host (Kannan et. al., 2010). Our results are consistent with this finding, indicating that loss of normal DBH function, as well as blockade of NE signaling, lead to a suppression of defensive behaviors that might increase a mouse's chance of escaping a predator. Early studies of *T. gondii* infection found decreased NE and increased DA in chronically infected animals (Stibbs, 1985). Infection by *T. gondii* has been shown to downregulate DBH in rats, resulting in chronic NE deficiency and excessive DA (Alsaady et. al., 2019). Humans can also be latently infected with *T. gondii*, and

individuals with schizophrenia and other neuropsychiatric disorders have been found to have higher levels of latent *T. gondii* infection than the general population (Torrey and Yolken, 2003). Altogether, our findings suggest that suppression of DBH might underlie the aberrant responses of rodents to predator threats; the loss of NE would remove the fearful/defensive responses, and the increased DA could even make predator odor pleasant or appetitive. We suggest further study of drugs that target NE and DA signaling as possible target therapies for Toxoplasma infection.

Additionally, although exposure to predator odor is not a full model of neuropsychiatric disorders such as obsessive-compulsive disorder, (OCD), Tourette's syndrome, and autism spectrum disorder (ASD), it may provide clues about the neurochemistry and neurocircuitry underlying repetitive behaviors that can be exacerbated by stress that are common in these disorders (Conelea and Woods, 2008; Adams et. al., 2018, Rodgers et. al., 2012, Gritti et. al., 2003). Integrating our results with our previous results and past literature, we propose that stressful situations activate LC neurons and increase NE transmission, exacerbating repetitive behaviors in people with TS, OCD, and ASD, and that anti-adrenergic drugs should be explored as potential therapeutics (Lustberg et. al., 2022).

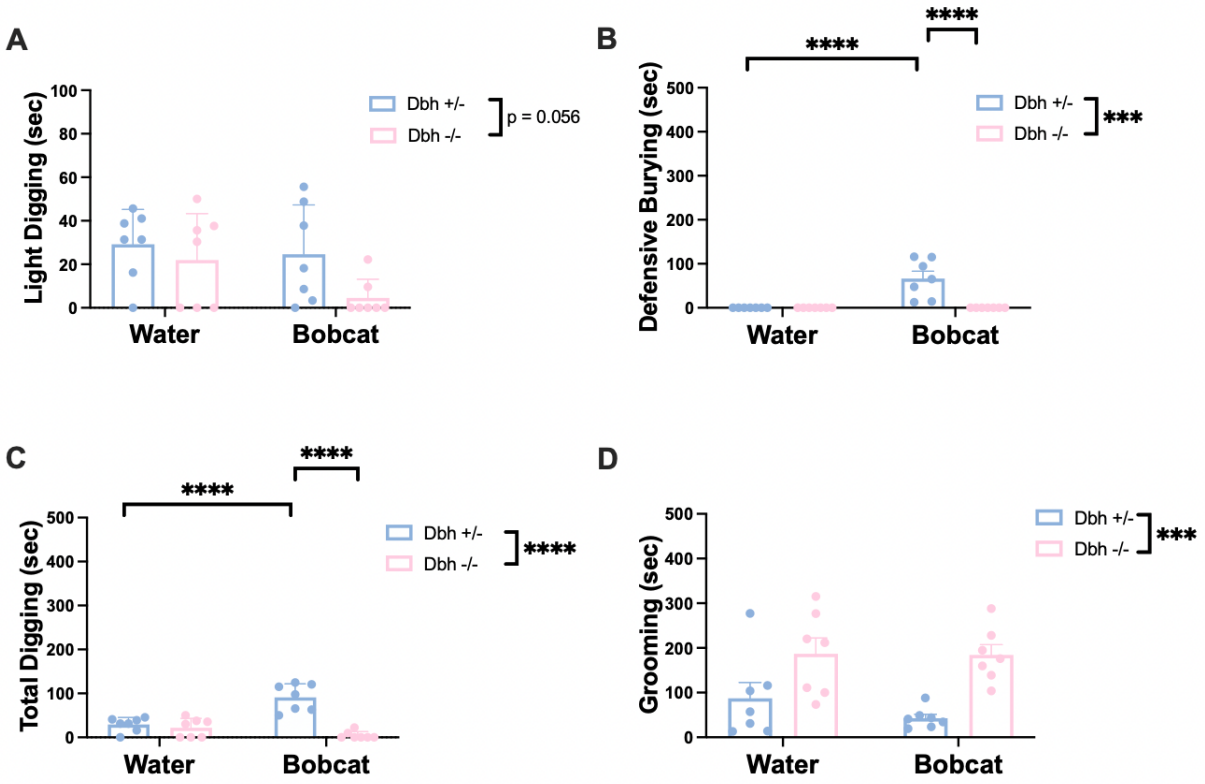

Figure 1. Effect of predator odor compared to water in a novel environment. Shown is time spent engaged in light digging (A), defensive burying (B), and total digging (C), which is the sum of light digging and defensive burying times. Shown is time spent engaged in grooming in (D). n = 14. Shown is mean ± SEM. *** $p<0.001$, ****$p<0.0001$.

Table 1: Comparison of *Dbh +/-* and *Dbh -/-* mice behavior in presence of predator odor vs. water

	Dbh +/-		*Dbh -/-*	
	Water	Bobcat	Water	Bobcat
Light Digging	—	—	—	—
Defensive Burying	—	↑	—	—
Total Digging	—	↑	—	—
Grooming	—	—	↑	↑

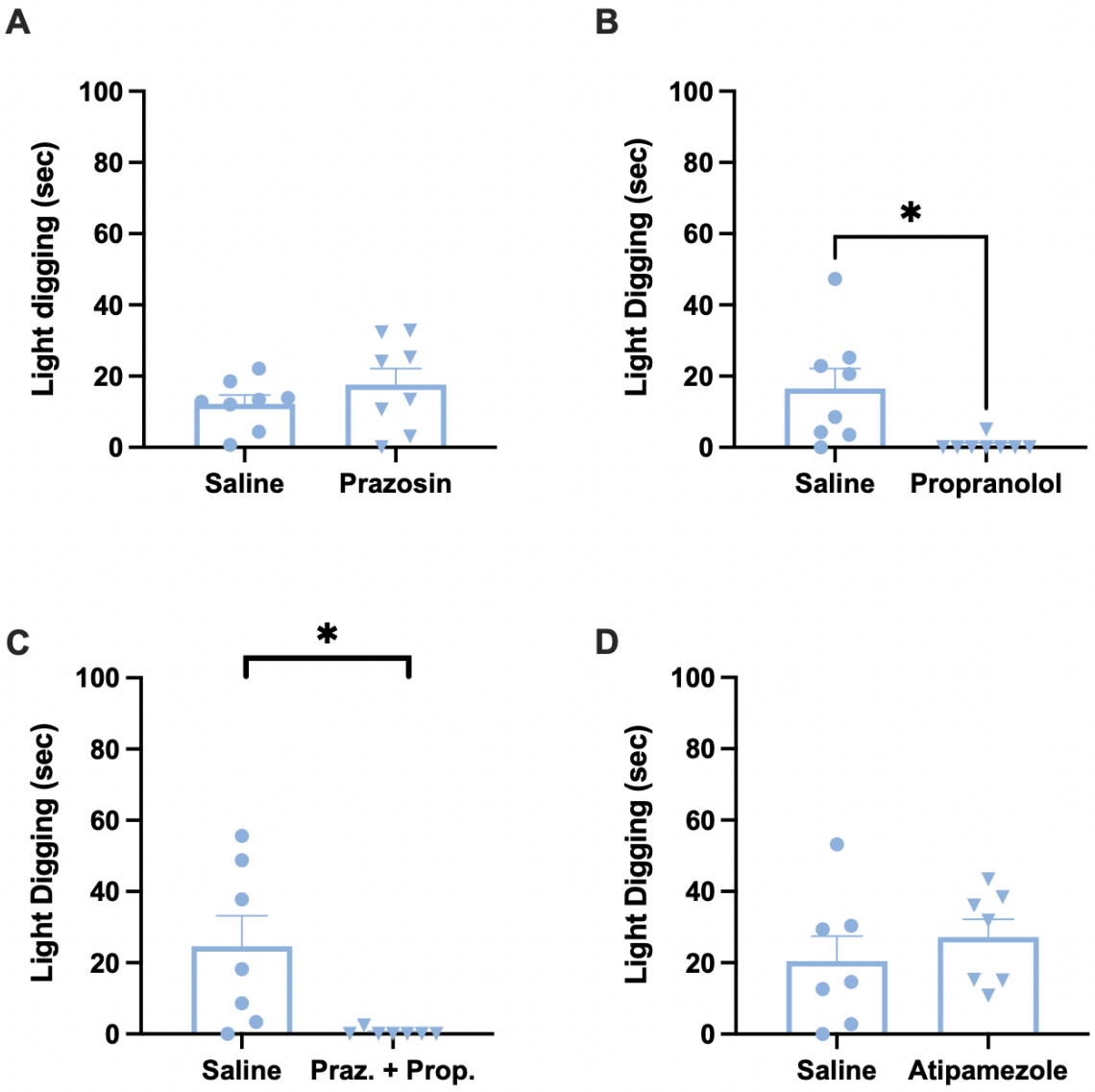

Figure 2. Effect of adrenergic receptor antagonists on light digging in the presence of predator odor in *Dbh* +/- mice. Shown is (A) α1AR antagonist prazosin, (B) βAR antagonist propranolol, (C) prazosin + propranolol, and (D) α2AR antagonist atipamezole. Shown is mean ± SEM. *p<0.05.

