Central norepinephrine transmission is required for stress-induced repetitive behavior in two rodent models of obsessive-compulsive disorder

*Daniel Lustberg¹, Alexa Iannitelli¹, Rachel P. Tillage¹, Molly Pruitt², L. Cameron Liles¹, David Weinshenker¹**

Affiliations:

¹Emory University, Department of Human Genetics, Atlanta, GA 30322

²University of Maryland School of Medicine, Baltimore, MD 21201

*Address correspondence to:

David Weinshenker, Ph.D.

Department of Human Genetics

Emory University School of Medicine

615 Michael St., Whitehead 301

Atlanta, GA 30322

Email: dweinsh@emory.edu

Acknowledgments: We thank Lundbeck for providing the DOPS, Synosia Therapeutics for providing the nepicastat, and C. Strauss for helpful editing of the manuscript. This work was supported by the National Institutes of Health (AG061175, NS102306, and DA038453 to DW; GM8602-22 to DL; MH116622 to RPT).

Conflicts of Interest: DW is co-inventor on a patent concerning the use of selective dopamine β -hydroxylase inhibitors for treatment of cocaine dependence (US-2010-0105748-A1; “Methods and Compositions for Treatment of Drug Addiction”). The other authors declare no conflicts of interest.

Abstract

Rationale: Obsessive-compulsive disorder (OCD) is characterized by repetitive behaviors exacerbated by stress. Many OCD patients do not respond to available pharmacotherapies, but neurosurgical ablation of the anterior cingulate cortex (ACC) can provide symptomatic relief. Although the ACC receives noradrenergic innervation and expresses adrenergic receptors (ARs), the involvement of norepinephrine (NE) in OCD has not been investigated.

Objective: To determine the effects of genetic or pharmacological disruption of NE neurotransmission on marble burying (MB) and nestlet shredding (NS) in two animal models of OCD.

Methods: We assessed NE-deficient (*Dbh* ^{-/-}) mice and NE-competent (*Dbh* ^{+/-}) controls in MB and NS tasks. We also measured the effects of anti-adrenergic drugs on NS and MB in control mice and the effects of pharmacological restoration of central NE in *Dbh* ^{-/-} mice. Finally, we compared c-fos induction in the locus coeruleus (LC) and ACC of *Dbh* ^{-/-} and control mice following both tasks.

Results: *Dbh* ^{-/-} mice virtually lacked MB and NS behaviors seen in control mice but did not differ in the elevated zero maze (EZM) model of general anxiety-like behavior. Pharmacological restoration of central NE synthesis in *Dbh* ^{-/-} mice completely rescued NS behavior, while NS and MB were suppressed in control mice by anti-adrenergic drugs. Expression of c-fos in the ACC was attenuated in *Dbh* ^{-/-} mice after MB and NS.

Conclusion: These findings support a role for NE transmission to the ACC in the expression of stress-induced compulsive behaviors and suggest further evaluation of anti-adrenergic drugs for OCD is warranted.

Keywords: norepinephrine; dopamine β-hydroxylase; obsessive-compulsive disorder; nestlet shredding; marble burying; guanfacine; adrenergic receptor; c-fos; locus coeruleus; anterior cingulate cortex; anxiety

Introduction

In patients with obsessive-compulsive disorder (OCD), intrusive thoughts drive repetitive and compulsive behaviors, among them checking, counting, and excessive hand washing. These obsessions are emotionally aversive and disproportionately command attentional resources, until the ritual that interrupts the obsession is completed ([Graybiel and Rauch 2000](#); [Pigott et al. 1994](#)). OCD is highly comorbid with other psychiatric disorders, such as attention deficit hyperactivity disorder (ADHD), anxiety, and Tourette's syndrome ([Abramovitch et al. 2015](#); [Carter et al. 2004](#); [Eapen and Robertson 2000](#); [Lombroso et al. 2008](#)). In OCD and other compulsive disorders, symptom severity increases during periods of psychological stress ([Adams et al. 2018](#); [Lin et al. 2007](#); [Rosso et al. 2012](#)). The neural circuitry and neurochemistry that drive compulsive behaviors have not been clearly delineated ([Micallef and Blin 2001](#); [Stein 2000](#)), but converging evidence from neuroimaging, neurosurgical, and neurobiological studies implicates a corticolimbic interface structure called the anterior cingulate cortex (ACC) in the pathophysiology of OCD ([Alarcon et al. 1994](#); [Brennan et al. 2013](#); [Fitzgerald et al. 2005](#); [McGovern and Sheth 2017](#)).

Data from human functional imaging studies have frequently identified ACC hyperactivity in OCD patients ([De Ridder et al. 2017](#); [Fitzgerald et al. 2005](#); [Riffkin et al. 2005](#); [Van Laere et al. 2006](#)). Both functional and structural abnormalities in the ACC of patients with OCD are correlated with disturbances in emotional reactivity, attention, and cognitive control ([Brennan et al. 2015](#); [McGovern and Sheth 2017](#); [Pauls et al. 2014](#)). The ACC receives glutamatergic and neuromodulatory input from a distributed network of forebrain and hindbrain structures and is well positioned to regulate attention, emotions, motivation, and action ([Margulies et al. 2007](#); [Yücel et al. 2003](#)). For instance, the ACC sends glutamatergic projections to the striatum to initiate voluntary behavior, and ACC projections to the orbitofrontal and medial prefrontal cortex support stimulus evaluation, decision-making, and behavioral flexibility under conditions of uncertainty or change ([Riffkin et al. 2005](#); [Stevens et al. 2011](#)). Findings from human and animal studies also demonstrate a functional role for the ACC in the response to environmental novelty ([Krebs et al. 2013](#); [Montag-Sallaz et al. 1999](#); [Struthers et al. 2005](#); [Tulving et al. 1994](#); [Weible et al. 2009](#)). In addition, neurons in the ACC may encode the aversive emotional component of pain sensation ([Navratilova et al. 2015](#); [Rainville et al. 1997](#); [Shackman et al. 2011](#)), and multiple studies have reported that hyperactivity of the ACC is associated with other aversive affective states ([Hayes and Northoff 2011](#); [Johansen and Fields 2004](#); [Stevens et al. 2011](#)).

Evidence from the neurosurgical literature suggests that ~40% of antidepressant-resistant OCD patients can achieve lasting remission of symptoms by undergoing a surgical procedure called a cingulotomy ([Brown et al. 2016](#); [Kim et al. 2003](#); [McGovern and Sheth 2017](#)), in which the ACC is lesioned bilaterally with gamma radiation ([Cosgrove and Rauch 2003](#); [Fodstad et al. 1982](#)). Despite the efficacy of this intervention for improving the quality of life in some treatment-resistant OCD patients, invasive surgical ablation of an important brain region

is an aggressive and irreversible treatment strategy that should only be considered as a last resort after pharmacological interventions have failed. Indeed, cingulotomy is associated with significant long-term cognitive and emotional side effects, including emotional apathy and deficits in attention ([Cohen et al. 1999a](#); [Cohen et al. 1999b](#); [Janer and Pardo 1991](#)).

Currently, the only drugs approved for the management of OCD are serotonergic antidepressants (tricyclics and selective serotonin reuptake inhibitors; SSRIs), which are ineffective for about half of patients and unfortunately associated with adverse side effects that limit compliance ([Dominguez 1992](#); [Mavissakalian et al. 1983](#); [Pizarro et al. 2014](#); [van Balkom et al. 1994](#)). Notably, very high antidepressant doses are required to improve OCD symptoms, and the latency for antidepressants to become effective is even longer for OCD patients than for patients with anxiety disorders or major depression ([Dougherty et al. 2004](#); [Fineberg and Gale 2005](#); [Pittenger and Bloch 2014](#)).

Because of the modest efficacy of SSRIs in alleviating OCD symptoms, most preclinical research on OCD pathophysiology has focused on the serotonin (5-HT) system and, to a lesser extent, the dopaminergic and glutamatergic systems ([Billett et al. 1998](#); [Goodman et al. 1990](#); [Pittenger et al. 2011](#); [Pittenger et al. 2006](#); [Zohar et al. 2000](#)). The development of more effective and tolerable pharmacotherapies has been impeded by an incomplete understanding of the neurobiological basis of OCD and other compulsive disorders ([Langen et al. 2011](#); [Micallef and Blin 2001](#); [Ting and Feng 2011](#)). Thus, there is an urgent need to develop and characterize better preclinical models of OCD to unravel the neurochemistry and neurocircuitry that drive compulsive behaviors.

Some advances in understanding the neurobiology of OCD have come from genetic and behavioral models of the disease, which have various degrees of face, construct, and predictive validity ([Ahmari et al. 2015](#); [Fineberg et al. 2011](#); [Joel 2006](#); [Ting and Feng 2011](#); [Witkin 2008](#)). The nestlet shredding (NS) and marble burying (MB) tasks are two rodent models of repetitive, compulsive behaviors that may be useful for testing novel pharmacotherapies for OCD ([Angoa-Pérez et al. 2013](#); [Li et al. 2006](#); [Wolmarans et al. 2016](#)). In these tasks, repetitive behaviors (digging for MB; shredding for NS) are elicited by cage-change stress and do not habituate even after repeated daily exposures to marbles or nestlets ([Thomas et al. 2009](#); [Witkin 2008](#)).

Multiple studies have found that routine laboratory animal husbandry practices increase serum corticosterone levels and other physiological measures of stress ([Balcombe et al. 2004](#); [Okano et al. 2005](#); [Rasmussen et al. 2011](#); [Schultz 1972](#)). Transferring mice to a clean cage or gently handling them with gloves, although seemingly innocuous, are associated with marked changes in affective behavior when animals are tested on the same day ([Rasmussen et al. 2011](#)). Sympathetic activation and endocrine stress responses to these procedures also do not habituate over a 15-day period ([Balcombe et al. 2004](#)).

