THE UNIVERSITY OF CHICAGO

MECHANISMS UNDERLYING OCD-RELEVANT BEHAVIORS IN MICE

A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE BIOLOGICAL SCIENCES
AND THE PRITZKER SCHOOL OF MEDICINE
IN CANDIDACY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

COMMITTEE ON NEUROBIOLOGY

BY
SUMMER LORETTA THOMPSON

CHICAGO, ILLINOIS
DECEMBER 2017

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

First and foremost, I would like to thank my advisor, Stephanie. I knew I wanted to join Stephanie's lab within days of starting my rotation. Stephanie took me on with enthusiasm despite the fact that I had never worked with animals before. She always takes my ideas seriously, no matter how naïve or far-fetched, which has helped me grow creatively as a scientist and which I appreciate immensely. Stephanie is great for a brainstorming session. Stephanie has been a fantastic mentor and I will be forever grateful for the way she has formed me as a scientist.

The team culture of Stephanie's lab is special. It made me instantly want to join their mission to solve the mysteries of psychiatric disorders using mouse models. I would like to thank all the people who made this team dynamic possible. I want thank Nancy Woehrle, for imparting immeasurable wisdom in the short time we worked together, and being patient with me as she taught me the most basic of lab skills (like making a pot of coffee). I would like to thank Mark Opal for showing me how to be organized and efficient in the lab setting. I would like to thank Stephanie Klenotich, for her unwavering support both as a labmate and as a friend, and for keeping things humorous at the most stressful of times. I want to thank Marcia Ramaker, for always being available for a consult or brainstorming session. In particular, I want to thank the two technicians without whom this project would not have been possible: Emily Ho and Amanda Welch. Emily, for her integral role in the launch of the BTBD3 project. Amanda, for her incredible work ethic, her uncanny ability to anticipate my every need, and her support from every angle. I also thank Will Katzka for his help and great attitude. I would also like to thank all of the volunteers that have helped with these projects over the years: Alphonsa Kunnel, Alaa Hajeissa, Mitra Sharifi, Nicole La Grange, and especially Julia Iourinets and Madeline Klinger.

I want to thank all of the contributors to this work. I especially want to thank Jared Young for introducing me to operant conditioning and giving me invaluable training. I also

want to thank Pete Sharp for always being on call for technical assistance with anything from coaching me in programming to troubleshooting hardware issues. I also want to thank Kayla Chase for her technical assistance with qPCR. I also want to thank Jessica Deslauriers and Jordan Kohn for their help troubleshooting western blots. I also want to thank Joanne Steinauer for generously sharing and teaching me how to use a variety of equipment. I also thank Nuno Sousa, Joao Bessa, John Neumaier, and James Knowles for their contributions and expertise.

I would like to thank my friends. I especially want to thank Meg for being my friend from day one of grad school, for treating me like a member of her family, and for being the best coffee date a girl could ask for. I want to thank Elaine for being there for me for the past ten years.

I want to thank Joe, for many many things, but especially for his support from afar after I moved to San Diego.

I want to thank my family. I want to thank my parents for believing in me, fostering my interests, and giving me the courage to pursue my dreams. I am incredibly grateful for my dad, who is always available to help and has saved me countless hours of work. I also want to thank my mom, and my sister, Willow, for their boundless emotional support.

Finally, I want to thank the members of my thesis committee, Abraham Palmer, Xiaoxi Zhuang, Daniel McGehee, and especially my thesis committee chair Ming Xu for generously giving me their time and invaluable feedback on this work.

ABSTRACT

Obsessive-compulsive disorder (OCD) is a psychiatric illness characterized by obsessions, compulsions, or both. Obsessions are intrusive thoughts, urges, or images that are ego dystonic and cause distress to the patient. Compulsions are repetitive behaviors or mental acts that are typically associated with obsessions. Limbic cortico-striato-thalamo-cortical circuitry is dysregulated in OCD and is thought to contribute to OCD symptomatology. However, effective treatment options are still lacking. Serotonin reuptake inhibitors (SRIs), made up of the selective SRIs and clomipramine, are the only effective pharmacological monotherapy for OCD. However, only about half of patients respond to SRIs, and symptom response is partial even in responders. Thus, a better understanding of OCD neurobiology will be needed in order to develop improved treatment options for this debilitating disorder.

Here, we used animal modeling as a tool to study mechanisms underlying OCD-relevant behavior. In chapter 2, we dissected signaling pathways underlying pharmacologically induced OCD-like behavior in the established 5-HT1BR-induced model of aspects of OCD. We found distinct effects of the canonical and noncanonical signaling pathways on OCD-like behavior, which could have implications for development of more selective therapeutics. In chapter 3, we assessed effects of the putative fast-acting anti-OCD treatment ketamine on the 5-HT1BR-induced model. We found dose-dependent effects of ketamine pretreatment on OCD-like behavior, with the lowest dose showing ameliorative effects on 5-HT1BR-induced OCD-like behavior. These findings reinforce the preliminary human results suggesting efficacy of ketamine as a treatment for OCD. These results also demonstrate that the 5-HT1BR-induced model is capable of identifying novel anti-OCD treatments. In chapter 4, we assessed putative OCD risk gene *BTBD3* for OCD-relevant phenotypes using a global *Btbd3* knockout mouse and viral-mediated knockdown of *Btbd3* expression in *Btbd3* floxed mice. We found robust effects of *Btbd3* expression on OCD-relevant behaviors, and that the hippocampus is a major driver of these effects. These results are the first to demonstrate a role for BTBD3

in behavior. These findings support the human data suggesting that BTBD3 may play a role in OCD etiology.

Together, these findings provide novel insights into mechanisms underlying OCD-relevant behaviors.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Obsessive-compulsive disorder

1.1.1 Background

Obsessive-compulsive disorder (**OCD**) is a psychiatric disorder characterized by rigid, repetitive patterns of thought and behavior that are distressing to the patient [156]. OCD has a lifetime prevalence of 1-3% [312]. The serotonin-reuptake inhibitors (**SRIs**) are the only effective pharmacological monotherapy for OCD [139], and only roughly half of patients respond [173], with partial response even in responders. Improved treatments for OCD are an unmet need. While some aspects of OCD neurobiology are known, many unanswered questions remain. Thus, a better understanding of the pathophysiology of OCD is needed in order to guide development of novel therapeutics.

1.1.2 Neural circuitry

OCD patients have aberrant activity in the limbic cortico-striato-thalamo-cortical (**CSTC**) circuit [243, 315]. The CSTC circuit is made up of two opposing pathways that regulate motor output: the direct pathway and the indirect pathway. The direct pathway promotes motor output, while the indirect pathway inhibits motor output. These two pathways balance each other out to properly execute selected behaviors (direct pathway) to the exclusion of other behaviors (indirect pathway). In the classic model of OCD pathophysiology, the balance of activity in these pathways is tipped toward the direct pathway, leading to aberrant behavior in the form of compulsions [315]. This theory is supported by neuroimaging studies that have identified hyperactivity in several brain regions in this circuit, including orbitofrontal cortex, anterior cingulate cortex, striatum, and mediodorsal thalamus, that is

exacerbated with symptom provocation and alleviated with treatment [315]. Importantly, cortico-striatal circuitry mediates goal-directed and habitual behavior [376], which are imbalanced in OCD [135].

However, it has been suggested that this CSTC model is an oversimplification [243, 245], in part by ignoring other brain structures that appear to play a role in OCD. For example, limbic structures such as the amygdala and hippocampus are proposed to modulate this CSTC circuit in OCD patients through inputs to prefrontal cortex and ventral striatum [192, 243], and the hippocampus has been suggested to play a primary role in OCD [299]. Thus, while the CSTC circuit is known to play a major role in OCD, the model of circuitry dysfunction in OCD is still being fine-tuned.

1.1.3 Psychiatric genetics and OCD

Both environmental and genetic factors contribute to the etiology of OCD. First-degree relatives are at higher risk for developing OCD [145], and twin studies estimate that genetics accounts for 45-65% of symptoms in childhood-onset populations and 27-47% in adult-onset populations [368]. OCD is predicted to be a highly polygenic disorder, with many genes contributing small proportions of the variance in symptoms [83, 351]. In fact, one study found no evidence of rare (minor allele frequency $<.05$) single nucleotide polymorphisms (**SNPs**) contributing to the genetic architecture of OCD [83], suggesting that much of the genetic component of OCD will be identifiable using genome-wide association studies, albeit with large sample sizes (**GWAS**).

The advent of GWAS revolutionized psychiatric genetics. While candidate gene studies often yielded unreliable results, perhaps due to insufficient control for multiple comparisons [198], GWAS are unbiased screens of the genome for common variants associated with a trait of interest [82]. A major benefit of GWAS is that its unbiased nature allows for identification of novel variants with no preconceived connection to the disorder of interest [198]. However,

as psychiatric disorders are highly polygenic, large sample sizes are needed in order to achieve genome-wide significance [124]. While individual variants identified in GWAS typically only account for a small portion of the variance in the disorder, they can still be highly biologically relevant. GWAS has identified genes already known to be of standalone importance to a disorder, such as receptors that serve as drug targets, suggesting that GWAS could identify variants that are essential to the disorder in and of themselves, in addition to revealing the broader genetic architecture of a disorder [148].

OCD is a psychiatric disorder for which GWAS has yet to achieve sufficient power to identify reliable genome-wide significant (**GWS**) hits [148]. The first GWAS for OCD identified one GWS SNP in the trio portion of the sample [342]. However, this SNP did not achieve significance in the case-control analysis and was not replicated in a second GWAS for OCD [235]. A pooled sample of these two studies also failed to identify any GWS SNPs [177]. A perhaps order of magnitude increase in sample size may be necessary before a substantial set of GWS hits can be identified in this patient population, based on the pattern of success for other psychiatric disorders such as schizophrenia [148] and the predicted high frequency of SNPs implicated in OCD [83]. However, it will take nontrivial time and effort to achieve this powered sample size. In the meantime, the top subthreshold hits can be characterized using knockout mice and screened for promising phenotypes of relevance to OCD.

Animal models are critical for determining the function of putative risk genes that have been identified in human studies [82]. Unlike in humans, gene expression can be manipulated in animal models, which allows for causal determination of gene function and can be a first step toward relating a gene back to the disorder of interest. One approach is to use knockout mice for genes of interest, which can be of use for efficient assessment of the function of a putative risk gene and its potential relevance to OCD. In sum, while OCD is known to have a substantial genetic component, confirmed risk genes have yet to be identified. In the meantime, putative risk genes can be evaluated using animal modeling approaches.

1.2 Animal models of OCD: a conceptual framework

Adapted from: Pittenger, C., Dulawa, S.C., & Thompson, S.L. (2017). Animal models of OCD: A conceptual framework. In C. Pittenger (Ed.), *Obsessive-compulsive disorder: Phenomenology, pathophysiology, and treatment* (pp. 323-331). New York, NY: Oxford University Press.

1.2.1 Can an animal have OCD?

The pathophysiology of neuropsychiatric disease, including obsessive-compulsive disorder (OCD) and related conditions, is extremely complex and remains, in most instances, frustratingly opaque. This limits our ability to develop new strategies for precise diagnosis, treatment, and prevention.

Studies in animal models have provided key insights into pathophysiology in many areas of medicine. The use of controlled experiments and invasive experimental techniques that are not possible in human subjects, for technical, practical, or ethical reasons, provides enormous power for the dissection of pathophysiology and the development of new somatic interventions. This has been perhaps less true in the study of neuropsychiatric disease than in many other branches of medicine, but progress is accelerating [242, 261, 125, 126]. Putative animal models of neuropsychiatric pathophysiology may arise spontaneously [303, 339], be fortuitously discovered in the course of other investigations [152, 380], or be generated through the targeted testing of specific pathophysiological hypotheses [7, 55, 323].

However, the increase in experimental precision and power that comes with studies in animal models is accompanied by countervailing interpretative challenges. While it is often easier to ask specific questions in an animal model, using rigorous experimental controls, powerful techniques, and large numbers of subjects, such studies must always contend with the question of relevance: if our goal is not to learn about mice but rather about humans, and in particular about humans with complex neuropsychiatric pathology, how are we to

know that the answers we get, in mice, ultimately matter?

Put another way: can mice (or other experimental animals) have OCD? And if an animal did have OCD, what would it look like? From one perspective it is obvious that mice cannot have OCD, and if they did, we would never be able to assess it adequately: core symptoms of OCD, especially the subjective experience of particular obsessions, probably cannot be recapitulated in the (presumably) simpler mind of a mouse; and even if they were recapitulated, we could not assess them in the absence of verbal report. But we humans share >95% of our genome with mice (and even more with non-human primates), and the overall organization of our brains is identical in many respects. Since OCD is substantially genetic and is associated with demonstrable brain abnormalities, it would be remarkable indeed if important disease-associated processes could not be captured in a mouse or other animal model.

Importantly, an animal study need not recapitulate all aspects of the pathophysiology of a disorder to be useful. Modeling an aspect of a complex disorder like OCD is far easier to accomplish. Studies in animals can help us understand particular aspects of brain functional organization or the normal function of a gene, for example, and such knowledge may be an essential foundation for an understanding of disease pathophysiology. The term 'animal model' is most broadly defined as any experimental preparation developed in an animal for the purpose of studying a human condition [126]. Interest in animal models of OCD pathophysiology has grown rapidly over the last decade [99, 8, 9, 52, 251], but such models raise vexing conceptual and interpretative issues. These are explored here.

1.2.2 Validity

There are two requirements for the successful use of an animal model of any disease process: careful validation and quantitative measurement of putatively disease-relevant phenomenology. **Validation criteria** seek to operationalize what conditions a model should satisfy to

convincingly tether it to the disease process it seeks to recapitulate[59]. To state that a model of a disease process is 'valid' is to claim that it has been convincingly demonstrated to capture key aspects of a clinical condition, and thus to express confidence that further studies in the model are likely to shed light on pathophysiology.

Validation criteria for animal modeling are adapted from concepts developed in the context of psychological testing [352, 78]. However, adaptation of these criteria to the study of animal models raises conceptual challenges. Recently, validation criteria have been defined inconsistently in the literature, and there has been contention as to which criteria need to be satisfied for a model to be considered adequately validated [256, 279, 356]. The key types of validity are face validity, etiological validity, predictive validity, and construct validity. These constitute a useful heuristic framework for the assessment of animal models. However, judgment of the validity of an animal model of a complex disorder such as OCD can be challenging and is sometimes ambiguous [286, 285].

Reliability is often not stated as a validity criterion, but is an essential characteristic of any successful model. Reliability refers to stability and low random variability of measurement in a model [126, 356]. A test or model cannot be valid without adequate reliability, as unreliable output measures will lead to a high probability of both type I and type II errors.

Face validity is simultaneously the most intuitive and the most problematic type of validity. It constitutes phenomenological similarity between the measured features of a model and the symptoms of the human disorder being modeled [256]. For example, in the case of OCD, claims to face validity have been based on the presence of repetitive behaviors that are argued to resemble compulsions [152, 380, 7, 327, 19, 347] chewing in a neat and symmetrical pattern[228], or various forms of behavioral inflexibility[19, 347] (although some of these models have other forms of validity as well). Face validity has been assessed as anywhere from useless[126, 256] to obligatory[241, 383] for the validation of an animal model of disease.

A problem with face validity as a criterion for judging animal models is the internal and

multiform nature of neuropsychiatric symptomatology; indeed, face validity has so many definitions and is so subject to interpretation it was recommended that the term be "banished to outer darkness" in the 1940s [256]. For example, excessive grooming in an animal model could well recapitulate symptomatology of OCD, [339, 152, 380, 7, 327] but it could as easily resemble trichotillomania and other grooming disorders [114], Tourette disorder [390, 389], an autism spectrum disorder[77, 272], a skin condition, or some rodent-specific condition with no direct homology to human disease. Although face validity is certainly not harmful to a model, it contributes little to overall confidence that the model has anything to tell us about disease.

It is important to note that virtually no behavioral phenotype or other biological finding is specific to any neuropsychiatric disorder. For example, deficits in prepulse inhibition (PPI), a measure of sensorimotor gating, are observed in OCD[323], Tourette disorder[55], and schizophrenia[328]. Greater specificity can be achieved by combining different phenotypes. For example, a model that exhibits elevated grooming, anxiety, and diminished PPI may be more convincingly specific to OCD than one that exhibits only one of these behaviors. This is true of other forms of validity as well – a constellation of characteristics, individually non-specific, may in concert produce greater specificity, and thus greater confidence that a model corresponds to a disease process.

Etiologic validity refers to the recapitulation in an animal model of the causal mechanisms underlying a particular disease [126]. Conceptually, there is little to complain about in a model with clear etiological validity; such models often have great potential power to identify pathophysiological mechanisms and novel treatment possibilities. The challenge here is that we know so little about the etiology of complex neuropsychiatric disease. High-penetrance genetic causes of complex disorders such as Alzheimer's disease[144] and Tourette syndrome[55] have been modeled, and these models have strong etiologic validity. However, such high-penetrance mutations are rare; modeling them is a valuable exercise, but it begs

the question of whether or not pathophysiological insights thus generated apply to the disorder more generally. Furthermore, recapitulation of well-established causal mutations does not always produce the expected pathological changes in an animal; for example, mutations associated with Huntington's disease that have high penetrance in humans do not reliably produce neurodegeneration in mice[381]. No animal models of OCD with unimpeachable etiologic validity have been described.

Predictive validity of a disease model refers to the accuracy with which the model predicts aspects of the human disorder that it seeks to recapitulate [261, 125]. Predictive validity in a disease model is sometimes used in the narrow sense of predicting response to a medication that is of benefit in the modeled disease [241, 382, 384]. Since serotonin reuptake inhibitors are the mainstay of the pharmacological treatment of OCD [206, 333], response of a model to these drugs is frequently presented as evidence of predictive validity[380, 323, 327, 184]. Dose and time course of response are also important criteria. An equally important aspect of pharmacological predictive validity is the *lack* of response to pharmacological treatments to which the modeled disorder is not responsive, conferring some selectivity to the response profile. In the case of OCD, response to a serotonin reuptake inhibitor and lack of response to other classes of antidepressant has been taken as a strong validator of animal models[323]. Non-pharmacological interventions are more difficult to recapitulate in an animal model – CBT is a mainstay of OCD treatment, for example, but it is difficult to see how it could be applied in a nonverbal animal system. Anatomically targeted treatments such as ablative neurosurgery and DBS are easier to apply with fidelity across species lines, and these have been investigated in some animal models of OCD. Predictive validity can also be applied to non-therapeutic interventions with a known effect on the modeled condition; for example, a pharmacological challenge[323] or an environmental manipulation such as stress[390, 389] that reliably makes symptoms worse in patients may be applied in an animal model.

A distinction can be drawn between predictive validation and the generation of novel predictions. Predictive validation entails the recapitulation in a model of aspects of a disease that are already well established, to increase confidence that the model is capturing relevant processes. The generation of novel predictions is typically a later step: after a model is accepted as adequately validated, it can be used to try to teach us something new.

Establishing predictive validity is complicated by the imprecise nature of psychiatric treatments. SSRI antidepressants, for example, are first-line pharmacotherapy for OCD; but they are also used in depression, generalized anxiety, bulimia nervosa, post-traumatic stress disorder, phobias, social anxiety, autism, and any number of other conditions. Furthermore, response rates to SSRI pharmacotherapy in OCD are not terribly impressive; only about half of patients achieve response in a typical study, and most have significant residual symptoms[208]. In light of these considerations, responsiveness of an animal model to SSRI pharmacotherapy is neither necessary nor sufficient to establish its validity. Examination of multiple phenotypes and responsiveness to several agents can strengthen the predictive validity of a model – for example, as described above, a valid OCD model might be expected to be responsive to SSRIs but not to other classes of antidepressant.

Construct validity is a more abstract concept. It denotes the extent to which a model accurately measures the conceptual notion, or construct, that it purports to capture [78]. Thus, in the case of prepulse inhibition as a behavioral model of sensorimotor gating, the construct validity of PPI reflects the extent to which it faithfully reflects the phenomenon of sensorimotor gating. For OCD models, 'perseveration' or 'motor impulsivity' could be constructs that the model seeks to capture. The challenge here, obviously, is the definition of relevant constructs in a testable manner. The theoretical constructs of most interest to psychiatrists and psychologists – obsessions, compulsions, neuroticism, depression – are difficult to operationalize in an animal system. As our conceptions of psychiatric disease evolve and the boundaries of particular clinical entities shift, the corresponding theoretical

constructs likewise change. Construct validity, in a strong sense, is therefore rarely achieved by models of neuropsychiatric disease.

Construct validity is made up of two inter-related concepts, convergent and discriminant validity. Convergent (or concurrent) validity is the high correlation between outputs that purport to measure the same underlying construct. Discriminant validity, on the other hand, is a lack of correlation among measures that purport to measure distinct constructs.

Synergy between different types of validity. These different criteria for validity can be mutually reinforcing, as noted above. For example, face validity is problematic both because it is non-specific and because it is inherently subjective – a mouse may groom excessively for many reasons, for example, and the presence of such a behavior may, on its own, be only a weak argument for the claim that the model captures core pathophysiological processes of OCD, Tourette syndrome, or any other particular condition. However, if an identical behavior is seen after experimental recapitulation of a hypothesized disease cause, and if it responds to pharmacological manipulations in a disease-relevant way, the disease model may be much more compelling – that is, etiological, predictive, and face validity may be mutually reinforcing.

1.2.3 Diagnostic boundaries and endophenotypes

An additional challenge to the animal modeling of OCD, and of other complex neuropsychiatric conditions, is the shifting and unresolved nature of psychiatric nosology. The categories of psychiatric diagnosis formalized in the Diagnostic and Statistical Manual (DSM) are based on observable symptomatology that may or may not reflect underlying etiology [15]; this diagnostic framework necessarily evolves over time, and the boundaries of individual disorders and groups of disorders shift. For example, hoarding symptoms have traditionally been thought of as a subtype of OCD, but recent advances have made it clear that hoarding is more properly considered a separate disorder [119]. Similarly, the categorization of OCD,

body dysmorphic disorder, hoarding, and grooming disorders into a single chapter in DSM-5 implies a relationship between these disorders that was not previously explicit (and that is not universally accepted [4]). The entire diagnostic strategy embodied in the DSM has recently come under criticism from the National Institute of Mental Health, which has espoused the development of a more biologically grounded dimensional approach to diagnosis[175].

Psychiatric nosology will continue to evolve. However well grounded in clinical research, such revisions complicate the project of developing informative animal models of disease. What, precisely, is to be modeled? It may be that a model will capture a subset of OCD features, or a latent biological or symptomatic construct that extends across several diagnoses; such models may be valid and informative, and yet not map cleanly onto any current disease entity. Too stringent a demand that an animal model faithfully recapitulate all aspects of a disease as currently conceptualized is likely to lead to the rejection of potentially valuable investigative tools. Indeed, capturing all aspects of a neuropsychiatric syndrome in an animal is surely impossible. In the case of OCD, one can imagine a model capturing mechanisms of anxiety, cognitive rigidity, a tendency to acquire habits or to repeat actions beyond what is adaptive, or any number of other component processes, without recapitulating all aspects of OCD. Such a model, while inevitably incomplete, may be highly informative.

A related consideration is that important, biologically grounded domains of symptomatology typically extend across diagnostic boundaries, and may be seen in *forme fruste* in individuals with no clinical diagnosis. For example, prepulse inhibition, anxiety, and compulsivity can all be conceptualized in dimensional terms and measured both in patients and in their healthy relatives. Where such constructs have a clear genetic underpinning they are termed **endophenotypes**[143]. It has been argued that attempting to model categorical disease entities in psychiatry is misguided, and that we are better off trying to capture transdiagnostic endophenotypes as continuous traits[261].

1.2.4 Mechanisms, disease models, and behavioral tests

While the human brain has unique complexities that endow us with all of the many features that set us apart from other animals, most of these are merely variations on themes that arose much earlier in evolution and are conserved between humans and other mammals. These conserved processes can be fruitfully studied in animals with substantial confidence that the principles thereby discerned, and many of the details, are likely to be applicable to humans as well. This principle applies to the study of both normal function and its perturbation in disease. For example, a rich literature over the past several decades has used elegant studies in animals to elucidate the circuitry that regulates fear, fear learning, and anxiety [84, 98, 181, 217, 321]; the mechanisms of fear and anxiety in humans – both adaptive and pathological – appear to be substantially similar. Studies of anxiety in animals are therefore likely to shed light on psychiatric conditions in which dysregulated anxiety is a prominent symptom, even if the animals under study do not directly recapitulate a specific human disease process. Another system of particular relevance to the neurobiology of OCD is the corticostriatal system and its involvement in motivated behavior, decision-making, and habit learning.

In contrast to these general studies of basic brain functions, a **disease model** may be defined as an attempt to recapitulate relevant brain processes in an animal system, for mechanistic or therapeutic study. (This is in contrast to animal models more generally, which have been defined as 'any experimental preparation developed in an animal for the purpose of studying a human condition'[126]). Disease models can take many forms, including spontaneous development of pathological behaviors, induced genetic abnormalities, pharmacological or behavioral manipulations, or direct manipulations of the brain. In each case the goal is to capture key elements of pathophysiology (though not the disorder in its entirety). The various validity criteria defined above are appropriately applied in judging the success of such models, as they attempt to judge the success of the modeling enterprise.

There is some potential ambiguity in the distinction between studies of physiological mechanism and of disease when one attempts to model disease-related endophenotypes, rather than discrete disease entities [261, 143]. If, for example, if one is studying pathologically enhanced habit learning or excessive anxiety in an animal model, is this best considered an instance of modeling a disease or of using an animal system to study a disease-related normal brain process? This is of course a semantic distinction; the important thing is to be clear on what is being studied – in this example, excess habit learning or increased anxiety, rather than OCD in all its complexity.

Disease-relevant **behavioral tests** are sometimes also referred to as models but are importantly distinct. A behavioral test is a concrete measure – ideally quantitative, continuous, and reliable –ŋ that seeks to capture a particular construct, which may be relevant to disease. For example, anxiety is typically measured in rodents using tests such as the elevated plus maze or the elevated zero maze[24]; but these tests are not models of any particular disease process. Other tests of relevance to OCD models include measures of exploratory behavior, repetitive behavior, stereotypy, grooming, startle and prepulse inhibition, motor learning, habit learning, and so forth. Plausible, validated behavioral tests that capture core features of clinically relevant symptomatology are critical for the assessment of animal models of disease, but they are not disease models in their own right.

1.2.5 Utility

From a clinical perspective, the ultimate value of an animal model of disease lies in its potential to identify salient aspects of human illness that would not otherwise have come to light: new concepts in pathophysiology; clarification of meaningful diagnostic boundaries; novel targets for treatment or prevention. A model could have robust validity, and yet be of limited practical value if it does not lead to clinical advances – although a highly validated model of disease is, in principle, likely to lead to translatable insights. (Of course, a model

could be of theoretical or ethological interest even in the absence of clinical implications; but the focus here is on relevance to human disease.)

It is important, therefore, to consider the **utility** of an animal model of disease: the particular disease-relevant question or questions it seeks to answer; its success or promise in addressing those questions; and the potential ultimate impact on clinically important issues such as diagnosis, prognosis, treatment, or prevention. To clarify this perspective on animal models, we consider major categories of question that animal models may attempt to address.

1. Testing etiologic and pathophysiological hypotheses. Clinical studies of epidemiology, genetics, or pathophysiology can generate etiologic hypotheses of disease, but in most cases these are inherently correlative. For example, the association of a particular allele with disease risk suggests a causal relationship, but even in the face of strong genetic evidence it remains possible that some other allele in linkage disequilibrium with the identified one is the causal factor, or (in some cases) that confounds such as population stratification in the genetic study have led to erroneous results. To establish the causality of such an association there is thus great value in recapitulating the disease-associated genetic abnormality in an animal model and examining the consequences, at the level of brain biology or of behavior[55, 144]. Non-genetic etiologic and pathophysiological hypotheses can also be tested: for example, the hypothesis that dysregulated corticostriatal activity is associated with OCD-relevant phenomenology[7, 264], or that abnormalities in particular serotonin receptors may be associated with disease[323]. Such tests of etiologic hypotheses are, of course, closely related to the concept of etiologic validity.

Testing hypotheses in this way depends on the ability to interpret disease-relevant consequences, at the level of brain biology and/or of behavior. For example, if recapitulation of an OCD-associated genetic abnormality produces anxiety and repetitive behaviors, the causal importance of the association is supported. Of course, the ability to interpret the con-

sequences of a modeled etiologic factor depends on the relevance to disease of the particular behavioral test being performed.