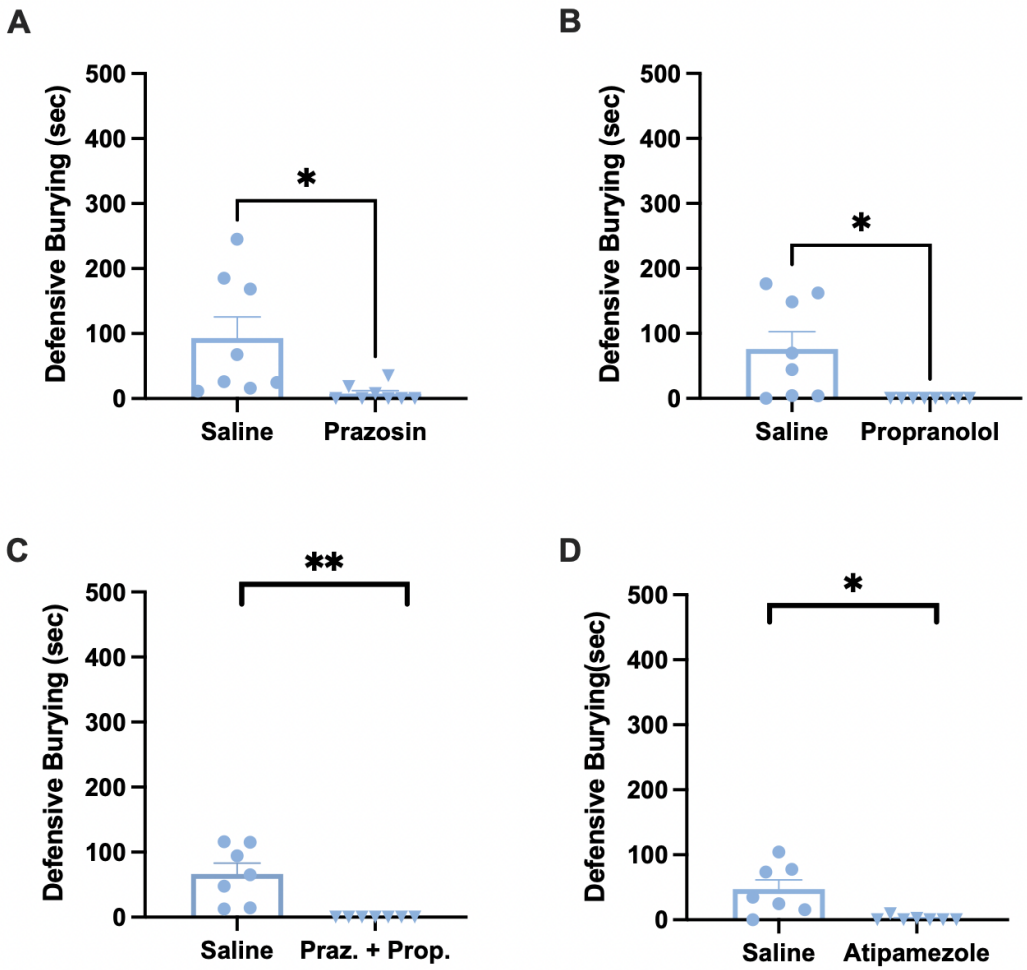

Figure 3. Effect of adrenergic receptor antagonists on defensive burying in the presence of predator odor in *Dbh* +/- mice. Shown is (A) α1AR antagonist prazosin, (B) βAR antagonist propranolol, (C) prazosin + propranolol, and (D) α2AR antagonist atipamezole. Shown is mean ± SEM. *p<0.05, **p<0.01.

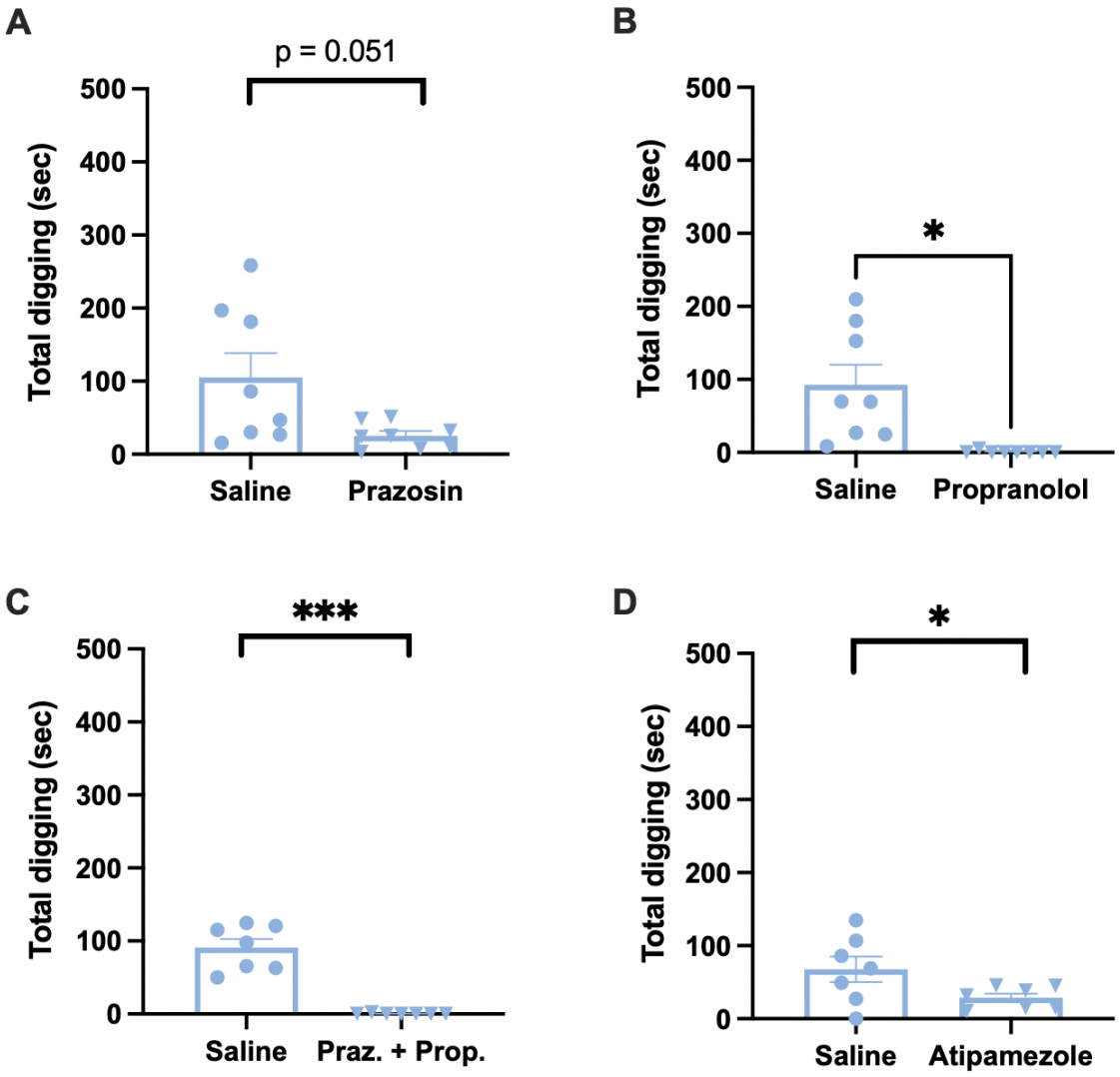

Figure 4. Effect of adrenergic receptor antagonists on total digging in the presence of predator odor in *Dbh* +/- mice. Shown is (A) α1AR antagonist prazosin, (B) βAR antagonist propranolol, (C) prazosin + propranolol, and (D) α2AR antagonist atipamezole. Shown is mean ± SEM. *p<0.05, ***p<0.001.