Digging and nest building are normal rodent behaviors that are augmented by stress ([Deacon 2006](#); [Kedia and Chattarji 2014](#); [Schultz 1972](#); [Umathe et al. 2008](#)). The fact that these behaviors do not habituate lends face validity to both tasks and distinguishes them from canonical models of novelty-induced anxiety ([Handley 1991](#); [Witkin 2008](#); [Wolmarans et al. 2016](#)). The construct validities of MB and NS are supported by studies using mice with genetic 5-HT depletion (*Tph2*^{-/-}). These mice display exaggerated MB and NS behaviors ([Angoa-Pérez et al. 2013](#); [Angoa-Pérez et al. 2012](#); [Kane et al. 2012](#)). MB and NS also show predictive validity in pharmacological experiments; SSRIs and tricyclic antidepressants reduce these repetitive behaviors ([Jimenez-Gomez et al. 2011](#); [Li et al. 2006](#)).

The central norepinephrine (NE) system mediates stress responses, attention, arousal, emotional state, and behavioral flexibility ([Aston-Jones et al. 2007](#); [Sara 2009](#)), all of which are disrupted in patients with OCD ([Adams et al. 2018](#); [Cocchi et al. 2012](#); [Gehring et al. 2000](#); [Kalanthorff et al. 2016](#); [Spitznagel and Suhr 2002](#)). The 5-HT and NE neuromodulatory systems regulate one another ([Kim et al. 2004](#); [O'Leary et al. 2007](#); [Pasquier et al. 1977](#); [Pudovkina et al. 2002](#); [Segal 1979](#)) and have overlapping terminal fields in forebrain regions, such as the ACC, where 5-HT exerts an inhibitory influence ([Czyrak et al. 2003](#); [Hajós et al. 2003](#); [Tanaka and North 1993](#)) and NE exerts an excitatory influence ([Berridge et al. 1993](#); [Gompf et al. 2010](#); [Marzo et al. 2014](#); [Stone et al. 2006](#)). Moreover, reciprocal excitatory communication between the noradrenergic locus coeruleus (LC) and the ACC is required to support sustained arousal during exposure to unfamiliar environments ([Gompf et al. 2010](#)), suggesting that the LC-ACC axis is a critical substrate of arousal and attention in response to contextual change ([Aston-Jones et al. 1999](#); [Vankov et al. 1995](#)).

Although studies on the subject are limited, there is some compelling evidence for abnormalities in central NE function and adrenergic receptor (AR) sensitivity in patients with compulsive disorders. Elevated plasma levels of NE metabolites ([Siever et al. 1983](#)), altered neuroendocrine responses to adrenergic drug challenges ([Brambilla et al. 1997](#); [Hollander et al. 1991](#); [Siever et al. 1983](#)), and genetic polymorphisms in the NE-metabolizing enzyme *COMT* ([Karayiorgou et al. 1999](#); [Pooley et al. 2007](#); [Schindler et al. 2000](#)) have been reported in OCD patients, but the role of the central NE system in OCD pathophysiology in humans has not been investigated thoroughly. Similarly, the effects of serotonergic drugs on NS and MB behavior have been extensively documented, but the effects of drugs targeting the NE system have only been described in a handful of studies ([Li et al. 2006](#); [Millan et al. 2000](#); [Sugimoto et al. 2007](#); [Young et al. 2006](#)).

Here we determined the effects of genetic or pharmacological disruption of central NE signaling on OCD-like behaviors in the NS and MB tasks using NE-deficient (*Dbh*^{-/-}) mice and their NE-competent (*Dbh*^{+/-}) counterparts ([Thomas et al. 1995](#)). To provide a contrast to canonical anxiety-like behavior, we also tested performance in the elevated zero maze (EZM). Finally, we assessed the effects of genetic NE deficiency on c-fos induction in the LC and ACC as a measure of task-specific neuronal activity during NS and MB tasks.

Materials and methods

Subjects

Dbh $-/-$ mice were maintained on a mixed 129/SvEv and C57BL/6J background, as previously described ([Thomas et al. 1998](#); [Thomas et al. 1995](#)). Pregnant *Dbh* $+/-$ dams were given drinking water that contained the β AR agonist isoproterenol and α 1AR agonist phenylephrine (20 μ g/ml each) + vitamin C (2 mg/ml) from E9.5–E14.5, and L-3,4-dihydroxyphenylserine (DOPS; 2 mg/ml + vitamin C 2 mg/ml) from E14.5-parturition to prevent the embryonic lethality associated with the homozygous *Dbh* deficiency ([Mitchell et al. 2008](#); [Thomas et al. 1995](#)). *Dbh* $-/-$ mice are readily identified by their ptosis phenotype, and genotypes were subsequently confirmed by PCR. *Dbh* $+/-$ littermates were used as controls because their behavior and catecholamine levels are indistinguishable from wild-type (*Dbh* $+/+$) mice ([Marino et al. 2005](#); [Mitchell et al. 2006](#); [Thomas et al. 1998](#); [Thomas et al. 1995](#)).

Male and female mice 3–8 months old were used in all experiments. Because no sex differences were reported in the literature or observed in pilot experiments, male and female mice of the same *Dbh* genotype were pooled. 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 light/12 h dark cycle with *ad libitum* access to food and water. Behavioral testing was conducted during the light cycle in a quiet room in which the animals were housed to minimize the stress of cage transport on test days.

Drugs

The following drugs were used for behavioral pharmacology experiments: the non-selective β -adrenergic receptor (β AR) antagonist DL-propranolol hydrochloride (Sigma-Aldrich, St. Louis, MO), the α 1AR antagonist prazosin hydrochloride (Sigma-Aldrich), the α 2AR agonists guanfacine hydrochloride (Sigma-Aldrich) and dexmedetomidine (Patterson Veterinary Supply, Greeley, CO), the peripheral non-selective β AR antagonist nadolol (Sigma-Aldrich), the DBH inhibitor nepicastat (Synosia Therapeutics, Basel, Switzerland), the β 1AR antagonist betaxolol (Sigma-Aldrich), the β 2AR antagonist ICI 118,551 (Sigma-Aldrich), the α 2AR antagonist atipamezole (Patterson Veterinary Supply), the peripheral aromatic acid decarboxylase inhibitor benserazide (Sigma-Aldrich), and the synthetic NE precursor L-3,4-dihydroxyphenylserine (DOPS; Lundbeck, Deerfield, IL). All drugs were dissolved in sterile saline (0.9% NaCl) except for prazosin, which was dissolved in saline

containing 1.5% DMSO + 1.5% Cremophor EL, and DOPS, which was dissolved in distilled water with 2% HCl, 2% NaOH, and 2 mg/kg vitamin C.

All compounds except DOPS were administered by i.p. injections at a volume of 10 ml/kg. Sterile saline vehicle was injected to control for any confounding effect of injection stress on behavior, and vehicle-treated animals were used for statistical comparison with anti-adrenergic compounds. For the DOPS rescue experiment, *Dbh* ^{-/-} mice were injected with DOPS (1 g/kg, s.c.) + benserazide (250 mg/kg; Sigma-Aldrich), then tested 5 h later once NE levels peaked. The vehicle control for the DOPS experiment was also administered 5 h before testing ([Rommelfanger et al. 2007](#); [Schank et al. 2008](#); [Thomas et al. 1998](#)). The doses for all compounds tested were based on previous studies ([Durcan et al. 1989](#); [Ji et al. 2014](#); [Kauppila et al. 1991](#); [Luttinger et al. 1985](#); [Millan et al. 2000](#); [Murchison et al. 2004](#); [Rudoy et al. 2007](#); [Schank et al. 2008](#); [Schroeder et al. 2013](#); [Stemmelin et al. 2008](#); [Van Der Laan et al. 1985](#)) and optimized in pilot experiments to ensure that behavioral effects were not due to sedation.

Elevated zero maze

Mice were exposed to the EZM (2" wide track, 20" diameter) under low light for 5 min ([Shepherd et al. 1994](#); [Tillage et al. 2019](#)). Time spent in the open and closed segments of the maze, entries into the open segments, distance traveled, and velocity were recorded on an overhead camera and measured using TopScan software (Clever Sys Inc., Reston, VA).

Nestlet shredding

Individual mice were removed from their home cages and placed into a new standard mouse cage (13" x 7" x 6") with clean bedding and a standard cotton nestlet square (5 cm x 5 cm, roughly 3g). The nestlets were pre-weighed before the start of testing to calculate the % shredded at the end of the task ([Angoa-Pérez et al. 2013](#); [Angoa-Pérez et al. 2012](#); [Li et al. 2006](#)). Mice were left undisturbed for 30, 60, or 90 min, after which they were returned to their home cages. The weights of the remaining non-shredded nestlet material were recorded, as previously described ([Angoa-Pérez et al. 2013](#); [Li et al. 2006](#)). In one experiment, mice were singly housed with nestlets for 24 h. For pharmacological experiments, all drugs except DOPS and nepicastat were administered 15 min before testing, and the task duration was set at 60 min. Nepicastat was administered 2 h prior to testing to allow for maximal NE depletion ([Schroeder et al. 2013](#)), and DOPS was administered 5 h before testing ([Schank et al. 2008](#); [Thomas et al. 1998](#)). To determine the effect of test cage habituation on NS behavior, individual *Dbh* ^{-/-} and control mice were moved into new, clean mouse cages without a nestlet. After 3 h of habituation, a nestlet

was introduced to the test cage, and NS within 60 min was measured. The NS behavior of habituated mice was compared with NS behavior in mice tested without habituation.