As a corollary, negative results from a test of an etiologic hypothesis are often of limited value. Modeling a hypothesized etiologic factor may fail to produce observable disease-relevant consequences for a variety of reasons. The model may not faithfully capture the etiologic factor as it exists in humans. The test being used to assess the effects of the manipulation may not capture the particular disease-relevant consequences. The modeled factor may only lead to disease in the presence of other causal processes not captured by the model (e.g. a genetic abnormality may only manifest in disease in the presence of a particular environmental challenge). Or, the consequences of the modeled abnormality may not be conserved between humans and the animals. Finally, of course, the etiologic hypothesis may be wrong – but it often difficult to draw this conclusion with confidence.

Importantly, not all etiologic hypotheses are readily testable in animal models. Many genetic findings, for example, purport to explain only a small fraction of the variance in disease risk in an individual carrier – that is, they are risk factors, but not sufficient causes. Recapitulating such risk factors in an animal model may be valuable for understanding their consequences, but it is unlikely to recapitulate disease pathophysiology. Some hypothesized etiologic factors may be unique to humans – if, for example, they depend on linguistic interactions, or on complex cognitive or social constructs that cannot readily be captured in animals. And some may simply be impractical to model in animals – for example, factors that unfold across many years.

2. Generating new etiologic hypotheses. Sometimes models arise fortuitously – for example, spontaneously occurring OCD-like behaviors in a variety of animals, or unanticipated OCD-like consequences of a genetic manipulation in mice[152, 380, 327]. Such models are, at the outset, based on face validity, and therefore they face a high bar to establish their relevance to human disease. However, they provide a unique opportunity to generate

new causal hypotheses. For example, the presence of elevated grooming after knockout of the developmental regulatory gene HoxB8 led to increased grooming that was proposed to have face validity as a model of OCD symptomatology[152]. Clarification of the mechanism by which this fascinating phenotype is generated has the potential to identify new potential etiological factors in OCD; in this example, it has cast light on the potential contribution of dysregulated microglia[69], which is a potentially fruitful area of ongoing clinical research.

3. Dissecting pathophysiology. Models with established etiologic or predictive validity can be immensely valuable to identify the consequences of pathogenic events on other aspects of development, brain biology, or behavior. For example, the recapitulation in an animal of a disease-associated, putatively causal genetic abnormality has immense potential value for the clarification of downstream consequences of that genetic abnormality. The experimental tools and rigorous experimental design that can be brought to bear in animal models permits the dissection of such consequences with a level of detail and rigor that is not generally possible in clinical studies.

4. Generating hypotheses for testing in clinical populations. The identification of new pathophysiological processes in an animal model with etiologic or predictive validity can lead to the generation of specific hypotheses that can then be tested in patients. For example, if a disease-associated genetic abnormality leads to measurable changes in the level of particular neurotransmitter receptors or regional brain activity in an animal model, these can potentially be tested in patients using imaging techniques. Such a return to clinical populations is essential to ensure the relevance of findings in the animal model – for example, in cases where a rare cause of disease is being modeled and its generalization to more heterogeneous disease is unclear. Pathophysiological alterations that are discovered in an animal model and then confirmed to also occur in patients may become potentially fruitful targets for therapeutic development.

5. Testing novel therapies. Testing established therapies is often part of the process

of characterizing an animal model – in particular, of establishing its predictive validity. Once a disease model has been accepted as recapitulating relevant pathophysiological processes, it becomes a potential valuable vehicle for the testing of novel therapies. These may be based on abnormalities discovered in that model, or they may be derived from other models, clinical observations, or other sources. There is particular strength in developing a potential novel therapeutic strategy in one disease model and then testing it another; success in such a test supports the generality of the intervention. This has rarely if ever been done in models of OCD. Importantly, testing of interventions, either established or novel, requires specification of one or more clinically relevant outcome variables (i.e. behavioral tests, or disease-relevant neurobiological measures). The specification of the test being used to assess an intervention will of course influence the success of the test and must be done with care.

6. Exploring fundamental disease-relevant physiological processes. As noted above, studies in animals can help clarify mechanisms of disease-relevant processes, though they cannot in general model a disorder in its entirety. For example, studies of the regulation of decision-making by the frontal cortex or of anxiety by the amygdala, or of the fundamental mechanisms underlying extinction learning, may help refine our understanding of these processes and thus refine a conceptual vocabulary for framing questions about disease pathophysiology. In the terminology developed above, such studies entail investigations of mechanism but do not constitute a disease model; nevertheless, they are of potentially great importance in advancing our knowledge.

Conclusion. Animal models of OCD and related disorders have received increasing attention over the past two decades, and a number of thought-provoking models have been produced. The gulf between manifest behaviors observed in a non-verbal animal and the complex, subjective, and heterogeneous symptomatology experienced by individuals with OCD (or with any neuropsychiatric disorder) is broad, and it is clear that no study in a non-verbal animal will recapitulate all aspects of this or any other neuropsychiatric disease.

Nevertheless, there is much to be learned from studying core features and pathophysiological processes in animal systems, in which important questions can often be addressed with a conceptual and technical rigor not possible in clinical investigations.

1.3 Pharmacological and behavioral rodent models of obsessive-compulsive disorder

Adapted from: Thompson, S.L. & Dulawa, S.C. (2017). Pharmacological and behavioral rodent models of obsessive-compulsive disorder. In C. Pittenger (Ed.), *Obsessive-compulsive disorder: Phenomenology, pathophysiology, and treatment* (pp. 385-400). New York, NY: Oxford University Press.

1.3.1 OCD background

Obsessive-compulsive disorder (**OCD**) is characterized by obsessions and/or compulsive behaviors [15]. Obsessions are intrusive thoughts, impulses, or images that cause distress [15]. Compulsions are repetitive, ritualistic behaviors or mental acts that patients feel driven to perform in association with obsessional thoughts. These symptoms are time consuming and disruptive to daily life [312]. Previously classified as an anxiety disorder, OCD is now central to a new class of disorders in DSM-5: 'Obsessive-Compulsive and Related Disorders', which also includes trichotillomania, body dysmorphic disorder, and hoarding disorder [15]. Some manifestation of obsessions or compulsions is a core feature of each of these disorders.

In addition to obsessions and compulsions, OCD has several associated features that are not part of the canonical symptomatology (Table 1.1). OCD patients exhibit heightened avoidance behavior [15, 304, 280, 227, 101, 110, 282, 258, 336, 106, 240, 162, 130, 133, 25] and decreases in novelty-seeking [280, 227, 212, 225, 10, 193]. OCD patients also tend to rely on habitual rather than goal-directed behavioral strategies, which may contribute to compul-

sive tendencies [25, 51, 135, 133, 132, 375]. OCD patients exhibit broad deficits in executive functions (see [3, 331] for meta-analyses), including response inhibition, cognitive flexibility, visuospatial working memory, and planning. Prepulse inhibition (**PPI**), an operational measure of sensorimotor gating [146], is also impaired in OCD patients [6, 45, 167, 316, 340, 345], particularly in females [340].

OCD patients have abnormalities in brain activity. OCD patients show resting-state hyperactivity in the orbitofrontal cortex, caudate, mediodorsal thalamus, and anterior cingulate cortex (see [315] for a review). Hyperactivity in some of these regions can be exacerbated by symptom provocation and attenuated by effective OCD treatment [315].

The first-line treatments for OCD are exposure and response prevention therapy [259, 407] and serotonin reuptake inhibitors (**SRIs**) [116]. Exposure and response prevention therapy consists of repeated exposure to fear-eliciting stimuli and inhibition of compulsive responses [147]. SRIs, comprising the selective SRIs [332] and the tricyclic antidepressant clomipramine [355], are the only effective pharmacological monotherapies for OCD [288]. However, only 40-60% of patients respond to first-line pharmacological treatment [153], and symptom relief in responders is typically incomplete [115].

The pharmacological treatment profile for OCD is distinguishable from that of major depression and anxiety disorders in that antidepressants other than SRIs are typically ineffective [27, 139, 220, 333], there is typically a longer latency to SRI treatment efficacy (approximately 4 to 12 weeks for OCD in contrast to 2 to 4 weeks for depression) [252], and higher SRI doses are often required [38]. Recent meta-analyses suggest that, contrary to conventional wisdom, SRI treatment may be effective in as little as one week for depression [350] and two weeks for OCD [179]. Regardless, there is a longer latency for OCD treatment efficacy. Adjunctive treatment with atypical antipsychotics to augment SRI treatment has shown efficacy in treatment-resistant OCD [93, 371]. High frequency deep brain stimulation (**DBS**) of the subthalamic nucleus [58], bed nucleus of the stria terminalis [178, 226, 300],

and particularly ventral capsule/ventral striatum [149, 178] have shown some promise in treating refractory OCD.

These associated features of OCD, including cognitive performance deficits, alterations in brain activity, and treatment profile, provide quantifiable and objectively measurable characteristics that facilitate research on etiology and treatment by providing an alternative to self-reported clinical symptoms. Importantly, although these features are not unique to OCD individually, in combination they make up an OCD-specific profile. See Table 1.1 for a list of associated features of OCD and tests used to measure these deficits.

1.3.2 Principles of animal modeling

An animal model is an experimental preparation used to study aspects of a condition of interest, such as human psychopathology [126, 125, 230, 242]. A major advantage of animal models is the ability to apply experimental manipulations that would be impossible to perform in humans [241]. On the other hand, our inability to access the internal mental state of animals presents a great challenge for modeling of psychiatric disorders, as self-report of internal state is often central to diagnosis [53]. Nevertheless, highly informative animal models can be generated by identifying and validating homologous measures between species that can be objectively quantified. The ability to model an aspect of a psychiatric disorder in animals is directly related to our ability to quantify the measure of interest in humans. Therefore, there are two requirements for successful use of animal models as effective translational tools: 1) identification of quantifiable phenotypes in psychiatric disorders [32] and 2) careful validation of animal models using the quantifiable human phenotypes.

Predictive validity refers to the ability of an animal model to make accurate predictions about a human phenomenon of interest [230, 126, 125, 352, 78, 205]. Implicit in the concept of predictive validity is reliability, which reflects stability and low chance of random variability in the measurement and output of a test [126, 230, 356]. A test or model cannot have

Construct	Human Paradigm(s)	Effect in OCD Patients	Rodent Equivalent	Other Rodent Paradigm(s)
Perseveration	DAT WCST	Increase in perseverative errors	DAT	5-CSRTT
Cognitive Flexibility	WCST ID/ED	Increase in errors, fewer categories completed Deficits in extradimensional shifting	ID/ED	DAT
Spatial Working Memory	DAT SWM	Increase in perseverative errors Strategy and between search errors	DAT	SAB, MWM, DNMS, win-shift
Response Inhibition	SST Go/No-Go Stroop	Deficient action cancellation: longer SSRT Deficient action restraint Deficient interference control	SST Go/No-Go Stroop	5-CSRTT (deficient action restraint)
Planning	TOL/TOH	Deficient		
Novelty-Seeking	TPQ	Decreased	N/A - self report	novel object test, SAB, dig test, hole board test
Avoidance	TPQ AAT SAP	Enhanced harm avoidance (self report) Slower approach to compulsion triggers Enhanced avoidance habits	N/A - self report SAP	OFT, L/D, EPM novel object test, OFT, L/D, NIH, SAB
Habit Formation	SAP, IDT	Excessive; reduced outcome devaluation	ODP	CD
Conditioned Fear	Fear Conditioning	Deficient extinction recall	Fear Conditioning	
Spatiotemporal Task Patterns	video telemetry	Increased return to key locations/objects Ritualistic set of actions performed at key locations/objects	Quinpirole-Induced Checking	
Sensorimotor Gating	PPI (eyeblink)	Decreased PPI	PPI (whole body vibration)	
	P50 suppression	Increased P50 T/C	P20-N40 gating	
Performance Monitoring	ERN (EEG)	Enhanced ERN during interference tasks (Stroop, Simon, Go/No-Go)	ERN (LFP)	
Brain Activity	resting state symptom-provocation treatment	Hyperactivity in OFC, CPu, ACC, MD Hyperactivity in OFC, CPu, ACC Reduction in resting state hyperactivity in OFC, CPu	rs-fMRI, PET, SPECT	electrophysiology, IEG expression

Table 1.1: This table lists constructs that are altered in OCD patients, the paradigms used to measure these constructs in humans, the effect of having OCD on the output of these paradigms, equivalent rodent paradigms, and other paradigms that measure the same construct in rodents. AAC: anterior cingulate cortex. AAT: approach avoidance task. CD: contingency degradation. CPu: caudate putamen. DAT: delayed alternation task. DNMS: delayed non-match to sample. ERN: error-related negativity. IDED: intradimensional/extradimensional shift. IDT: instrumental discrimination task. IEG: immediate early gene. MD: mediodorsal nucleus of the thalamus. MWM: Morris water maze. ODP: outcome devaluation procedure. OFC: orbitofrontal cortex. PET: positron emission tomography. PPI: prepulse inhibition. rs-fMRI: resting state functional magnetic resonance imaging. SAB: spontaneous alternation behavior. SAP: shock avoidance paradigm. SPECT: Single-photon emission computed tomography. SSRT: stop signal reaction time. SST: stop signal task. TOL/TOH: tower of London/tower of Hanoi. TPQ: tridimensional personality questionnaire. SWM: visuospatial working memory task. WCST: Wisconsin card sorting task.

predictive validity without reliability [126, 230, 356], as unreliable output measures lead to unreliable predictions. The predictive validity of an animal model can be established by identifying output measures in the model that recapitulate a reliable trait of the disorder. For example, PPI deficits in an animal model of OCD would recapitulate the PPI deficits that have been reliably observed in patients. The more specific the output measure is to the disorder of interest, the more convincing the animal model will be. Unfortunately, virtually no phenotypes have been identified that are exclusive to one psychiatric disorder. For example, PPI deficits may be predictive of OCD, but they are also seen in schizophrenia [45], autism [204], and Tourette syndrome [204]. Greater specificity in a model can be achieved if it recapitulates several phenotypes observed in the disorder of interest. For example, a mouse model exhibiting PPI deficits and perseverative behaviors that are reduced by chronic SRI treatment shows good specificity as a model for OCD. However, modeling a disorder in its entirety is virtually impossible, not to mention problematic because diagnostic categories often evolve over time [126].

Demonstrating that a manipulation with known effects in the disorder has the same effects in the model also strengthens predictive validity for the model. Drug effects are frequently the most accessible manipulations for assessing predictive validity. If using drugs to assess predictive validity of a new model of a psychiatric disorder, the type of drug, the dose [382], and the time course of effects should be predictive of what is seen in the human condition.

Validation using manipulation of key phenotypes is particularly valuable for measures that do not have good **construct validity**, which refers to the degree of confidence that what is observed in an animal faithfully reflects the underlying construct that one is seeking to investigate. For example, an over-grooming phenotype in an animal model may appear OCD-like, but this phenotype could reflect a different process entirely, such as an itchy skin condition, rather than compulsivity. Evidence supporting that over-grooming reflects

OCD-like behavior could be provided by evidence that chronic SRI treatment, but not NRI treatment, ameliorates the excessive grooming. Yet, drug treatments are not the only means for establishing predictive validity through manipulation. Any manipulation, including environmental factors, surgical interventions, or developmental insults, can be used to show that a model makes accurate predictions. For example, attenuation of over-grooming following DBS of the ventral capsule/ventral striatum, bed nucleus of the stria terminalis, or subthalamic nucleus would contribute predictive validity to the over-grooming model. Thus, reliable characteristics of a disorder and established effects of manipulations of these characteristics in patients can be modeled in animals and used to determine predictive validity of a model.[1]

Because the ability to predict is the essence of scientific understanding, predictive validity has been proposed as the only requirement for the initial validation and use of a model [126, 125, 205, 230]. However, this approach has not been applied throughout the field of animal modeling [383], and many models have gone into general use without predictive validation. Many models instead have gone into general use on the basis of only construct or **face validity**, the phenomenological similarity of a phenotype to the human condition. In this review, predictive validity is emphasized as the primary prerequisite for a valid animal model of a psychiatric disorder.

Development of valid animal models of psychiatric disorders is constrained by our knowledge of psychiatric disorders in humans. This fact highlights the need for human research assessing objective traits associated with disorders of interest that are amenable to study in animals, such as many of the associated features of OCD listed above and detailed in Table 1.1. Some researchers have taken an even more direct approach to developing this platform for animal models by performing 'reverse translational' research, or work assessing behaviors in humans that are classically studied in animals [392]. One example of this type of work is

1. The standards for predictive validity detailed here are within the context of models intended for the purpose of studying underlying mechanisms. Models with other purposes exist, such as assays screening for novel treatments, but are outside the scope of this review.

the human Behavioral Pattern Monitor employed by Geyer and colleagues [277, 392], which assesses human locomotor activity in a manner analogous to that performed in rodents. Eilam, Szechtman and colleagues [104, 103, 402, 404, 403, 405] have performed a series of studies assessing the spatiotemporal motor patterns underlying compulsive behaviors. This work has begun to quantify previously self-reported features of a disorder, leading to post hoc attribution of predictive validity to preexisting animal work, and creating a foundation of human work upon which to build novel animal models. These innovative strategies are particularly pertinent in the context of translational potential of animal models of OCD.

Currently, there are relatively few extensively validated measures for OCD-like behavior in animals. Some behaviors have been labeled as OCD-like due to phenomenological similarity, but remain unsubstantiated. The identification of more objectively quantifiable phenotypes in OCD patients are needed as tools to establish novel animal models. Select models of OCD are reviewed below.

1.3.3 Pharmacologically induced models of obsessive-compulsive disorder

Animal models of disease processes may be induced or spontaneous. Induced models may use environmental, genetic, pharmacological, or other manipulations to generate a phenotype of interest, whereas spontaneous models employ naturally occurring behaviors of the animal. Pharmacologically induced, environmentally induced, and spontaneous rodent models of OCD are reviewed here. Models produced by genetic modifications or by direct manipulations of the brain are reviewed elsewhere in this volume, as are spontaneously occurring models in species other than rodents.

RU24969-induced

Triptans are non-selective serotonin agonists with high affinity at the 5-HT1B receptor (previously known as 5-HT1Dβ) [161, 377]. They have been found to exacerbate OCD symptoms

in OCD patients [155, 206, 338, 401]. Dulawa and colleagues found that acute administration of a 5-HT1B/1A receptor agonist, RU24969, induces several OCD-like phenotypes in mice [165, 323, 324, 386].

Mice treated acutely with RU24969 have PPI deficits [323, 324], similar to those seen in patients with OCD [6, 45, 167, 316, 345]. PPI deficits lend predictive validity to the model because PPI is an objectively measureable trait across species. RU24969-treated mice also display a hyperactive, perseverative pattern of behavior in the open field test, termed 'route stereotypy' [165, 323, 324]. Mice treated acutely with RU24969 also rear less in the open field, indicative of reduced exploration or response to novelty [221]. Importantly, all of these deficits are attenuated by chronic treatment (4 weeks for all phenotypes except hyperactivity in the open field, which was reduced by 3 weeks) with the SRIs fluoxetine and clomipramine, but not the tricyclic antidepressant desipramine. Thus, the pharmacological profile for attenuation of these behavioral deficits generally follows the OCD treatment profile.

Mice treated acutely with RU24969 also show deficits in the delayed alternation paradigm [386], a task that measures spatial working memory [95] and perseveration [254] in rodents and humans. OCD patients also exhibit deficits in delayed alternation that are driven by perseverative errors [1, 2, 56, 154, 253, 255, 254, 297, 373]. OCD patients also have spatial working memory deficits more broadly, as assessed in other spatial working memory paradigms that do not measure perseveration [36, 62, 195, 196, 233, 260, 294, 295, 308, 358, 366, 367]. Delayed alternation deficits induced by RU24969 thus recapitulate deficits found in OCD patients, lending predictive validity to the finding. This effect is attenuated by chronic (4 weeks) treatment with fluoxetine [386], contributing additional predictive validity to the delayed alternation deficits.

Acute RU24969 treatment also induces expression of Fos, an immediate early gene used as a marker for neuronal activity [326], in the caudate-putamen [165]. Since OCD patients

exhibit increased activation in the caudate [315], RU24969-induced Fos expression in the dorsal striatum contributes predictive validity to the model. Four weeks of treatment with fluoxetine, but not desipramine, prevented RU24969-induced Fos expression in the dorsal striatum, further lending predictive validity to the model. Since predictive validity serves as the main prerequisite for initial use of a model [126], these various strands of evidence establish the RU24969-induced model as suitable to be used for studying the neurobiological underpinnings of OCD.

Quinpirole-induced checking

Szechtman, Eilam and colleagues found that chronic administration (twice weekly for a total of 10 injections) of the dopamine D2/D3 agonist quinpirole to rats induces compulsive-like checking of objects in the open field [347]. This checking behavior has a stereotyped spatiotemporal structure that is characterized by: 1) locations or objects in the subject's territory to which the subject returns with great frequency, 2) short intervals between returns to these key locations or objects, 3) few stop locations during return to key objects/locations, 4) a characteristic battery of actions comprising the checking ritual and 5) a shift in spatiotemporal structure when the key locations/objects are changed [347, 346, 102, 33, 393]. In the model, chronic (5 weeks) treatment with SRI paroxetine mitigates some measures (#1, #3) of the quinpirole-induced checking [71]. Since SRIs are first-line pharmacological treatment for OCD [117], this result lends predictive validity to the model. However, acute treatment with non-selective serotonin agonist *meta*-chlorophenylpiperazine (**mCPP**), a drug that exacerbates symptoms in OCD patients [155, 400, 399, 171, 169, 170, 284, 283, 48, 108, 86] (although see [65, 138, 281, 199]), also reduces frequency of returns to key locations (#1) [360]. Some of the phenotypes are reduced by clomipramine treatment administered during development of quinpirole-induced excessive checking behaviors (#1 and #2), but this effect wanes over time for time to return to key locations (#2) [347]. In contrast, clomipramine is

an effective long-term treatment for OCD [116]. Thus, the quinpirole-induced checking model has an inconsistent pharmacological treatment profile for quinpirole-induced high frequency of stops at key locations (#1) and short intervals between returns to key locations (#2). Interestingly, high-frequency DBS in the subthalamic nucleus [385] and nucleus accumbens [257], two brain regions where DBS has shown efficacy in treatment-refractory OCD, robustly reverse all three core measures of quinpirole-induced checking (criteria #1-3 listed above). The DBS treatment findings provide strong predictive validity for the core features of the quinpirole-induced checking model as OCD-like. The combination of pharmacological and DBS findings data support quinpirole-induced reductions in stop locations during return to key objects (#3) as the best measure of OCD-like behavior.

In support of this model, Eilam and colleagues quantified the spatiotemporal structure of compulsions in humans using video telemetry [104, 103, 404, 402, 405, 403]. This work corroborates the first and supports the fourth proposed criteria for compulsive checking, greater frequency of return to key locations/objects and a characteristic set of actions performed at key locations/objects, respectively (Table 1.1) [104, 347, 404]. These findings contribute predictive validity to quinpirole-induced checking because the human condition of interest, a compulsive ritual, was objectively quantified, and the animal model recapitulates aspects of that condition. Together with the OCD-specific DBS treatment effects, these findings support the quinpirole-induced checking model of OCD. However, the mixed pharmacological profile reduces confidence in some of the quinpirole-induced phenotypes.

Neonatal clomipramine

The neonatal clomipramine model is based on the broad hypothesis that administering a drug neonatally can have opposite effects to those found when the same drug is administered in adulthood [18]. Although clomipramine can be used to successfully treat OCD [116], neonatal treatment in rats induces a variety of behavioral deficits during adulthood that

may be OCD-like [19]. For example, these rats show spatial working memory deficits in the win-shift paradigm, a test that measures spatial working memory using the natural tendency of rodents to spontaneously alternate in a radial maze [121, 267]. In the version of the task used in this study, half the arms of the maze are initially baited with reward. After a delay, the previously unbaited arms are baited, requiring subjects to remember which arms were previously baited. While a human equivalent of the win-shift paradigm has not been assessed in OCD patients, since OCD patients have spatial working memory deficits [3, 331], the spatial working memory deficits lend some predictive validity to the model. Similarly, neonatal clomipramine-treated rats show deficits in spontaneous alternation in a T-maze. Spontaneous alternation is a measure of novelty-seeking or exploration [91, 214] and an index of spatial working memory [214]. Since OCD patients have reduced novelty-seeking [280, 227, 212, 225, 10, 193] and deficits in spatial working memory [3, 331], reduced spontaneous alternation lends some predictive validity to the model.

Neonatal clomipramine-treated rats show increased anxiety in the elevated plus maze, a well-validated test for unconditioned anxiety [273]. However, anxiolytic compounds, such as alprazolam [337] or lorazepam [207], and anxiogenic compounds, such as yohimbine [298] or sodium lactate [141], are not clinically effective monotherapies for OCD. Most OCD patients report anxiety, discomfort, or disgust in association with their obsessional thoughts [296]; but generalized anxiety is not a primary feature of OCD, and is certainly not a specific characteristic of the disorder [176]. Indeed, OCD was removed from the anxiety disorders in the recent DSM-5 [15]. Thus, the anxiogenic effects of neonatal clomipramine treatment do not add much predictive validity to the neonatal clomipramine model.

Rats receiving neonatal clomipramine hoard food pellets in the home cage. Hoarding is no longer considered a subtype of OCD, but rather a distinct disorder [15] with its own symptom, treatment, and neurobiological profiles [120]. However, hoarding has high comorbidity with OCD and can present as an obsession-driven behavior in OCD patients [278]. making food

hoarding in the neonatal clomipramine model still potentially relevant to OCD.

In conclusion, the neonatal clomipramine model has some predictive validity for OCD, driven by spatial working memory deficits and reduced novelty seeking. However, the remaining behavioral deficits have a less clear relationship with OCD and would require additional validation measures in order to demonstrate that these behaviors are OCD-like. Assessing these behavioral deficits for sensitivity to treatments would potentially strengthen the model. Of interest, neonatal clomipramine treatment also induces depression-like [374] and binge-eating phenotypes [118]. conditions that are often comorbid with OCD. Since none of the phenotypes described to date is specific to any one disorder, future studies should further examine whether neonatal clomipramine induces any deficits that are more specific to one or another of these disorders.

8-OH-DPAT-induced

Bridger and colleagues found that a 5-HT1A receptor agonist, 8-OH-DPAT, induces deficits in spontaneous alternation in rats [391] and in mice, resulting in perseverative selection of one arm of a T-maze [21]. 8-OH-DPAT-induced spontaneous alternation is rescued by chronic (3 weeks) treatment with SRIs fluoxetine [21, 391], lending some predictive validity to the model. However, subchronic (3 once-daily injections) clomipramine also alleviates the spontaneous alternation deficit, detracting from the predictive validity of the model; more chronic treatment is required in OCD [113]. Electrical stimulation of nucleus accumbens exacerbates rather than alleviates the spontaneous alternation deficit, also detracting from the predictive validity of this model [370]. Overall, the 8-OH-DPAT-induced spontaneous alternation deficit currently has poor predictive validity as a model of OCD.

mCPP-induced ritualistic chewing

The non-selective serotonin agonist mCPP induces ritualistic chewing behavior in rats, a phenomenon in which rats perform repetitive chewing or gaping mouth movements in the absence of food stimuli [211]. This phenotype is attenuated by chronic (3 weeks), but not acute, pretreatment with SRIs. However, this time course for treatment efficacy is closer to the profile of major depression (2 to 4 weeks), and no negative control antidepressant treatments (non-SRIs) were assessed in the chronic study. Thus, the model has no clear link to a specific disorder at this time.

1.3.4 Environmentally induced models

A number of models with potential relevance to OCD have been described, in which an environmental manipulation, rather than a pharmacological one, is used to induce putatively OCD-like behavior.

Schedule-induced polydipsia

Schedule-induced polydipsia (**SIP**) is a phenomenon in which rats develop an excessive drinking, or polydipsic, phenotype following scheduled food restriction [111]. Specifically, rats will drink large quantities of water (up to half their body weight) when kept on a fixed-time feeding schedule of food pellets administered 60 - 180 seconds apart, and maintained at 80% of free-feeding bodyweight (Falk, 1971). Woods and colleagues found that chronic SRI treatment selectively reduces SIP in rats [388]. Specifically, the SRIs fluoxetine, fluvoxamine, and clomipramine reduced SIP with a time course ranging from 15 to 22 days for onset of treatment efficacy, whereas chronic treatment with desipramine, haloperidol, or diazepam did not [388]. However, the time course for efficacy of SRIs was shorter than that seen in OCD patients [252], and later studies found even earlier effects [168, 232], ranging from acute to six-day chronic treatment, detracting from the predictive validity of the model. In

addition, the selectivity of the treatment profile also did not replicate in later studies. For example, antipsychotics such as raclopride and flupentixol attenuated SIP behavior [92], and antipsychotics are generally ineffective as monotherapy in OCD [238]. Deep-brain stimulation of nucleus accumbens or bed nucleus of the stria terminalis attenuates the SIP phenotype [369], as in OCD patients, contributing predictive validity to the model [149, 178, 226, 300]. Overall, findings in the SIP model map inconsistently onto treatment response in OCD.