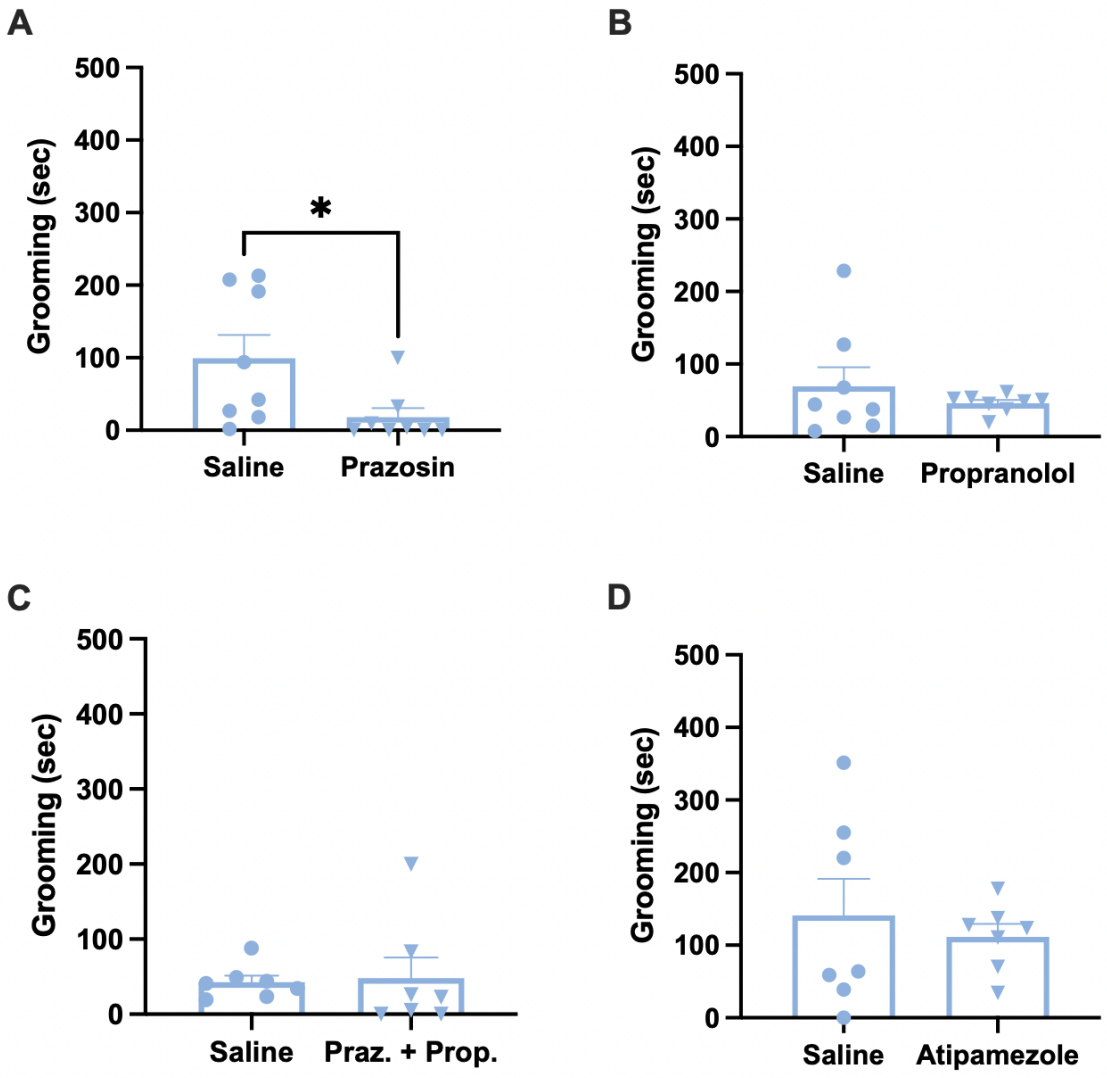

Figure 5. Effect of adrenergic receptor antagonists on grooming in the presence of predator odor in *Dbh* +/- mice. Shown is (A) α1AR antagonist prazosin, (B) βAR antagonist propranolol, (C) prazosin + propranolol, and (D) α2AR antagonist atipamezole. Shown is mean ± SEM. *p<0.05.

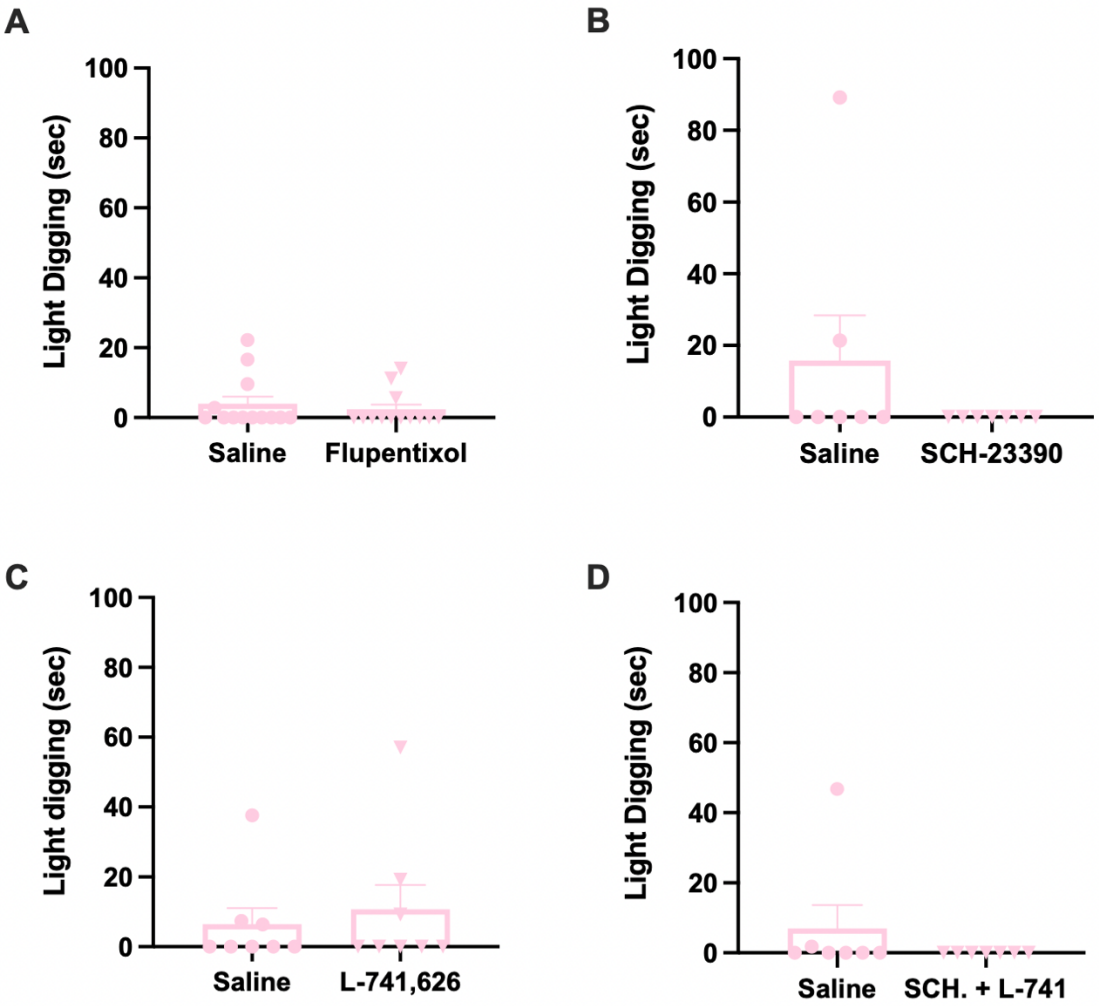

Figure 6. Effects of dopamine receptor antagonists on light digging in *Dbh* -/- mice in the presence of predator odor. Shown is (A) the non-selective DA receptor antagonist flupenthixol, (B) the D1 antagonist SCH-23390, (C) the D2 antagonist L-741,626, and (D) SCH-23390 + L-741,626. Shown is mean ± SEM.