Marble burying

Individual mice were removed from their home cages and placed into a large novel cage (10" x 18" x 10") with 20 black glass marbles arranged in a 4 x 5 grid pattern on top of 2" of normal bedding substrate. The test cage had no lid, and the room was brightly lit. Mice were left undisturbed in the test cage for 30 min. At the end of the test, mice were returned to their home cages. Digital photographs of each test cage were captured at uniform angles and distances. The number of marbles buried was determined by counting the marbles that remained unburied and subtracting this number from 20. A marble was counted as "buried" if at least 2/3 of it was submerged by bedding ([Angoa-Pérez et al. 2013](#); [Tillage et al. 2019](#)). For pharmacological experiments, all drugs were administered by i.p. injection 30 min prior to testing, as described ([Jimenez-Gomez et al. 2011](#); [Sugimoto et al. 2007](#)).

c-fos immunohistochemistry

Dbh ^{-/-} and control mice were exposed to MB or NS tasks, after which they were left undisturbed in the test cage. 90 min following the start of the task, mice were euthanized with an overdose of sodium pentobarbital (Fatal Plus, 150 mg/kg, i.p.; Med-Vet International, Mettawa, IL) and transcardially perfused with cold 4% paraformaldehyde in 0.01 M PBS. After extraction, brains were postfixed for 24 h in 4% paraformaldehyde at 4°C, and then transferred to cryoprotectant 30% sucrose/PBS solution for 72 h at 4°C. Brains were then embedded in OCT medium (Tissue-Tek) and sectioned by cryostat into 40-um coronal slices at the level of the ACC and LC. Another group of *Dbh* ^{-/-} and control mice was selected for baseline comparisons of c-fos induction in the LC and ACC; these animals were naïve to the tasks and were removed from their home cages and immediately euthanized and perfused.

Brain sections were blocked for 1 h at room temperature in 5% normal goat serum (NGS) in 0.01 M PBS/0.1% Triton-X permeabilization buffer. Sections were then incubated for 48 h at 4°C in NGS blocking/permeabilization buffer, including primary antibodies raised against c-fos (rabbit anti-c-fos, Millipore, Danvers, MA, ABE457; 1:5000) and NET (mouse anti-NET; MAb Technologies, Neenah, WI, NET05-2; 1:1000). After washing in 0.01 M PBS, sections were incubated for 2 h in blocking buffer, including goat anti-rabbit AlexaFluor 568 and goat anti-mouse AlexaFluor 488 (Invitrogen, Carlsbad, CA; 1:500). After washing,

the sections were mounted onto Superfrost Plus slides and coverslipped with Fluoromount-G plus DAPI (Southern Biotech, Birmingham, AL).

Fluorescent imaging and c-fos quantification

Fluorescent micrographs of immunostained sections were acquired on a Leica DM6000B epifluorescent upright microscope at 20x magnification with uniform exposure parameters. One representative atlas-matched section of the LC and the ACC was selected from each animal for c-fos quantification. An identically sized region of interest was drawn for all images to delineate the borders of both structures in all animals after both behaviors. Image processing and analysis were performed using ImageJ software. Our analysis pipeline includes background subtraction, intensity thresholding (Otsu method), and automated quantification within defined regions of interest, guided by size and shape criteria for c-fos⁺ cells (size: 50–100 μm^2 , circularity: 0.6–1.0). The NET antibody was used to define LC cell bodies and identify axon terminals in apposition to ACC neurons.

Statistical analysis

For NS experiments, the effects of time on shredding in *Dbh* ^{-/-} and *Dbh* ^{+/-} mice were compared using a two-way repeated measures ANOVA (genotype x time), with post hoc Sidak's test for multiple comparisons. The effects of pre-habitation to the test cage on shredding were also compared by two-way repeated measures ANOVA for each genotype (genotype x habituation time), with post hoc Sidak's test for multiple comparisons. NS behavior between genotypes after 24 h was compared using an unpaired t-test. NS behavior in DOPS- and vehicle-treated *Dbh* ^{-/-} mice was also assessed by unpaired t-test. The effects of anti-adrenergic drugs on NS compared to vehicle were assessed using a one-way ANOVA, with post hoc Dunnett's test for multiple comparisons. For the MB experiments, genotype differences in burying were assessed by unpaired t-test. The effects of anti-adrenergic drugs on MB compared to vehicle were assessed by one-way repeated-measures ANOVA, with post hoc Dunnett's test for multiple comparisons.

For c-fos quantification, genotype differences were compared in the LC and ACC at baseline and after MB or NS. Comparisons were made within behavioral tasks and between genotypes by multiple t-tests using the Holm-Sidak correction for multiple comparisons. The threshold for adjusted significance was set at $p < 0.05$, and two-tailed variants of tests were used throughout. Graphical data are presented as group mean $\pm$ SEM. Statistical analyses were conducted and graphs were made using Prism Version 7 (GraphPad Software, San Diego, CA).

Results

Central norepinephrine is necessary and sufficient for stress-induced nestlet shredding behavior

A cohort of age- and sex-matched *Dbh* $-/-$ and *Dbh* $+/-$ control mice were compared in the NS task, with three different task durations on three different test days: 30 min on Day 1, 60 min on Day 2, and 90 min on Day 3. A two-way repeated measures ANOVA (genotype x task duration) showed a main effect of time ($F(2,24) = 20.93$, $p < 0.0001$), a main effect of genotype ($F(1,12) = 171.2$, $p < 0.0001$), and a time x genotype interaction ($F(2,24) = 15.81$, $p < 0.001$). Post hoc analyses revealed that control mice shredded more nestlets at 60 min (75.44%) and 90 min (96.16%) than at 30 min (22.82%) [$p < 0.0001$], while shredding was minimal in *Dbh* $-/-$ mice at all time points ($p > 0.05$ for all *Dbh* $-/-$ time point comparisons) (Fig. 1a). After animals were individually housed with nestlets for 24 h, all mice of both genotypes shredded 100% of their nestlets ($t(14) = 0$, $p > 0.99$) (Fig. 1b). Acute restoration of central NE synthesis in *Dbh* $-/-$ mice with DOPS + benserazide increased shredding to control levels at the 60-min time point (81.68% vs 6.88%; $t(9) = 10.97$, $p < 0.0001$) (Fig. 1c). Finally, the effect of a 3-h habituation to the test cage on NS (60-min time point) was compared between genotypes. A repeated measures two-way ANOVA (genotype x habituation status) showed a main effect of habituation status ($F(1,10) = 9.47$, $p = 0.01$), a main effect of genotype ($F(1,10) = 23.62$, $p < 0.001$), and a strong trend for a genotype x habituation status interaction [$F(1,10) = 4.71$, $p = 0.06$]. Post hoc comparisons revealed that habituation reduced NS in control mice compared to no habituation (74.1% vs 34.84%; $p < 0.01$), but NS in *Dbh* $-/-$ mice did not differ significantly after habituation (14.19% vs 7.41%; $p > 0.05$) (Fig. 1d).

Nestlet shredding can be suppressed in control mice by anti-adrenergic drugs

Because *Dbh* $+/-$ mice demonstrated robust but not maximal NS behavior at 60 min (Fig. 1a,d), we used this task duration for pharmacological experiments. Control mice were administered either saline vehicle or experimental compounds in their home cages 15 min prior to testing. The test period began once the mouse was placed into a new clean cage with a pre-weighed cotton nestlet square. The experimental compounds used here were the following anti-adrenergic drugs and their mechanisms of action: the α_1 AR antagonist prazosin (0.5 mg/kg), the β AR antagonist propranolol (10 mg/kg), the α_2 AR antagonist atipamezole (0.5 mg/kg), the α_2 AR agonists guanfacine (0.3 mg/kg) and dexmedetomidine (0.02 mg/kg), and the DBH inhibitor nepicastat (100 mg/kg). A one-way ANOVA showed a main effect of treatment ($F(6,41) = 126.3$, $p < 0.0001$). Post hoc comparisons revealed that propranolol ($p < 0.0001$), guanfacine ($p < 0.0001$), dexmedetomidine ($p < 0.0001$), and nepicastat ($p < 0.0001$) reduced NS compared to vehicle. There was no significant difference in NS in the prazosin

group compared to saline ($p > 0.05$). By contrast, atipamezole significantly increased NS behavior compared to vehicle ($p = 0.03$) (Fig. 2a).

To rule out the contribution of peripheral β ARs and determine whether different subtypes of central β AR regulate the expression of NS, another group of *Dbh* +/- mice was tested in a separate series of experiments. A one-way ANOVA of treatment efficacy showed a main effect of treatment ($F(4, 28) = 10.91$, $p < 0.0001$). Post hoc analyses revealed that the peripheral β AR antagonist nadolol (10 mg/kg) was ineffective at reducing NS compared to vehicle ($p > 0.05$), as was the selective β_1 antagonist betaxolol (5 mg/kg) ($p > 0.05$) and the selective β_2 antagonist ICI 118,551 (1 mg/kg) ($p > 0.05$). However, a cocktail of the β_1 and β_2 AR antagonists was sufficient to reduce NS compared to vehicle ($p < 0.001$), consistent with a partially redundant role for β_1 and β_2 ARs in NS behavior (Fig. 2b).

Genetic or pharmacological suppression of norepinephrine transmission attenuates marble burying

Dbh -/- and *Dbh* +/- control mice were assessed in the MB task. Compared to control mice, *Dbh* -/- mice buried fewer marbles ($t(26) = 2.37$, $p = 0.03$) (Fig. 3a). Because NE is important for arousal and wakefulness, a subset of mice was filmed to determine whether *Dbh* -/- mice were not burying marbles because they were falling asleep. Neither control nor *Dbh* -/- mice fell asleep during the MB task, and mice of both genotypes engaged in typical behaviors, including grooming, rearing, and sniffing. We did notice that some *Dbh* -/- mice occasionally exhibited qualitatively unusual behaviors not observed in control mice, such as sitting on top of the marbles (data not shown).

Dbh +/- control mice were used for the pharmacological characterization of anti-adrenergic drug effects on MB. The experimental compounds used here were prazosin (0.5 mg/kg), propranolol (10 mg/kg), a cocktail of prazosin and propranolol at the same doses, and guanfacine (0.3 mg/kg). A one-way repeated measures ANOVA of treatment efficacy revealed a main effect of treatment ($F(2,18) = 12.11$, $p < 0.001$). Post hoc comparisons demonstrated that, compared to vehicle, prazosin ($p < 0.05$), prazosin + propranolol ($p < 0.001$), and guanfacine ($p < 0.001$) potently suppressed MB compared to vehicle, while propranolol alone was not effective ($p > 0.05$) (Fig. 3b).