Signal attenuation

The signal attenuation paradigm is based on the concept that compulsive behaviors may result from deficient feedback associated with goal-directed behavior [190]. Joel and colleagues found that in an operant conditioning paradigm, degradation of the contingency between a feedback stimulus signal and food reward induces a compulsive-like lever pressing phenotype [190, 185]. Specifically, the paradigm consists of four stages that lead to compulsive-like lever pressing: 1) food retrieval training, in which rats are trained to associate a compound stimulus (light + sound) with food delivery in the absence of levers, 2) lever-press training, in which pressing one of two levers (the reinforced lever) leads to the compound stimulus and food delivery, 3) signal attenuation, in which levers are absent and the compound stimulus is presented but does not lead to food delivery, and 4) test, in which levers are present and the compound stimulus is induced by lever pressing but does not result in food delivery [190]. Inclusion of the signal attenuation stage (#3 above) leads to excessive, uncompleted lever presses in the test phase in which rats compulsively press the reinforced lever without searching for food in the magazine. Thus, post-signal attenuation lever presses in the test stage (#4 above) are considered the compulsive-like output measure in this paradigm.

Uncompleted excessive lever presses are attenuated by acute pretreatment with SRIs paroxetine and fluvoxamine but not tricyclic antidepressant desipramine [186]. However, uncompleted excessive lever pressing is also attenuated by acute haloperidol [188] and di-

azepam at some doses [186]. Thus, the pharmacological profile of the signal attenuation paradigm is not selective to therapeutics for OCD and does not follow the time course for effective OCD treatment, detracting from the predictive validity of the model.

Non-pharmacological treatments have also been assessed in the signal attenuation model. DBS of the subthalamic nucleus [201], external segment of globus pallidus [202], or entopeduncular nucleus (rodent homolog of internal segment of globus pallidus) [202] reduce compulsive-like lever-pressing in the model. Subthalamic nucleus DBS has shown some efficacy in OCD [58], contributing predictive validity to the model. Interestingly, in the signal attenuation paradigm, orbitofrontal cortex lesions increase compulsive-like lever pressing in rats [189, 187, 190], and this effect is attenuated by acute systemic [187] or intrastriatal [317] paroxetine treatment. Overall, the signal attenuation model is theoretically interesting but lacks consistent evidence supporting its predictive validity as a model of OCD.

1.3.5 Spontaneous models

A number of spontaneous behaviors in rodents have been interpreted as OCD-like. The criteria for establishing such behaviors as valid recapitulations of OCD-like processes are the same as for induced models: face validity must be treated with great caution, while predictive validity is key to accepting a model as potentially informative about the pathophysiology of OCD.

Deer mouse

Deer mice (*Peromyscus maniculatus bairdii*) reared in laboratory conditions exhibit spontaneous stereotypies consisting of somersaulting, jumping, and pattern running; these are exacerbated by lack of environmental enrichment [293, 292, 362, 361]. Korff, Harvey and colleagues found that confinement-induced stereotypies in deer mice are attenuated by chronic (3 weeks) fluoxetine [209, 210] but not desipramine treatment [209]. These findings were cor-

roborated by a follow-up study showing the efficacy of chronic (4 weeks), but not subchronic (1 to 3 weeks), treatment with SRI escitalopram in attenuating the stereotypies [387]. The response of deer mouse stereotypies to these treatments lends some predictive validity to this model of OCD.

Reinforced spatial alternation

Tsaltas and colleagues found that when spatial alternation in a T-maze is reinforced, a minority of rats continue to select one arm, rather than alternate arms, in spite of extensive training with reward for alternation [359]. This 'directional persistence' is enhanced by acute treatment with the non-selective serotonin agonist mCPP and reversed by chronic (20-day) pretreatment with fluoxetine, but not desipramine or diazepam [359]. There is ample evidence that mCPP exacerbates OCD symptoms in humans [155, 400, 399, 171, 169, 170, 284, 283, 48, 108, 86] (although see [65, 138, 281, 199]). This exacerbation can be blocked by chronic (3.5 to 5 months) clomipramine [399] or fluoxetine (12 weeks or more) [169] pretreatment in humans. Thus, this model has substantial predictive validity in the selectivity of the pharmacological response profile.

Marble burying

Marble burying is a phenomenon in which rodents spontaneously bury marbles under bedding material in a cage. Unlike defensive burying [357], there is no preexisting aversive quality to the stimulus [46]. Marble burying was initially used in conjunction with another behavioral output measure, swim-induced grooming [70], to distinguish between anxiolytic and antipsychotic drugs. Broekkamp and colleagues found that acute administration of either an anxiolytic or antipsychotic drug reduced both marble burying and swim-induced grooming [46]. Thus, neither behavior was selectively responsive to one class of drug. However, anxiolytics had a greater effect on marble burying than swim-induced grooming in mice, and

antipsychotics had a larger effect on swim-induced grooming than marble burying. Thus, the ratio of the ED_{50} for grooming inhibition to the ED_{50} for burying inhibition can be a predictive measure: a ratio greater than one predicts an anxiolytic compound, whereas a ratio below one predicts an antipsychotic compound (with the exception of anti-cholinergic compounds) [46].

Marble burying has been regarded as a compulsive behavior because it is inhibited by SRIs [164], does not habituate to repeated testing [46, 223, 262], and marbles do not show any aversive properties that would indicate anxiety [262, 354]. However, the effects of SRI treatment on marble burying are acute [164], detracting from the predictive validity of the paradigm. In addition, marble burying is affected by both anxiolytic and antipsychotic [50] drugs, neither of which are effective monotherapy for OCD. Thus, the construct measured by marble burying is very ambiguous; little information can be gained from use of the marble burying model, and there is little evidence of its relevance to OCD, or compulsive behaviors in general.

Nest-building

Nest-building has also been proposed as an animal model of OCD. Nest-building is a species-typical behavior in which rodents will use provided materials to create a nest, a behavior generally thought to be indicative of well-being [123, 183]. Greene-Schloesser and colleagues assessed nest-building behavior in the 'BIG' strain of mice, which is genetically selected for excessive nest building behavior [150]. They found that clomipramine and fluoxetine, but not desipramine, successfully reduced nest-building in BIG mice. However, these treatments show efficacy in as little as one week [150]; this short time course detracts from the predictive validity of the model. Reduced nest-building is also found in numerous genetic models of other disorders in which repetitive behavior is a core feature, including autism [105, 109, 314] and schizophrenia [158, 348]. Thus, there is little evidence supporting excessive nest-building

as a behavioral measure for studying compulsive behaviors in OCD.

Food restriction-induced hyperactivity

Food restriction-induced hyperactivity (**FRIH**), also termed activity-based anorexia, has been proposed as a rodent model of OCD [12]. FRIH is a phenomenon in which rodents provided with both a running wheel and scheduled feeding results in paradoxical hyperactivity and reduction in food intake [160], which can lead to severe weight loss and even death [12]. Altemus and colleagues found that eight days of treatment with fluoxetine, but not imipramine, decreased FRIH, increased food intake, and ameliorated weight loss in rats [12], whereas serotonin depletion worsened all phenotypes [11]. However, chronic fluoxetine (10 to 24 days) has also been reported to have no effects on FRIH in mice [203]. Although SRIs are effective in OCD, the subchronic time course of these effects in the model detracts from the predictive validity. The serotonin depletion effects are also not reflective of OCD, as acute tryptophan depletion in OCD patients does not worsen symptoms [28, 330, 35, 213]. In addition, treatments that are ineffective in OCD, such as the atypical antipsychotic olanzapine, have shown efficacy in treating the FRIH model at doses that do not affect feeding or wheel running alone [203]. Therefore, studies to date suggest that FRIH does not provide a model of OCD.

1.3.6 Conclusions

In summary, a wide variety of rodent models and experimental approaches have been used to study aspects of OCD. Many of these models have some predictive validity, which should be a requirement for any model.

Animal models are in a constant state of development and reevaluation. The models discussed here, and others, are best considered on a continuum of validity, rather than simply "valid" or "invalid". Evaluation of the validity of a model may change, with new

studies quantifying phenotypes in OCD patients, or new studies building on preexisting animal models.

A few of the models reviewed here are particularly robust. The RU24969-induced and reinforced spatial alternation models have particularly good predictive validity, making them good candidates for studying the etiology and pathophysiology of OCD. Other models have characteristics that substantially detract from their predictive validity for OCD, suggesting they are not optimal for studying the etiology and pathophysiology of OCD. These include the 8-OH-DPAT-induced, marble burying, and nest-building models. These models have generated results that differ substantially from what is observed in OCD patients, making their relevance to OCD tenuous. Still other models have not undergone sufficient testing for their validity to be evaluated. For example, if the behavioral phenotypes generated by neonatal clomipramine treatment were shown to be selectively sensitive to OCD-effective treatments, the model would be more robust. The mCPP-induced ritualistic chewing model also remains to be convincingly linked to OCD via validation experiments.

Moving forward, the development of new models should be guided with an emphasis on obtaining predictive validity. Animal models exhibiting substantial predictive validity can be used with confidence to identify neurobiological mechanisms underlying the aspect of OCD that is being modeled. Development of such models can be greatly assisted by human research that increases the battery of quantifiable traits associated with OCD that can then be recapitulated in future animal models.

CHAPTER 2

DISTINCT ROLES FOR β-ARRESTIN2 AND GSK-3 SIGNALING IN 5-HT1BR-MEDIATED PERSEVERATIVE BEHAVIOR AND PREPULSE INHIBITION DEFICITS IN MICE

2.1 Introduction

Serotonin-1B receptors (**5-HT1BRs**), previously termed 5-HT1Dβ in humans [161], modulate perseverative behaviors and prepulse inhibition (**PPI**) in humans [155, 206, 287, 338] and mice [165, 324, 323, 386]. 5-HT1BRs are G-protein-coupled receptors (**GPCRs**) that bind $G_{i\alpha 2}$ to inhibit adenylyl cyclase and cAMP production [129, 224]. 5-HT1BRs are primarily localized on axon terminals of serotonergic and non-serotonergic neurons, where they reduce neurotransmitter release when activated [142]. 5-HT1BRs signal through not only a canonical, G-protein-coupled pathway, but also at least one noncanonical G-protein-independent pathway that involves beta-arrestin2 (β**-arrestin2**) [68]. Canonical 5-HT1BR signaling through $G_{i\alpha 2}$ is mediated by direct interaction with glycogen synthase-3 beta **(GSK-3**β), a constitutively active serine/threonine protein kinase that stabilizes the complex between 5-HT1BRs and $G_{i\alpha 2}$, and is integral for downstream G-protein-mediated signaling [67, 68]. This interaction is specific; GSK-3β does not modulate signaling of similar serotonin receptor 5-HT1A, and the other GSK-3 isoform, GSK-3α, does not modulate 5-HT1BR signaling [68]. On the other hand, the intracellular adaptor protein β-arrestin2 interacts with 5-HT1BRs in an activity-dependent manner, and mediates noncanonical signaling [67, 218]. Whether canonical or noncanonical 5-HT1BR signaling induces perseverative behavior and PPI deficits remains unknown. Given the recent identification of biased ligands that differentially stimulate canonical versus β-arrestin2-mediated 5-HT1BR signaling [307], it is of

great interest to identify the pathway mediating 5-HT1BR-induced perseverative behavior and PPI deficits.

Perseverative behavior, which refers to inflexible and repetitive behavior, is a core feature of several neuropsychiatric disorders including obsessive-compulsive disorder (**OCD**) and autism spectrum disorders (**ASD**)[15]. Indeed, inappropriate repetition of actions is a defining feature of the complex rituals that comprise compulsive behaviors in OCD [402], and restricted, repetitive patterns of behavior are a core feature of ASD [15]. OCD and ASD are also characterized by PPI deficits, which correlate with perseverative behaviors [6, 276]. PPI is an operational measure of sensorimotor gating, and refers to the reduction in startle magnitude that occurs when an abrupt startling stimulus is preceded by a brief prepulse [146]. Sensorimotor gating impairments in OCD may contribute to the inability to filter out obsessive thoughts or inhibit compulsive urges [6]. Currently, chronic treatment with serotonin reuptake inhibitors (**SRIs**) provides the only pharmacological monotherapy for perseverative symptoms in OCD and ASD [140, 288, 334]; yet, approximately 50% of patients are nonresponders [288]. Thus, novel treatments for these disorders are a major unmet need.

5-HT1BR activation may play a role in the perseverative behaviors and PPI deficits observed in OCD or autism. 5-HT1BR agonists exacerbate OCD symptoms [155, 206, 338] in some, but not all, studies [42, 281]. 5-HT1BR agonists also induce unusually high growth hormone responses associated with baseline repetitive behaviors in ASD [172]. The relationship between 5-HT1BR availability and PPI is altered in OCD patients [287]. Furthermore, acute treatment with 5-HT1A/1B receptor agonist RU24969 induces PPI deficits and a highly perseverative pattern of locomotion in the open field in mice [165, 324, 323]. These effects are primarily mediated through 5-HT1BRs, not 5-HT1ARs, as indicated by loss of effects after pretreatment with antagonists at 5-HT1BRs, but not 5-HT1ARs. This perseverative locomotor pattern is characterized by a constellation of features including hyperactivity, minimal

vertical rearing, and a rigid circling path, as measured by the spatial scaling exponent d (**spatial *d***). Spatial d describes the smoothness of the path of the animal in the open field, where paths with many directional changes have high spatial d, and paths with few directional changes have low spatial d, which is characteristic of locomotor perseveration [269]. This locomotor perseveration is further illustrated by the lack of directional changes made by RU24969-treated animals in a spontaneous alternation task [263]. This repetitive behavioral syndrome is highly inflexible, performed to the exclusion of species-typical behaviors; both eating and drinking are reduced in RU24969-treated animals [20, 159]. RU24969-induced behavioral deficits in the open field and PPI can be ameliorated by four weeks of treatment with SRIs, but not norepinephrine reuptake inhibitors [165, 324, 323], paralleling treatment effects on perseverative features of OCD and ASD [139, 140]. However, little is known about the downstream intracellular pathways mediating these behavioral effects.

Both canonical and noncanonical 5-HT1BR signaling have been reported to mediate some of the behavioral effects of 5-HT1BR agonists. For example, GSK-3β knockout in serotonin neurons (**snGSK-3β KO**) prevents 5-HT1A/1B agonist anpirtoline-induced reductions in rearing and center activity, but not hyperactivity, in the open field [397]. On the other hand, knockout of the β-arrestin2 gene (***Arrb2***) abolishes anpirtoline-induced hyperactivity, but has not been assessed for effects on other anpirtoline-induced changes in open field phenotypes [67]. However, neither the role of canonical nor noncanonical 5-HT1BR signaling has been dissected with respect to perseverative behaviors or PPI. Here, we investigated the role of canonical versus noncanonical 5-HT1BR signaling in RU24969-induced perseverative hyperlocomotion and PPI deficits. To determine the contribution of canonical 5-HT1BR signaling to behavior, mice received pretreatment with a GSK-3 inhibitor, and then treatment with RU24969 before assessment of open field behavior and PPI. To assess the contribution of noncanonical signaling, *Arrb2* wild-type (**WT**), heterozygous (**HT**), and knockout (**KO**) mice received RU24969 challenge and then underwent open field and PPI testing. Identifying

a signaling bias at 5-HT1BRs for the induction of perseverative behaviors and PPI deficits could lead to novel treatments for disorders characterized by these phenotypes.

2.2 Materials and methods

2.2.1 Animals

Experiment-naïve female, 8-week old, Balb/cJ mice (Experiments 1-4) were purchased from Jackson Laboratories (Bar Harbor, ME) and acclimated to the animal facility for 1 week prior to undergoing experimental procedures. Male and female *Arrb2* WT, HT, and KO mice on a C57BL/6J background, aged 7 to 11 weeks, (Experiment 5) were bred in-house through heterozygous crossings from mice purchased from Jackson Laboratories (Stock #: 023852; Bar Harbor, ME). Animals were housed in a climate-controlled room maintained on a 12 hour:12 hour light:dark cycle. All testing was performed during the light cycle. Mice had ad libitum access to standard chow and water. All procedures were conducted in accordance with the National Institutes of Health laboratory animal care guidelines and with the Institutional Animal Care and Use Committee of The University of Chicago or University of California San Diego.

2.2.2 Drugs

All drugs were administered via intraperitoneal injection. 5-HT1A/B agonist RU24969 (Tocris Bioscience, Bristol, UK) was dissolved in saline (0.9% NaCl) and administered at 0 or 3 mg/kg (Experiments 1 and 3), 0 or 10 mg/kg (Experiments 2 and 4) or 0, 3, or 10 mg/kg (Experiment 5) at 5 ml/kg injection volume. Doses were selected based on previous studies [165, 323, 324]. The GSK-3 inhibitor SB216763 (Tocris Bioscience, Bristol, UK) was dissolved in 4% DMSO/15% Tween-80 in saline and injected at 20 ml/kg injection volume. SB216763 was administered at 0, 5, or 10 mg/kg based on previous studies

[107, 194, 248]. GSK-3 inhibitor AR-A014418 (Tocris Bioscience, Bristol, UK) was dissolved in 4% DMSO/15% Tween-80 in saline and injected at 20 ml/kg injection volume. AR-A014418 was administered at 0, 10, or 20 mg/kg based on previous studies [191, 309]. SB216763 and AR-A014418 have high selectivity for GSK-3 inhibition, modulate 5-HT1BR signaling, and cross the blood-brain barrier [37, 67, 73, 322].

2.2.3 Behavioral testing

Open field

The open field test was performed as described previously [324]. Briefly, mice were placed in a corner of the open field and activity was monitored for 20 minutes. All measures were automatically generated by the Versamax program (Accuscan, Columbus, OH) with the exception of the spatial scaling exponent "spatial *d*," which was calculated using Python (Python Software Foundation, Beaverton, OR), NightOwl (custom software), and BMDP software. Spatial *d* describes the geometric pattern of activity, with higher values indicating a more circumscribed path and lower values indicating a straighter path characteristic of locomotor perseveration [269].

Prepulse inhibition

PPI was assessed as described previously [324]. Briefly, mice were placed in startle chambers (San Diego Instruments, San Diego, CA) and amplitude of the startle response was measured for 65 ms in response to five types of trials lasting 40 ms each for a total of 62 trials: pulse alone (40 ms at 120 dB), three different prepulse inhibition trials (20 ms prepulses 3, 6, or 12 dB above background followed 100 ms later by a 120 dB pulse), or no stimulus, in which only background noise was presented. The test session was 20 minutes long, comprised of a 5 min acclimation period, a block of six startle trials (Block 1), two blocks of 25 intermixed

trial types (Blocks 2 and 3), then a final block of six startle trials (Block 4). Percent PPI was calculated as follows: 100*(startle response - prepulse-inhibited startle response)/startle response. For all experiments assessing PPI, PPI testing occurred directly following open field testing.

2.2.4 Experiments

Experiment 1

Mice were pseudorandomly assigned to receive one of three pretreatments (0, 5, or 10 mg/kg SB216763) and one of two treatments (0 or 3 mg/kg RU24969; n = 12-14/group) in a between-subject design. SB216763 was administered 30 minutes prior to open field testing. RU24969 was administered 5 minutes prior to open field testing. Animals underwent PPI testing directly following open field testing.

Experiment 2

Mice were pseudorandomly assigned to receive one of three pretreatments (0, 5, or 10 mg/kg SB216763) and one of two treatments (0 or 10 mg/kg RU24969; n = 14/group) in a between-subject design. Treatment timing and behavioral assessment were as in Experiment 1.

Experiment 3

Mice and treatments were as in Experiment 1. SB216763 was administered 60 minutes prior to open field testing. RU24969 was administered 35 minutes prior to open field testing.

Experiment 4

Mice were pseudorandomly assigned to receive one of three pretreatments (0, 10, or 20 mg/kg AR-A014418) and one of two treatments (0 or 10 mg/kg RU24969; n = 14/group) in

a between subject design. Treatment timing and behavioral testing were as in Experiment 1.

Experiment 5

Male and female *Arrb2* WT, HT, and KO mice (n=13-15/group) all received 0, 3, and 10 mg/kg RU24969 in a counterbalanced fashion, in a within-subject design. Treatment timing and behavioral testing were as in Experiment 1.

2.2.5 Statistical analysis

For all experiments, dependent measures were analyzed using repeated measures analysis of variance (**ANOVA**). Significant interactions were resolved by assessing simple main effects in ANOVAs. Significant simple main effects were resolved by tests for simple contrasts: post hoc ANOVAs for within-subject variables or Student Newman-Keuls tests for between-subject variables. P-values from post hoc ANOVAs were assessed for significance using the Bonferroni correction. Alpha was set at 0.05. Open field measures were analyzed with bin (4×5 minutes) as a repeated measure. PPI was analyzed with block and prepulse intensity as repeated measures, and startle was also analyzed with block as a repeated measure. Analyses for these repeated measures (bin, block, prepulse intensity) are only stated in the text and represented in figures for which interactions with factors of interest (genotype, pretreatment, treatment) occurred. In Experiment 5, RU24969 treatment was analyzed as an additional repeated measure. Furthermore, a secondary analysis was performed, in which only mice with a matched genotype control for total distance traveled within the saline condition were included, where matched mice had activity levels disparities of less than 100 cm or 5% of total distance traveled. Then, the same analyses were performed. Effect sizes were assessed using Cohen's d. Pearson's correlations and simple regressions were assessed.

2.3 Results

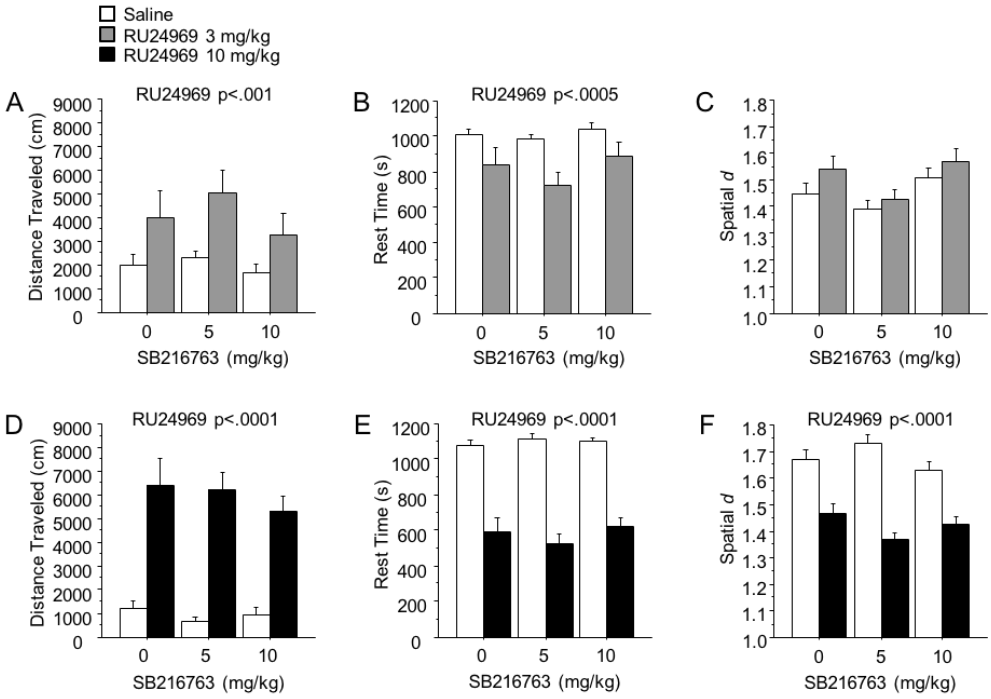

Figure 2.1: GSK-3 inhibition did not affect RU24969-induced changes in activity in the open field test. A - C show distance traveled (A), time spent resting (B), and spatial d (C) for Experiment 1. D - F show distance traveled (D), time spent resting (E), and spatial d (F) for Experiment 2. Results expressed as mean ± SEM

2.3.1 GSK-3 inhibition does not affect RU24969-induced open field phenotypes

In the open field, RU24969 treatment increased distance traveled across pretreatment groups at the low dose, 3 mg/kg ($F_{(1,72)} = 12.12$; p<.001; Figure 2.1a), and at the high dose, 10 mg/kg ($F_{(1,78)} = 96.83$; p<.0001; Figure 2.1d). SB216763 pretreatment had no effect on distance traveled. RU24969 also reduced time spent resting across pretreatment groups for low-dose ($F_{(1,72)} = 14.55$; p<.0005; Figure 2.1b) and high-dose treatment ($F_{(1,78)} = 158.58$; p<.0001; Figure 2.1e), whereas SB216763 had no effect on rest time. 10 mg/kg RU24969 decreased spatial d across SB216763 groups ($F_{(1,78)} = 24.26$; p<.0001; Figure 2.1f), whereas

3 mg/kg RU24969 had no effect on spatial *d* (Figure 2.1c). SB216763 had no effect on spatial *d* in Experiments 1 and 2. Similarly, high-dose, but not low-dose, RU24969 affected center measures with no effect of SB216763 (Figure 2.6; Supplemental Results). The absence of GSK-3 inhibitor interaction with RU24969 (high-dose) to affect open field measures was confirmed using a second GSK-3 inhibitor, AR-A014418 (Figure 2.7; Supplemental Results).

2.3.2 GSK-3 inhibition mitigates RU24969-induced PPI deficits

In Experiment 1, there was a three-way interaction among RU24969, SB216763 and Block for startle amplitude ($F_{(2,72)} = 4.53$; $p<.05$; Figure 2.2a). Post hoc analysis revealed that within saline-treated mice, 10 mg/kg SB216763 increased startle amplitude over 0 or 5 mg/kg SB216763-pretreated groups in Block 3 but not Block 2. SB216763 pretreatment mitigated low-dose RU24969-induced PPI deficits in the second half of testing, as revealed by a three-way interaction among SB216763, RU24969, and Block ($F_{(2,70)} = 3.89$; $p<.05$; Figure 2.2b). Post hoc analyses revealed that RU24969 reduced PPI collapsed across block in saline-pretreated mice ($F_{(1,22)} = 18.19$; $p<.0005$), whereas RU24969 reduced PPI in Block 2 but not Block 3 for 5 mg/kg SB216763-pretreated mice. In addition, for 5 mg/kg SB216763-pretreated mice, PPI was reduced in Block 3 relative to Block 2 in saline-treated mice ($F_{(1,12)} = 14.98$; $p<.005$). RU24969 reduced PPI in both blocks for 10 mg/kg SB216763-pretreated mice, but the 3 mg/kg RU24969 group had increased PPI in Block 3 relative to Block 2 ($F_{(1,11)} = 9.79$; $p<.01$). Because of this later onset effect of SB216763 on PPI, effects of SB216763 pretreatment on low-dose RU24969-induced open field phenotypes were assessed at a later time point to coincide with the onset of the above effects on PPI. There were no interactions between SB216763 and RU24969 for any open field measure at this later time point (Figure 2.8; Supplemental Results).