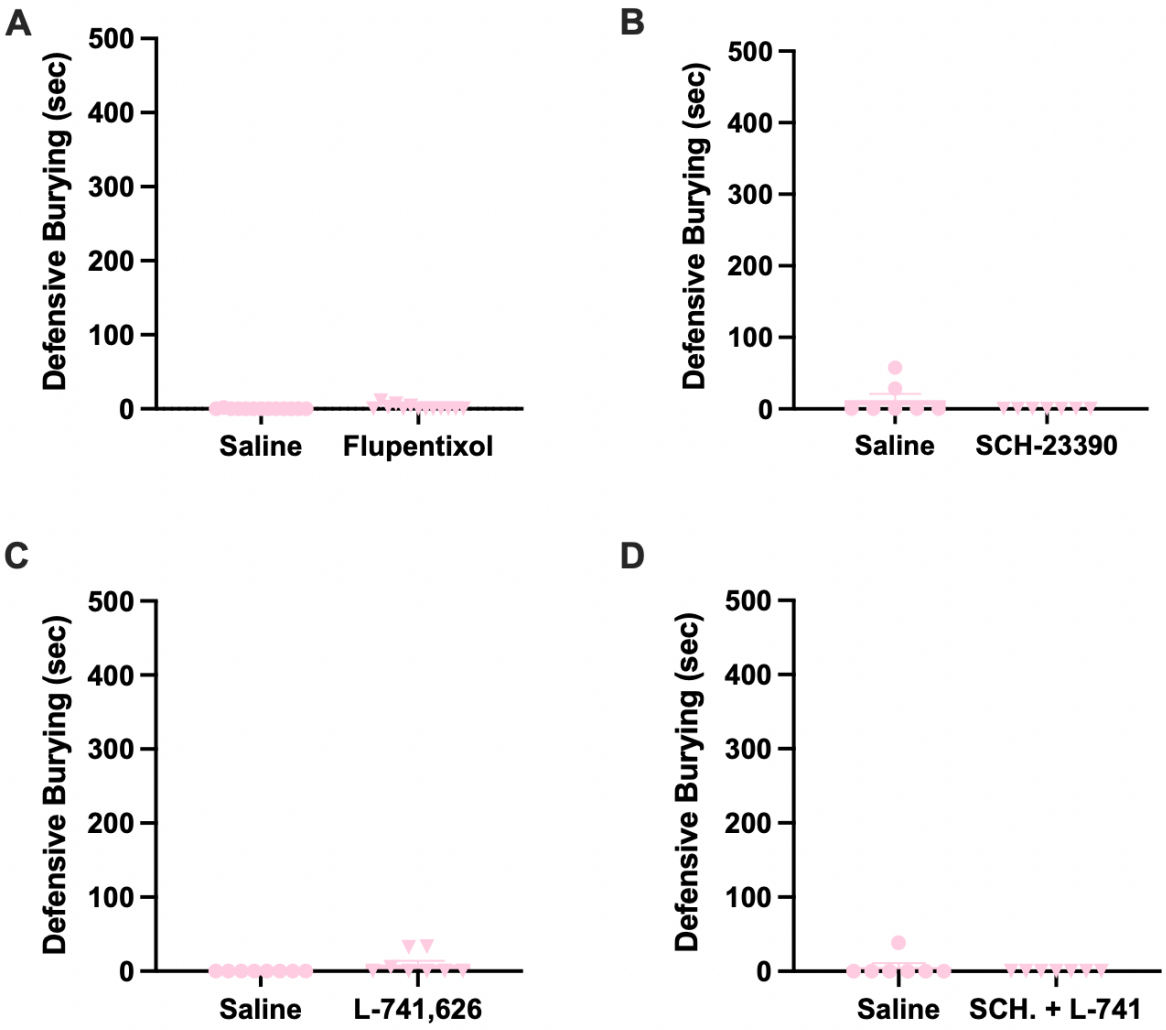

Figure 7. Effects of dopamine receptor antagonists on defensive burying in *Dbh -/-* mice in the presence of predator odor. Shown is (A) the non-selective DA receptor antagonist flupenthixol, (B) the D1 antagonist SCH-23390, C) the D2 antagonist L-741,626, and (D) SCH-23390 + L-741,626. Shown is mean ± SEM.

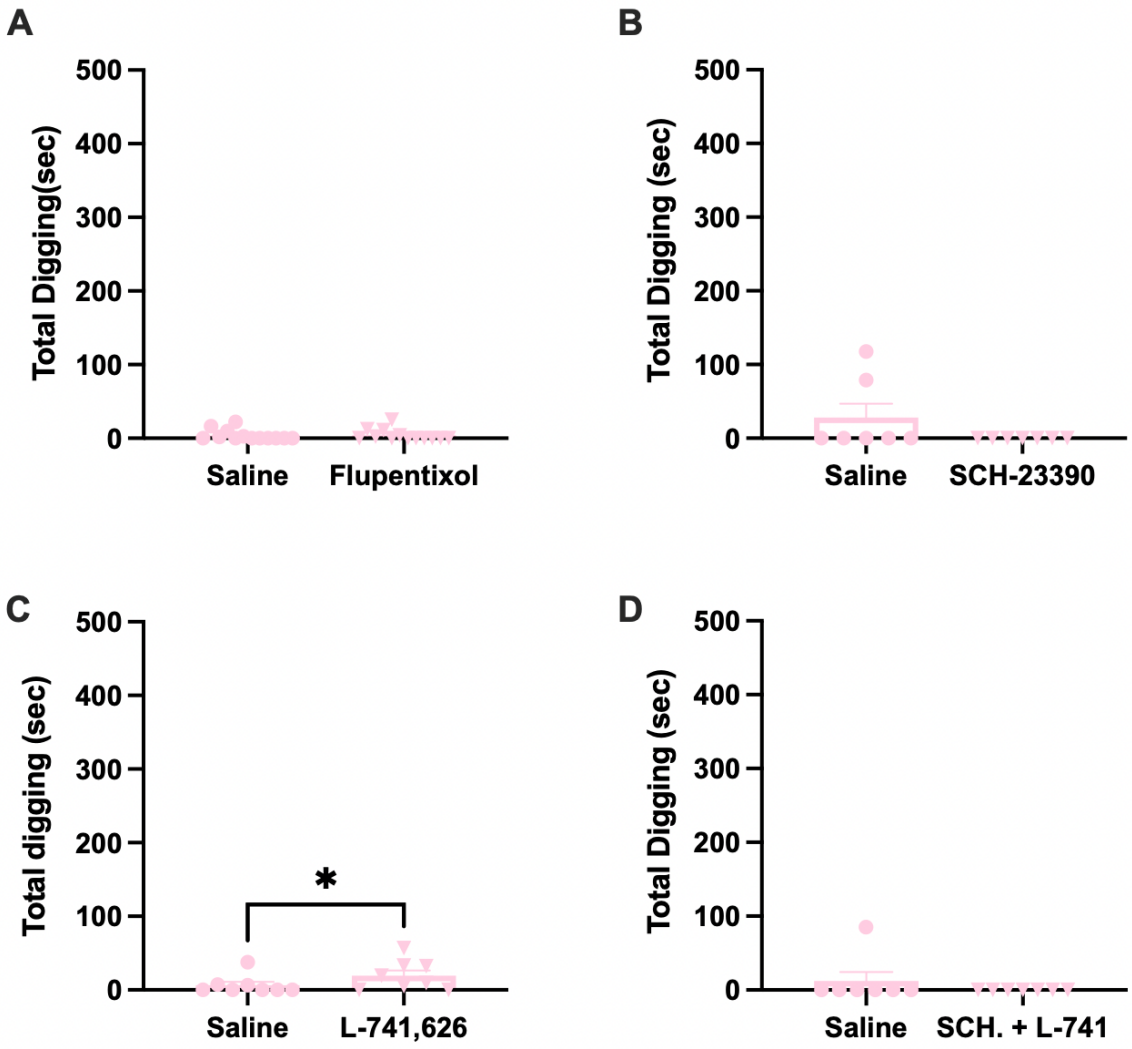

Figure 8. Effects of dopamine receptor antagonists on total digging in *Dbh -/-* mice in the presence of predator odor. Shown is (A) the non-selective DA receptor antagonist flupenthixol, (B) the D1 antagonist SCH-23390, C) the D2 antagonist L-741,626, and (D) SCH-23390 + L-741,626. Shown is mean ± SEM. *p<0.05.