Dbh -/- mice exhibit normal anxiety-like behavior in the elevated zero maze

Dbh -/- and *Dbh* +/- mice have previously been compared in conflict-based anxiety tasks, including the elevated plus maze (EPM), open field test, and light/dark box, and no knockout phenotypes have been observed (Marino et al. 2005; Schank et al. 2008). To confirm that their MB and NS phenotypes were unique to stress-

induced repetitive behaviors and unrelated to general anxiety, we evaluated the performance of knockouts and controls in the EZM, another canonical anxiety assay. There were no statistically significant genotype differences on anxiety-like measures in this task, including the percent of time spent in the open segment ($t(17) = 0.01, p > 0.05$) (Fig. 4a) or open segment entries ($t(17) = 1.10, p > 0.05$) (Fig. 4b). *Dbh*^{-/-} mice did exhibit diminished locomotor activity during the EZM task; they traveled at a reduced average velocity compared to controls ($t(17)=2.34, p = 0.03$) (Fig. 4c) and tended to travel shorter distances, although the latter did not reach statistical significance ($t(17) = 1.92, p = 0.07$) (Fig. 4d). Attenuation of these locomotor measures of arousal in *Dbh*^{-/-} mice is consistent with previous reports that these animals exhibit attenuated locomotor responses to novel environments ([Porter-Stransky et al. 2019](#); [Weinshenker et al. 2002](#)).

NE-deficient mice show diminished marble burying- and nestlet shredding-induced c-fos expression in anterior cingulate cortex

Dbh^{-/-} and *Dbh*^{+/-} control mice were euthanized for quantification of c-fos⁺ cells in the LC and ACC at baseline and following either NS or MB. At baseline, c-fos expression was minimal and there were no significant genotype differences in c-fos⁺ cells in either the LC ($t(4) = 0.76, p > 0.05$) or the ACC ($t(4)=0.39, p > 0.05$) (Fig. 5a). Both MB and NS induced robust c-fos expression in both regions, but genotype differences emerged. Following MB, *Dbh*^{-/-} mice had significantly fewer c-fos⁺ cells in the ACC ($t(7) = 6.38, p < 0.001$), but normal c-fos⁺ induction in the LC ($t(7) = 0.07, p > 0.05$) (Fig. 5b). Following NS, *Dbh*^{-/-} mice had significantly fewer c-fos⁺ cells in the ACC ($t(10) = 5.77, p < 0.001$) and the LC ($t(10) = 5.66, p < 0.001$) compared to control mice (Fig. 5c).

Discussion

Central norepinephrine is necessary and sufficient for stress-induced nestlet shredding behavior

In this study, we compared NS behavior induced by cage-change stress in NE-deficient and control mice. We found that over the course of 90 min, NE-deficient mice demonstrated virtually no NS, whereas their NE-competent littermates vigorously shredded nearly 100% of their nestlets. Importantly, we observed no genotype differences in NS behavior when mice were given 24 h to shred, indicating that the NE-deficient mice are capable of shredding but simply do not do so with any urgency when placed in a new cage. This finding provides evidence that NE is necessary for rapid NS behavior following cage change, but not for typical nest-building behavior in general. When we acutely restored central NE to *Dbh*^{-/-} mice using DOPS + benserazide, rapid NS behavior was

rescued to control levels. This key finding demonstrates that transient pharmacological restoration of central NE synthesis and transmission is sufficient to support stress-induced NS behavior in NE-deficient animals.

Norepinephrine-deficient mice bury fewer marbles in the marble burying task

Although MB is often interpreted as an anxiety-like behavior, performance in the MB task does not correlate with behavioral measures in other models of anxiety, such as the EPM or open field test ([Jimenez-Gomez et al. 2011](#); [Thomas et al. 2009](#)). However, MB behavior is highly correlated with NS behavior and does not habituate after repeated exposures, indicating that it better reflects compulsive behavior ([Angoa-Pérez et al. 2013](#); [Li et al. 2006](#); [Witkin 2008](#)). Indeed, NE-deficient mice exhibited normal anxiety-like behavior in the EZM but buried fewer marbles than controls in the MB task. This dissociation in behavioral phenotypes is consistent with previously published reports of normal *Dbh* $-/-$ behavior in canonical conflict-based anxiety paradigms ([Marino et al. 2005](#); [Schank et al. 2008](#)) and suggests that NE may play a more important role in the expression of stress-induced compulsive behaviors than in innate anxiety ([Brady 1994](#); [Kedia and Chattarji 2014](#); [Mantsch et al. 2010](#); [McCall et al. 2015](#); [Valentino et al. 1993](#)).

Nestlet shredding and marble burying can be suppressed in control mice by anti-adrenergic drugs

NE acts primarily through $\alpha 1$ and β ARs to exert neuromodulatory effects on target cells, and NE transmission can be decreased by activation of $\alpha 2$ inhibitory autoreceptors. To determine which ARs are involved in the expression of stress-induced NS behavior, we treated control mice with a battery of anti-adrenergic drugs that block ARs on target cells ($\alpha 1$ and β AR antagonists), activate inhibitory autoreceptors ($\alpha 2$ AR agonists), or prevent NE biosynthesis (DBH inhibitors). Prazosin, an $\alpha 1$ AR antagonist, did not significantly affect NS compared to saline vehicle. However, disruption of NE synthesis with nepicastat, reduction of NE release with the $\alpha 2$ AR agonists guanfacine and dexmedetomidine, or antagonism of β ARs with propranolol profoundly suppressed NS behavior in control mice. In a separate set of experiments to determine the requirement of central $\beta 1$ and $\beta 2$ AR activation for NS behavior, we found that blockade of β ARs outside the brain with the peripherally restricted antagonist nadolol did not suppress NS. We also found that selective antagonism of either $\beta 1$ ARs or $\beta 2$ ARs alone was not sufficient to suppress NS, but NS could be reduced by co-administration of $\beta 1$ - and $\beta 2$ AR-selective antagonists. Thus, the effect of propranolol on NS behavior is likely to be mediated by central $\beta 1$ and $\beta 2$ ARs, which have overlapping distributions in some brain regions like the ACC, amygdala, and hippocampus, and may serve partially redundant signaling functions ([Abraham et al. 2008](#); [Qu et al. 2008](#); [Rainbow et al. 1984](#); [Zheng et al. 2015](#); [Zhou et al. 2013](#)).

NS behavior was increased when NE transmission was enhanced via the α 2AR antagonist atipamezole, which blocks α 2 inhibitory autoreceptors. In addition, we found that anti-adrenergic drugs potently suppressed MB behavior in control mice. Guanfacine and prazosin, but not propranolol, dramatically reduced MB. These results are consistent with the literature implicating α 1AR activation in behavioral reactivity to novelty ([Stone et al. 2006](#)), maintenance of generalized arousal ([Broese et al. 2012](#); [Porter-Stransky et al. 2019](#); [Stone et al. 2003](#)), and stress-induced anxiety ([Rasmussen et al. 2016](#); [Skelly et al. 2014](#)).

NE-deficient mice show diminished c-fos induction in anterior cingulate cortex following nestlet shredding and marble burying

The ACC is implicated in the pathophysiology of OCD ([Brennan et al. 2015](#); [McGovern and Sheth 2017](#)), and surgical ablation of the ACC can relieve OCD symptoms ([Jung et al. 2006](#); [Kim et al. 2003](#)). Furthermore, the ACC expresses all subtypes of ARs ([Crino et al. 1993](#); [Rainbow et al. 1984](#)) and is bidirectionally connected to the LC ([Gompf et al. 2010](#); [Loughlin et al. 1982](#)). Baseline genotype differences in c-fos induction in the LC and ACC were not seen when animals were euthanized immediately after removal from their home cages. However, c-fos induction was dramatically reduced in the ACC of NE-deficient mice after NS and MB compared to controls. After NS, but not MB, c-fos induction was reduced in the LC of NE-deficient mice compared to controls.

The reason for the emergence of genotype differences in LC activity following NS but not MB is unclear but could be related to task duration and complexity, or possibly to engagement of other structures and neurotransmitter systems that were not considered in our analysis. Given that hyperactivity of the ACC ([Fitzgerald et al. 2005](#); [Mavrogiorgou et al. 2002](#)), reduced endocrine response to clonidine ([Hollander et al. 1991](#); [Siever et al. 1983](#)), and abnormal catecholamine metabolism ([Benkelfat et al. 1991](#); [Schindler et al. 2000](#)) have all been reported in patients with OCD, we propose that excessive NE transmission to the ACC may account for some of the cognitive and affective symptoms of OCD ([De Geus et al. 2007](#)).

Clinical implications

Although clinical studies examining the effects of α 1AR blockade on OCD symptoms are scarce ([Feenstra et al. 2016](#)), our findings suggest that further clinical trials with prazosin for OCD patients are justified. The ability of guanfacine to suppress MB is consistent with evidence from the limited number of studies showing that other α 2AR agonists like clonidine and dexmedetomidine can reduce MB ([Millan et al. 2000](#); [Young et al. 2006](#)). However, clonidine and dexmedetomidine have potent sedative properties, which can hamper the interpretation

of their behavioral effects in rodents and constrains their tolerability for psychiatric patients. At the dose used in this study, guanfacine had no sedative or motor-impairing effects; in fact, the sedative and motor-impairing ED₅₀ for guanfacine in rodents is roughly five-fold higher than the dose used in the present study ([Luttinger et al. 1985](#); [Scholtysik 1980](#); [Van Der Laan et al. 1985](#)). Clinically, guanfacine is preferred over clonidine for treatment of pediatric ADHD due to its lack of sedative effects and superior tolerability ([Arnsten et al. 1988](#); [Posey and McDougale 2007](#); [Sallee and Eaton 2010](#); [Sallee et al. 2009](#)). Propranolol and guanfacine are well tolerated by patients and have been used both on- and off-label for the treatment of other psychiatric disorders ([Chappell et al. 1995](#); [Cummings et al. 2002](#); [Lederman 1999](#); [Steenen et al. 2016](#)). Nepicastat and guanfacine are effective for attenuating compulsive drug-seeking behavior in animal models of addiction ([Colombo et al. 2014](#); [Le et al. 2011](#); [Schroeder et al. 2013](#)), as well as relapse in patients with substance abuse disorders ([De La Garza II et al. 2015](#)). In scattered psychiatric case studies, the α 2AR agonists guanfacine and clonidine have substantially improved OCD symptoms in certain patients ([Knesevich 1982](#); [Lipsedge and Prothero 1987](#); [Taormina et al. 2016](#)).