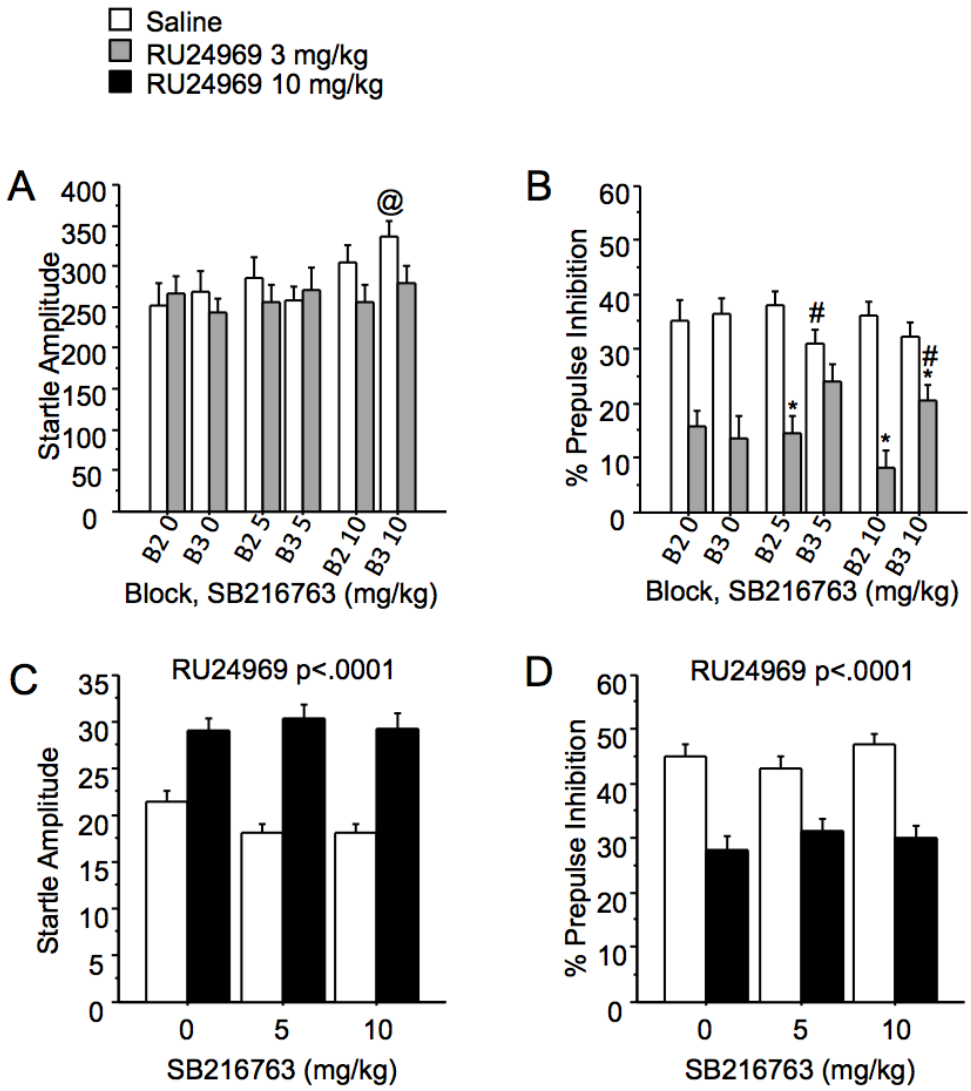

Figure 2.2: GSK-3 inhibition mitigated low dose, but not high dose, RU24969-induced changes in prepulse inhibition. A - B show startle amplitude (A) and percent PPI (B) for Experiment 1. C - D show startle amplitude (C) and percent PPI (D) for Experiment 2. Results expressed as mean ± SEM. *Significant difference from saline treated group within pretreatment and block. #Significant difference from block 2 within pretreatment and treatment group. @Significant difference from both other pretreatment groups within treatment group within block.

In Experiment 2, RU24969 increased startle amplitude overall ($F_{(1,78)} = 57.50$; $p<.0001$; Figure 2.2c), whereas SB216763 had no effect. High-dose RU24969 treatment decreased PPI overall ($F_{(1,78)} = 25.07$; $p<.0001$; Figure 2.2d), whereas SB216763 had no effect. The lack

of interaction between GSK-3 inhibition and high-dose RU24969 treatment for PPI measures was confirmed using another GSK-3 inhibitor, AR-A014418 (Figure 2.7; Supplemental Results).

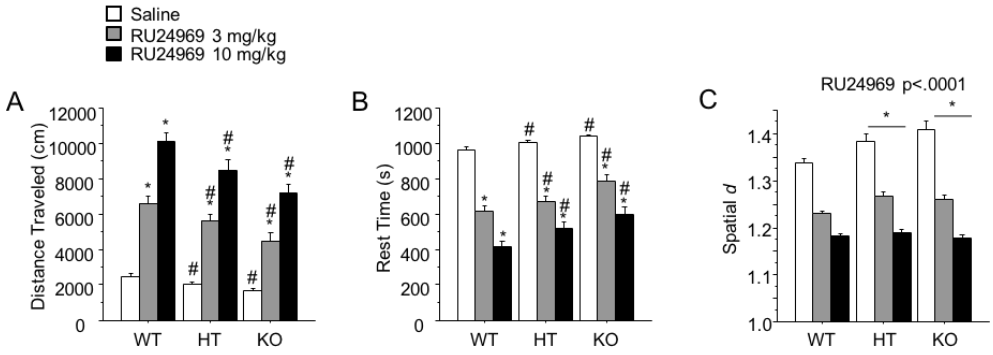

Figure 2.3: *Arrb2* genotype affects RU24969-induced changes in activity in the open field test. A - C show distance traveled (A), time spent resting (B), and spatial *d* (C) for Experiment 3. Results expressed as mean ± SEM. *Significant difference from saline treatment group within genotype. #Significant difference from WT within treatment condition. *Significant difference from WT collapsed across treatment groups.

2.3.3 β-arrestin2 expression affects RU24969-induced open field phenotypes

A genotype by treatment interaction was found for distance traveled ($F_{(4,156)} = 3.09$; $p<.05$; Figure 2.3a). Post hoc tests revealed that both 3 mg/kg and 10 mg/kg RU24969 increased distance traveled for WT (3 mg/kg: $F_{(1,28)} = 89.52$; $p<.0001$; 10 mg/kg: $F_{(1,28)} = 241.40$; $p<.0001$), HT (3 mg/kg: $F_{(1,27)} = 87.13$; $p<.0001$; 10 mg/kg: $F_{(1,27)} = 141.17$; $p<.0001$) and KO mice (3 mg/kg: $F_{(1,26)} = 52.48$; $p<.0001$; 10 mg/kg: $F_{(1,27)} = 127.49$; $p<.0001$). Post hoc tests also revealed that *Arrb2* HT and KO mice traveled less distance than WT mice at each RU24969 dose. To assess the relationship between saline- and RU24969-induced activity levels, within-subject correlation and regression analyses were performed between saline and RU24969 conditions. These tests revealed no relationship between saline and RU24969-induced activity levels within any genotype (Supplemental Results).

A trend for a genotype by treatment interaction for rest time was found ($F_{(4,156)} = 1.99$;

p = .099; Figure 2.3b). Post hoc tests revealed that both 3 mg/kg and 10 mg/kg RU24969 decreased rest time within each genotype and that HT and KO mice rested more than WT mice within each RU24969 dose. To assess the relationship between saline- and RU24969-induced rest times, within-subject correlation and regression analyses were performed between saline and RU24969 conditions. This assessment identified no relationship between saline- and RU24969-induced rest times within each genotype (Supplemental Results).

There was a main effect of genotype on spatial d ($F_{(2,75)} = 3.45$; $p<.05$; Figure 2.3c). Post hoc tests revealed that both HT and KO mice had higher spatial d than WT mice. There was a main effect of RU24969 on spatial d ($F_{(2,150)} = 150.30$; $p<.0001$). Post hoc tests revealed that both 3 mg/kg and 10 mg/kg RU24969 reduced spatial d.

Next, mice were matched for saline-induced distance traveled in the open field in order to assess genotype by treatment interactions independent of a baseline effect of genotype on distance traveled. These activity-matched results confirmed that *Arrb2* KO mice traveled a shorter distance than WT or HT mice receiving 10 mg/kg RU24969 (Supplemental Results; Figure 2.10).

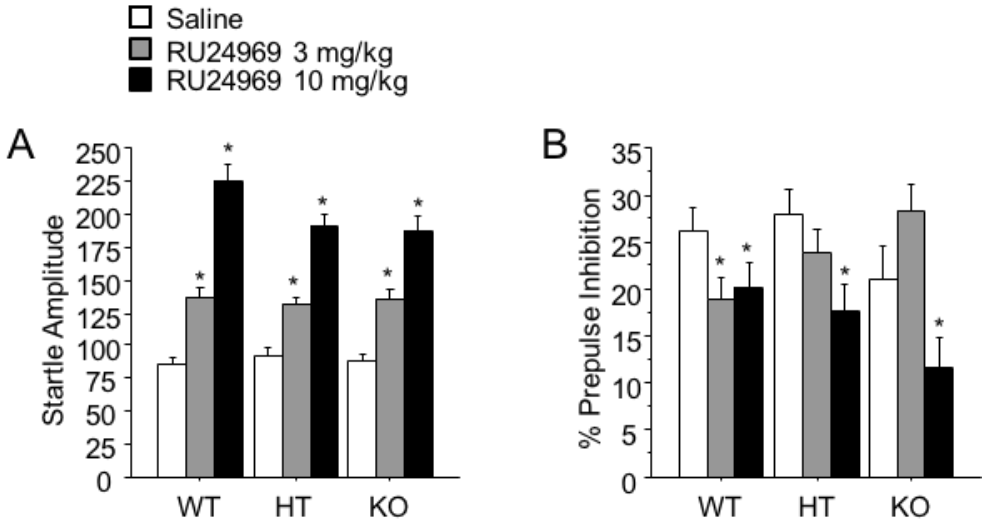

Figure 2.4: *Arrb2* genotype affects RU24969-induced changes in prepulse inhibition. A - B show startle amplitude (A) and PPI (B) for Experiment 3. Results expressed as mean ± SEM. *Significant difference from saline treatment group within genotype.

2.3.4 β-arrestin2 expression affects RU24969-induced PPI deficits

A trend was found for a genotype by treatment interaction for startle amplitude ($F_{(4,164)}$ = 2.08; p = .09; Figure 2.4a). Post hoc tests revealed that 3 mg/kg and 10 mg/kg RU24969 increased startle for WT (3 mg/kg: $F_{(1,29)}$ = 29.74; p<.0001; 10 mg/kg: $F_{(1,29)}$ = 86.58; p<.0001), HT (3 mg/kg: $F_{(1,29)}$ = 22.83; p<.0001; 10 mg/kg: $F_{(1,29)}$ = 98.13; p<.0001), and KO mice (3 mg/kg $F_{(1,27)}$ = 20.57; p = .0001; 10 mg/kg: $F_{(1,27)}$ = 57.56; p<.0001) whereas *Arrb2* genotype had no effect at any dose of RU24969.

A genotype by treatment interaction was observed for PPI ($F_{(4,164)}$ = 2.66; p<.05; Figure 2.4b). Post hoc tests revealed that 10 mg/kg RU24969 reduced PPI in KO mice ($F_{(1,27)}$ = 56.59; p<.0001), whereas simple main effects of RU24969 treatment missed significance in WT ($F_{(2,58)}$ = 2.73; p = .07) and HT mice ($F_{(2,58)}$ = 3.11; p = .05). Since RU24969 has consistently been reported to reduce PPI [324, 323], planned comparisons were performed for the effects of each RU24969 dose within genotype. Planned comparisons revealed that 3 mg/kg RU24969 reduced PPI in WT, but not HT or KO, mice. Planned comparisons also revealed that 10 mg/kg RU24969 reduced PPI within each genotype.

2.4 Discussion

Here we show that GSK-3 inhibition and *Arrb2* knockout both reduce 5-HT1BR-induced PPI deficits, while *Arrb2* knockout also reduces 5-HT1BR-induced hyperlocomotion, a key component of the perseverative pattern of locomotor activity induced by RU24969 administration. Specifically, GSK-3 inhibition did not mitigate RU24969-induced open field phenotypes (Figure 2.1; 2.6-2.8) but did attenuate RU24969-induced PPI deficits (Figure 2.2b). These findings suggest that blockade of canonical 5-HT1BR signaling reduces RU24969-induced PPI deficits. Alternatively, *Arrb2* knockout reduced RU24969-induced hyperactivity (Figure 2.3a-b) and mitigated RU24969-induced PPI deficits (Figure 2.4b), indicating that noncanonical 5-HT1BR signaling may play a role in RU24969-induced PPI deficits

and perseverative behavior. These findings may have implications for development of novel therapeutics for disorders characterized by these phenotypes.

GSK-3 inhibition had no effect on RU24969-induced activity measures in the open field. RU24969 induced robust hyperactivity concomitant with decreases in rest time (Figure 2.1; Figures 2.7-2.8), paralleling our previous work [165, 324, 323]. This hyperactivity occurred regardless of GSK-3 inhibitor pretreatment, in accordance with previous evidence that pharmacological or genetic inhibition of GSK-3 failed to mitigate anpirtoline-induced hyperactivity [67, 397]. Similarly, GSK-3 inhibition did not modulate RU24969-induced changes in center measures (Figure 2.6c-d; Figure 2.8d-e). In contrast, a recent report showed that snGSK-3β KO prevented anpirtoline-induced thigmotaxis [397]. However, in the present experiments, center measures were substantially related to distance traveled, complicating their interpretation (Supplemental Results). snGSK-3β KO mice have has also been reported to lack anpirtoline-induced reductions in vertical rearing [397]. However, we were unable to assess this effect due to low baseline levels of rearing in the Balb/cJ strain [17] relative to C57BL/6J mice [165]. Interestingly, we found that AR-A014418 decreased spatial d across treatments (Figure 2.7c; 2.5c).

Furthermore, SB216763 had no such effect with a thirty-minute pretreatment (Figure 2.1c; Figure 2.1f), but decreased spatial d at the sixty-minute pretreatment time point (Figure 2.8c; 2.5f). Since GSK-3 inhibition did not potentiate RU24969-induced reductions in spatial d, these findings indicate that GSK-3 modulates spatial d independent from 5-HT1BR signaling, and that GSK-3 signaling might be protective against locomotor perseveration [269]. Overall, GSK-3 does not appear to play a substantial role in RU24969-induced behavioral effects in the open field within these experimental conditions.

We found that GSK-3 inhibition mitigated RU24969-induced PPI deficits (Figure 2.2b). These effects were specific to the later testing block, indicating a delay in the interaction between RU24969 and GSK-3 inhibitors. Therefore, we tested the effects of GSK-3 inhibition

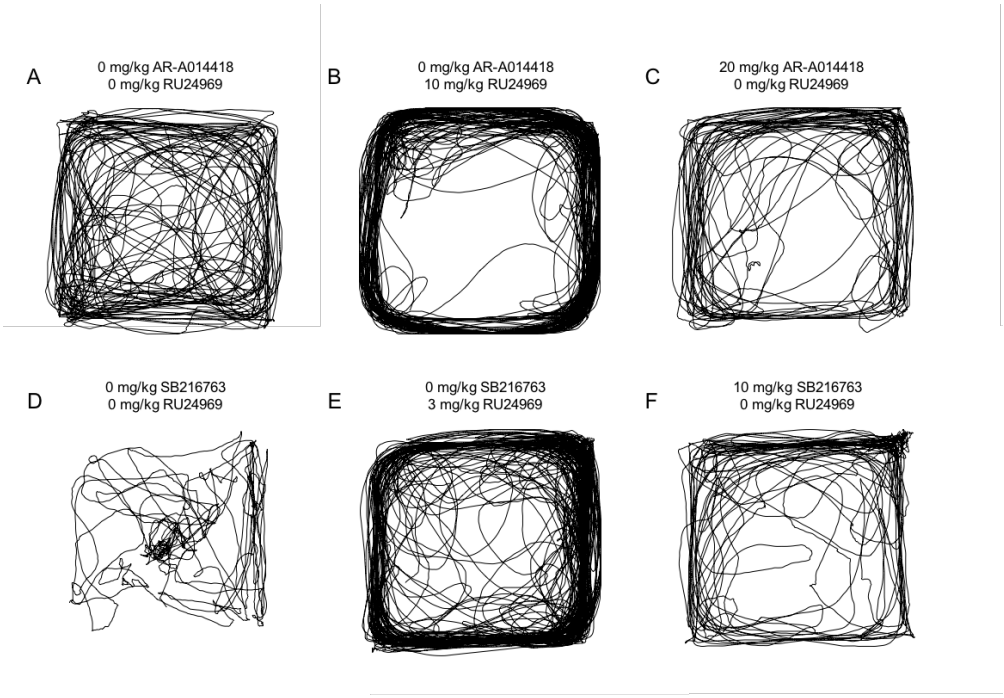

Figure 2.5: Representative paths mice took in the open field in Experiments 4 (A-C) and 3 (D-F). RU24969-treated (B, E) and GSK-3 inhibitor-treated (C, F) mice have a similar pattern of activity, in contrast to mice treated only with vehicle (A, D).

on RU24969-induced open field phenotypes at this later time point (Experiment 3), to confirm that the lack of effect of SB216763 on total distance traveled in Experiment 1 was due to delayed onset of drug effects. However, no interactions between SB216763 and RU24969 were observed (Figure 2.8). The attenuation of RU24969-induced PPI deficits by SB216763 were not due to any confounding effects on startle magnitude (Figure 2.2a), since the effects of GSK-3 inhibition on startle amplitude were dissociable from those on PPI. Furthermore, GSK-3 inhibition by SB216763 reduced 3 mg/kg, but not 10 mg/kg, RU24969-induced PPI deficits (Figure 2.2). Most likely, the lack of effect of AR-A014418 on RU24969-induced PPI deficits is due to the fact that only the high dose of RU24969 was used in this study (Figure 2.7), but this should be confirmed with the lower dose of RU24969 in future studies. The absence of effects of SB216763 alone on PPI conflicts with previous reports, indicating that experimental factors such as route of administration, testing time point, strain, or sex may

influence these effects [63, 194]. In fact, GSK-3 activity was previously shown to positively correlate with PPI across a panel of mouse strains [14]. In sum, GSK-3 inhibition reduces the PPI disruption induced by low-dose RU24969.

In contrast to GSK-3 inhibition, *Arrb2* knockout mitigated RU24969-induced hyperactivity in the open field. Genotype affected baseline locomotor activity levels (Figure 2.3a-b), with modest reductions in locomotion observed in HT and KO mice, consistent with previous reports [29, 30, 41, 75]. However, the reduction in RU24969-induced hyperlocomotion observed in HT and KO mice was not attributable to blunted baseline activity levels; no relationship was found between saline- and RU24969-induced locomotor activity within any genotype, such that mice less active in the saline condition were not necessarily less active in the RU24969-treated conditions (Supplemental Results). In addition, effect sizes for RU24969 treatment on distance traveled were substantially larger in WT than HT or KO mice. We further confirmed that the effect of RU24969 on locomotion was independent of baseline activity level by creating matched groups across genotypes for baseline locomotion; *Arrb2* HT and KO mice still showed blunted RU24969-induced hyperactivity (Figure 2.10a) and reductions in rest time after receiving 10 mg/kg RU24969 (Figure 2.10b). Our findings parallel previous reports that *Arrb2* knockout prevents anpirtoline-induced hyperactivity [67]. While several previous reports have resolved the confounding hypoactivity of *Arrb2* KO mice by extended habituation to the open field prior to drug challenge [30, 67], our activity-matched control analysis does not interfere with the effects of novelty on behavioral measures in the open field. Overall, *Arrb2* KO and HT mice show diminished RU24969-induced changes in locomotor activity, regardless of baseline activity levels.

Arrb2 knockout also affected spatial d and vertical rearing in the open field. *Arrb2* HT and KO mice showed increases in spatial d (Figure 2.3c), suggesting more circumscribed paths of locomotion, and less locomotor perseveration, than in WT mice [269]. However, this effect was lost in the activity-matched subset analysis (Figure 2.10c). Baseline spatial d in

WT mice was notably lower in Experiment 5 than Experiments 1-4, consistent with previous spatial *d* levels found in C57BL/6J [165] versus Balb/cJ mice [324, 323]. Our finding that *Arrb2* KO showed decreased rearing (Figure 2.9a) conflicts with one previous report that found no effect of *Arrb2* genotype on rearing, perhaps attributable to the low baseline level of rearing in that study [30]. Thus, to our knowledge, this is the first report of reduced rearing in *Arrb2* HT and KO mice, suggesting reduced exploration or response to novelty [221]. Although this effect was lost in the activity-matched subset analysis, this was likely due to loss of sufficient sample size (Figure 2.10d). RU24969 robustly decreased rearing within each genotype (Figure 2.9a)[165], although differing genotypic levels of baseline rearing and a floor effect precluded interpretation of the interaction between genotype and RU24969 (Figure 2.9a). Furthermore, this interaction is lost in the activity-matched subset analysis (Figure 2.10d).

Arrb2 genotype modulated center activity as well. *Arrb2* HT and KO mice spent less time and traveled a lower proportion of their total distance in the center of the open field than WT mice within the saline condition (Figure 2.9), in accordance with previous findings [81]. This reduced exploration of the center by HT and KO mice aligns with the observed reductions in rearing, indicating reduced exploration. However, these genotypic effects may have resulted from observed differences in distance traveled (Supplemental Results). *Arrb2* genotype also modulated the effects of RU24969 on center measures (Figure 2.9b-c). Interestingly, in the activity-matched analysis, the baseline effect of genotype on center time was eliminated, yet center time was increased by 3 mg/kg RU24969 only in WT mice (Figure 2.10e). Thus, *Arrb2* KO and HT mice show diminished RU24969-induced changes in time spent in the center of the open field, regardless of baseline activity levels.

The dose-dependent mitigation of RU24969-induced PPI deficits we found in *Arrb2* HT and KO mice parallels our findings with GSK-3 inhibitors. In contrast to WT mice, *Arrb2* HT and KO mice did not exhibit PPI deficits following treatment with 3 mg/kg RU24969.

However, 10 mg/kg RU24969 treatment reduced PPI in all genotypes (Figure 2.4). These effects on PPI were not artifacts of changes in startle magnitude, since RU24969 increased startle comparably in all genotypes. PPI levels in Experiment 5 were notably lower than in Experiments 1-4, likely due to the low PPI levels characteristic of the C57BL/6J strain [271]. Thus, reduction in β-arrestin2-mediated signaling appears to reduce RU24969-mediated PPI deficits at lower, but not higher, doses.

Some aspects of the RU24969-induced behavioral syndrome were not modulated by GSK-3 inhibition or *Arrb2* KO, such as reduced spatial *d*, or were incompletely blocked by inhibiting either pathway, such as hyperactivity. One possible explanation for these findings is that yet another noncanonical signaling pathway mediates the effects of 5-HT1BR activation. G-protein coupled-receptor kinases (**GRK**s) classically phosphorylate GPCRs to recruit arrestins for desensitization or endocytosis. However, emerging evidence indicates that GRKs also modulate activity of key intracellular signaling molecules, such as ERK and Akt [157]. Another possibility is that the effects of blocking β-arrestin2 signaling were obscured by developmental compensations in the *Arrb2* KO mouse.

The constitutive *Arrb2* KO mice used in the present studies likely have compensatory developmental changes, such that results obtained with these mice may differ from those obtained using acute treatment with a β-arrestin2 inhibitor. Indeed, the compensatory developmental consequences of lacking a gene throughout life can cause effects that are opposite to those of acute drug action at the gene product [54]. This caveat highlights the need for development of selective β-arrestin2 inhibitors. One recently identified candidate, Barbadin, selectively blocks the interaction between β-arrestins and AP-2, thus disrupting β-arrestin-mediated endocytosis of GPCRs and endocytosis-associated signaling in vitro [31]. This agent could be useful to determine which components of noncanonical β-arrestin signaling are mediated through internalized GPCRs. Furthermore, the recently developed floxed β-arrestin2 mouse [363], and viral approaches for altering β-arrestin2 expression pro-

vide future tools for identifying the role of β-arrestin2 signaling. Our present results provide initial evidence for an important role for 5-HT1BR noncanonical intracellular signaling in both perseverative behavior and PPI deficits. Future work should confirm these effects using β-arrestin2 inhibitors, inducible *Arrb2* mice, and viral approaches.

Our findings may have therapeutic implications for neuropsychiatric disorders characterized by perseverative behaviors and PPI deficits, including OCD and ASD. Pharmacological 5-HT1BR challenge exacerbates OCD symptoms [155, 206, 338] and induces a larger growth hormone response in ASD that correlates with baseline levels of repetitive behaviors [172]. Furthermore, the 5-HT1A/1B antagonist pindolol potentiates the effects of chronic SRI treatment in treatment-resistant OCD patients [43, 80, 85]. Thus, 5-HT1BR inhibition may ameliorate perseverative behavior. Our data suggest that ligands inhibiting canonical, G-protein-coupled signaling or noncanonical, β-arrestin2-dependent signaling for 5-HT1BRs might be effective for mitigating PPI deficits seen in disorders such as OCD and ASD. However, GSK-3 inhibitors did not alleviate 5-HT1BR-mediated perseverative behavior, and reduced spatial *d* overall (Figure 2.7c; 2.8c), mimicking one aspect of RU24969-induced locomotor perseveration (Figure 2.5). These effects suggest that neither drugs blocking 5-HT1BR-mediated canonical signaling, nor nonspecific GSK-3 inhibitors reduce perseverative behavior. Alternatively, *Arrb2* knockout mitigated both RU24969-induced PPI deficits and certain aspects of perseverative hyperlocomotion, suggesting that inhibition of noncanonical 5-HT1BR-mediated signaling might provide a novel and selective therapeutic target for reducing perseverative behaviors and PPI deficits. However, *Arrb2* knockout did not mitigate all aspects of the RU24969-induced perseverative behavioral pattern in the open field, and produced a modest reduction of hyperactivity. Furthermore, our findings in *Arrb2* KO mouse will require confirmation using a selective β-arrestin2 inhibitor when one becomes available. In sum, 5-HT1BR antagonists or inverse agonists biased toward β-arrestin2 blockade may be useful agents in OCD and ASD, and should be developed and tested in animals.

In conclusion, both GSK-3- and β-arrestin2-dependent intracellular signaling pathways mediate aspects of 5-HT1BR-induced behavioral deficits. Our findings demonstrate that GSK-3 inhibitors attenuate 5-HT1BR-induced PPI deficits, but not perseverative hyperlocomotor behavior. Additionally, GSK-3 inhibitors were found to increase locomotor perseveration, and thus might be detrimental to patients with OCD and related disorders. On the other hand, *Arrb2* knockout reduces 5-HT1BR-induced locomotor perseveration and PPI deficits. Thus, β-arrestin2-biased antagonists or inverse agonists at 5-HT1BR could be prospective candidates for treating perseverative behaviors and PPI deficits, and should be further examined.

2.5 Supplemental material

2.5.1 Results

No effect of SB216763 on RU24969-induced changes in open field center measures

RU24969 treatment increased time spent in the center of the open field overall at the high dose ($F_{(1,78)}$ = 10.73; p<.005; Figure 2.6c) but not the low dose (Figure 2.6a). Similarly, high-dose ($F_{(1,73)}$ = 13.01; p<.001; Figure 2.6d) but not low-dose (Figure 2.6b) RU24969 increased proportion of distance traveled in the center across pretreatments. However, time spent in the center was significantly correlated with total distance traveled for low-dose RU24969 (r = .35; 95% CI: .13 to .53; p<.005) and high-dose RU24969 (r = .54; 95% CI: .36 to .67; p<.0001). A simple linear regression analysis identified a significant regression for low dose RU24969 ($F_{(1,76)}$ = 10.29; p<.005; r^2 = .12), indicating that 12% of the variance in time spent in the center is explained by the total distance traveled. There was also a significant simple linear regression for high dose RU24969 ($F_{(1,82)}$ = 32.80; p<.0001; r^2 = .29). Proportion of distance traveled in the center also showed substantial correlation with total distance traveled for the high dose of RU24969 (r = .51; 95% CI: .33 to .66; p<.0001) but not the low dose. Simple linear regression analysis revealed a significant regression for proportion of distance in the center for high-dose RU24969 ($F_{(1,77)}$ = 27.05; p<.0001; r^2 = .26) but not low-dose RU24969. Virtually no vertical activity was observed in either Experiment 1 or 2 (data not shown); thus, statistical analysis was not performed.