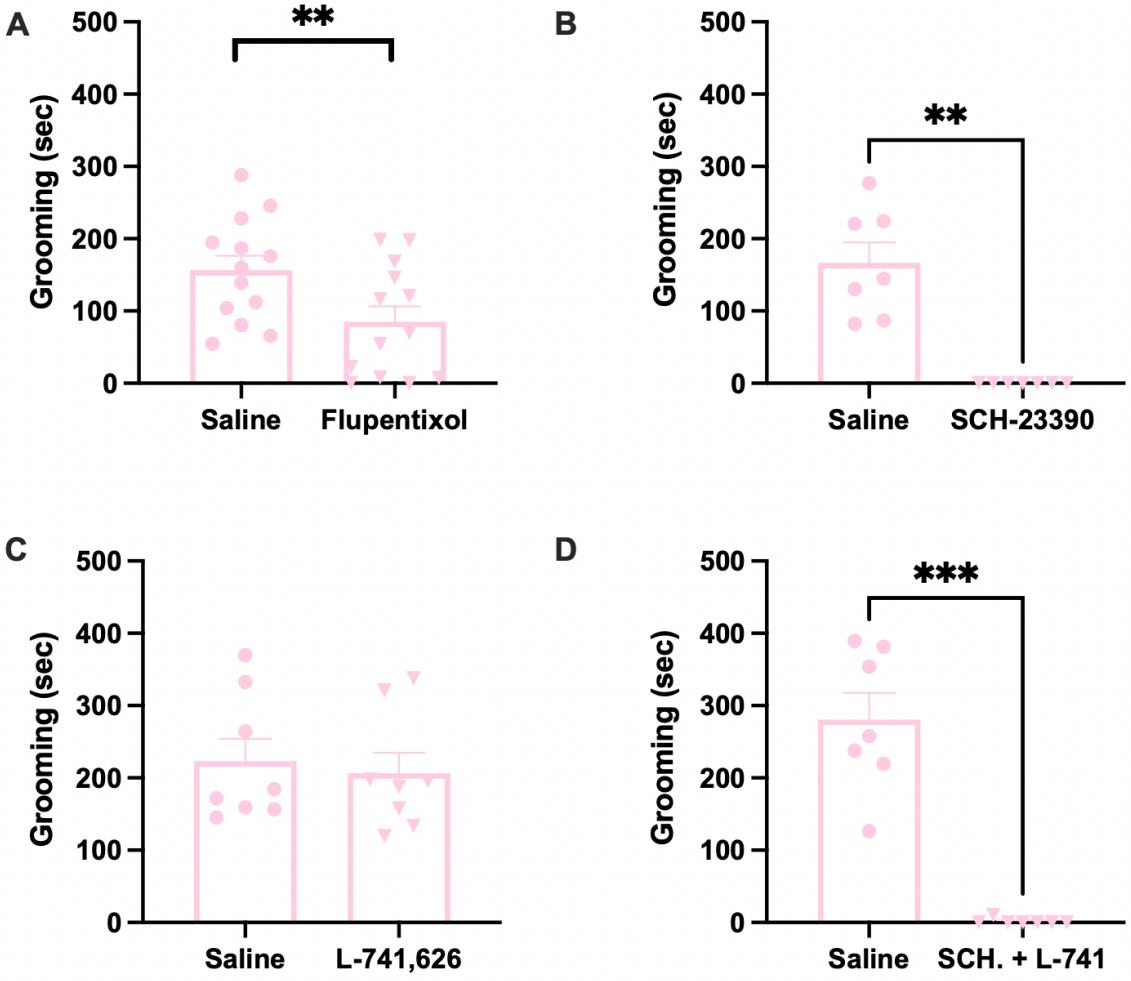

Figure 9. Effects of dopamine receptor antagonists on grooming in *Dbh* -/- mice in the presence of predator odor. Shown is (A) the non-selective DA receptor antagonist flupenthixol, (B) the D1 antagonist SCH-23390, (C) the D2 antagonist L-741,626, and (D) SCH-23390 + L-741,626. Shown is mean ± SEM. **p<0.01, ***p<0.001.

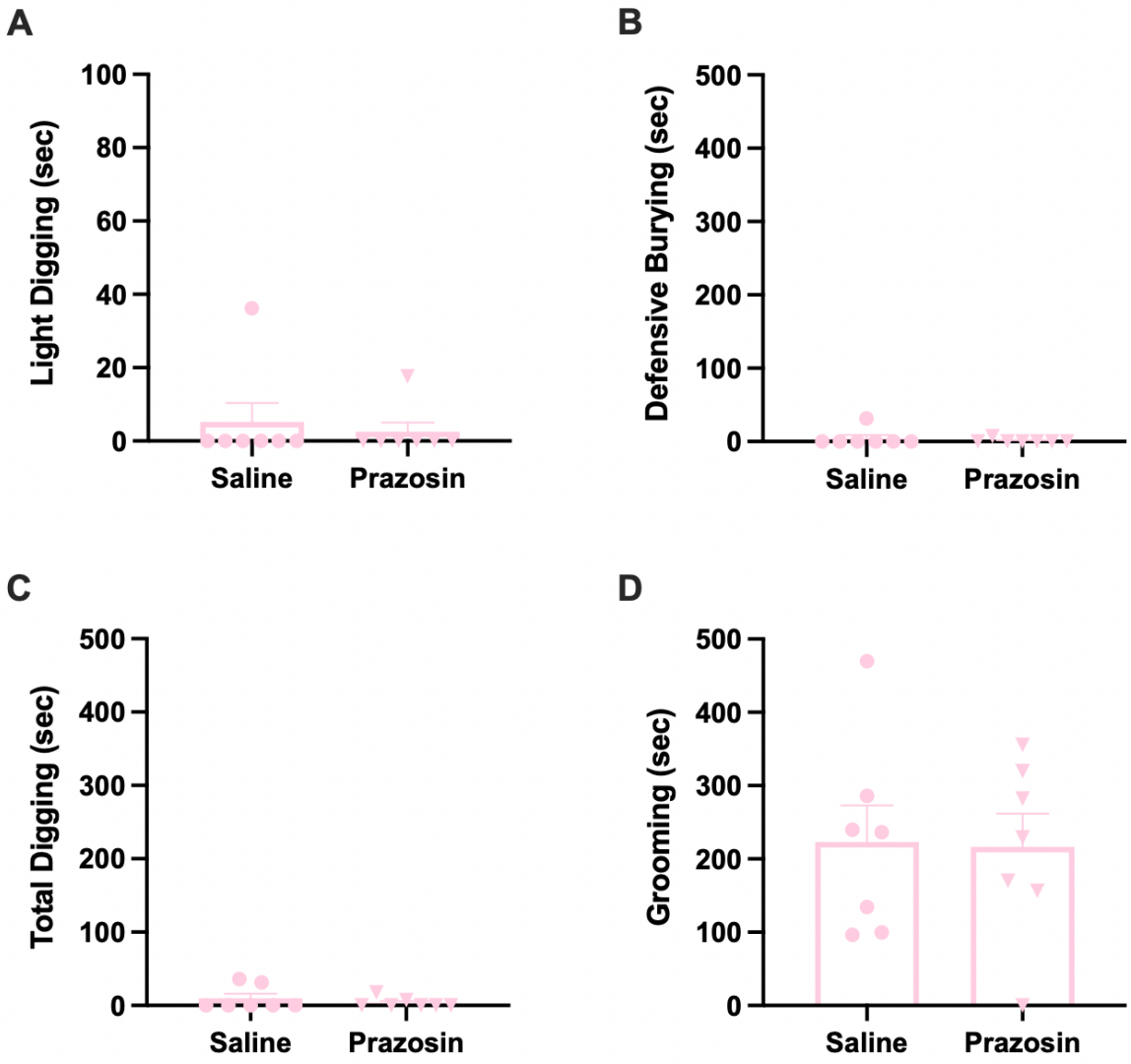

Figure 10. Effects of α1-adrenergic receptor antagonism receptor on behavior in *Dbh -/-* mice in the presence of predator odor. Shown is time spent (A) light digging, (B) defensive burying, (C) total digging, and (D) grooming. Shown is mean ± SEM.