Limitations and future directions

It is noteworthy that 5-HT-deficient and NE-deficient mice have diametrically opposite phenotypes in the MB and NS tasks ([Angoa-Pérez et al. 2013](#); [Angoa-Pérez et al. 2012](#)). Disentangling the role of the NE and 5-HT neuromodulatory systems in the expression of stress-induced compulsive behaviors will be a challenging but worthwhile endeavor. From a circuitry standpoint, it is generally agreed that serotonergic neurons in the raphe nuclei exert a tonic inhibitory influence on the LC, while the LC exerts a tonic excitatory influence on serotonergic neurons in the dorsal raphe ([Kim et al. 2004](#); [Pudovkina et al. 2002](#); [Segal 1979](#); [Szabo and Blier 2001](#)). Thus, a possible explanation for the excessive MB and NS behavior observed in 5-HT-deficient mice is that the LC may be hyperactive in the absence of serotonergic modulation. This hypothesis could be tested by administering anti-adrenergic agents, such as nepicastat or guanfacine, to 5-HT-deficient mice and measuring NS and MB behavior when NE transmission is reduced.

A limitation of our study is that we cannot infer a causal role for LC-NE transmission to the ACC in the expression of NS and MB behaviors. More sophisticated studies using tools to manipulate distinct circuits will be required to functionally dissect the NE-dependent network that governs OCD-like behavior. Furthermore, our finding that β ARs mediate NS, while α 1ARs mediated MB, suggests that different cell types and/or circuits may support these correlated but distinct behaviors. The requirement of α 1AR activation for MB but not NS may be related to the size and complexity of the test cage for each task. For MB, testing occurred in large cages “enriched” with marbles, whereas NS was performed in a normal mouse cage with clean bedding and a standard nestlet square. The relative complexity of the MB test environment might activate arousal-promoting α 1ARs that are not

engaged in the simpler NS environment ([Stone et al. 1999](#); [Stone et al. 2005](#); [Stone et al. 2004](#); [Stone et al. 2006](#)). The requirement of β ARs for NS but not MB may be related to the longer duration of the task (60 min for NS vs 30 min for MB), which could allow for synergistic actions of corticosterone on β AR signaling in stress-responsive brain regions ([Gorman et al. 1993](#); [Roozendaal et al. 2004](#); [Roozendaal et al. 2006](#)).

Finally, this study focused on neuronal activity in two brain regions, the LC and ACC, following MB and NS. The central NE system is anatomically and functionally complex ([Robertson et al. 2013](#); [Robertson et al. 2016](#); [Uematsu et al. 2015](#)), and discrete manipulations of genetically defined subpopulations of NE neurons can produce unique or even opposite effects on behavior ([Aston-Jones and Waterhouse 2016](#); [Chen et al. 2019](#); [Flavin and Winder 2013](#); [McCall et al. 2017](#)). Moving forward, it will be important to consider the contribution of noradrenergic brainstem groups besides the LC, such as the A2 neurons residing in the nucleus of the solitary tract, to the expression of these behaviors ([Itoi and Sugimoto 2010](#); [Rinaman 2010](#)).

Conclusions

In summary, these findings support an expanded model of OCD pathophysiology that incorporates dysregulation of central NE signaling, possibly between the LC and ACC. In addition, we propose that anti-adrenergic agents should be assessed for clinical efficacy in treating OCD, since many of these drugs are already used both on- and off-label for the treatment of related psychiatric diseases. Given the high cost and failure rate of psychiatric drug testing, the possibility of repurposing an approved drug such as guanfacine to treat OCD represents an attractive alternative to new drug development ([Lee et al. 2016](#)).

References

- Abraham PA, Xing G, Zhang L, Eric ZY, Post R, Gamble EH, Li HJBr (2008) β 1-and β 2-adrenoceptor induced synaptic facilitation in rat basolateral amygdala 1209:65-73
- Abramovitch A, Dar R, Mittelman A, Wilhelm SJHrop (2015) Comorbidity between attention deficit/hyperactivity disorder and obsessive-compulsive disorder across the lifespan: a systematic and critical review 23:245
- Adams TG, Kelmendi B, Brake CA, Gruner P, Badour CL, Pittenger CJCS (2018) The role of stress in the pathogenesis and maintenance of obsessive-compulsive disorder 2:2470547018758043
- Ahmari SE, Dougherty DDJD, anxiety (2015) Dissecting OCD circuits: from animal models to targeted treatments 32:550-562
- Alarcon RD, Libb J, Boll TJJoN, Neurosciences C (1994) Neuropsychological testing in obsessive-compulsive disorder: A clinical review 6:217-217
- Angoa-Pérez M, Kane MJ, Briggs DI, Francescutti DM, Kuhn DMJJ (2013) Marble burying and nestlet shredding as tests of repetitive, compulsive-like behaviors in mice:e50978
- Angoa-Pérez M et al. (2012) Genetic depletion of brain 5HT reveals a common molecular pathway mediating compulsivity and impulsivity 121:974-984
- Arnsten A, Cai JX, Goldman-Rakic PSJJoN (1988) The alpha-2 adrenergic agonist guanfacine improves memory in aged monkeys without sedative or hypotensive side effects: evidence for alpha-2 receptor subtypes 8:4287-4298
- Aston-Jones G, Iba M, Clayton E, Rajkowski J, Cohen JJBnN, therapeutics (2007) The locus coeruleus and regulation of behavioral flexibility and attention: Clinical implications:196-235
- Aston-Jones G, Rajkowski J, Cohen JJBp (1999) Role of locus coeruleus in attention and behavioral flexibility 46:1309-1320
- Aston-Jones G, Waterhouse BJBr (2016) Locus coeruleus: from global projection system to adaptive regulation of behavior 1645:75-78
- Balcombe JP, Barnard ND, Sandusky CJJotAAfLAS (2004) Laboratory routines cause animal stress 43:42-51
- Benkelfat C, Mefford IN, Masters CF, Nordahl TE, King AC, Cohen RM, Murphy DLJPr (1991) Plasma catecholamines and their metabolites in obsessive-compulsive disorder 37:321-331
- Berridge C, Arnsten A, Foote SJPM (1993) Noradrenergic modulation of cognitive function: clinical implications of anatomical, electrophysiological and behavioural studies in animal models 23:557-564
- Billett E et al. (1998) Investigation of dopamine system genes in obsessive-compulsive disorder
- Brady LSJBrb (1994) Stress, antidepressant drugs, and the locus coeruleus 35:545-556

- Brambilla F et al. (1997) Noradrenergic receptor sensitivity in obsessive compulsive disorder: II. Cortisol response to acute clonidine administration 69:163-168
- Brennan BP, Rauch SL, Jensen JE, Pope Jr HGJBp (2013) A critical review of magnetic resonance spectroscopy studies of obsessive-compulsive disorder 73:24-31
- Brennan BP et al. (2015) An examination of rostral anterior cingulate cortex function and neurochemistry in obsessive-compulsive disorder 40:1866
- Broese M, Riemann D, Hein L, Nissen CJP (2012) α -Adrenergic receptor function, arousal and sleep: mechanisms and therapeutic implications 45:209-216
- Brown LT, Mikell CB, Youngerman BE, Zhang Y, McKhann GM, Sheth SAJJon (2016) Dorsal anterior cingulotomy and anterior capsulotomy for severe, refractory obsessive-compulsive disorder: a systematic review of observational studies 124:77-89
- Carter AS, Pollock RA, Suvak MK, Pauls DLJD, Anxiety (2004) Anxiety and major depression comorbidity in a family study of obsessive-compulsive disorder 20:165-174
- Chappell PB et al. (1995) Guanfacine treatment of comorbid attention-deficit hyperactivity disorder and Tourette's syndrome: preliminary clinical experience 34:1140-1146
- Chen Y-W et al. (2019) Genetic identification of a population of noradrenergic neurons implicated in attenuation of stress-related responses Molecular Psychiatry 24:710-725 doi:10.1038/s41380-018-0245-8
- Cocchi L, Harrison BJ, Pujol J, Harding IH, Fornito A, Pantelis C, Yücel MJHbm (2012) Functional alterations of large-scale brain networks related to cognitive control in obsessive-compulsive disorder 33:1089-1106
- Cohen R, Kaplan R, Moser D, Jenkins M, Wilkinson HJN (1999a) Impairments of attention after cingulotomy 53:819-819
- Cohen RA et al. (1999b) Alteration of intention and self-initiated action associated with bilateral anterior cingulotomy 11:444-453
- Colombo G et al. (2014) The dopamine β -hydroxylase inhibitor, nepicastat, reduces different alcohol-related behaviors in rats 38:2345-2353
- Cosgrove GR, Rauch SLJNCoNA (2003) Stereotactic cingulotomy 14:225-235
- Crino PB, Morrison JH, Hof PR (1993) Monoaminergic innervation of cingulate cortex. In: Neurobiology of cingulate cortex and limbic thalamus. Springer, pp 285-310
- Cummings DD, Singer HS, Krieger M, Miller TL, Mahone EMJCn (2002) Neuropsychiatric effects of guanfacine in children with mild tourette syndrome: a pilot study 25:325-332
- Czyrak A, Czepiel K, Maćkowiak M, Chocyk A, Wędzony KJBr (2003) Serotonin 5-HT_{1A} receptors might control the output of cortical glutamatergic neurons in rat cingulate cortex 989:42-51