No effect of AR-A014418 on high dose RU24969-induced changes in behavior

In the open field, RU24969 treatment increased total distance traveled across pretreatment groups ($F_{(1,78)}$ = 85.57; p<.0001; Figure 2.7a). AR-A014418 pretreatment did not interact

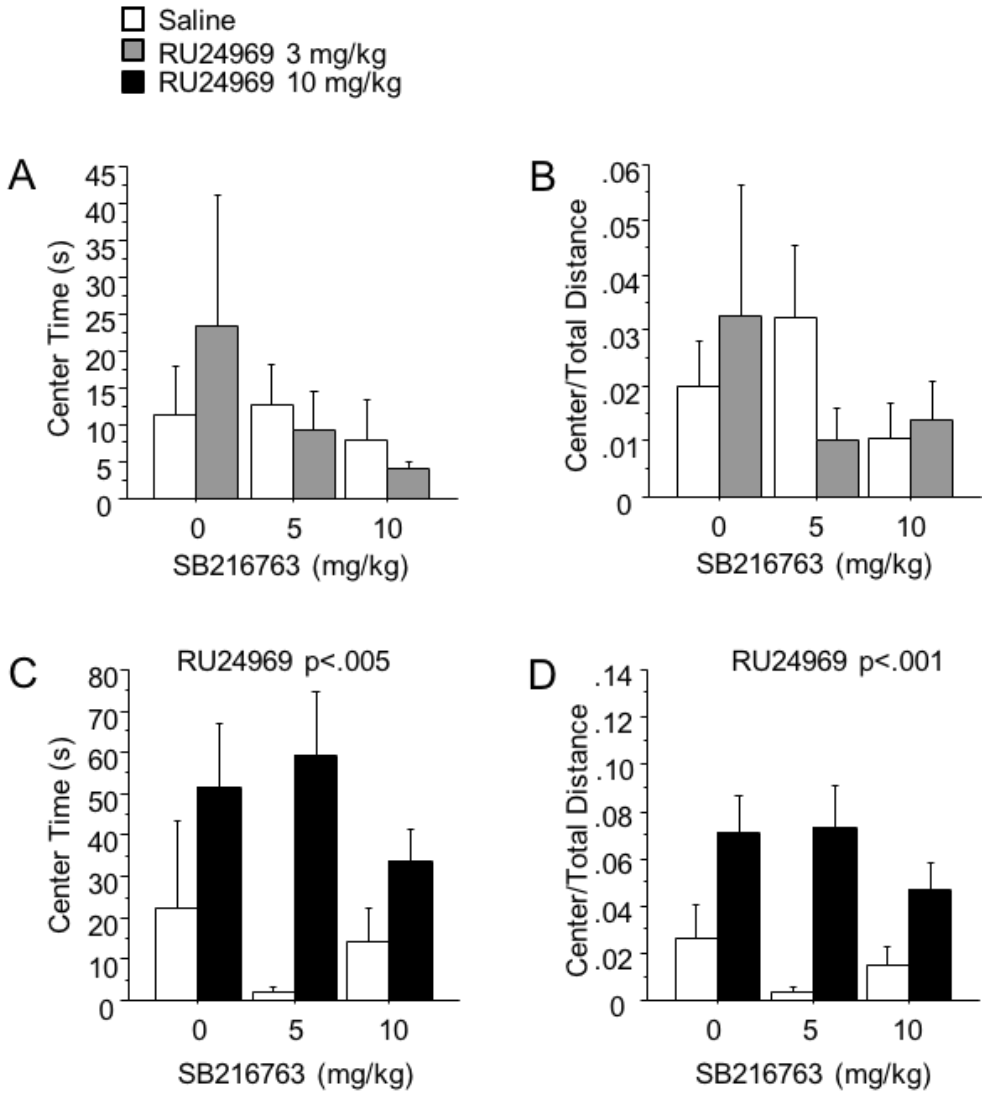

Figure 2.6: GSK-3 inhibition did not affect RU24969-induced changes in additional measures of activity in the open field test. A - B show time spent in the center of the open field (A) and proportion of distance traveled in the center (B) for Experiment 1. C - D show time spent in the center (C) and proportion of distance traveled in the center (D) for Experiment 2. Results expressed as mean ± SEM.

with RU24969 treatment or have a main effect on distance traveled. RU24969 also reduced time spent resting across pretreatment groups ($F_{(1,78)} = 136.45$; $p<.0001$; Figure 2.7b), while AR-A014418 had no effect on rest time. RU24969 reduced spatial d across pretreatment doses ($F_{(1,75)} = 5.54$; $p<.05$; Figure 2.7c).

AR-A014418 also reduced spatial d ($F_{(2,75)} = 3.91$; $p<.05$; Figure 2.8c) at both the 10

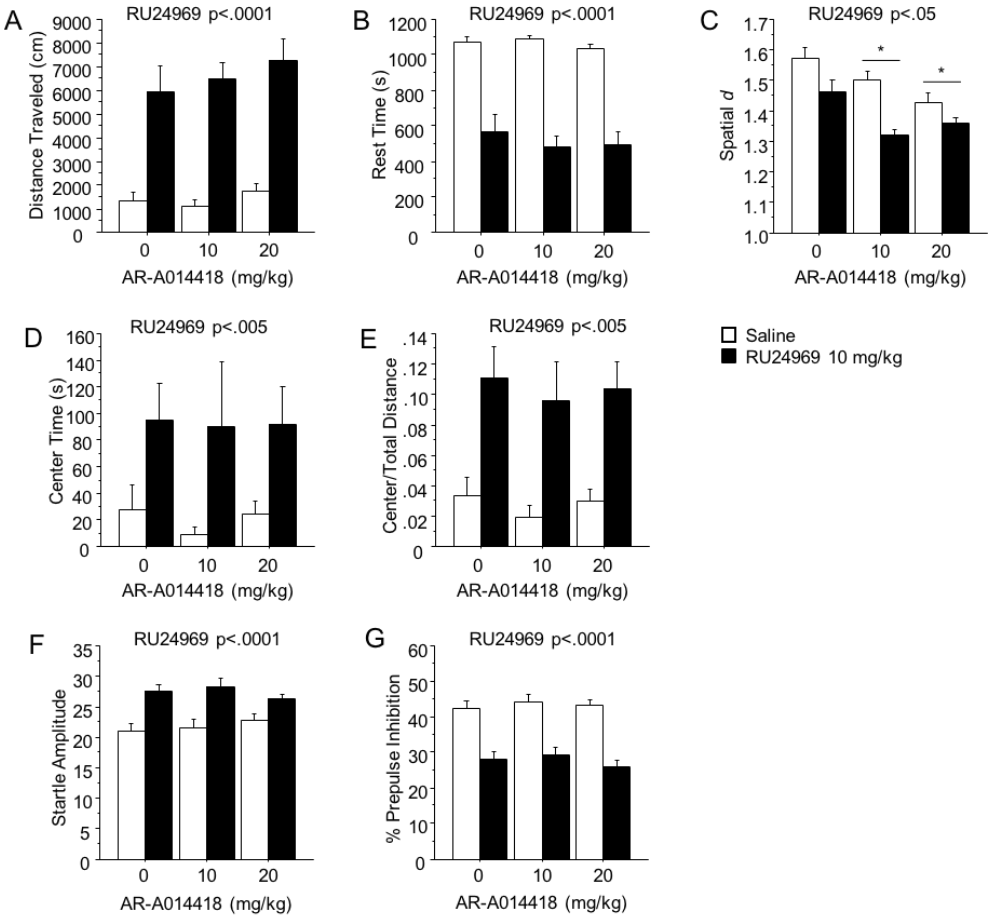

Figure 2.7: GSK-3 inhibition with a second GSK-3 inhibitor did not affect high dose RU24969-induced behavior in the open field or prepulse inhibition. A - E show effects of AR-A014418 and RU24969 on open field measures: total distance traveled (A), time spent resting (B), spatial *d* (C), time spent in the center of the open field (D), and proportion of total distance traveled spent in the center of the open field (E). F - G show effects of AR-A014418 and RU24969 on startle amplitude (F) and percent prepulse inhibition (G). Results expressed as mean ± SEM. *Significantly different from vehicle pretreatment across treatment groups.

mg/kg and 20 mg/kg doses, but did not interact with RU24969 treatment to affect spatial *d* (Figure 2.10a-c). RU24969 treatment increased time spent in the center of the open field overall ($F_{(1,78)} = 10.70$; p<.005; Figure 2.7d), whereas AR-A014418 had no effect. Similarly, RU24969 increased the proportion of the total distance traveled in the center across pretreatments ($F_{(1,74)} = 8.77$; p<.005; Figure 2.7e) with no effect of AR-A014418. However, time spent in the center was significantly correlated with total distance traveled

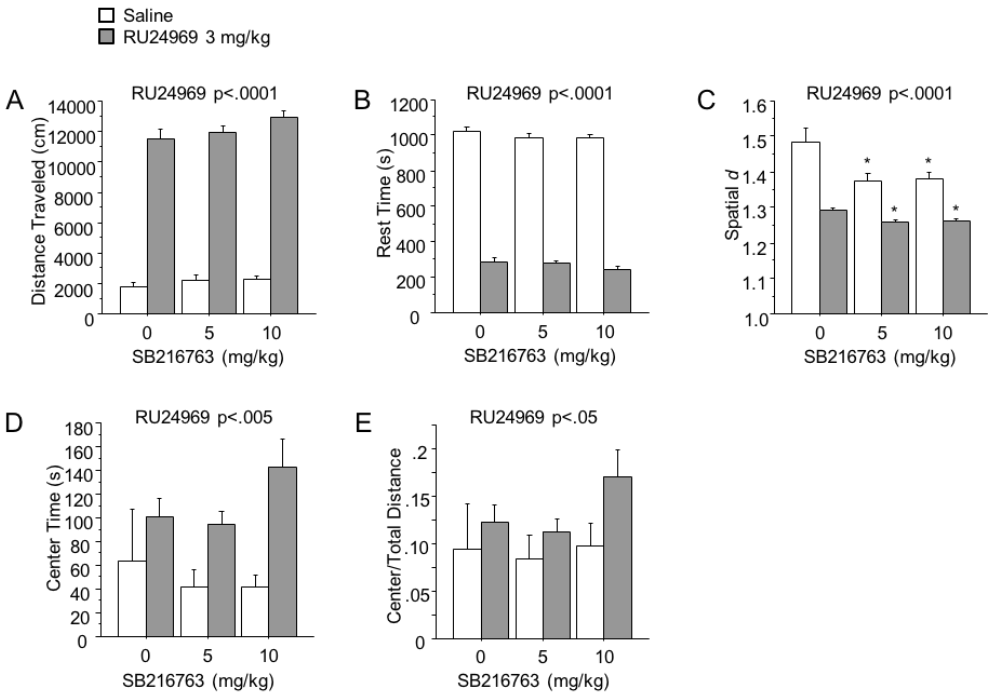

Figure 2.8: No effect of delayed GSK-3 inhibition on low dose RU24969-induced behavior in the open field. A - E show effect of SB216763 and RU24969 on open field measures: total distance traveled (A), time spent resting (B), spatial d (C), time spent in the center of the open field (D), and proportion of distance traveled in the center of the open field (E). Results expressed as mean ± SEM. *Significantly different from vehicle pretreatment within treatment group.

(r = .56; 95% CI: .39 to .69; p<.0001) when pooled across groups. Simple linear regression analysis revealed a significant regression for time spent in the center of the open field ($F_{(1,82)}$ = 36.54; p<.0001; r^2 = .31), indicating that 31% of the variance in time spent in the center is explained by the total distance traveled. Proportion of distance traveled in the center also showed substantial correlation with total distance traveled (r = .54; 95% CI: .37 to .68; p<.0001). Simple linear regression analysis revealed significant regressions for proportion of distance in the center for ($F_{(1,78)}$ = 32.49; p<.0001; r^2 = .29). Virtually no vertical activity was observed in Experiment 4 (data not shown); thus, statistical analysis was not performed. RU24969 treatment increased startle amplitude overall ($F_{(1,78)}$ = 20.38; p<.0001; Figure 2.8f), whereas AR-A014418 had no effect on startle. RU24969 treatment decreased

PPI overall ($F_{(1,78)}$ = 47.24; p<.0001; Figure 2.8g), whereas AR-A014418 had no effect on PPI.

Effect of SB216763 on low dose RU24969-induced open field behaviors at an extended time point

To confirm that the negative results in Experiment 1 did not simply miss the optimal time point for the interaction, SB216763 pretreatment with low-dose RU24969 treatment effects on open field measures were assessed at an extended time point that mirrors the effects found in PPI (Figure 2b). RU24969 increased total distance traveled across pretreatment groups ($F_{(1,76)}$ = 846.10; p<.0001; Figure 2.8a). SB216763 pretreatment had a trend for an effect on total distance traveled ($F_{(2,76)}$ = 2.79; p=.07), but had no interaction with RU24969 treatment. RU24969 decreased time spent resting in the open field ($F_{(1,76)}$ = 1613.13; p<.0001; Figure 2.8b), whereas SB216763 treatment had no main effect or interaction with RU24969 for rest time. RU24969 decreased spatial *d* ($F_{(1,74)}$ = 23.32; p<.0001; Figures 2.8c), whereas there was no main effect of SB216763 pretreatment on spatial *d*. However, due to the effect of AR-A014418 on spatial *d* (Figure 2.7c; 2.10c), planned comparisons were performed to assess the effect of SB216763 on spatial *d*. Planned comparisons revealed that both 5 mg/kg and 10 mg/kg SB216763 reduced spatial *d* in both the saline and 3 mg/kg RU24969-treated groups (Figure 2.8c; 2.10f). RU24969 also induced an increase in time spent in the center of the open field ($F_{(1,76)}$ = 11.00; p<.005; Figure 2.8d) and proportion of distance traveled in the center of the open field ($F_{(1,74)}$ = 5.82; p<.05; Figure 2.8e). However, correlation and regression analyses revealed that these measures were affected by total distance traveled. Time spent in the center was correlated with total distance traveled (r = .40; 95% CI: .20 to .57; p<.0005) overall. Simple linear regression analysis revealed a significant regression ($F_{(1,80)}$ = 15.10; p<.0005; r^2 = .16) indicating that 16% of the variance in time spent in the center is attributable to the total distance traveled. Similarly, proportion

of distance traveled in the center was correlated with total distance traveled (r = .28; 95% CI: .06 to .46; p<.05) and there was a significant regression ($F_{(1,80)}$ = 6.54; p<.05; r^2 = .08).

Relationship between saline and RU24969-treated activity levels in the open field within *Arrb2* genotype

None of the genotypes had a significant correlation between distance traveled in the saline and 3 mg/kg RU24969 conditions (WT: r = .27; 95% CI: -.11 to .58; p = .16; HT: r = .16; 95% CI: -.23 to .50; p = .42; KO: r = .30; 95% CI: -.09 to .61; p = .12) or between the saline and 10 mg/kg RU24969 conditions (WT: r = .017; 95% CI: -.35 to .38; p = .93; HT: r = .27; 95% CI: -.11 to .58; p = .16; KO: r = .027; 95% CI: -.35 to .40; p = .89). This lack of relationship was further supported by simple linear regression models assessing RU24969-induced distance traveled based on saline-induced distance traveled. For WT mice, there was no significant regression between saline and 3 mg/kg RU24969 distance traveled ($F_{(1,27)}$ = 2.08; p = .16; r^2 = .07) or between saline and 10 mg/kg RU24969 ($F_{(1,27)}$ = .01; p = .93; r^2 = .0003). Likewise for HT mice, there was no significant regression between saline and 3 mg/kg RU24969 ($F_{(1,26)}$ = .69; p = .41; r^2 = .03) or 10 mg/kg RU24969 ($F_{(1,27)}$ = 2.15; p = .15; r^2 =.07). Similarly for KO mice, there was no significant regression for 3 mg/kg ($F_{(1,25)}$ = 2.55; p = .12; r^2 = .09) or 10 mg/kg RU24969 ($F_{(1,26)}$ = .02; p = .89; r^2 = .001). Furthermore, the effect size of 10 mg/kg RU24969 treatment on distance traveled was substantially larger in WT mice (d = -3.43) than in HT (d = -2.54) or KO (d = -2.53).

None of the genotypes had a significant correlation between time spent resting in the saline and 3 mg/kg RU24969 conditions (WT: r = .083; 95% CI: -.29 to .44; p = .67; HT: r = .011; 95% CI: -.36 to .38; p = .95; KO: r = .058; 95% CI: -.33 to .43; p = .78) or between the saline and 10 mg/kg RU24969 conditions (WT: r = .023; 95% CI: -.340 to .380; p = .91; HT: r = .291; 95% CI: -.078 to .589; p = .12; KO: r = .027; 95% CI: -.350 to .396; p = .89).

This lack of relationship was further supported by simple linear regression models between saline and RU24969-treated conditions. For WT mice, there was no significant regression between saline and 3 mg/kg RU24969 time spent resting ($F_{(1,27)} = .19$; $p = .67$; $r^2 = .007$) or between saline and 10 mg/kg RU24969 ($F_{(1,27)} = .07$; $p = .79$; $r^2 = .003$). Similarly for HT mice, neither 3 mg/kg ($F_{(1,26)} = .003$; $p = .95$; $r^2 = .0001$) nor 10 mg/kg ($F_{(1,27)} = .05$; $p = .83$; $r^2 = .002$) had significant regressions. Finally, KO mice did not have a significant regression for 3 mg/kg ($F_{(1,25)} = .09$; $p = .77$; $r^2 = .003$) or for 10 mg/kg RU24969 ($F_{(1,26)} = .49$; $p = .49$; $r^2 = .02$). Furthermore, the effect size of 10 mg/kg RU24969 treatment on rest time was larger in the WT mice ($d = 3.31$) than in the HT ($d = 2.59$) or KO mice ($d = 2.45$).

Effect of *Arrb2* genotype and RU24969 on vertical and center measures in the open field

A genotype by RU24969 treatment interaction was observed for vertical activity ($F_{(4,156)} = 4.73$; $p<.005$) (Figure 2.9a). Post hoc tests revealed that both 3 mg/kg and 10 mg/kg RU24969 reduced the number of vertical rearings within each genotype. Post hoc tests also revealed that HT and KO mice had fewer vertical rearings than WT mice within the saline condition. ANOVA revealed a genotype by treatment interaction for time spent in the center of the open field ($F_{(4,156)} = 2.87$; $p = .03$; Figure 2.9b). Post hoc tests revealed that 3 mg/kg RU24969 increased time spent in the center for HT but not WT or KO mice, whereas 10 mg/kg RU24969 decreased time spent in the center for WT mice only. Post hoc tests also revealed that HT and KO mice spent less time in the center than WT mice within the saline condition, and KO but not HT mice spent less time in the center within the 3 mg/kg RU24969 condition. No difference was observed among the genotypes within the 10 mg/kg RU24969 condition. To determine the relationship between time spent in the center and total distance traveled in the open field, within-subject correlation analyses were performed

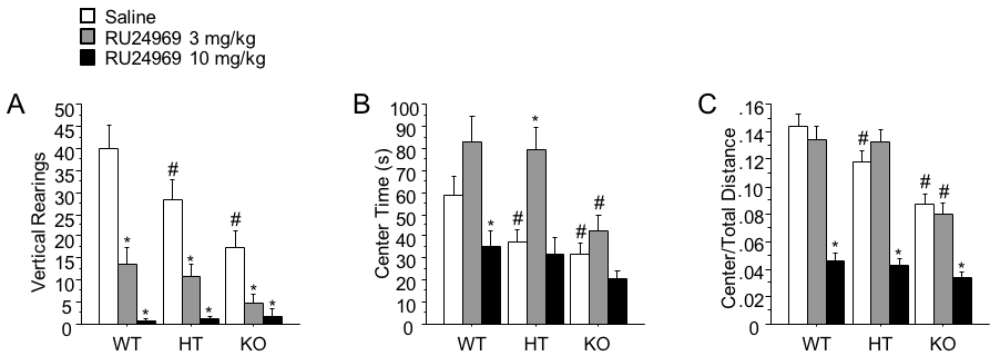

Figure 2.9: *Arrb2* genotype affected RU24969-induced changes in additional measures of activity in the open field test. A - C show the number of instances of vertical rearing (A), time spent in the center of the open field (B) and proportion of distance traveled in the center (C) for Experiment 5. Results expressed as mean ± SEM. *Significant difference from saline treatment group within genotype. #Significant difference from WT within treatment condition.

for each genotype in the saline condition. All three genotypes showed substantial correlation between distance traveled and time spent in the center (WT: r = .79; 95% CI: .59 to .89; p<.0001; HT: r = .74; 95% CI: .50 to .87; p<.0001; KO: r = .550; 95% CI: .22 to .77; p<.005). This relationship was further supported by significant simple linear regressions for all three genotypes (WT: $F_{(1,27)} = 43.23$; p<.0001; $r^2 = .62$; HT: $F_{(1,27)} = 31.64$; p<.0001; $r^2 = .54$; KO: $F_{(1,26)} = 11.29$; p<.005; $r^2 = .30$). Similarly, there was a genotype by treatment interaction for proportion of distance traveled in the center ($F_{(4,146)} = 2.61$; p<.05; Figure 2.9c). Post hoc tests revealed that 10 mg/kg RU24969 reduced distance traveled in the center for all three genotypes, whereas 3 mg/kg RU24969 had no effect for any genotype. Post hoc tests also revealed that HT and KO mice traveled a shorter distance in the center of the open field than WT mice in the saline condition. KO but not HT mice traveled a shorter distance than WT mice in the 3 mg/kg RU24969 condition, and there was no difference among the genotypes in the 10 mg/kg RU24969 condition. To determine the relationship between distance traveled in the center and total distance traveled in the open field, within-subject correlation analyses were performed for each genotype in the saline condition. WT mice showed a trend for a correlation between center and total distance (r = .35; 95% CI:

-.03 to .64; p = .07). HT (r = .58; 95% CI: .27 to .78; p<.001) and KO (r = .52; 95% CI: .17 to .76; p<.01) mice had significant correlations between center and total distance traveled. This relationship was further supported by simple linear regressions. For WT mice, there was a trend for a significant regression ($F_{(1,26)}$ = 3.54; p = .07; r^2 = .12). For HT mice, there was a significant regression ($F_{(1,26)}$ = 13.2; p<.005; r^2 = .34), as well as for KO mice ($F_{(1,24)}$ = 8.95; p<.01; r^2 = .27).

β-arrestin 2 expression effects on RU24969-induced open field phenotypes after saline activity matching

To assess the relationship between genotype and treatment in the absence of a baseline effect of genotype on distance traveled, mice were matched by genotype for total distance traveled in the saline condition. Mice without matches were excluded. This matching procedure resulted in an elimination of the effect of genotype on distance traveled in the open field in the saline condition ($F_{(2,29)}$ = .003; p = .99). The behavior of this subset of mice was then reanalyzed for all effects. A genotype by treatment interaction was found for distance traveled in the matched set of mice ($F_{(4,50)}$ = 3.28; p<.05; Figure 2.10a). Post hoc tests revealed that both 3 mg/kg and 10 mg/kg RU24969 still increased distance traveled for WT (3 mg/kg: $F_{(1,10)}$ = 69.02; p<.0001; 10 mg/kg: $F_{(1,10)}$ = 162.15; p<.0001), HT (3 mg/kg: $F_{(1,8)}$ = 15.19; p<.005; 10 mg/kg: $F_{(1,9)}$ = 58.76; p<.0001) and KO mice (3 mg/kg: $F_{(1,10)}$ = 21.332; p = .001; 10 mg/kg: $F_{(1,10)}$ = 47.84; p<.0001). Post hoc tests also revealed that *Arrb2* KO mice traveled a shorter total distance than WT or HT mice, within the 10 mg/kg RU24969 condition, but not within the 3 mg/kg condition after Bonferroni correction ($F_{(2,28)}$ = 3.54; p = .04). Using the same set of mice matched for distance traveled in the saline condition, rest time was reassessed. As expected, there was no longer an effect of genotype on time spent resting in the saline condition ($F_{(2,29)}$ = .47; p = .63). A genotype by treatment interaction was observed for time spent resting ($F_{(4,50)}$ = 2.85; p<.05; Figure

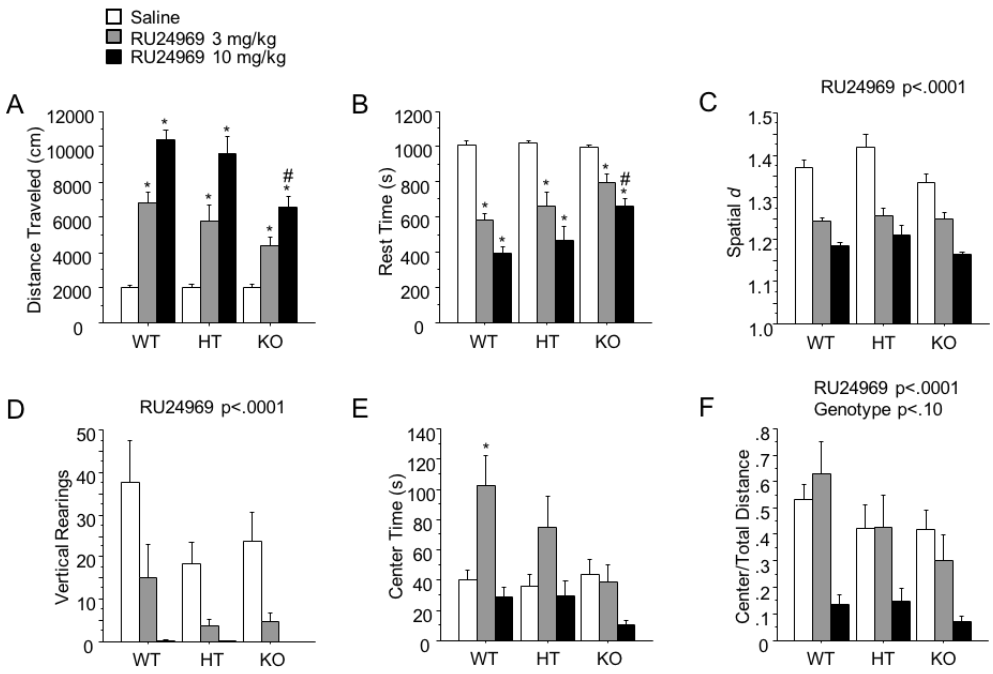

Figure 2.10: *Arrb2* genotype affects RU24969-induced changes in open field behavior regardless of baseline activity level. A - F show total open field measures: total distance traveled (A), time spent resting (B), spatial d (C), the number of instances of vertical rearing (D), time spent in the center (E), and proportion of total distance traveled in the center (F) for Experiment 5 after secondary analysis with activity-matched controls. Results expressed as mean ± SEM. *Significant difference from saline treatment group within genotype. #Significant difference from WT within treatment condition.

2.10b). Post hoc tests revealed that 3 mg/kg and 10 mg/kg RU24969 decreased rest time in WT (3 mg/kg: $F_{(1,10)} = 96.12$; p<.0001; 10 mg/kg: $F_{(1,10)} = 161.66$; p<.0001), HT (3 mg/kg: $F_{(1,8)} = 16.83$; p<.005; 10 mg/kg: $F_{(1,9)} = 42.41$; p = .0001) and KO mice (3 mg/kg: $F_{(1,10)} = 15.00$; p<.005; 10 mg/kg: $F_{(1,10)} = 40.78$; p<.0001). Post hoc tests also revealed that KO mice rested more than WT or HT mice in the 10 mg/kg RU24969 condition, but not after Bonferroni correction in the 3 mg/kg RU24969 condition ($F_{(2,28)} = 3.71$; p = .04).

Using the same set of mice as above, spatial d was reanalyzed. The previous main effect of genotype lost significance with this subset of mice ($F_{(2,24)} = 2.09$; p = .15). A main effect of RU24969 treatment was observed ($F_{(2,48)} = 65.60$; p<.0001; Figure 2.10c). Post hoc tests

revealed that both 3 mg/kg RU24969 ($F_{(1,29)} = 61.00$; $p<.0001$) and 10 mg/kg RU24969 decreased spatial d ($F_{(1,30)} = 100.78$; $p<.0001$).

Using the saline activity-matched cohort, vertical activity was reassessed. There was no longer an effect of genotype on vertical rearing in the saline-treated condition ($F_{(2,29)} = 1.518$; $p = .24$). A main effect of RU24969 treatment was observed ($F_{(2,50)} = 23.41$; $p<.0001$; Figure 2.10d). Post hoc tests revealed that both 3 mg/kg RU24969 ($F_{(1,30)} = 23.87$; $p<.0001$) and 10 mg/kg RU24969 decreased vertical activity ($F_{(1,31)} = 33.41$; $p<.0001$).

Center measures were then reanalyzed. First, there was no longer an effect of genotype on time spent in the center in the saline condition ($F_{(2,29)} = .24$; $p = .79$). A trend for a genotype by RU24969 treatment interaction was observed ($F_{(4,50)} = 2.24$; $p = .08$). Planned comparisons revealed a significant effect of RU24969 in WT mice ($F_{(2,20)} = 10.08$; $p<.001$), where 3 mg/kg RU24969 induced greater time spent in the center than both saline ($F_{(1,10)} = 9.36$; $p<.05$) and 10 mg/kg RU24969 groups ($F_{(1,10)} = 13.49$; $p<.005$), with no difference between saline and 10 mg/kg RU24969 ($F_{(1,10)} = 1.03$; $p = .33$)(Figure 2.10e). In contrast, there was no effect of RU24969 treatment in HT ($F_{(2,16)} = 4.71$; $p = .03$) or KO mice ($F_{(2,20)} = 4.03$; $p = .03$) after Bonferroni correction. For the proportion of distance traveled in the center (Figure 2.10f), there was no effect of genotype in the saline-treated condition ($F_{(2,27)} = .78$; $p = .47$). A trend for a main effect of genotype on proportion of distance traveled in the center was observed ($F_{(2,23)} = 3.22$; $p = .06$). Planned comparisons revealed that WT mice traveled a greater proportion of their distance in the center than KO mice (Figure 2.10f). A main effect of RU24969 treatment was also observed for proportion of distance traveled in the center ($F_{(2,46)} = 24.63$; $p<.0001$). Post hoc tests revealed that 3 mg/kg RU24969 had no effect on proportion of distance traveled in the center ($F_{(1,28)} = .05$; $p = .81$), whereas 10 mg/kg RU24969 decreased proportion of distance traveled in the center ($F_{(1,29)} = 63.79$; $p<.0001$).