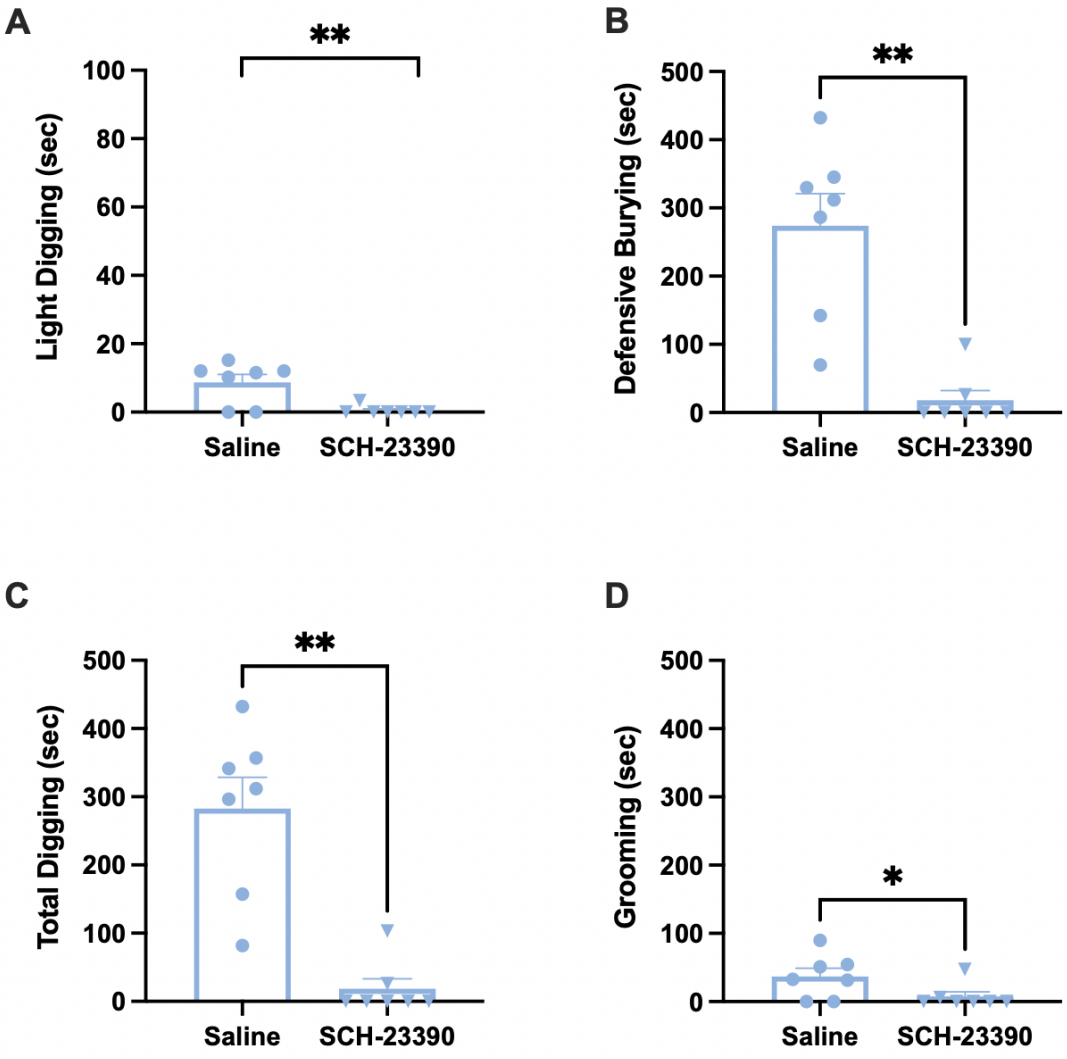

Figure 11. Effects of the D1 antagonist SCH-23390 on behaviors in *Dbh* +/- mice in the presence of predator odor. Shown is time spent (A) light digging, (B) defensive burying, (C) total digging, and (D) grooming. *p<0.05, **p<0.01.

References

1. Adams, T. G., Kelmendi, B., Brake, C. A., Gruner, P., Badour, C. L., & Pittenger, C. (2018). The role of stress in the pathogenesis and maintenance of obsessive-compulsive disorder. *Chronic Stress*, *2*, 247054701875804. https://doi.org/10.1177/2470547018758043
2. Aghajanian, G. K., & VanderMaelen, C. P. (1982). Alpha2-adrenoceptor-mediated hyperpolarization of locus coeruleus neurons: Intracellular studies in vivo. *Science*, *215*(4538), 1394–1396. https://doi.org/10.1126/science.6278591
3. Alsaady, I., Tedford, E., Alsaad, M., Bristow, G., Kohli, S., Murray, M., Reeves, M., Vijayabaskar, M. S., Clapcote, S. J., Wastling, J., & McConkey, G. A. (2019). Downregulation of the central noradrenergic system by *toxoplasma gondii* infection. *Infection and Immunity*, *87*(2). https://doi.org/10.1128/iai.00789-18
4. Arriza, J. L., Dawson, T. M., Simerly, R. B., Martin, L. J., Caron, M. G., Snyder, S. H., & Lefkowitz, R. J. (1992). The G-protein-coupled receptor kinases beta ARK1 and beta ark2 are widely distributed at synapses in rat brain. *The Journal of Neuroscience*, *12*(10), 4045–4055. https://doi.org/10.1523/jneurosci.12-10-04045.1992
5. Berridge, C. W., Mitton, E., Clark, W., & Roth, R. H. (1999). Engagement in a non-escape (displacement) behavior elicits a selective and lateralized suppression of frontal cortical dopaminergic utilization in stress. *Synapse*, *32*(3), 187–197. https://doi.org/10.1002/(sici)1098-2396(19990601)32:3<187::aid-syn5>3.0.co;2-9
6. Berridge, K. C., & Aldridge, J. W. (2000). Super-stereotypy I: Enhancement of a complex movement sequence by systemic dopamine D1 agonists. *Synapse*, *37*(3), 194–204. https://doi.org/10.1002/1098-2396(20000901)37:3<194::aid-syn3>3.0.co;2-a
7. Berridge, C. W., & Dunn, A. J. (1989). Restraint-stress-induced changes in exploratory behavior appear to be mediated by norepinephrine-stimulated release of CRF. *The Journal of Neuroscience*, *9*(10), 3513–3521. https://doi.org/10.1523/jneurosci.09-10-03513.1989
8. Blackburn, J. R., Pfaus, J. G., & Phillips, A. G. (1992). Dopamine functions in appetitive and defensive behaviours. *Progress in Neurobiology*, *39*(3), 247–279. https://doi.org/10.1016/0301-0082(92)90018-a
9. Bourdélat-Parks, B. N., Anderson, G. M., Donaldson, Z. R., Weiss, J. M., Bonsall, R. W., Emery, M. S., Liles, L. C., & Weinshenker, D. (2005). Effects of dopamine β-hydroxylase genotype and disulfiram inhibition on catecholamine homeostasis in mice. *Psychopharmacology*, *183*(1), 72–80. https://doi.org/10.1007/s00213-005-0139-8
10. Cabib, S., Castellano, C., Cestari, V., Filibeck, U., & Puglisi-Allegra, S. (1991). D1 and D2 receptor antagonists differently affect cocaine-induced locomotor hyperactivity in the mouse. *Psychopharmacology*, *105*(3), 335–339. https://doi.org/10.1007/bf02244427
11. Chabre, O., Conklin, B. R., Brandon, S., Bourne, H. R., & Limbird, L. E. (1994). Coupling of the alpha 2A-adrenergic receptor to multiple G-proteins. A simple approach for estimating receptor-G-protein coupling efficiency in a transient expression system. *Journal of Biological Chemistry*, *269*(8), 5730–5734. https://doi.org/10.1016/s0021-9258(17)37522-1