- De Geus F, Denys DA, Sitskoorn MM, Westenberg HGJP, neurosciences c (2007) Attention and cognition in patients with obsessive-compulsive disorder 61:45-53
- De La Garza II R et al. (2015) Evaluation of the dopamine β -hydroxylase (D β H) inhibitor nopicastat in participants who meet criteria for cocaine use disorder 59:40-48
- De Ridder D, Leong SL, Manning P, Vanneste S, Glue PJWn (2017) Anterior cingulate implant for obsessive-compulsive disorder 97:754. e757-754. e716
- Deacon RMJNp (2006) Digging and marble burying in mice: simple methods for in vivo identification of biological impacts 1:122
- Dominguez RAJTJocp (1992) Serotonergic antidepressants and their efficacy in obsessive compulsive disorder
- Dougherty DD, Rauch SL, Jenike MAJJocp (2004) Pharmacotherapy for obsessive-compulsive disorder 60:1195-1202
- Durcan MJ, Lister RG, Linnoila MJP (1989) Behavioral effects of alpha 2 adrenoceptor antagonists and their interactions with ethanol in tests of locomotion, exploration and anxiety in mice 97:189-193
- Eapen V, Robertson MMJCD (2000) Comorbid obsessive-compulsive disorder and Tourette syndrome 13:173-183
- Feenstra MG, Klompmakers A, Figee M, Fluitman S, Vulink N, Westenberg HG, Denys DJEN (2016) Prazosin addition to fluvoxamine: A preclinical study and open clinical trial in OCD 26:310-319
- Fineberg N, Chamberlain S, Hollander E, Boulougouris V, Robbins TJBjop (2011) Translational approaches to obsessive-compulsive disorder: from animal models to clinical treatment 164:1044-1061
- Fineberg NA, Gale TMJIJoN (2005) Evidence-based pharmacotherapy of obsessive-compulsive disorder 8:107-129
- Fitzgerald KD, Welsh RC, Gehring WJ, Abelson JL, Himle JA, Liberzon I, Taylor SFJBp (2005) Error-related hyperactivity of the anterior cingulate cortex in obsessive-compulsive disorder 57:287-294
- Flavin SA, Winder DGJN (2013) Noradrenergic control of the bed nucleus of the stria terminalis in stress and reward 70:324-330
- Fodstad H, Strandman E, Karlsson B, West KJAn (1982) Treatment of chronic obsessive compulsive states with stereotactic anterior capsulotomy or cingulotomy 62:1-23
- Gehring WJ, Himle J, Nisenson LGJPs (2000) Action-monitoring dysfunction in obsessive-compulsive disorder 11:1-6
- Gompf HS, Mathai C, Fuller PM, Wood DA, Pedersen NP, Saper CB, Lu JJJoN (2010) Locus ceruleus and anterior cingulate cortex sustain wakefulness in a novel environment 30:14543-14551
- Goodman WK, McDougle CJ, Price LH, Riddle MA, Pauls D, Leckman JJTJocp (1990) Beyond the serotonin hypothesis: a role for dopamine in some forms of obsessive compulsive disorder?

- Gorman AL, Dunn AJPB, Behavior (1993) β -Adrenergic receptors are involved in stress-related behavioral changes 45:1-7
- Graybiel AM, Rauch SLJN (2000) Toward a neurobiology of obsessive-compulsive disorder 28:343-347
- Hajós M, Gartside SE, Varga V, Sharp TJN (2003) In vivo inhibition of neuronal activity in the rat ventromedial prefrontal cortex by midbrain-raphe nuclei: role of 5-HT1A receptors 45:72-81
- Handley SL (1991) Evaluation of marble-burying behavior as a model of anxiety 38:63-67
- Hayes DJ, Northoff GJFiin (2011) Identifying a network of brain regions involved in aversion-related processing: a cross-species translational investigation 5:49
- Hollander E et al. (1991) Noradrenergic function in obsessive-compulsive disorder: behavioral and neuroendocrine responses to clonidine and comparison to healthy controls 37:161-177
- Itoi K, Sugimoto NJJon (2010) The brainstem noradrenergic systems in stress, anxiety and depression 22:355-361
- Janer KW, Pardo JVJJoCN (1991) Deficits in selective attention following bilateral anterior cingulotomy 3:231-241
- Ji M-H et al. (2014) Dexmedetomidine alleviates anxiety-like behaviors and cognitive impairments in a rat model of post-traumatic stress disorder 54:284-288
- Jimenez-Gomez C, Osentoski A, Woods JHJBp (2011) Pharmacological evaluation of the adequacy of marble burying as an animal model of compulsion and/or anxiety 22:711
- Joel D (2006) Current animal models of obsessive compulsive disorder: a critical review 30:374-388
- Johansen JP, Fields HLJNn (2004) Glutamatergic activation of anterior cingulate cortex produces an aversive teaching signal 7:398
- Jung HH, Kim C-H, Chang JH, Park YG, Chung SS, Chang JWJS, neurosurgery f (2006) Bilateral anterior cingulotomy for refractory obsessive-compulsive disorder: Long-term follow-up results 84:184-189
- Kalanthroff E, Linkovski O, Weinbach N, Pascucci O, Anholt GE, Simpson HBJJobt, psychiatry e (2016) What underlies the effect of sleep disruption? The role of alertness in obsessive-compulsive disorder (OCD) 57:212-213
- Kane MJ, Angoa-Peréz M, Briggs DI, Sykes CE, Francescutti DM, Rosenberg DR, Kuhn DMJPo (2012) Mice genetically depleted of brain serotonin display social impairments, communication deficits and repetitive behaviors: possible relevance to autism 7:e48975
- Karayorgou M et al. (1999) Family-based association studies support a sexually dimorphic effect of COMT and MAOA on genetic susceptibility to obsessive-compulsive disorder 45:1178-1189

- Kaupilla T, Tanila H, Carlson S, Taira TJEjop (1991) Effects of atipamezole, a novel α 2-adrenoceptor antagonist, in open-field, plus-maze, two compartment exploratory, and forced swimming tests in the rat 205:177-182
- Kedia S, Chattarji SJJonm (2014) Marble burying as a test of the delayed anxiogenic effects of acute immobilisation stress in mice 233:150-154
- Kim CH, Chang J, Koo MS, Kim J, Suh H, Park I, Lee HJAPS (2003) Anterior cingulotomy for refractory obsessive-compulsive disorder 107:283-290
- Kim M-A, Lee HS, Lee BY, Waterhouse BDJBr (2004) Reciprocal connections between subdivisions of the dorsal raphe and the nuclear core of the locus coeruleus in the rat 1026:56-67
- Knesevich JWJTAjop (1982) Successful treatment of obsessive-compulsive disorder with clonidine hydrochloride
- Krebs RM, Fias W, Achten E, Boehler CNJN (2013) Picture novelty attenuates semantic interference and modulates concomitant neural activity in the anterior cingulate cortex and the locus coeruleus 74:179-187
- Langen M, Durston S, Kas MJ, van Engeland H, Staal WGJN, Reviews B (2011) The neurobiology of repetitive behavior:... and men 35:356-365
- Le A, Funk D, Juzysch W, Coen K, Navarre BM, Cifani C, Shaham YJP (2011) Effect of prazosin and guanfacine on stress-induced reinstatement of alcohol and food seeking in rats 218:89-99
- Lederman RJJa (1999) Medical treatment of performance anxiety 1
- Lee H-M, Kim YJSr, treatment (2016) Drug repurposing is a new opportunity for developing drugs against neuropsychiatric disorders 2016
- Li X, Morrow D, Witkin JMJLs (2006) Decreases in nestlet shredding of mice by serotonin uptake inhibitors: comparison with marble burying 78:1933-1939
- Lin H et al. (2007) Psychosocial stress predicts future symptom severities in children and adolescents with Tourette syndrome and/or obsessive-compulsive disorder 48:157-166
- Lipsedge M, Prothero WJTAjop (1987) Clonidine and clomipramine in obsessive-compulsive disorder
- Lombroso PJ, Scahill LJB, Development (2008) Tourette syndrome and obsessive-compulsive disorder 30:231-237
- Loughlin SE, Foote SL, Fallon JHJBrb (1982) Locus coeruleus projections to cortex: topography, morphology and collateralization 9:287-294
- Luttinger D, Ferrari R, Perrone M, Haubrich DJJoP, Therapeutics E (1985) Pharmacological analysis of alpha-2 adrenergic mechanisms in nociception and ataxia 232:883-889

- Mantsch JR, Weyer A, Vranjkovic O, Beyer CE, Baker DA, Caretta HJN (2010) Involvement of noradrenergic neurotransmission in the stress-but not cocaine-induced reinstatement of extinguished cocaine-induced conditioned place preference in mice: role for β -2 adrenergic receptors 35:2165
- Margulies DS, Kelly AC, Uddin LQ, Biswal BB, Castellanos FX, Milham MPJN (2007) Mapping the functional connectivity of anterior cingulate cortex 37:579-588
- Marino MD, Bourdlat-Parks BN, Liles LC, Weinshenker DJBbr (2005) Genetic reduction of noradrenergic function alters social memory and reduces aggression in mice 161:197-203
- Marzo A, Totah NK, Neves RM, Logothetis NK, Eschenko OJJOn (2014) Unilateral electrical stimulation of rat locus coeruleus elicits bilateral response of norepinephrine neurons and sustained activation of medial prefrontal cortex 111:2570-2588
- Mavissakalian M, Michelson LJJoN, Disease M (1983) Tricyclic antidepressants in obsessive-compulsive disorder: Antiobsessional or antidepressant agents?
- Mavroggiorgou P et al. (2002) P300 subcomponents in obsessive-compulsive disorder 36:399-406
- McCall JG, Al-Hasani R, Siuda ER, Hong DY, Norris AJ, Ford CP, Bruchas MRJN (2015) CRH engagement of the locus coeruleus noradrenergic system mediates stress-induced anxiety 87:605-620
- McCall JG, Siuda ER, Bhatti DL, Lawson LA, McElligott ZA, Stuber GD, Bruchas MRJE (2017) Locus coeruleus to basolateral amygdala noradrenergic projections promote anxiety-like behavior 6:e18247
- McGovern RA, Sheth SAJJOn (2017) Role of the dorsal anterior cingulate cortex in obsessive-compulsive disorder: converging evidence from cognitive neuroscience and psychiatric neurosurgery 126:132-147
- Micallef J, Blin OJCn (2001) Neurobiology and clinical pharmacology of obsessive-compulsive disorder 24:191-207
- Millan MJ et al. (2000) S18616, a highly potent spiroimidazoline agonist at α 2-adrenoceptors: II. Influence on monoaminergic transmission, motor function, and anxiety in comparison with dexmedetomidine and clonidine 295:1206-1222
- Mitchell HA, Ahern TH, Liles LC, Javors MA, Weinshenker DJBp (2006) The effects of norepinephrine transporter inactivation on locomotor activity in mice 60:1046-1052
- Mitchell HA et al. (2008) Behavioral responses of dopamine β -hydroxylase knockout mice to modafinil suggest a dual noradrenergic–dopaminergic mechanism of action 91:217-222
- Montag-Sallaz M, Welzl H, Kuhl D, Montag D, Schachner MJJon (1999) Novelty-induced increased expression of immediate-early genes c-fos and arg 3.1 in the mouse brain 38:234-246
- Murchison CF, Zhang X-Y, Zhang W-P, Ouyang M, Lee A, Thomas SAJC (2004) A distinct role for norepinephrine in memory retrieval 117:131-143