CHAPTER 3

DOSE-DEPENDENT EFFECTS OF KETAMINE ON 5-HT1BR-INDUCED OCD-LIKE BEHAVIOR IN MICE

3.1 Introduction

Obsessive-compulsive disorder (OCD) is a psychiatric illness with a lifetime prevalence of 1-3% [312] that is characterized by obsessions, compulsions, or both [15]. Serotonin reuptake inhibitors (SRIs) provide the only pharmacological monotherapy for OCD [115], although approximately half of patients do not respond to SRIs [153], and symptom reduction is only partial even in responders [115]. Furthermore, therapeutic response to SRIs has a long latency in OCD, often in the range of four to twelve weeks [252](but see Issaria et al., 2016[179]). Augmenting SRI treatment with atypical antipsychotics has proven effective in some treatment refractory patients [93, 371], but with the downside of associated side effects. Improved and faster-onset pharmacological therapies for OCD remain an unmet need.

Recently, the noncompetitive NMDA receptor antagonist ketamine was reported to induce rapid therapeutic effects in OCD patients [39, 305, 306]. As in depressed patients [34, 396], a single infusion of ketamine reduced symptoms in OCD patients within minutes to hours [39, 305, 306]. While the antidepressant effects of ketamine have proven to be long lasting in depressed patients [396], the durability of anti-OCD effects is still under investigation, with findings ranging from primarily acute effects [39] to effects lasting four weeks when combined with exposure-based cognitive therapy [306]. In sum, ketamine is emerging as a potential new treatment for refractory OCD, although more controlled studies are needed to replicate these findings. Thus, it is of critical importance to determine the scope of ketamine's efficacy in OCD and the neural mechanisms underlying the therapeutic effects of ketamine treatment, and to use this information to improve treatment.

The effects of ketamine treatment in patients and rodent behavioral models exhibit strong

consistency. Ketamine shows both acute and sustained antidepressant-like effects in the forced swim test [23, 66, 127, 229](but see [291]), and has shown rapid efficacy in models typically sensitive only to chronic treatment with classical antidepressants, including novelty suppressed feeding [335], chronic unpredictable stress [222], and olfactory bulbectomy (our unpublished findings). Recently, prophylactic ketamine treatment has shown efficacy in a rodent model of posttraumatic stress disorder (PTSD)[239], another psychiatric disorder for which ketamine shows promise as a novel treatment [112]. Importantly, such rodent models provide a platform for identifying the neural mechanisms of ketamine's therapeutic effects, and could yield information for optimizing treatment parameters, such as dose and timing. However, no studies to date have assessed the effects of ketamine treatment in an animal model of aspects of OCD.

In this study, we sought to determine whether 5-HT1B receptor (5-HT1BR) agonist-induced perseverative behavior and PPI deficits, two phenotypes observed in OCD patients [6, 341], would be responsive to acute ketamine treatment. Specifically, the 5-HT1BR-induced mouse model of aspects of OCD consists of acute challenge with the 5-HT1A/1B agonist RU24969, which induces perseverative hyperlocomotion in the open field, deficits in prepulse inhibition, and striatal activation, all of which are alleviated by 4 weeks of pretreatment with SRIs, but not desipramine [165, 323, 324]. Here, we assessed the immediate and sustained effects of acute ketamine treatment on RU24969-induced perseverative behavior and PPI deficits in mice. Our findings provide novel information regarding the effects of ketamine on these behavioral phenotypes, and support the use of the 5-HT1BR-induced model to further investigate the neural mechanisms underlying the anti-OCD effects of ketamine.

3.2 Materials and methods

3.2.1 Animals

Experimentally naïve 8-week-old female, Balb/cJ mice were purchased from Jackson Laboratories (Stock #: 000651; Bar Harbor, ME) for all experiments. Animals were acclimated to the vivarium for one week before undergoing experimental procedures. Animals were housed in a climate-controlled room on a 12 hour : 12 hour light : dark cycle and had ad libitum access to standard chow and water. All experiments were performed in accordance with the Institutional Animal Care and Use Committee of The University of Chicago or University of California San Diego.

3.2.2 Drugs

All drugs were administered via intraperitoneal injection with an injection volume of 5 ml/kg bodyweight. 5-HT1A/1B agonist RU24969 (Tocris Bioscience, Bristol, UK) was dissolved in saline (0.9% NaCl) and administered at 0 or 10 mg/kg (Experiments 1, 2, 5, and 6) or at 0, 3, or 10 mg/kg (Experiments 3 and 4). Doses were selected based on previous work [165, 323, 324]. The noncompetitive NMDA receptor (NMDAR) antagonist ketamine hydrochloride (Sigma Aldrich, St. Louis, MO, USA) was dissolved in saline and administered at 0, 3, or 10 mg/kg (Experiments 1 and 2) or at 0 or 30 mg/kg (Experiments 3 and 4). Doses were selected based on previous studies [23, 66, 239, 291] and our own findings in the forced swim test (unpublished observations). The SRI fluoxetine (LKT Laboratories, Inc., St. Paul, MN, USA) was dissolved in water and administered at 0 or 10 mg/kg (Experiment 5) to match previous studies [324].

3.2.3 Open field

The open field test was performed as described previously [324]. Briefly, mice were placed in a corner of the open field and activity was measured for twenty minutes. Output measures were automatically generated by the Versamax software (Accuscan, Columbus, OH) except for the spatial scaling exponent, spatial *d*. Spatial *d* was calculated using a combination of Python (Python Software Foundation, Beaverton, OR, USA), BMDP, and NightOwl (custom) software. Spatial *d* describes the geometric pattern of activity in the open field, where higher values indicate a more meandering path and lower values indicate a smoother and straighter path, characteristic of route stereotypy [269].

3.2.4 Prepulse inhibition

PPI was assessed as described previously [324]. Briefly, mice were placed in startle chambers (San Diego Instruments, San Diego, CA). The amplitude of the startle response was measured for a 65-millisecond (ms) window after five types of trials, each lasting 40 milliseconds: a startle trial (120 dB for 40 ms, no prepulse), a 3 dB above background prepulse trial (20 ms prepulse, followed 100 ms later by 120 dB pulse for 40 ms), a 6 dB above background prepulse trial, a 12 dB above background prepulse trial, or no stimulus, in which only background noise was presented. For all experiments, PPI testing took place directly following open field testing.

3.2.5 Experiment 1

Mice were pseudorandomly assigned to receive ketamine pretreatment (0, 3, or 10 mg/kg) and RU24969 treatment (0 or 10 mg/kg; n = 13-15/group). Ketamine pretreatment was administered 30 minutes prior to open field testing. In all studies, RU24969 treatment was administered 5 minutes prior to open field testing. Mice underwent PPI testing immediately after open field testing.

3.2.6 Experiment 2

Drug administration and behavioral testing were as in Experiment 1, except that pretreatment was administered 24 ± 2 hours prior to RU24969 administration (n = 12-13/group).

3.2.7 Experiment 3

Mice were pseudorandomly assigned to receive ketamine pretreatments (0 or 30 mg/kg) and RU24969 treatment (0, 3, or 10 mg/kg; n = 14/group). Timing of drug administration and behavioral testing were as in Experiment 1.

3.2.8 Experiment 4

Drug administration and behavioral testing were as in Experiment 3, except that pretreatment was administered 24 ± 2 hours prior to RU24969 administration (n = 12-14/group).

3.2.9 Experiment 5

Mice were pseudorandomly assigned to receive fluoxetine pretreatment (0 or 10 mg/kg) and RU24969 treatment (0 or 10 mg/kg). Timing of drug administration and behavioral testing were as in Experiment 1.

3.2.10 Statistical analysis

Dependent measures were analyzed using repeated measures analysis of variance (ANOVA). Alpha was set at 0.05. Interactions reaching significant or trend-level ($p<0.10$) p-values (for a priori predictions only) were resolved by assessing simple main effects in ANOVAs for factors with more than two groups. Student Newman-Keuls post hoc tests were used for assessment of final pair-wise contrasts. Open field measures were analyzed with bin as

a repeated measure (bin size set at five minutes). PPI and startle analysis used block as a repeated measure.

3.3 Results

3.3.1 Mitigating effects of low-dose ketamine on RU24969-induced OCD-like behavior in the open field

A combined analysis was performed in addition to analyzing studies for each pretreatment time point separately (ketamine pretreatment at 30 minutes or 24 hours). First, the two datasets were combined, and each dependent measure was assessed for a significant effect of time point within the vehicle/vehicle group, and for ketamine by time point interactions. No such effects were identified (with the exception of startle amplitude, which varies between experiments due to arbitrary units adjusted during calibration), justifying the analysis of the combined dataset.

In the combined analysis, a significant interaction between ketamine pretreatment and RU24969 treatment was identified for distance traveled in the open field ($F_{(2,146)} = 4.23$; $p<.05$; Figure 3.1A). Post hoc tests revealed that RU24969 treatment significantly increased distance traveled in all three pretreatment conditions. Planned comparisons revealed that within RU24969-treated groups, mice receiving 3 mg/kg ketamine traveled less distance than mice receiving 0 or 10 mg/kg ketamine pretreatment. While the same direction of effects was also apparent within the 30-minute and 24-hour time points (Figure 3.1B; 3.1C), the interactions were not significant (30-minute: $F_{(2,78)} = 1.88$; $p = .16$; 24-hour: $F_{(2,68)} = 2.31$; $p = .11$). Rather, each study showed a main effect of RU24969 treatment (30-minute: $F_{(1,78)} = 62.28$; $p<.0001$; 24-hour: $F_{(1,68)} = 82.62$; $p<.0001$).

Similarly, an interaction was found between ketamine pretreatment and RU24969 treatment for time spent resting in the open field for the combined analysis ($F_{(2,146)} = 4.65$; $p<.05$;

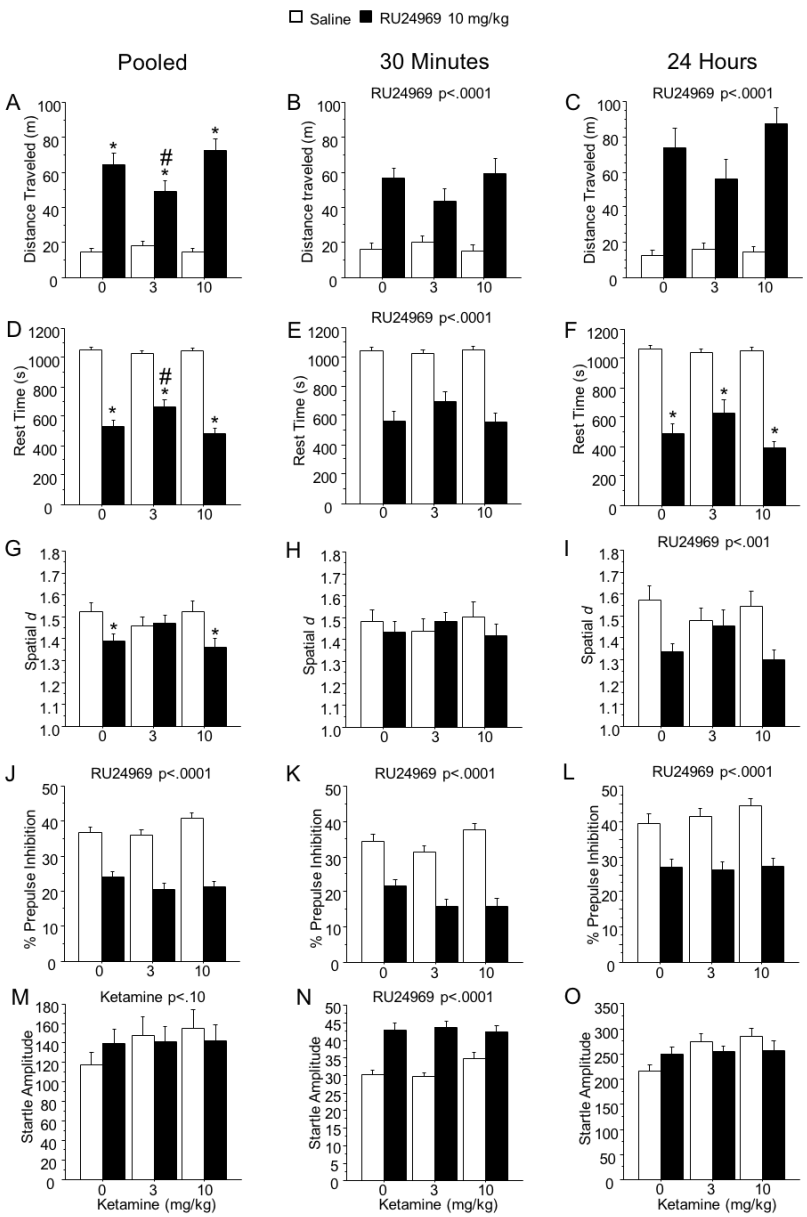

Figure 3.1: Low-dose ketamine pretreatment mitigated RU24969-induced locomotor perseveration. A - C show distance traveled in the open field for pooled (A), 30-minute (B), and 24-hour (C) pretreatments. D - F show rest time for pooled (D), 30-minute (E), and 24-hour (F) pretreatments. G - I show spatial *d* for pooled (G), 30-minute (H), and 24-hour (I) pretreatments. J - L show PPI for combined (J), 30-minute (K), and 24-hour (L) pretreatments. M - O show startle amplitude for pooled (M), 30-minute (N), and 24-hour (O) pretreatments. Results expressed as mean ± SEM. *Significant difference from saline-treated group within pretreatment condition. #Significant difference from saline within treatment condition.

Figure 3.1D). Post hoc analysis revealed that the 3 mg/kg ketamine group spent more time resting than the 0 mg/kg or 10 mg/kg ketamine groups within 10 mg/kg RU24969-treated mice. Post hoc tests also revealed that 10 mg/kg RU24969 treatment decreased rest time in all three pretreatment groups. The direction of effects was the same in both the 30-minute and 24-hour time point studies (Figure 3.1E, 3.1F), but neither achieved a significant interaction between ketamine and RU24969 (30-minute: $F_{(2,78)} = 1.89$; $p = .16$; 24-hour: $F_{(1,68)} = 2.83$; $p = .07$). Planned comparisons to resolve the trend for a ketamine by RU24969 interaction at the 24-hour time point revealed that RU24969 reduced time spent resting within each ketamine pretreatment group, consistent with the main effect of RU24969 ($F_{(1,68)} = 158.94$; $p<.0001$). However, there were no differences between RU24969-treated groups. A main effect of RU24969 was also identified at the 30-minute time point ($F_{(1,78)} = 122.38$; $p<.0001$).

For spatial *d*, a strong trend was found for an interaction between ketamine pretreatment and RU24969 treatment for the combined analysis ($F_{(2,146)} = 2.87$; $p = .06$; Figure 3.1G). Planned comparisons revealed that RU24969 significantly decreased spatial *d* in the 0 mg/kg and 10 mg/kg, but not 3 mg/kg, ketamine pretreatment groups. While the pattern of effects was consistent in the 30-minute and 24-hour time point studies (Figure 3.1H, 3.1I), the interactions were not significant for either (30-minute: $F_{(2,78)} = .77$; $p = .47$; 24-hour: $F_{(2, 68)} = 2.24$; $p = .11$). However, a main effect of RU24969 was observed in the 24-hour ($F_{(1,68)} = 12.32$; $p<.001$) but not 30-minute study ($F_{(1,78)} = .47$; $p = .49$). There was too little vertical activity to sufficiently power an analysis of treatment effects (data not shown).

3.3.2 No effect of low-dose ketamine on RU24969-induced PPI deficits

The combined analysis for Experiments 1 and 2 revealed a main effect of RU24969 treatment on PPI ($F_{(1,146)} = 70.04$; $p<.0001$; Figure 3.1J). However, ketamine pretreatment did not alter PPI. Similarly, Experiments 1 ($F_{(1,78)} = 43.14$; $p<.0001$; Figure 3.1K) and 2 ($F_{(1,68)}$

= 28.28; p<.0001 Figure 3.1L) showed main effects for RU24969 to decrease PPI. A trend for a main effect of ketamine pretreatment to increase startle amplitude was found in the combined analysis ($F_{(2,146)}$ = 2.86; p = .06; Figure 3.1M), but planned comparisons did not identify any significant contrasts. No effect of RU24969 treatment was found on startle in the combined analysis. RU24969 treatment increased startle in Experiment 1 ($F_{(1,78)}$ = 34.32; p<.0001; Figure 3.1N), but not Experiment 2 (Figure 3.1O).

3.3.3 Differential effects of high-dose ketamine on RU24969-induced behavioral effects in the open field by time point

Using the same pretreatment time points, we next assessed effects of high dose (30 mg/kg) ketamine pretreatment on RU24969-induced behavioral effects, based on the effectiveness of higher doses of ketamine in animal models of depression [291] and posttraumatic stress disorder [239], using the same ketamine pretreatment time points.

These two studies (Experiments 3 and 4) did not meet criteria for a combined analysis. All open field measures showed robust ketamine by time point (30-minute versus 24-hour pretreatment) interactions (total distance traveled: $F_{(1,152)}$ = 15.55; p = .0001; rest time: $F_{(1,152)}$ = 18.32; p<.0001; spatial d : $F_{(1,152)}$ = 4.38; p<.05) with the exception of vertical rearing, which was unable to be analyzed due to very few instances of vertical activity (data not shown). Therefore, these studies were analyzed separately.

In the 30-minute pretreatment study, a pretreatment by treatment interaction was identified for total distance traveled in the open field ($F_{(2,78)}$ = 4.07; p<.05; Figure 3.2A). Post hocs revealed simple main effects of RU24969 treatment within the 0 mg/kg pretreatment condition ($F_{(2,39)}$ = 4.43; p = .019) and within the 30 mg/kg ketamine condition ($F_{(2,39)}$ = 21.44; p<.0001). Both 3 mg/kg and 10 mg/kg RU24969 treatment significantly increased total distance traveled compared to 0 mg/kg RU24969 treatment within both the 0 mg/kg pretreatment and 30 mg/kg ketamine conditions. Furthermore, 30 mg/kg ketamine treat-

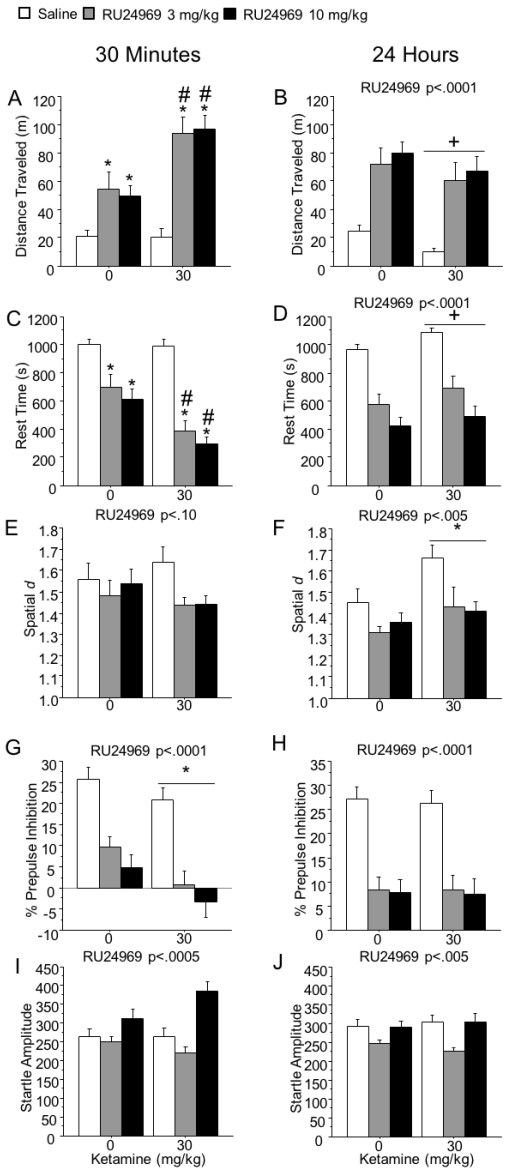

Figure 3.2: High-dose ketamine pretreatment acutely exacerbated RU24969-induced locomotor perseveration. A - B show distance traveled in the open field for 30-minute (A), and 24-hour (B) pretreatments. C - D show rest time for 30-minute (C), and 24-hour (D) pretreatments. E - F show spatial d for 30-minute (E), and 24-hour (F) pretreatments. G - H show PPI for 30-minute (G), and 24-hour (H) pretreatments. I - J show startle amplitude for 30-minute (I), and 24-hour (J) pretreatments. Results expressed as mean ± SEM. *Significant difference from saline treated group within pretreatment condition. #Significant difference from saline within treatment condition. $\underline{*}$Significant difference from saline pretreatment collapsed across treatment groups. +Trend for difference from saline pretreatment collapsed across treatment groups.

ment increased total distance traveled in the 3 mg/kg and 10 mg/kg, but not 0 mg/kg, RU24969 conditions. In the 24-hour pretreatment study, a main effect of RU24969 treatment was identified ($F_{(2,740)} = 21.46$; $p<.0001$; Figure 3.2B) and post hoc tests revealed that both 3 mg/kg and 10 mg/kg RU24969 increased distance traveled in the open field. A trend was identified for a main effect of ketamine pretreatment ($F_{(1,74)} = 2.92$; $p = .09$) on distance traveled. No ketamine pretreatment by RU24969 interaction was found.

Similarly, a pretreatment by treatment interaction was identified for time spent resting in the open field ($F_{(2,78)} = 4.01$; $p<.05$; Figure 3.2C) in the 30-minute pretreatment study. Post hocs revealed that both 3 and 10 mg/kg RU24969 treatment decreased time spent resting within the 0 mg/kg and 30 mg/kg ketamine pretreatment conditions. Furthermore, 30 mg/kg ketamine pretreatment reduced rest time in the 3 mg/kg and 10 mg/kg, but not 0 mg/kg, RU24969 conditions. In contrast, in the 24-hour pretreatment study, a main effect of RU24969 treatment was identified for time spent resting in the open field ($F_{(2,74)} = 39.79$; $p<.0001$; Figure 3.2D). Post hoc tests revealed that both 3 mg/kg and 10 mg/kg RU24969 treatment reduced time spent resting. A trend was found for a main effect of ketamine pretreatment to increase rest time ($F_{(1,74)} = 3.57$; $p = .06$).

In the 30-minute pretreatment study, a trend for a main effect of RU24969 treatment to reduce spatial d was found ($F_{(2,78)} = 2.55$; $p = .09$; Figure 3.2E), but planned comparisons did not reveal any significant contrasts. In the 24-hour pretreatment study, a main effect of ketamine pretreatment to increase spatial d was found ($F_{(1,72)} = 6.64$; $p<.05$; Figure 3.2F). Furthermore, a main effect for RU24969 treatment to reduce spatial d was also identified ($F_{(2,72)} = 5.11$; $p<.01$). Post hoc tests revealed that both 3 mg/kg and 10 mg/kg RU24969 reduced spatial d across pretreatment groups.

3.3.4 Differential effects of high-dose ketamine on RU24969-induced PPI deficits by time point

In Experiment 3, a main effect indicated that 30 mg/kg ketamine pretreatment reduced PPI ($F_{(1,78)} = 5.24$; $p<.05$; Figure 3.2G). A main effect of RU24969 treatment was also identified ($F_{(2,78)} = 18.65$; $p<.0001$). Post hoc tests revealed that 3 mg/kg and 10 mg/kg RU24969 treatment reduced PPI levels. A main effect of RU24969 treatment was also identified for baseline startle amplitude in Experiment 3 ($F_{(2,78)} = 9.74$; $p<.0005$; Figure 3.2I), with 10 mg/kg RU24969 treatment significantly increasing startle response. In Experiment 4, a main effect of RU24969 treatment on PPI was identified ($F_{(2,73)} = 14.57$; $p<.0001$; Figure 3.2H); both 3 mg/kg and 10 mg/kg RU24969 decreased PPI levels across pretreatment groups. A main effect of RU24969 treatment was also identified for baseline startle amplitude in Experiment 4 ($F_{(2,73)} = 6.15$; $p<.005$; Figure 3.2J), with 3 mg/kg RU24969 reducing baseline startle response.

3.3.5 Acute fluoxetine pretreatment does not mitigate RU24969-induced OCD-like behavior

In the open field, a main effect was found for RU24969 treatment to increase total distance traveled ($F_{(1,52)} = 33.41$; $p<.0001$; Figure 3.3A); there was no effect of fluoxetine pretreatment on distance traveled. Similarly, RU24969 treatment decreased time spent resting in the open field ($F_{(1,52)} = 66.48$; $p<.0001$; Figure 3.3B) across pretreatment groups, while fluoxetine pretreatment had no effect on rest time. There were no effects of fluoxetine or RU24969 on spatial d (Figure 3.3C). For PPI, a fluoxetine by RU24969 interaction was identified ($F_{(1,52)} = 10.12$; $p<.005$; Figure 3.3D). Post hoc tests revealed that RU24969 reduced PPI within the 0 mg/kg and 10 mg/kg fluoxetine pretreatment groups; furthermore, 10 mg/kg fluoxetine increased PPI in the 0 mg/kg RU24969 condition, and reduced PPI in the 10

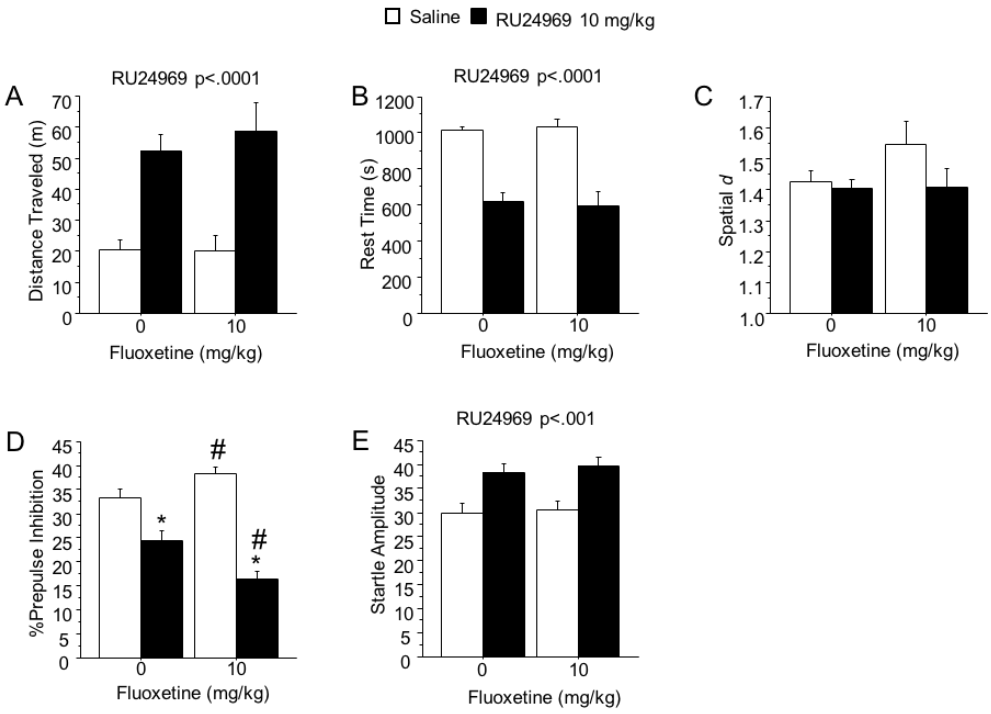

Figure 3.3: Acute fluoxetine pretreatment did not mitigate RU24969-induced locomotor perseveration or prepulse inhibition deficits. A - C show distance traveled (A), rest time (B), and spatial *d* in the open field (C). D - E show PPI (D) and baseline startle amplitude. Results expressed as mean ± SEM. *Significant difference from saline-treated group within pretreatment condition. #Significant difference from saline within treatment condition.

mg/kg RU24969 condition. A main effect of RU24969 treatment was identified for baseline startle amplitude ($F_{(1,52)} = 12.36$; $p<.001$; Figure 3.3E).