12. Chinen, C., & Frussa-Filho, R. (1999). Conditioning to injection procedures and repeated testing increase SCH 23390-induced catalepsy in mice. *Neuropsychopharmacology*, *21*(5), 670–678. https://doi.org/10.1016/s0893-133x(99)00061-5
13. Cilz, N. I., Kurada, L., Hu, B., & Lei, S. (2013). Dopaminergic modulation of GABAergic transmission in the entorhinal cortex: Concerted roles of 1 adrenoreceptors, inward rectifier K+, and T-type ca2+ channels. *Cerebral Cortex*, *24*(12), 3195–3208. https://doi.org/10.1093/cercor/bht177
14. Conelea, C. A., & Woods, D. W. (2008). The influence of contextual factors on TIC expression in Tourette's Syndrome: A review. *Journal of Psychosomatic Research*, *65*(5), 487–496. https://doi.org/10.1016/j.jpsychores.2008.04.010
15. Daniel, S. E., & Rainnie, D. G. (2015). Stress modulation of opposing circuits in the bed nucleus of the Stria terminalis. *Neuropsychopharmacology*, *41*(1), 103–125. https://doi.org/10.1038/npp.2015.178
16. De Boer, S. F., Slangen, J. L., & Van der Gugten, J. (1990). Plasma catecholamine and corticosterone levels during active and passive shock-prod avoidance behavior in rats: Effects of chlordiazepoxide. *Physiology & Behavior*, *47*(6), 1089–1098. https://doi.org/10.1016/0031-9384(90)90357-a
17. den Hartog, C. R., Blandino, K. L., Nash, M. K. L., Sjogren, E. R., Grampetro, M. A., Moorman, D. E., & Vazey, E. M. (2020). Noradrenergic tone mediates marble burying behavior after chronic stress and ethanol. *Psychopharmacology*, *237*(10), 3021–3031. https://doi.org/10.1007/s00213-020-05589-7
18. Dixit, P. V., Sahu, R., & Mishra, D. K. (2020). Marble-burying behavior test as a murine model of compulsive-like behavior. *Journal of Pharmacological and Toxicological Methods*, *102*, 106676. https://doi.org/10.1016/j.vascn.2020.106676
19. Do Monte, F. H., Canteras, N. S., Fernandes, D., Assreuy, J., & Carobrez, A. P. (2008). New Perspectives on β-adrenergic mediation of innate and learned fear responses to Predator Odor. *The Journal of Neuroscience*, *28*(49), 13296–13302. https://doi.org/10.1523/jneurosci.2843-08.2008
20. Dreher, J. K., & Jackson, D. M. (1989). Role of D1 and D2 dopamine receptors in mediating locomotor activity elicited from the nucleus accumbens of rats. *Brain Research*, *487*(2), 267–277. https://doi.org/10.1016/0006-8993(89)90831-7
21. Drago, F., Contarino, A., & Busà, L. (1999). The expression of neuropeptide-induced excessive grooming behavior in dopamine D1 and D2 receptor-deficient mice. *European Journal of Pharmacology*, *365*(2-3), 125–131. https://doi.org/10.1016/s0014-2999(98)00877-2
22. Endres, T., & Fendt, M. (2008). Inactivation of the lateral septum blocks fox odor-induced fear behavior. *NeuroReport*, *19*(6), 667–670. https://doi.org/10.1097/wnr.0b013e3282fb78d9
23. Ferrero, D. M., Lemon, J. K., Fluegge, D., Pashkovski, S. L., Korzan, W. J., Datta, S. R., Spehr, M., Fendt, M., & Liberles, S. D. (2011). Detection and avoidance of a carnivore odor by prey. *Proceedings of the National Academy of Sciences*, *108*(27), 11235–11240. https://doi.org/10.1073/pnas.1103317108
24. Finlay, J. M., Zigmond, M. J., & Abercrombie, E. D. (1995). Increased dopamine and norepinephrine release in medial prefrontal cortex induced by acute and chronic stress: Effects of diazepam. *Neuroscience*, *64*(3), 619–628. https://doi.org/10.1016/0306-4522(94)00331-x

25. Garbe, C. M., Kemble, E. D., & Rawleigh, J. M. (1993). Novel odors evoke risk assessment and suppress appetitive behaviors in mice. *Aggressive Behavior*, *19*(6), 447–454. https://doi.org/10.1002/1098-2337(1993)19:6<447::aid-ab2480190605>3.0.co;2-y
26. Grittim A., Bove, D., Di Sarno, A. M., D'Addio, A. A., Chiapparo, S., and Bove, R. M. (2003). Stereotyped movements in a group of autistic children. *Functional Neurology,* 18(2), 89-94.
27. Harris, G. C., & Aston-Jones, G. (1993). B-adrenergic antagonists attenuate withdrawal anxiety in cocaine-and morphine-dependent rats. *Psychopharmacology*, *113*(1), 131–136. https://doi.org/10.1007/bf02244345
28. Irwin, J., Ahluwalia, P., Zacharko, R. M., & Anisman, H. (1986). Central norepinephrine and plasma corticosterone following acute and chronic stressors: Influence of social isolation and handling. *Pharmacology Biochemistry and Behavior*, *24*(4), 1151–1154. https://doi.org/10.1016/0091-3057(86)90471-5
29. Janitzky, K., D′Hanis, W., Kröber, A., & Schwegler, H. (2015). TMT predator odor activated neural circuit in C57BL/6J mice indicates TMT-stress as a suitable model for uncontrollable intense stress. *Brain Research*, *1599*, 1–8. https://doi.org/10.1016/j.brainres.2014.12.030
30. Jhang, J., Lee, H., Kang, M. S., Lee, H.-S., Park, H., & Han, J.-H. (2018). Anterior cingulate cortex and its input to the basolateral amygdala control innate fear response. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-05090-y
31. Kannan, G., Moldovan, K., Xiao, J.-C., Yolken, R. H., Jones-Brando, L., & Pletnikov, M. V. (2010). Toxoplasma gondii strain-dependent effects on mouse behaviour. *Folia Parasitologica*, *57*(2), 151–155. https://doi.org/10.14411/fp.2010.019
32. Kedia, S., & Chattarji, S. (2014). Marble burying as a test of the delayed anxiogenic effects of acute immobilisation stress in mice. *Journal of Neuroscience Methods*, *233*, 150–154. https://doi.org/10.1016/j.jneumeth.2014.06.012
33. Kemble, E. D., & Bolwahnn, B. L. (1997). Immediate and long-term effects of novel odors on risk assessment in mice. *Physiology & Behavior*, *61*(4), 543–549. https://doi.org/10.1016/s0031-9384(96)00499-4
34. Ketenci, S., Acet, N. G., Sarıdoğan, G. E., Aydın, B., Cabadak, H., & Gören, M. Z. (2020). The neurochemical effects of prazosin treatment on fear circuitry in a rat traumatic stress model. *Clinical Psychopharmacology and Neuroscience*, 18(2), 219–230. https://doi.org/10.9758/cpn.2020.18.2.219
35. Korte, S. M., Buwalda, B., Bouws, G. A. H., Koolhaas, J. M., Maes, F. W., & Bohus, B. (1992). Conditioned neuroendocrine and cardiovascular stress responsiveness accompanying behavioral passivity and activity in aged and in Young rats. *Physiology & Behavior*, *51*(4), 815–822. https://doi.org/10.1016/0031-9384(92)90120-q
36. Kuhar MJ, Couceyro PR, Lambert PD. α- and β-Adrenergic Receptors. (1999). In: Siegel GJ, Agranoff BW, Albers RW, et al., editors. Basic Neurochemistry: Molecular, Cellular and Medical Aspects. 6th edition. Philadelphia: Lippincott-Raven. Available from: https://www.ncbi.nlm.nih.gov/books/NBK28138/
37. Lewis, M.H. and Bodfish, J.W. (1998), Repetitive behavior disorders in autism. Ment. Retard. Dev. *Disabil. Res. Rev*., 4: 80-89. https://doi.org/10.1002/(SICI)1098-2779(1998)4:2<80::AID-MRDD4>3.0.CO;2-0
38. Lewis, S. (2011). Grooming mice to model autism. *Nature Reviews Neuroscience*, *12*(5), 248–249. https://doi.org/10.1038/nrn3033