- Navratilova E et al. (2015) Endogenous opioid activity in the anterior cingulate cortex is required for relief of pain 35:7264-7271
- O'Leary OF, Bechtholt AJ, Crowley JJ, Valentino RJ, Lucki IJEn (2007) The role of noradrenergic tone in the dorsal raphe nucleus of the mouse in the acute behavioral effects of antidepressant drugs 17:215-226
- Okano S, Nagaya H, Inatomi NJJops (2005) Novelty stress increases fecal pellet output in mongolian gerbils: effects of several drugs 98:411-418
- Pasquier DA, Kemper TL, Forbes WB, Morgane PJJBrb (1977) Dorsal raphe, substantia nigra and locus coeruleus: interconnections with each other and the neostriatum 2:323-339
- Pauls DL, Abramovitch A, Rauch SL, Geller DAJNRN (2014) Obsessive-compulsive disorder: an integrative genetic and neurobiological perspective 15:410-424
- Pigott TA, L'Heureux F, Dubbert B, Bernstein S, Murphy DLJTJocp (1994) Obsessive compulsive disorder: comorbid conditions 55:15-27; discussion 28-32
- Pittenger C, Bloch MH, Williams KJP, therapeutics (2011) Glutamate abnormalities in obsessive compulsive disorder: neurobiology, pathophysiology, and treatment 132:314-332
- Pittenger C, Bloch MHJPC (2014) Pharmacological treatment of obsessive-compulsive disorder 37:375-391
- Pittenger C, Krystal JH, Coric VJN (2006) Glutamate-modulating drugs as novel pharmacotherapeutic agents in the treatment of obsessive-compulsive disorder 3:69-81
- Pizarro M, Fontenelle LF, Paravidino DC, Yücel M, Miguel EC, de Menezes GBJEoop (2014) An updated review of antidepressants with marked serotonergic effects in obsessive-compulsive disorder 15:1391-1401
- Pooley E, Fineberg N, Harrison PJMp (2007) The met 158 allele of catechol-O-methyltransferase (COMT) is associated with obsessive-compulsive disorder in men: case-control study and meta-analysis 12:556
- Porter-Stransky KA et al. (2019) Noradrenergic Transmission at Alpha1-Adrenergic Receptors in the Ventral Periaqueductal Gray Modulates Arousal 85:237-247
- Posey DJ, McDougale CJJCDr (2007) Guanfacine and guanfacine extended release: treatment for ADHD and related disorders 13:465-474
- Pudovkina OL, Cremers TI, Westerink BHJEjop (2002) The interaction between the locus coeruleus and dorsal raphe nucleus studied with dual-probe microdialysis 445:37-42
- Qu LL, Guo NN, Li BMJH (2008) β 1- and β 2-Adrenoceptors in basolateral nucleus of amygdala and their roles in consolidation of fear memory in rats 18:1131-1139
- Rainbow TC, Parsons B, Wolfe BBJPotNAoS (1984) Quantitative autoradiography of beta 1- and beta 2- adrenergic receptors in rat brain 81:1585-1589
- Rainville P, Duncan GH, Price DD, Carrier B, Bushnell MCJS (1997) Pain affect encoded in human anterior cingulate but not somatosensory cortex 277:968-971

- Rasmussen DD, Kincaid CL, Froehlich JCJA, Alcoholism (2016) Prazosin prevents increased anxiety behavior that occurs in response to stress during alcohol deprivations:1-7
- Rasmussen S, Miller MM, Filipski SB, Tolwani RJJotAAfLAS (2011) Cage change influences serum corticosterone and anxiety-like behaviors in the mouse 50:479-483
- Rifkin J et al. (2005) A manual and automated MRI study of anterior cingulate and orbito-frontal cortices, and caudate nucleus in obsessive-compulsive disorder: comparison with healthy controls and patients with schizophrenia 138:99-113
- Rinaman L (2010) Hindbrain noradrenergic A2 neurons: diverse roles in autonomic, endocrine, cognitive, and behavioral functions 300:R222-R235
- Robertson SD, Plummer NW, De Marchena J, Jensen PJNn (2013) Developmental origins of central norepinephrine neuron diversity 16:1016
- Robertson SD, Plummer NW, Jensen PJBr (2016) Uncovering diversity in the development of central noradrenergic neurons and their efferents 1641:234-244
- Rommelfanger K, Edwards G, Freeman K, Liles L, Miller G, Weinshenker DJPotNAoS (2007) Norepinephrine loss produces more profound motor deficits than MPTP treatment in mice 104:13804-13809
- Roozendaal B, Hahn EL, Nathan SV, Dominique J-F, McGaugh JLJJon (2004) Glucocorticoid effects on memory retrieval require concurrent noradrenergic activity in the hippocampus and basolateral amygdala 24:8161-8169
- Roozendaal B, Okuda S, Van der Zee EA, McGaugh JLJPotNAoS (2006) Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala 103:6741-6746
- Rosso G, Albert U, Asinari GF, Bogetto F, Maina GJPr (2012) Stressful life events and obsessive-compulsive disorder: clinical features and symptom dimensions 197:259-264
- Rudoy C, Van Bockstaele EJPiN-P, Psychiatry B (2007) Betaxolol, a selective β 1-adrenergic receptor antagonist, diminishes anxiety-like behavior during early withdrawal from chronic cocaine administration in rats 31:1119-1129
- Sallee FR, Eaton KJEoop (2010) Guanfacine extended-release for attention-deficit/hyperactivity disorder (ADHD) 11:2549-2556
- Sallee FR et al. (2009) Guanfacine extended release in children and adolescents with attention-deficit/hyperactivity disorder: a placebo-controlled trial 48:155-165
- Sara SJJNrn (2009) The locus coeruleus and noradrenergic modulation of cognition 10:211
- Schank JR, Liles LC, Weinshenker DJBp (2008) Norepinephrine signaling through β -adrenergic receptors is critical for expression of cocaine-induced anxiety 63:1007-1012

- Schindler KM, Richter M, Kennedy JL, Pato MT, Pato CNJAjomg (2000) Association between homozygosity at the COMT gene locus and obsessive compulsive disorder 96:721-724
- Scholtysik GJBjocp (1980) Pharmacology of guanfacine 10:21S-24S
- Schroeder JP, Epps SA, Grice TW, Weinshenker DJN (2013) The selective dopamine β -hydroxylase inhibitor nepicastat attenuates multiple aspects of cocaine-seeking behavior 38:1032
- Schultz DJPS (1972) The effects of novelty on laboratory rat digging behavior 29:303-304
- Segal MJTJop (1979) Serotonergic innervation of the locus coeruleus from the dorsal raphe and its action on responses to noxious stimuli 286:401-415
- Shackman AJ, Salomons TV, Slagter HA, Fox AS, Winter JJ, Davidson RJJNRN (2011) The integration of negative affect, pain and cognitive control in the cingulate cortex 12:154
- Shepherd JK, Grewal SS, Fletcher A, Bill DJ, Dourish CTJP (1994) Behavioural and pharmacological characterisation of the elevated “zero-maze” as an animal model of anxiety 116:56-64
- Siever LJ, Insel TR, Jimerson DC, Lake CR, Uhde TW, Aloï J, Murphy DLJTBJoP (1983) Growth hormone response to clonidine in obsessive-compulsive patients 142:184-187
- Skelly MJ, Weiner JLJB, behavior (2014) Chronic treatment with prazosin or duloxetine lessens concurrent anxiety-like behavior and alcohol intake: evidence of disrupted noradrenergic signaling in anxiety-related alcohol use 4:468-483
- Spitznagel MB, Suhr JAJPr (2002) Executive function deficits associated with symptoms of schizotypy and obsessive-compulsive disorder 110:151-163
- Steenen SA, van Wijk AJ, Van Der Heijden GJ, van Westrhenen R, de Lange J, de Jongh AJJoP (2016) Propranolol for the treatment of anxiety disorders: Systematic review and meta-analysis 30:128-139
- Stein DJJBp (2000) Neurobiology of the obsessive-compulsive spectrum disorders 47:296-304
- Stemmelin J et al. (2008) Stimulation of the β 3-adrenoceptor as a novel treatment strategy for anxiety and depressive disorders 33:574
- Stevens FL, Hurley RA, Taber KHJTJon, neurosciences c (2011) Anterior cingulate cortex: unique role in cognition and emotion 23:121-125
- Stone E, Zhang Y, Rosengarten H, Yeretsian J, Quartermain DJN (1999) Brain alpha 1-adrenergic neurotransmission is necessary for behavioral activation to environmental change in mice 94:1245-1252
- Stone EA, Lin Y, Ahsan MR, Quartermain DJP (2005) α 1-Adrenergic and α 2-adrenergic balance in the dorsal pons and gross behavioral activity of mice in a novel environment 183:127
- Stone EA, Lin Y, Ahsan R, Quartermain DJBbr (2004) Gross mapping of α 1-adrenoceptors that regulate behavioral activation in the mouse brain 152:167-175