3.4 Discussion

3.4.1 Overall

Our results indicate that acute treatment with ketamine reduces 5-HT1BR-induced perseverative behavior. We show that a single injection of low-dose (3 mg/kg) ketamine prior to RU24969 administration mitigated perseverative hyperlocomotion in the open field (Figure 3.1), whereas higher doses were ineffective, or worsened the 5-HT1BR-induced behavioral syndrome (Figure 3.2). Specifically, across the 30-minute and 24-hour pretreatment time

points, 3 mg/kg ketamine attenuated RU24969-induced hyperactivity and reductions in spatial *d*. However, the 10 mg/kg ketamine dose did not alter RU24969-induced perseverative behavior at any time point. In contrast, high-dose (30 mg/kg) ketamine pretreatment exacerbated RU24969-induced hyperactivity (Figure 3.2A;C) when administered 30 minutes prior, and did not prevent any RU24969-induced effects 24 hours later (Figure 3.2B; 3.2D). Interestingly, high-dose ketamine independently reduced locomotor perseveration at 24 hours (Figure 3.2F), warranting further study. These data suggest that acute low doses of ketamine reduce perseverative behaviors in mice, and are consistent with several reports that acute ketamine treatment reduces symptoms in OCD patients [39, 305, 306].

3.4.2 Effects of low-dose ketamine on perseverative hyperlocomotion

Ketamine reduced RU24969-induced perseverative hyperlocomotion only at 3 mg/kg, the lowest dose tested. This ketamine-mediated reduction in perseverative hyperlocomotion was indicated by reduced distance traveled (Figure 3.1A), increased rest time (Figure 3.1D), and increased spatial *d* (Figure 3.1G). Alternatively, pretreatment with 10 mg/kg ketamine did not alter any measure of 5-HT1BR-induced perseverative hyperlocomotion. This ability of 3 mg/kg ketamine to reduce perseverative hyperlocomotion was observed when data was pooled across the 30-minute and 24-hour time points.

3.4.3 Effects of high-dose ketamine on perseverative hyperlocomotion

Surprisingly, a higher, but still subanesthetic, dose of ketamine worsened RU24969-induced perseverative hyperlocomotion. We assessed effects of pretreatment with a higher (30 mg/kg) dose of ketamine in light of recent work suggesting that this dose is prophylactic treatment in a rodent PTSD model [239], and our own findings in the forced swim test and olfactory bulbectomy paradigms (unpublished observations). We found that 30 mg/kg ketamine potentiated RU24969-induced hyperactivity and reductions in rest time (Figure 3.2). These

findings contrast with previous reports of similar doses of ketamine inducing hyperactivity in mice [64, 63], whereas in the present study, ketamine had no effect on control activity levels. However, the potentiation of 5-HT1BR-induced hyperactivity by ketamine were not sustained at 24 hours (Figure 3.2B;3.2D). Rather, ketamine pretreatment increased spatial *d*, and produced a trend to reduce distance traveled and increase rest time, all across treatment groups. Thus, even in the absence of 5-HT1BR-induced perseverative behaviors, acute 30 mg/kg ketamine treatment reduced perseverative locomotor patterns in the open field. Thus, while 3 mg/kg ketamine reduced 5-HT1BR-induced perseverative hyperlocomotion across timepoints, the 30 mg/kg dose of ketamine worsened RU24969-induced perseverative hyperlocomotion after 30 minutes, but reduced perseverative locomotor patterns overall by 24 h. These findings suggest that studies of acute ketamine treatment in OCD patients might obtain opposite findings on symptom outcomes depending on dosage used.

3.4.4 Effects of ketamine on PPI

In contrast to perseverative hyperlocomotion, ketamine pretreatment did not mitigate RU24969-induced PPI deficits at any dose. The lower ketamine doses, 3 and 10 mg/kg, had no effects on PPI (Figure 3.1J-L), in agreement with previous studies [395]. In contrast, acute pretreatment with 30 mg/kg ketamine reduced PPI (Figure 3.2G-H), in line with previous findings at this dose [64, 94, 395, 394]. However, this effect was lost by 24 hours. In sum, ketamine did not modulate 5-HT1B-mediated PPI deficits across a range of subanesthetic doses. Our findings suggest that the therapeutic effect of acute ketamine treatment in OCD patients may not be mediated by increasing PPI, which is reduced in this patient population [6].

3.4.5 Effects of fluoxetine on the 5-HT1BR-induced model

We also tested the effects of acute fluoxetine treatment on 5-HT1BR-induced perseverative hyperlocomotion and PPI deficits. Since acute fluoxetine does not reduce perseverative

behaviors in OCD patients, we predicted that acute fluoxetine treatment would not reduce 5-HT1BR-induced behavioral deficits, and would provide a negative control. As expected, acute fluoxetine pretreatment did not mitigate RU24969-induced locomotor perseveration or PPI deficits (Figure 3.3). In fact, 10 mg/kg fluoxetine pretreatment worsened RU24969-induced PPI deficits (Figure 3.3D). The 5-HT1BR-induced model of OCD-like behavior has been shown to be sensitive to chronic (4 weeks), but not subchronic treatment with SRIs [323, 324]. However, the effects of acute fluoxetine treatment have never previously been reported in the model. Here, we demonstrate that acute ketamine, but not fluoxetine, treatment reduces 5-HT1BR-induced perseverative hyperlocomotion, consistent with clinical reports in OCD patients.

3.4.6 Relation to human trials

Trials assessing therapeutic efficacy of a single dose of ketamine for OCD [39, 305, 306] or depression [34, 396] have typically used one dosing regimen for ketamine: 0.5 mg/kg, administered as a continuous intravenous infusion over the course of 40 minutes.Our findings suggest that a dose range of 3-30 mg/kg induces substantially different effects on the 5-HT1BR-induced behavioral syndrome. While 3 mg/kg ketamine attenuated 5-HT1BR-induced perseverative locomotion (Figure 3.1A; 3.1D; 3.1G), 30 mg/kg ketamine increased spatial d across RU24969 treatment (0 – 10 mg/kg) at 24 h, indicating reduced perseverative locomotor patterns (Figure 3.2D). The ability of low-dose ketamine treatment to reduce 5-HT1BR-induced perseverative hyperlocomotion is consistent with the ability of other effective OCD treatments, like chronic SRI treatment [115], to reduce OCD-like behavior in the model. However, the ability of 30 mg/kg ketamine to increase spatial d across RU24969 treatment (0 – 10 mg/kg) was unexpected, and might also contribute to the therapeutic effects of ketamine in OCD patients. Furthermore, 30 mg/kg ketamine also reduced hyperlocomotion across treatment, but only at the trend level (Figure 3.2B,D). Rodent models

of depression-like behavior have shown antidepressant-like effects of ketamine across this range of doses [23, 66, 127, 128, 229, 291, 301, 313]. More preclinical work will be required to clarify potential therapeutic effects of acute ketamine treatment on baseline versus 5-HT1BR-induced perseverative behaviors..

The endurance of ketamine's therapeutic effects in OCD is currently unknown. One trial found primarily acute effects in treatment refractory patients, with loss of effects within one to three days [39]. However, another trial found effects lasting through the final measurement at one week post-infusion [305], and another found effects lasting through their final assessment at four weeks, when ketamine treatment was combined with two weeks of cognitive behavioral therapy [306]. More trials in OCD patients with extended assessment time points will be needed to clarify this point. The 5-HT1BR-induced mouse model could be used to identify the durability of ketamine's palliative effects on locomotor perseveration, which showed similar effects at 24 hours to those seen at 30 minutes in the current studies. Further studies should examine more extended time points to identify the temporal limits of ketamine's therapeutic effects at various doses. Such studies might predict the timeframe for ketamine's therapeutic effects in OCD patients.

3.4.7 5-HT1BR-induced model

Successful mitigation of perseverative hyperlocomotion by acute ketamine treatment has important implications for the 5-HT1BR-induced mouse model of aspects of OCD. One of the strengths of the 5-HT1BR-induced model is its pharmacological response profile. OCD-like deficits in the model are responsive to chronic SRI treatment, but not other classes of antidepressants [323, 324], like in OCD patients. Importantly, the present findings indicate that validating a model using currently known pharmacotherapies does not preclude discovery of novel therapeutics, a common criticism brought against animal models. Specifically, our results suggest that the 5-HT1BR-induced model can also identify novel treatments for OCD

such as ketamine. Furthermore, since the 5-HT1BR-induced model is sensitive to therapeutic onset, the model could be particularly useful for identifying additional fast-acting anti-OCD compounds.

3.4.8 Limitations

There are several limitations to the work presented here. These studies might not have been sufficiently powered to identify all time point-specific effects of low-dose ketamine pretreatment in Experiments 1 and 2, and should be confirmed with larger sample sizes. Future work should also extend these findings to later time points to determine the durability of ketamine's effects. Finally, future studies should examine the effects of lower doses of ketamine on perseverative hyperlocomotion in the 5-HT1BR-induced model in order to identify an optimal dose. Despite these caveats, these studies present preliminary evidence for therapeutic-like effects of ketamine in the 5-HT1BR-induced model of aspects of OCD-like behavior.

3.4.9 Conclusions

To our knowledge, this study is the first to identify a therapeutic-like effect of ketamine in an animal model of OCD-like behavior. Here, we found that a single, low-dose (3 mg/kg), pretreatment with ketamine mitigated 5-HT1BR-induced perseverative hyperlocomotion in the open field. The present findings contribute to the validity of the 5-HT1BR-induced model and demonstrate that it may be viable for identifying novel treatments. Finally, this model may be used to identify the mechanisms by which ketamine produces rapid reductions in OCD-like behavior.

CHAPTER 4

BTBD3 EXPRESSION IN HIPPOCAMPUS MODULATES OCD-RELEVANT BEHAVIORS

4.1 Introduction

BTBD3 is a transcription factor that guides dendrites toward active axon terminals during development [234]. The *BTBD3* gene was recently implicated in the first genome-wide association study (**GWAS**) for obsessive-compulsive disorder (**OCD**), but remains to be verified as an OCD risk gene [342]. It is of great interest to identify whether BTBD3 plays a role in OCD-relevant behaviors.

BTBD3 is a member of the BTB/POZ family of transcription factors that play a role in protein degradation, immune function, and development [275, 329]. BTBD3 preferentially guides dendrites of layer IV spiny stellate neurons toward active thalamocortical afferents in mouse somatosensory barrel cortex [234]. Relatedly, BTBD3 is integral for proper polarity of the dendritic arbor of these neurons and *Btbd3* knockdown induces dendritic hypertrophy in barrel cortex spiny stellate neurons. *BTBD3* expression increases rapidly early in postnatal development and is then stable through adulthood in both humans [247] and mice [219, 234]. Therefore, BTBD3 is vital for circuit formation during postnatal development, but the extent of this function outside barrel cortex is unknown. Furthermore, behavioral effects of this role for BTBD3 in neural circuit formation have not been explored.

While OCD is known to have a substantial genetic component [368], specific risk genes reliably associated with OCD have yet to emerge. The first GWAS for OCD identified one genome-wide significant single nucleotide polymorphism (**SNP**) in the trio portion of the sample [342]. This SNP is 89 kilobases downstream of the *BTBD3* gene and is an expression quantitative trait locus for *BTBD3*, suggesting that *BTBD3* may be an OCD risk gene that rs6131295 marks [342]. However, this SNP was not identified in a follow-up GWAS [235]

or a GWAS combining these two samples [177]. However, neither of these studies identified any genome-wide significant SNPs. Thus, it remains to be determined whether *BTBD3* will emerge as a true risk gene for OCD once better powered sample sizes are attained in human studies.

Interestingly, *BTBD3* expression has significant overlap with key brain regions implicated in OCD. Namely, *BTBD3* is robustly expressed in hippocampus, mediodorsal thalamus, and anterior cingulate cortex (**ACC**) [219, 247]. OCD is characterized by aberrant activity in the limbic cortico-striato-thalamo-cortical (**CSTC**) circuit [315]. This circuit includes mediodorsal thalamus and ACC and is modulated by hippocampus via inputs to the ventral striatum [192]. These brain regions are key players in goal-directed decision-making [44, 180, 365, 249, 268], which is impaired in OCD patients and thought to underlie compulsive behavior [135, 132, 130, 136]. Thus, BTBD3 could contribute to formation of aspects of the CSTC circuit that regulate goal-directed behavior, presenting a potential connection to OCD.

Here, we used a constitutive *Btbd3* knockout (**KO**) mouse as a tool to assess behavioral effects of *Btbd3* expression. Upon identifying several behavioral deficits, we sought to identify mechanisms underlying these behavioral effects of *Btbd3* expression using pharmacological, neurohistological, and viral-mediated gene transfer approaches. We present evidence of a role for hippocampal *Btbd3* expression in exploration and goal-directed behavior.

4.2 Materials and methods

4.2.1 Animals

Animals were housed in a climate controlled vivarium on a 12 hour: 12 hour light:dark cycle. Animals were group housed and given ad libitum access to standard chow and water unless otherwise stated. *Btbd3* KO mice on a mixed 129/B6 background were originally obtained

from Riken (Hirosawa, Wako, Saitama, Japan). Exons 1 and 2 of the *Btbd3* gene were replaced with a neomycin-resistant cassette. Heterozygous (**HT**) *Btbd3* mice were bred in house to generate cohorts of wild-type (**WT**), HT, and KO mice. All experiments used *Btbd3* WT, HT, and KO mice unless explicitly stated otherwise. Furthermore, we generated *Btbd3* floxed (***Btbd3*$_{\mathbf{flox}}$**) mice on a pure C57BL/6J background (Transviragen, Research Triangle Park, NC, USA). *Btbd3*$_{\text{flox}}$ mice were custom designed using CRISPR/Cas9 technology with loxP sites inserted flanking exon 2 of the *Btbd3* gene. Homozygous *Btbd3*$_{\text{flox}}$ mice were crossed to generate all *Btbd3*$_{\text{flox}}$ cohorts. C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). All procedures were performed in accordance with the local Institutional Animal Care and Use Committee.

4.2.2 Drugs

Drugs were administered continuously in the home cage drinking water in opaque bottles. Fluoxetine was administered at 80 mg/L to achieve a dose of 10 mg/kg/day, and desipramine was administered at 215 mg/L to achieve a dose of 20 mg/kg/day [97, 165]. Fluoxetine was changed weekly, and desipramine biweekly. Vehicle groups received unadulterated water as the control.

4.2.3 Open field

Open field testing was performed as previously described [324]. Briefly, mice were placed in a corner of the open field (Accuscan, Columbus, OH, USA) and activity was monitored for thirty to forty-five minutes depending upon the experiment. Versamax software (Accuscan, Columbus, OH, USA) automatically generated primary output measures except for spatial *d*, which was calculated using NightOwl software (Custom) and Python (Python Software Foundation, Beaverton, OR, USA).

4.2.4 Barbering

Mice were pair-housed by sex and genotype. Mice were checked live for evidence of barbering, characterized by bald patches in the fur or clipped whiskers. During chronic drug treatment, cages were live checked weekly for evidence of barbering and photographed. Photographs were checked to confirm live assessment of barbering.

4.2.5 Wheel-running

Animals were singly housed and cages were equipped with wireless running wheels (Med Associates, St. Albans, VT, USA). Wheel revolution counts were transmitted to a computer running Wheel Manager Software (Med Associates) continuously throughout the seven-day testing period.

4.2.6 Dig test

The dig test was performed as described previously [165]. Briefly, mice were placed in a novel cage with fresh bedding (1" deep) and video recorded for 3 minutes. Videos were later scored for digging behavior, defined by significant movement of bedding with the limbs.

4.2.7 Marble-burying

Marble-burying was performed as described previously [88]. Briefly, cages were filled with 5 cm bedding and twelve marbles placed on top in a 3× 4 grid, 4 cm center-to-center marble spacing. Mice were placed in a cage with marbles for 30 minutes. The number of marbles buried to 2/3 depth was recorded live at the end of the session. Cages were photographed at the end of each session.

4.2.8 Nest building

Nest building was performed as described previously [87]. Briefly, compressed cotton nestlets (Ancare, Bellmore, NY, USA) were weighed prior to testing. Mice were singly housed and given a nestlet in the home cage. In the eight-hour variation of the test, nestlets were removed 8 hours later and any part of the nestlet remaining in its original compressed form was weighed. In the overnight version of the test, nestlets were received just before initiation of the dark cycle. 14 hours later, remaining nestlet was weighed the following morning.

4.2.9 Cognitive testing

Male *Btbd3* WT, HT, and KO mice were food-restricted to 85% of baseline bodyweight. Training and testing occurred in five-hole operant chambers (Med Associates). Chambers consisted of 5 nosepoke holes with recessed LEDs, an additional light over the central nosepoke hole, a house light, and a food delivery magazine on the opposite wall from the nosepoke holes. Responses in the nosepoke holes and magazine were detected using infrared beams. Liquid reinforcement was administered using peristaltic pumps (Lafayette Instruments, Lafayette, IN, USA) and consisted of strawberry milkshake (Nesquik mixed with nonfat milk) in 25 μl increments. Tasks were programmed in MED-PC for Windows (Med Associates). Mice underwent general training to respond for reward, followed by Go/No-Go, Progressive Ratio Breakpoint (**PRBP**) testing, and finally the Probabilistic Learning Task (**PLT**). The Go/No-Go paradigm is a task used to measure response inhibition in humans [100] and has been adapted for rodents [237]. The PRBP task assesses motivation by assessing how hard animals are willing to work for food reward [166]. The Probabilistic Learning Task measures goal-directed learning and response strategies [16].

Mice were initially trained to retrieve reward from the magazine (Phase I). To achieve this aim, the magazine light was lit and administered reward every 15 seconds in a 20-minute session. Once animals reliably retrieved reward (~60 rewards earned per session), they moved

on to the subsequent training phase. In Phase II, mice were trained to respond to the central nosepoke hole. To achieve this aim, the central nosepoke hole was lit constantly until the animal poked its nose in the hole. Then, reward was administered in the magazine. Once the mouse retrieved the reward, a variable intertrial interval (**ITI**) was employed, followed by initiation of the next trial for a total of 30 minutes per session. Once animals consistently responded in the central hole, they were trained in the Go/No-Go paradigm. In Phase III, animals were trained to respond to the central hole during a limited stimulus interval in which they initially had 8 seconds to respond, then were trained to respond within 5 seconds, then finally 3 seconds, in a 30-minute session. During this phase, animals were also punished for incorrect responses (responses in unlit holes), for omissions (lack of response during the stimulus window), and for premature responses (responses prior to initiation of the trial indicated by light in the central hole). Punishment consisted of a 4 second time out with the house light on. Animals had to retrieve reward from the magazine to initiate the subsequent trial. Initially, a variable ITI of 5 - 17 seconds was used. However, this resulted in high levels of premature responding; thus the ITI was shortened to 3 - 7 seconds. The stimulus interval was 8 seconds for the first 5 sessions, then 5 seconds for 5 sessions, then ultimately 3 seconds for 5 sessions. Animals that did not reach criterion (20 successful trials within a session) by the end of the fifth training session at the 3-second stimulus interval were excluded from analysis for the Go/No-Go paradigm. Next, animals underwent 15 30-minute Go/No-Go test sessions. Here, a "Go" trial consisted of the central hole being lit during the 3-second stimulus interval, as during training, except that responses in incorrect holes were not punished during the test phase. "No-Go" trials consisted of lighting the central hole concomitant with an additional, green, light just above the central hole for the 3-second stimulus interval, creating a compound stimulus. Animals were rewarded for refraining from responding to this stimulus, and were punished with a time out if they did respond (a "false alarm"). The first five days were training for the No-Go stimulus. The remaining ten days

of testing were used for analysis. The false alarm rate was the primary outcome measure for response inhibition, where a high rate of false alarms indicates impairments in action restraint, a type of impulsivity [100]. Premature responding was the secondary response inhibition measure. The sensitivity index d-prime (d') measures how well animals learn the task by comparing the hit rate and false alarm rate.

Following completion of Go/No-Go testing, animals moved on to the PRBP task. First, animals did a refresher training session at Phase II (see above). The next day, animals underwent the 60-minute PRBP test session. PRBP testing was performed as previously described [246]. Briefly, mice had to respond in the central nosepoke hole progressively more times in order to earn a reward in the following steps: 1, 2, 4, 7, 11, 16, 22, 29, 37, 46, 56 and 67 responses required for reward. The "breakpoint" was the primary outcome measure, the highest number of responses the animal would perform to obtain reward.

Finally, animals underwent PLT testing. First, animals underwent one day of training. This session was a modified version of the Phase II training session, in which all five holes were lit and active for earning reward rather than only the central hole. The following day, mice underwent PLT testing in 3 blocks of 60 trials for a maximum 60-minute session. Two holes were lit for each trial, and the two lit holes were counterbalanced across groups. Within a block, one hole was the "target" hole and the other the "non-target" hole. The target hole was commonly rewarded (90, 80, or 70% of the trials for blocks 1, 2, and 3 respectively) and the non-target hole was uncommonly rewarded (10, 20, or 30% of the trials). After completing the 60 trials for block 1 (90 or 10% reward probabilities), the two previously lit holes were extinguished and two new holes were lit, one with 80% and the other with a 20% reward probability. Similarly, for the third block, the previously lit holes were extinguished and two new holes were lit, one with 70% and one with 30% reward probability. "Win-stay" and "lose-shift" strategies on the target and non-target holes were the primary outcome measures, where "win-stay" denotes a trial in which an animal returns to the same hole in

which they were rewarded on the previous trial. "Lose-shift" refers to a trial in which an animal shifts responding to the opposite hole after receiving punishment on the previous trial. Accuracy was also a primary outcome measure, defined as the percentage of trials in which the animal responded on the target hole.

4.2.10 Viral-mediated knockdown of Btbd3 expression

Neonatal *Btbd3*$_{\text{flox}}$ mouse pups received intracranial infusions of a custom adeno-associated virus expressing Cre recombinase: AAV2/8-CMV-Cre-P2A-tdTomato-WPRE (Viral Vector Core Facility, University of Iowa, Iowa City, IA, USA) or control: AAV2/8-CMV-tdTomato-WPRE, targeting the whole hippocampus (0.3 mm anterior, 2.0 mm lateral, 2.5 mm ventral of lambda) or dorsal hippocampus (1.0 mm anterior, 0.3 mm lateral, 2.7 mm ventral of lambda) at postnatal day 2 (**P2**). The AAV2/8 serotype was used because of its efficient gene delivery to the central nervous system of neonatal mice [47, 60]. Cetalomegalovirus (**CMV**) was selected as the promoter because of its transduction efficiency in the central nervous system in neonatal rodents [236] and long-term expression [353]. A woodchuck post-trascriptional regulatory element (**WPRE**) was used to enhance viral transduction efficiency [378]. The self-cleaving P2A peptide was used to create a bicistronic vector that would separately express Cre recombinase and tdTomato, such that tdTomato would traffic similarly in Cre-containing and control virus-infused animals [200]. TdTomato was used as the reporter because of its brightness and photostability [325]. P2 mice were removed from the home cage and placed on a heating pad. Animals were cryoanesthetized on ice prior to surgery. The torso was secured with lab tape and the head was placed on a chin rest custom fitted into a stereotaxic device (Stoelting, Wood Dale, IL, USA). The head was secured using modeling clay (Crayola, Easton, PA, USA). Virus was administered using a syringe pump (Harvard Apparatus, Holliston, MA, USA) holding a 10 μl syringe (#1701, Hamilton Company, Reno, NV, USA) attached to plastic tubing and a 33 gauge cannula (Plastics One, Roanoke, VA,

USA) that was secured into the stereotaxic device. Coordinates were measured relative to lambda. Infusions were performed bilaterally at a rate of 0.1 μl/minute for a total infusion of 0.25 μl per side. The cannula was left in place for an additional minute for diffusion of virus prior to slowly drawing up the cannula. Animals were then removed from the stereotaxic device, and paws were tattooed for identification before returning to the heating pad. Once fully recovered from anesthesia, animals were rolled in soiled bedding from the home cage and returned to the home cage. Mice were assessed for behavior during adulthood (8-13 weeks). Brains were then extracted, fixed in 4% paraformaldehyde, and stored at -80°C. Brains were sectioned on a cryostat (Model 3050S; Leica Biosystems, Nussloch, Eisfeld, Germany). Sections were collected at 30 μm thickness onto Superfrost slides (Fisher) and mounted with Vectashield Hardset Antifade Mounting Medium with DAPI (Cat# H-1500; Vector Laboratories, Burlingame, CA, USA). Sections were then visualized under a fluorescent microscope (BX51; Olympus, Center Valley, PA, USA). Brains were assessed for infusion location by visualizing tdTomato and only included in analyses if the majority (>50%) of fluorescence was within the confines of the hippocampus.

4.2.11 Dendritic morphology

Mice were transcardially perfused with 0.9% NaCl. Brains were then processed for Golgi-Cox staining as previously described [57]. Briefly, brains were extracted and immersed in Golgi-Cox solution, consisting of a 1:1 solution of 5% potassium dichromate and 5% mercuric chloride diluted 4:10 with potassium chromate for 14 days at room temperature [137]. Brains were then transferred to a 30% sucrose solution and stored at 4°C until shipped to Dr. Nuno Sousa's laboratory (University of Minho, Braga, Portugal) for processing. Brains were cut coronally at 200 μm section thickness on a vibratome. Sections were collected in 6% sucrose, blotted dry, and mounted onto gelatin-coated slides. Slides were then alkalinized in 18.7% ammonia. Next, slides were developed in Dektol (Kodak, Rochester, NY, USA) and

fixed in Kodak Rapid Fix. Slides were dehydrated in ethanol and cleared in xylene before coverslipping.

Neurons were imaged and reconstructed as previously described [57]. Briefly, images were taken of each selected neuron at 600× magnification using a motorized microscope (Axioplan 2, Carl Zeiss, Germany) and a camera (DXC-390, Sony Corporation, Tokyo, Japan) and reconstructed using Neurolucida software (Microbrightfield, VT, USA) to obtain the complete dendritic tree. Neurons were assessed for dendritic branching using Sholl analysis at 20 μm radius intervals, which was separated by apical and basal dendrites for pyramidal neurons. Neurons in all regions were assessed for total dendritic length, also separated by apical and basal dendrites for pyramidal neurons. Spine density was assessed for all dendrites in dentate granule cells, and specifically for apical dendrites in anterior cingulate cortex layer II/III pyramidal neurons. The spine density was not assessed in thalamic stellate neurons due to insufficient resolution in the limited dendritic tree of these neurons.

4.2.12 Statistical analysis

Continuous dependent measures were analyzed using repeated measures analysis of variance (**ANOVA**) or factorial ANOVAs. Alpha was set at 0.05. Significant interactions were resolved by assessing simple main effects in post hoc ANOVAs for factors with more than two groups, corrected for multiple comparisons using the Bonferroni method. Post hoc tests were performed using Student Newman-Keuls. Where appropriate, Kruskal-Wallis one-way ANOVAs were performed for effects of genotype following omnibus ANOVA analysis. Mann-Whitney U tests with Bonferroni correction were used as post hoc tests for Kruskal-Wallis ANOVAs. Categorical dependent measures were analyzed using chi-square tests for endpoint factors and Kaplan-Meier survival curves for repeated measures analyses. Electrophysiology data was analyzed using unpaired t-tests. Statistical analysis was performed using Statview

software (SAS Institute, Inc., Cary, NC, USA). Effects of secondary factors (such as sex) were only mentioned if they had significant interactions with primary factors (genotype, virus).

4.3 Results

4.3.1 Btbd3 KO mice have deficits in compulsive-like behavior

Adult *Btbd3* male and female WT, HT, and KO mice were assessed for behavioral deficits in a variety of paradigms. The first phenotype observed in the *Btbd3* KO mice was a high frequency of cage-mate barbering (Figure 4.1A). Thus, animals were pair-housed by sex and genotype and barbering behavior was monitored (n = 78-165/genotype/sex). An uneven distribution of instances of barbering was identified across genotypes (Figure 4.1B)($\mathcal{X}^2$ (2, n = 647) = 11.38; p<.005). Thus, *Btbd3* HT ($\mathcal{X}^2$ (1, n = 486) = 7.73; p<.01) and KO ($\mathcal{X}^2$ (1, n = 332) = 11.77; p<.0001) mice were found to have increased incidence of barbering compared to WT.