39. Londei, T., Valentini, A. M. V., & G. Leone, V. (1998). Investigative burying by laboratory mice may involve non-functional, compulsive, behaviour. *Behavioural Brain Research*, *94*(2), 249–254. https://doi.org/10.1016/s0166-4328(97)00162-9
40. Lustberg, D., Iannitelli, A. F., Tillage, R. P., Pruitt, M., Liles, L. C., & Weinshenker, D. (2020). Central norepinephrine transmission is required for stress-induced repetitive behavior in two rodent models of obsessive-compulsive disorder. *Psychopharmacology*, *237*(7), 1973–1987. https://doi.org/10.1007/s00213-020-05512-0
41. Lustberg, D., Tillage, R. P., Bai, Y., Pruitt, M., Liles, L. C., & Weinshenker, D. (2020). Noradrenergic circuits in the forebrain control affective responses to novelty. *Psychopharmacology*, *237*(11), 3337–3355. https://doi.org/10.1007/s00213-020-05615-8
42. Lustberg, D. J., Liu, J. Q., Iannitelli, A. F., Vanderhoof, S. O., Liles, L. C., McCann, K. E., & Weinshenker, D. (2022). Norepinephrine and dopamine contribute to distinct repetitive behaviors induced by novel odorant stress in male and female mice. *Hormones and Behavior*, *144*, e105205. https://doi.org/10.1016/j.yhbeh.2022.105205
43. Meltzer, H. Y., & Stahl, S. M. (1976). The dopamine hypothesis of schizophrenia: A review. *Schizophrenia Bulletin, 2*(1), 19–76. https://doi.org/10.1093/schbul/2.1.19
44. Miura, H., Qiao, H., & Ohta, T. (2002). Attenuating effects of the isolated rearing condition on increased brain serotonin and dopamine turnover elicited by novelty stress. *Brain Research*, *926*(1-2), 10–17. https://doi.org/10.1016/s0006-8993(01)03201-2
45. Murray, A. M., & Waddington, J. L. (1989). The induction of grooming and vacuous chewing by a series of selective D-1 dopamine receptor agonists: Two directions of D-1:D-2 interaction. *European Journal of Pharmacology*, *160*(3), 377–384. https://doi.org/10.1016/0014-2999(89)90093-9
46. Murchison, C. F., Zhang, X.-Y., Zhang, W.-P., Ouyang, M., Lee, A., & Thomas, S. A. (2004). A distinct role for norepinephrine in memory retrieval. *Cell*, *117*(1), 131–143. https://doi.org/10.1016/s0092-8674(04)00259-4
47. Neve, K.A. (2013). Dopamine Receptors. In: Encyclopedia of Biological Chemistry: Second Edition. Elsevier Inc, pp. 169–173. doi:10.1016/B978-0-12-378630-2. 00326-1.
48. Nordstrom, E. J., Bittner, K. C., McGrath, M. J., Parks, C. R., & Burton, F. H. (2015). "Hyperglutamatergic Cortico-Striato-Thalamo-Cortical Circuit" Breaker Drugs Alleviate tics in a Transgenic Circuit Model of Tourette's Syndrome. *Brain Research*, *1629*, 38–53. https://doi.org/10.1016/j.brainres.2015.09.032
49. Nordstrom, E. J., & Burton, F. H. (2002). A transgenic model of comorbid Tourette's syndrome and obsessive-compulsive disorder circuitry. *Molecular Psychiatry*, *7*(6), 617–625. https://doi.org/10.1038/sj.mp.4001144
50. Olson, V. G., Rockett, H. R., Reh, R. K., Redila, V. A., Tran, P. M., Venkov, H. A., DeFino, M. C., Hague, C., Peskind, E. R., Szot, P., & Raskind, M. A. (2011). The role of norepinephrine in differential response to stress in an animal model of posttraumatic stress disorder. *Biological Psychiatry*, *70*(5), 441–448. https://doi.org/10.1016/j.biopsych.2010.11.029
51. Özkan, M., Johnson, N. W., Sehirli, U. S., Woodhall, G. L., & Stanford, I. M. (2017). Dopamine acting at D1-like, D2-like and α1-adrenergic receptors differentially modulates theta and gamma oscillatory activity in primary motor cortex. *PLOS ONE*, *12*(7). https://doi.org/10.1371/journal.pone.0181633

52. Paladini, C. A., Fiorillo, C. D., Morikawa, H., & Williams, J. T. (2001). Amphetamine selectively blocks inhibitory glutamate transmission in dopamine neurons. *Nature Neuroscience*, *4*(3), 275–281. https://doi.org/10.1038/85124
53. Rodgers, J., Glod, M., Connolly, B., & McConachie, H. (2012). The relationship between anxiety and repetitive behaviours in autism spectrum disorder. *Journal of Autism and Developmental Disorders*, *42*(11), 2404–2409. https://doi.org/10.1007/s10803-012-1531-y
54. Scalia, F., & Winans, S. S. (1975). The differential projections of the olfactory bulb and accessory olfactory bulb in mammals. *The Journal of Comparative Neurology*, *161*(1), 31–55. https://doi.org/10.1002/cne.901610105
55. Schank, J. R., Liles, L. C., & Weinshenker, D. (2008). Norepinephrine signaling through β-adrenergic receptors is critical for expression of cocaine-induced anxiety. *Biological Psychiatry*, *63*(11), 1007–1012. https://doi.org/10.1016/j.biopsych.2007.10.018
56. Shanks, N., Zalcman, S., Zacharko, R. M., & Anisman, H. (1991). Alterations of central norepinephrine, dopamine and serotonin in several strains of mice following acute stressor exposure. *Pharmacology Biochemistry and Behavior*, *38*(1), 69–75. https://doi.org/10.1016/0091-3057(91)90591-o
57. Smolinsky, A. N., Bergner, C. L., LaPorte, J. L., & Kalueff, A. V. (2009). Analysis of grooming behavior and its utility in studying animal stress, anxiety, and Depression. *Mood and Anxiety Related Phenotypes in Mice*, 21–36. https://doi.org/10.1007/978-1-60761-303-9_2
58. Stibbs H.H. (1985). Changes in brain concentrations of catecholamines and indoleamines in Toxoplasma gondii infected mice. *Ann Trop Med Parasitol,* 79(2):153-7. doi: 10.1080/00034983.1985.11811902. PMID: 2420295.
59. Stone, E. A., Zhang, Y., Rosengarten, H., Yeretsian, J., & Quartermain, D. (1999). Brain alpha 1-adrenergic neurotransmission is necessary for behavioral activation to environmental change in mice. *Neuroscience*, *94*(4), 1245–1252. https://doi.org/10.1016/s0306-4522(99)00394-2
60. Szot, P., Weinshenker, D., White, S. S., Robbins, C. A., Rust, N. C., Schwartzkroin, P. A., & Palmiter, R. D. (1999). Norepinephrine-deficient mice have increased susceptibility to seizure-inducing stimuli. *The Journal of Neuroscience*, *19*(24), 10985–10992. https://doi.org/10.1523/jneurosci.19-24-10985.1999
61. Thomas, S. A., Marck, B. T., Palmiter, R. D., & Matsumoto, A. M. (2002). Restoration of norepinephrine and reversal of phenotypes in mice lacking dopamine β-hydroxylase. *Journal of Neurochemistry*, *70*(6), 2468–2476. https://doi.org/10.1046/j.1471-4159.1998.70062468.x
62. Tillage, R. P., Foster, S. L., Lustberg, D., Liles, L. C., McCann, K. E., & Weinshenker, D. (2021). Co-released norepinephrine and Galanin Act on different timescales to promote stress-induced anxiety-like behavior. *Neuropsychopharmacology*, *46*(8), 1535–1543. https://doi.org/10.1038/s41386-021-01011-8
63. Tillage, R. P., Wilson, G. E., Liles, L. C., Holmes, P. V., & Weinshenker, D. (2020). Chronic environmental or genetic elevation of galanin in noradrenergic neurons confers stress resilience in mice. *The Journal of Neuroscience*, *40*(39), 7464–7474. https://doi.org/10.1523/jneurosci.0973-20.2020

64. Timmermans P.B,, van Zwieten P.A. (1982). alpha 2 adrenoceptors: Classification, Localization, Mechanisms, and Targets for Drugs. *J Med Chem*, 25(12):1389-401. doi: 10.1021/jm00354a001.
65. Torrey, E. F., & Yolken, R. H. (2003). Toxoplasma gondii and schizophrenia. *Emerging Infectious Diseases*, *9*(11), 1375–1380. https://doi.org/10.3201/eid0911.030143
66. Valenti, O., Lodge, D. J., & Grace, A. A. (2011). Aversive stimuli alter ventral tegmental area dopamine neuron activity via a common action in the ventral hippocampus. *The Journal of Neuroscience*, *31*(11), 4280–4289. https://doi.org/10.1523/jneurosci.5310-10.2011
67. Weinshenker, D., Miller, N. S., Blizinsky, K., Laughlin, M. L., & Palmiter, R. D. (2002). Mice with chronic norepinephrine deficiency resemble amphetamine-sensitized animals. *Proceedings of the National Academy of Sciences*, *99*(21), 13873–13877. https://doi.org/10.1073/pnas.212519999
68. Welch, J. M., Lu, J., Rodriguiz, R. M., Trotta, N. C., Peca, J., Ding, J.-D., Feliciano, C., Chen, M., Adams, J. P., Luo, J., Dudek, S. M., Weinberg, R. J., Calakos, N., Wetsel, W. C., & Feng, G. (2007). Cortico-striatal synaptic defects and OCD-like behaviours in SAPAP3-mutant mice. *Nature*, *448*(7156), 894–900. https://doi.org/10.1038/nature06104
69. Winans, S. S., & Scalia, F. (1970). Amygdaloid nucleus: New Afferent input from the Vomeronasal Organ. *Science*, *170*(3955), 330–332. https://doi.org/10.1126/science.170.3955.330
70. Wood, J., LaPalombara, Z., & Ahmari, S. E. (2018). Monoamine abnormalities in the SAPAP3 knockout model of obsessive-compulsive disorder-related behaviour. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1742), 20170023. https://doi.org/10.1098/rstb.2017.0023
71. Wu, D., Katz, A., Lee, C. H., & Simon, M. I. (1992). Activation of phospholipase C by alpha 1-adrenergic receptors is mediated by the alpha subunits of GQ family. *Journal of Biological Chemistry*, *267*(36), 25798–25802. https://doi.org/10.1016/s0021-9258(18)35680-1