- Stone EA, Lin Y, Rosengarten H, Kramer HK, Quartermain DJN (2003) Emerging evidence for a central epinephrine-innervated α 1-adrenergic system that regulates behavioral activation and is impaired in depression 28:1387
- Stone EA, Yan L, Ahsan MR, Lehmann ML, Yeretsian J, Quartermain DJS (2006) Role of CNS α 1-adrenoceptor activity in central fos responses to novelty 59:299-307
- Struthers WM, DuPriest A, Runyan JJEbr (2005) Habituation reduces novelty-induced FOS expression in the striatum and cingulate cortex 167:136-140
- Sugimoto Y, Tagawa N, Kobayashi Y, Hotta Y, Yamada JJB, Bulletin P (2007) Effects of the serotonin and noradrenaline reuptake inhibitor (SNRI) milnacipran on marble burying behavior in mice 30:2399-2401
- Szabo ST, Blier PJBr (2001) Functional and pharmacological characterization of the modulatory role of serotonin on the firing activity of locus coeruleus norepinephrine neurons 922:9-20
- Tanaka E, North RAJJoN (1993) Actions of 5-hydroxytryptamine on neurons of the rat cingulate cortex 69:1749-1757
- Taormina SP, Galloway MP, Rosenberg DRJJod, JDBP bp (2016) Treatment Efficacy of Combined Sertraline and Guanfacine in Comorbid OCD and ADHD: Two Case Studies 37:491
- Thomas A, Burant A, Bui N, Graham D, Yuva-Paylor LA, Paylor RJP (2009) Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety 204:361-373
- Thomas SA, Marck BT, Palmiter RD, Matsumoto AMJJon (1998) Restoration of norepinephrine and reversal of phenotypes in mice lacking dopamine β -hydroxylase 70:2468-2476
- Thomas SA, Matsumoto AM, Palmiter RDJN (1995) Noradrenaline is essential for mouse fetal development 374:643
- Tillage RP et al. (2019) Elimination of galanin synthesis in noradrenergic neurons reduces galanin in select brain areas and promotes active coping behaviors:651943
- Ting JT, Feng GJCoin (2011) Neurobiology of obsessive-compulsive disorder: insights into neural circuitry dysfunction through mouse genetics 21:842-848
- Tulving E, Markowitsch HJ, Kapur S, Habib R, Houle SJNAIJfRCoRiN (1994) Novelty encoding networks in the human brain: positron emission tomography data
- Uematsu A, Tan BZ, Johansen JPJL, memory (2015) Projection specificity in heterogeneous locus coeruleus cell populations: implications for learning and memory 22:444-451
- Umathe S, Bhutada P, Dixit P, Shende VJEjop (2008) Increased marble-burying behavior in ethanol-withdrawal state: modulation by gonadotropin-releasing hormone agonist 587:175-180
- Valentino RJ, Foote SL, Page MEJAotNYAoS (1993) The Locus Coeruleus as a Site for Integrating Corticotropin-Releasing Factor and Noradrenergic Mediation of Stress Responses a 697:173-188

- van Balkom AJ, van Oppen P, Vermeulen AW, van Dyck R, Nauta MC, Vorst HCJCpr (1994) A meta-analysis on the treatment of obsessive compulsive disorder: A comparison of antidepressants, behavior, and cognitive therapy 14:359-381
- Van Der Laan JW, Van Veenendaal W, Voorthuis P, Weick G, Hillen FCJEjop (1985) The effects of centrally acting adrenergic agonists on temperature and on explorative and motor behaviour. Relation with effects on quasi-morphine withdrawal behaviour 107:367-373
- Van Laere K, Nuttin B, Gabriels L, Dupont P, Rasmussen S, Greenberg BD, Cosyns PJJoNM (2006) Metabolic imaging of anterior capsular stimulation in refractory obsessive-compulsive disorder: a key role for the subgenual anterior cingulate and ventral striatum 47:740-747
- Vankov A, Hervé-Minvielle A, Sara SJJEJoN (1995) Response to novelty and its rapid habituation in locus coeruleus neurons of the freely exploring rat 7:1180-1187
- Weible AP, Rowland DC, Pang R, Kentros CJJon (2009) Neural correlates of novel object and novel location recognition behavior in the mouse anterior cingulate cortex 102:2055-2068
- Weinshenker D, Miller NS, Blizinsky K, Laughlin ML, Palmiter RDJPotNAoS (2002) Mice with chronic norepinephrine deficiency resemble amphetamine-sensitized animals 99:13873-13877
- Witkin JMJCPin (2008) Animal models of obsessive-compulsive disorder 45:9.30. 31-39.30. 39
- Wolmarans DW, Stein DJ, Harvey BHJC, Affective,, Neuroscience B (2016) Of mice and marbles: Novel perspectives on burying behavior as a screening test for psychiatric illness 16:551-560
- Young R, Batkai S, Dukat M, Glennon RAJPB, Behavior (2006) TDIQ (5, 6, 7, 8-tetrahydro-1, 3-dioxolo [4, 5-g] isoquinoline) exhibits anxiolytic-like activity in a marble-burying assay in mice 84:62-73
- Yücel M, Wood SJ, Fornito A, Riffkin J, Velakoulis D, Pantelis CJJoP, Neuroscience (2003) Anterior cingulate dysfunction: implications for psychiatric disorders? 28:350
- Zheng J, Luo F, Guo N-n, Cheng Z-y, Li B-mJBr (2015) β 1-and β 2-adrenoceptors in hippocampal CA3 region are required for long-term memory consolidation in rats 1627:109-118
- Zhou H-C et al. (2013) Activation of β 2-adrenoceptor enhances synaptic potentiation and behavioral memory via cAMP-PKA signaling in the medial prefrontal cortex of rats 20:274-284
- Zohar J, Chopra M, Sasson Y, Amiaz R, Amital DJTWJoBP (2000) Obsessive compulsive disorder: serotonin and beyond 1:92-100

Figure Legends:

Fig. 1 Assessment of nestlet shredding behavior in *Dbh* $-/-$ and *Dbh* $+/-$ mice. **a** Regardless of task duration, NS behavior was profoundly reduced in *Dbh* $-/-$ mice ($n = 7$) compared to controls ($n = 7$). In control mice, NS increased when task duration was extended to 60 or 90 min compared to 30 min, but NS behavior in *Dbh* $-/-$ mice ($n = 7$) did not increase as a function of task duration. **b** When mice were singly housed with nestlets overnight, NS reached 100% and did not differ between *Dbh* $-/-$ ($n = 8$) and control mice ($n = 8$). **c** Restoring central NE levels in *Dbh* $-/-$ mice by treating them with DOPS (1 g/kg) + benserazide (250 mg/kg) 5 h prior to testing ($n = 5$) increased NS compared to vehicle ($n = 6$). **d** 3-h habituation to the test cage significantly reduced NS in control mice ($n = 6$), indicating that shredding was mediated by acute cage-change stress. Habituation did not affect NS in *Dbh* $-/-$ mice ($n = 6$). **** $p < 0.0001$, ** $p < 0.01$, n.s. = not significant.

Fig. 2 Anti-adrenergic drugs suppress nestlet shredding in control mice. **a** Compared to vehicle-treated mice (VEH; $n = 7$), NS was reduced by propranolol (10 mg/kg; PROP; $n = 7$), guanfacine (0.3 mg/kg; GUAN; $n = 7$), dexmedetomidine (0.02 mg/kg; DEX; $n = 7$), and nepicastat (100 mg/kg, NEP; $n = 7$). NS was enhanced by atipamezole (0.5 mg/kg; ATIP; $n = 7$) and unaffected by prazosin (0.5 mg/kg; PRAZ; $n = 6$). **b** Compared to VEH-treated mice ($n = 6$), nadolol (10 mg/kg; NAD; $n = 6$), betaxolol (5 mg/kg; BETAX; $n = 7$), and ICI-118,551 (1 mg/kg; ICI; $n = 7$) were ineffective at reducing NS, but the combination of BETAX and ICI (B+I; $n = 7$) reduced NS compared to VEH. *** $p < 0.001$.

Fig. 3 Consequences of genetic and pharmacological disruption of norepinephrine transmission on marble burying. **a** Compared to *Dbh* $+/-$ controls ($n = 15$), *Dbh* $-/-$ ($n = 13$) mice buried fewer marbles. **b** In control mice, PROP (10 mg/kg) was ineffective, but PRAZ (0.5 mg/kg), co-administration of PRAZ + PROP, or GUAN (0.3 mg/kg) reduced MB compared to VEH ($n = 10$, repeated measures). * $p < 0.05$, *** $p < 0.001$.

Fig. 4 Assessment of *Dbh* $-/-$ and *Dbh* $+/-$ mice in the elevated zero maze. *Dbh* $-/-$ mice did not differ from controls on anxiety-like measures, including **a** % time in the open (anxiogenic) segment and **b** entries into the open segment. There was a significant genotype difference in **c** average velocity and a trend for **d** total distance traveled. $N = 9-10$ per group, * $p < 0.05$.

Fig. 5 Comparison of nestlet shredding- and marble burying-induced c-fos expression in anterior cingulate cortex and locus coeruleus between *Dbh* $-/-$ and *Dbh* $+/-$ mice. **a** In experimentally naïve mice at baseline, c-fos

induction was minimal in the ACC (top row) and LC (bottom row), and there were no differences in c-fos⁺ cells between *Dbh* ^{-/-} (n = 3) and control mice (n = 3) in either region. **b** After MB, fewer c-fos⁺ cells were detected in the ACC of *Dbh* ^{-/-} mice (n = 4) compared to control mice (n = 5), but no genotype differences were found in the LC. **c** After NS, fewer c-fos⁺ cells were detected in both the ACC and LC of *Dbh* ^{-/-} mice (n = 6) compared to control mice (n = 6). ****p* < 0.001, n.s. = not significant.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5