Barbering is associated with impaired shifting [122], a common impairment in OCD [61, 260, 265, 364, 379], and has been identified in conjunction with excessive wheel-running in a mouse model of OCD [163]. Thus, activity was tested on running wheels for seven days. In the wheel-running paradigm (n = 9-10/genotype/sex), a day by cycle by genotype interaction was identified for number of wheel revolutions run ($F_{(12,312)}$ = 2.32; p<.01). Post hoc ANOVAs revealed a significant main effect of genotype in the dark cycle ($F_{(2,55)}$ = 4.57; p<.05). Post hoc tests revealed that *Btbd3* KO mice ran more wheel revolutions than WT or HT mice overall (Figure 4.1C).

Next, we tested goal-directed decision making in the PLT (n = 13-17/genotype, all male). In this task, only the third block of testing showed any differential genotypic effects. Therefore, block 3 was analyzed independently. A main effect of genotype was identified for

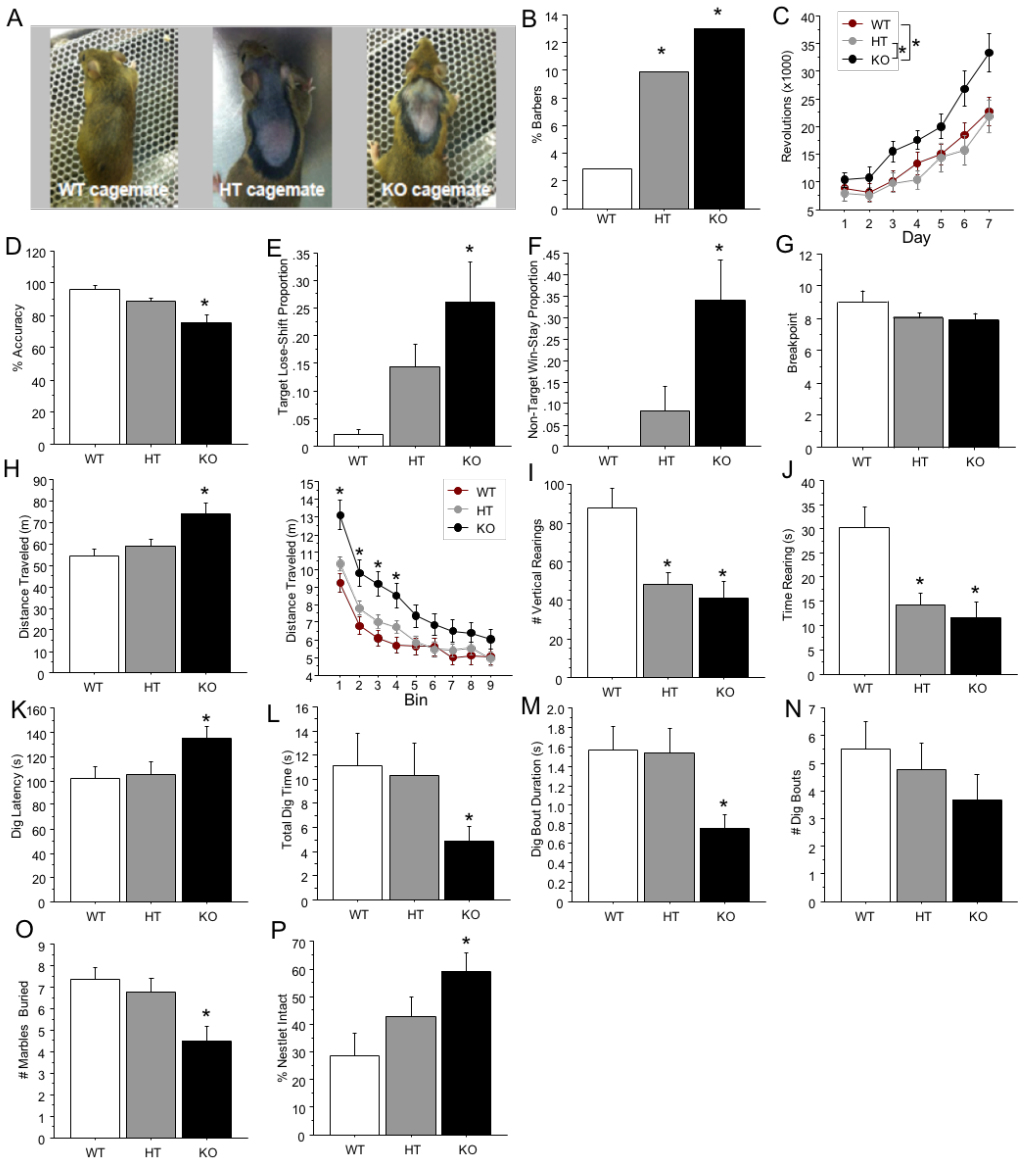

Figure 4.1: *Btbd3* knockout mice have deficits in exploration and goal-directed behavior. A-B show instances of cage-mate barbering. C shows wheel-running in the dark cycle. D-F show select measures from the probabilistic learning task. G shows the number of rewards animals were willing to work for in the progressive ratio breakpoint task. H shows distance traveled in the open field. I-J show vertical rearing in the open field. K-N show measures of digging behavior in the dig test. O shows the number of marbles buried in the marble-burying task. P shows the remaining portion of the nestlet by weight left unused for building a nest at the end of the task. Results are expressed as mean ± SEM, with the exception of panel B, which is categorical data and shows instances. *Significant difference from *Btbd3* WT mice. WT: wild-type; HT: heterozygous; KO: knockout.

accuracy ($F_{(2,35)}$ = 8.01; p<.005). Post hoc tests revealed that *Btbd3* KO mice were less accurate at responding in the target hole than WT or HT mice (Figure 4.1D). A main effect of genotype was found for the proportion of lose-shift responses on the target hole ($F_{(2,35)}$ = 4.96; p<.05). Post hoc tests revealed a higher proportion of target lose-shift responses in *Btbd3* KO mice compared to WT mice (Figure 4.1E). A main effect of genotype was also identified for proportion of win-stay responses on the non-target hole ($F_{(2,23)}$ = 4.24; p<.05). Post hoc tests revealed that *Btbd3* KO mice were more likely to return to the non-target hole after being rewarded at that hole than WT mice (Figure 4.1F). Genotype did not have significant effects on target win-stay or non-target lose-shift proportions (Supplemental Results). To identify any potential effect of genotype on motivation for reward, mice were then tested in the PRBP paradigm. Genotype did not affect breakpoint (Figure 4.1G)($F_{(2,43)}$ = 1.58; p = .22).

4.3.2 Btbd3 KO mice have behavioral deficits in measures of exploration

Since exploration is a key part of goal-directed behavior [74], we next assessed *Btbd3* mice in paradigms that assess species-typical exploratory behaviors. In a 45-minute open field test (n = 11-34/genotype/sex), several behavioral differences were found among genotypes. A main effect of genotype on distance traveled was identified ($F_{(2,130)}$ = 6.18; p<.005). Post hoc tests revealed that KO mice traveled a greater total distance than either HT or WT mice (Figure 4.1H). A genotype by bin interaction was also identified ($F_{(16,1040)}$ = 2.57; p<.0001). Post hoc tests revealed that *Btbd3* KO mice traveled a greater total distance than WT or HT mice in bins 1-4. Post hoc ANOVAs showed main effects of bin within each genotype. Thus, subsequent bins were analyzed for effects within each genotype to assess habituation. Post hoc tests revealed a reduction in distance traveled from bin 1 to bin 2 within each genotype. No other subsequent bins showed significant changes, with the exception of a reduction in activity from bin 4 to bin 5 in *Btbd3* HT mice. Distance traveled was higher in bins 1 and

2 than in the final bin within each genotype. *Btbd3* HT and KO mice also showed greater distance traveled in bins 3 and 4 versus bin 9, whereas *Btbd3* WT mice did not.

For vertical rearing, a main effect of genotype was identified ($F_{(2,130)} = 6.97$; $p<.005$). Post hoc tests revealed that *Btbd3* HT and KO mice had reduced instances of rearing relative to WT mice (Figure 4.1I). Similarly, a main effect of genotype was found for time spent rearing ($F_{(2,130)} = 7.18$; $p<.005$). Post hoc tests revealed that *Btbd3* HT and KO mice spent less time rearing than WT mice (Figure 4.1J). No effects of genotype were identified for center measures or spatial *d* (Supplemental Results).

To further test exploration [5], *Btbd3* mice were assessed in the dig test (n = 13-20/genotype/sex). A trend was identified for a main effect of genotype on latency to start digging ($F_{(2,85)} = 2.65$; $p = .08$). As there was no interaction between genotype and sex, a Kruskal-Wallis one-way ANOVA for effects of genotype on latency to dig was performed. This analysis revealed a main effect of genotype on latency to dig ($H_{(2)} = 6.43$; $p<.05$). Post hoc tests revealed a greater latency to dig in *Btbd3* KO mice than WT mice (Figure 4.1K)($U = 289$; $p<.025$). No effect of genotype was identified for total digging duration ($F_{(2,85)} = 1.84$; $p = .16$) in the main ANOVA. As there was no interaction between genotype and sex, a Kruskal-Wallis one-way ANOVA was performed and revealed a main effect of genotype on total time spent digging ($H_{(2)} = 5.93$; $p<.05$). Post hoc tests revealed significantly less time spent digging in *Btbd3* KO mice than WT mice (Figure 4.1L)($U = 286$; $p<.025$). A main effect of genotype was found for average bout duration ($F_{(2,85)} = 3.79$; $p<.05$. Post hoc tests revealed that *Btbd3* KO mice had shorter digging bouts than either WT or HT mice (Figure 4.1M). No effect of genotype was identified for number of digging bouts (Figure 4.1N) in the main ANOVA ($F_{(2,85)} = .63$; $p = .54$) nor in the Kruskal-Wallis one-way ANOVA ($H_{(2)} = 4.36$; $p = .11$).

To corroborate findings in the dig test, marble-burying was tested, as marble-burying is considered a measure of digging behavior in mice [88, 90, 354]. In the marble-burying

paradigm (n = 9-14/genotype/sex), a main effect of genotype was identified for the number of marbles buried ($F_{(2,66)} = 5.56$; $p<.01$). Post hoc tests revealed that *Btbd3* KO mice buried fewer marbles than WT or HT mice (Figure 4.1O).

Several of the behavioral deficits identified thus far mirror those found in mice receiving lesions throughout the hippocampus in adulthood [89, 90]. Thus, nest-building was measured, which is impaired in hippocampus-lesioned mice [90]. In the 8-hour nest building task (n = 12/genotype/sex), a main effect of genotype was identified for percent of original nestlet weight intact at the end of the test ($F_{(2,65)} = 4.33$; $p<.05$). Post hoc tests revealed that *Btbd3* KO mice left significantly more nestlet untouched at the end of the task than WT mice (Figure 4.1P).

4.3.3 Barbering behavior is preventable by chronic OCD-effective, but not -ineffective treatment in Btbd3 WT and HT but not KO mice

Barbering behavior was then assessed for responsiveness to OCD-effective treatment (fluoxetine) or -ineffective treatment (desipramine). Barbering-naïve animals were pair-housed by genotype and sex and treated with fluoxetine, desipramine, or vehicle in the drinking water for fourteen weeks (n = 12-19/genotype/sex/treatment). Animals were monitored for onset of barbering behavior weekly. Mantel-Cox log rank tests revealed significant effects of fluoxetine treatment compared to vehicle when pooled across genotypes (Figure 4.2A) at weeks 4 ($\mathcal{X}^2$ (1, n = 185) = 6.05; $p<.05$), 5 ($\mathcal{X}^2$ (1, n = 185) = 5.45; $p<.05$), 6 ($\mathcal{X}^2$ (1, n = 185) = 6.47; $p<.05$), 9 ($\mathcal{X}^2$ (1, n = 185) = 4.84; $p<.05$), 10 ($\mathcal{X}^2$ (1, n = 185) = 6.33; $p<.05$), 11 ($\mathcal{X}^2$ (1, n = 185) = 6.18; $p<.05$), 12 ($\mathcal{X}^2$ (1, n = 185) = 7.71; $p<.01$), 13 ($\mathcal{X}^2$ (1, n = 185) = 8.60; $p<.005$), and 14 ($\mathcal{X}^2$ (1, n = 185) = 7.58; $p<.01$). Weeks 2 ($\mathcal{X}^2$ (1, n = 185) = .003; p = .95) and 3 ($\mathcal{X}^2$ (1, n = 185) = 1.48; p = .2245) did not show significant effects of fluoxetine treatment. Weeks 7 ($\mathcal{X}^2$ (1, n = 185) = 2.88; p = .09) and 8 ($\mathcal{X}^2$ (1, n = 185) = 3.46; p = .06) had trend level effects of fluoxetine treatment on barbering. In

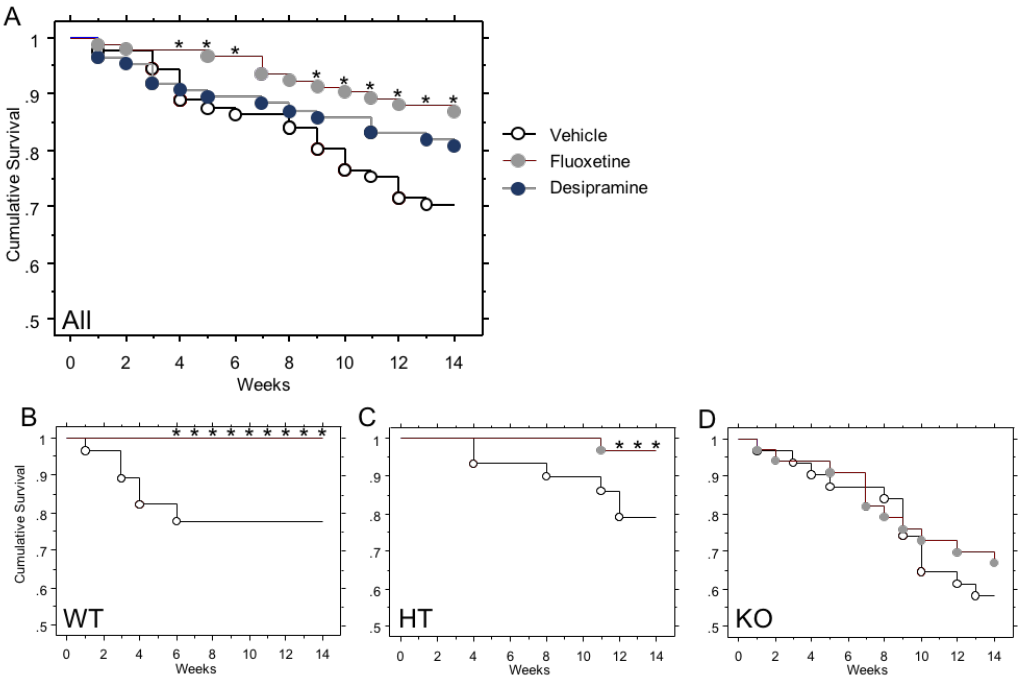

Figure 4.2: Barbering behavior is selectively prevented by fluoxetine treatment except in *Btbd3* KO mice. A shows onset of barbering behavior across genotypes. B-D compare onset of barbering behavior in fluoxetine versus vehicle-treated mice split by genotype: WT (B), HT (C), and KO (D). *Significant difference from vehicle treatment. WT: wild-type; HT: heterozygous; KO: knockout.

contrast, desipramine did not affect onset of barbering pooled across genotypes at any time point (week 14: ($\mathcal{X}^2$ (1, n = 177) = 2.09; p = .15)). Based on the effects of fluoxetine across genotypes, effect of fluoxetine within each genotype was examined. Within *Btbd3* WT mice, fluoxetine completely prevented the onset of barbering behavior (Figure 4.2B), precluding accurate analysis. To estimate effects, data were tested at each time point with one fluoxetine-treated WT mouse artificially marked as becoming a barber. With this simulated analysis, fluoxetine reached trend-level significance at four weeks of treatment ($\mathcal{X}^2$ (1, n = 56) = 3.03; p = .08) and became significant by six weeks of treatment ($\mathcal{X}^2$ (1, n = 56) = 4.59; p<.05), which was then sustained through the remaining weeks. Within *Btbd3* HT mice, fluoxetine became effective at twelve weeks of treatment (Figure 4.2C)($\mathcal{X}^2$ (1, n = 64) = 4.50; p<.05) and remained significant through week 14 ($\mathcal{X}^2$ (1, n = 64) = 4.50; p<.05).

In contrast, within *Btbd3* KO mice, fluoxetine did not affect onset of barbering behavior at any time point (Figure 4.2D)(week 14: $\mathcal{X}^2$ (1, n = 65) = .40; p = .53).

4.3.4 Btbd3 knockdown in hippocampus during early development recapitulates KO deficits in adulthood

Whole hippocampal lesions in adulthood [89, 90] or neonatal ventral hippocampal lesions [216] cause behavioral deficits that overlap with those found in the global *Btbd3* KO mouse. In addition, *Btbd3* is robustly expressed in hippocampus. Thus, the effect of loss of *Btbd3* expression in hippocampus early in postnatal development on behavior was tested. $Btbd3_{\text{flox}}$ mice were infused with a Cre recombinase-expressing or control virus (see Materials and Methods) at postnatal day 2 (P2) and assessed for select behavioral deficits in adulthood (n = 13 - 26/virus/sex). No effect of virus was identified for wheel-running (Figure 4.3A). In the open field (45-minute test), a main effect of virus was identified for total distance traveled (Figure 4.3B)($F_{(1,70)}$ = 48.85; p<.0001). A virus by sex interaction was also identified ($F_{(1,70)}$ = 15.19; p<.0005). Post hoc tests revealed that Cre virus increased total distance traveled in both males and females. Post hoc tests also revealed significantly more activity in females than males in the Cre, but not control, virus condition. A virus by bin interaction was also identified for total distance traveled ($F_{(8,560)}$ = 4.22; p<.0001). Post hoc tests revealed higher activity levels in the Cre condition than the control condition within each bin. Post hoc ANOVAs revealed a main effect of bin within each virus condition (see Supplemental Results). For instances of vertical rearing (Figure 4.3C), there was no main effect of virus ($F_{(1,70)}$ = .81; p = .37). However, an interaction between bin and virus was identified ($F_{(8,560)}$ = 3.93; p<.0005). Post hoc tests revealed a reduction in instances of vertical rearing in the Cre condition only within bin 1. Post hoc ANOVAs revealed a main effect of bin within each virus condition (see Supplemental Results). A main effect of virus was identified for time spent rearing (Figure 4.3D)($F_{(1,70)}$ = 6.17; p<.05). An interaction between virus and

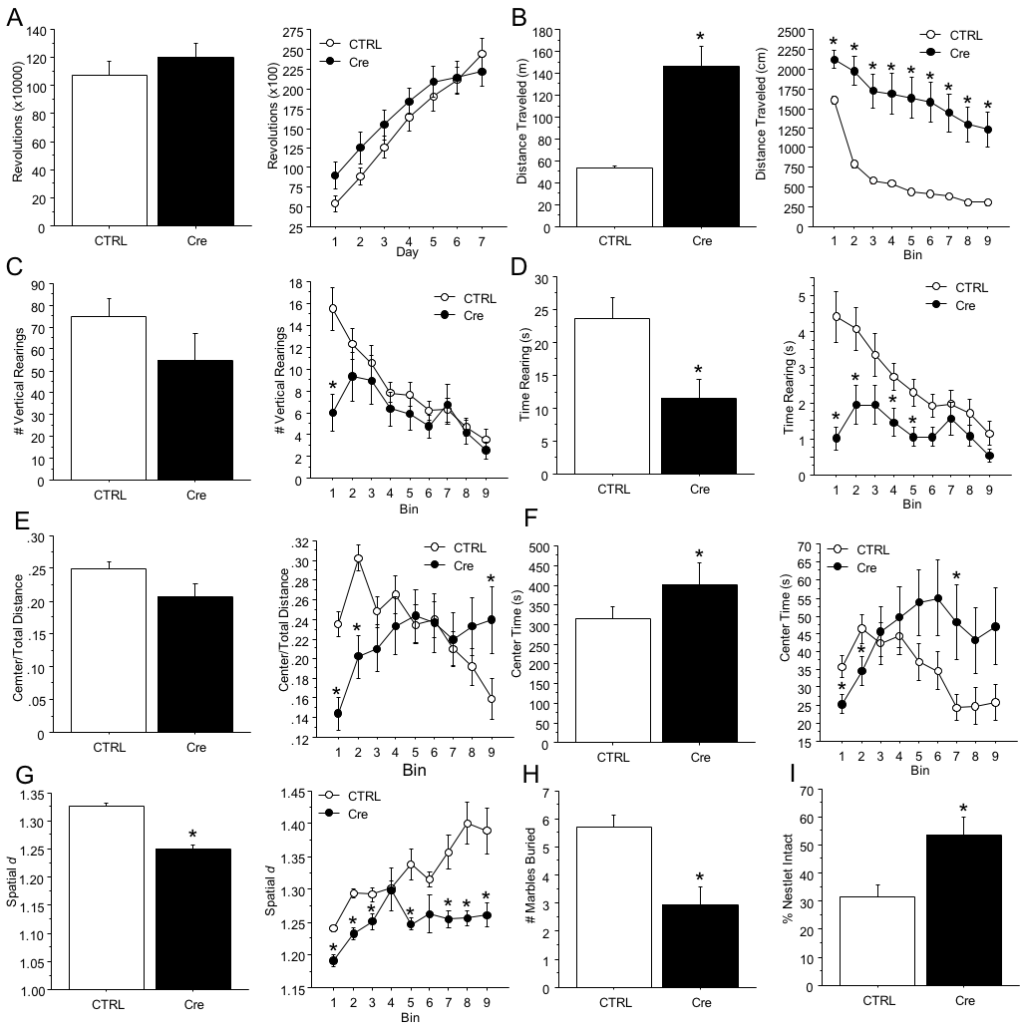

Figure 4.3: Hippocampal *Btbd3* knockdown mice recapitulate some of the behavioral deficits found in the global *Btbd3* knockout mouse. A shows wheel-running in the dark cycle. B shows distance traveled in the open field. C-D show vertical rearing in the open field. E-F show center activity in the open field. G shows spatial *d* in the open field. H shows the number of marbles buried in the marble-burying task. I shows the remaining portion of the nestlet by weight left unused for building a nest at the end of the task. Results are expressed as mean ± SEM. *Significant difference from control virus. CTRL: Control.

bin was also identified ($F_{(8,560)} = 3.49$; $p<.001$). Post hoc tests revealed reduced time spent rearing in the Cre condition for bins 1, 2, 4, and 5 relative to the control condition. Post hoc ANOVAs identified a main effect of bin within each virus condition (see Supplemental Results). No main effect of virus was identified for proportion of distance traveled in the center (Figure 4.3E), but an interaction was found between virus and bin ($F_{(8,504)} = 6.90$;

p<.0001). Post hoc tests revealed lower proportion of distance traveled in the center in the Cre relative to control condition for bins 1 and 2, and a higher proportion of distance traveled in the center than control in bin 9. Post hoc ANOVAs revealed a main effect of bin within each virus condition (see Supplemental Results). A main effect of virus was identified for time spent in the center (Figure 4.3F)($F_{(1,70)} = 4.19$; p<.05). An interaction was identified between virus and bin ($F_{(8,560)} = 2.76$; p<.01). Post hoc tests revealed lower center time in the Cre condition relative to control for bins 1 and 2, and elevated center time in the Cre versus control condition for bin 7. Post hoc ANOVAs revealed a main effect of bin within each virus condition (see Supplemental Results). A main effect of virus was identified for spatial *d* (Figure 4.3G)($F_{(1,63)} = 23.84$; p<.0001). An interaction was observed between virus and bin ($F_{(8,504)} = 2.32$; p<.05). Post hoc tests revealed decreased spatial *d* in the Cre relative to control condition for each bin except bins 4 and 6. A main effect of bin was identified within each virus condition (see Supplemental Results).

A main effect of virus was identified for the number of marbles buried in the marble-burying test ($F_{(1,70)} = 10.87$; p<.005), with mice in the Cre condition burying fewer marbles than controls (Figure 4.3H). In the nest-building test (overnight version), a main effect of virus was found for the percentage of original nestlet weight remaining intact at the end of the test (Figure 4.3I)($F_{(1,70)} = 6.47$; p<.05).

4.3.5 Btbd3 KO mice have altered dendritic morphology in select brain regions

Dendritic morphology was assessed in the dentate gyrus and CA1 subregions of the hippocampus, anterior cingulate cortex (ACC), and mediodorsal thalamus (n = 4-6/genotype, all female). In the dentate gyrus, dentate granule cells were assessed. No effect of genotype was identified in the dentate gyrus for total dendritic length (Figure 4.4A)($F_{(2,12)} = 1.88$; p = .20), dendritic crossings in the sholl analysis (Figure 4.4B)($F_{(2,12)} = 1.49$; p = .26),

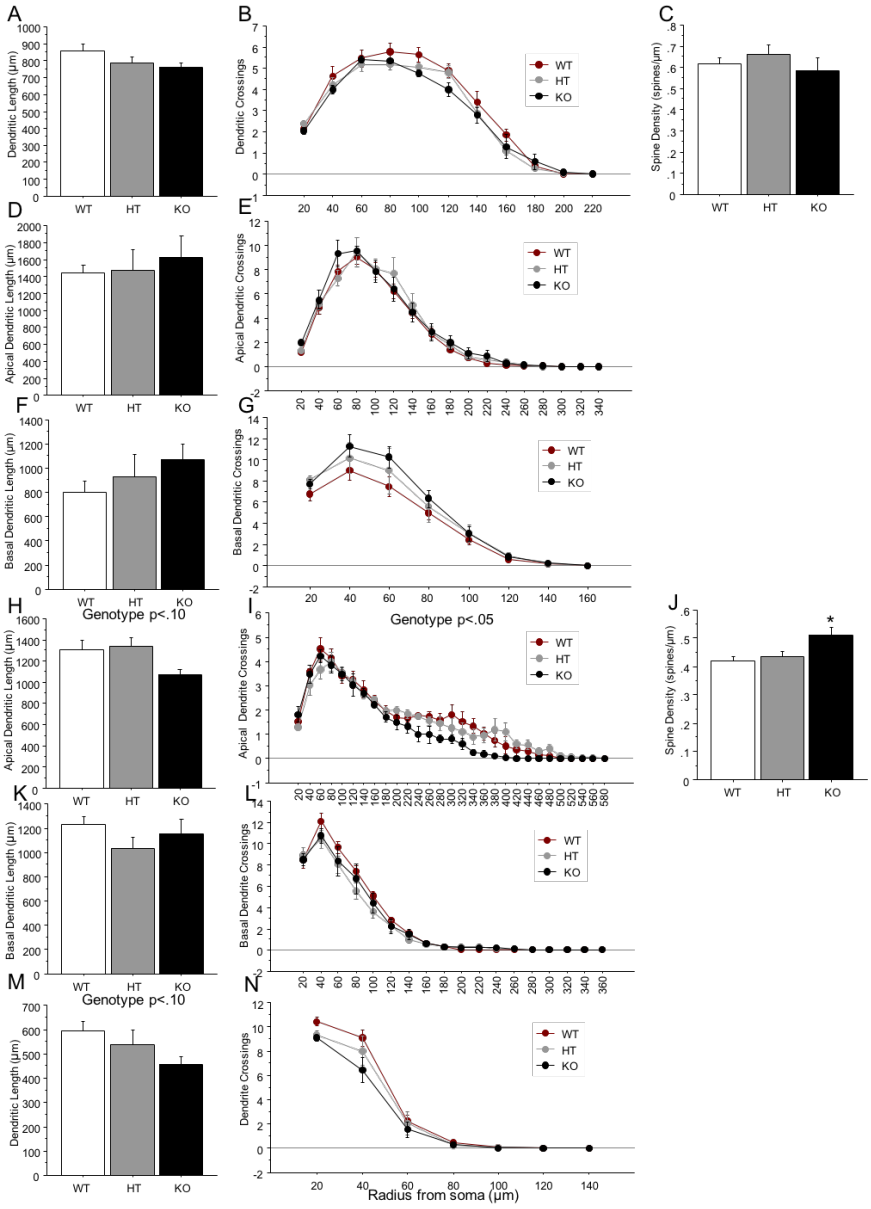

Figure 4.4: *Btbd3* expression modulates dendritic morphology in key brain regions. Dendritic morphology is shown for dentate granule cells (A-C), CA1pyramidal neurons (D-G), anterior cingulate layer II/III pyramidal neurons (H-L), and mediodorsal thalamus spiny stellate neurons (M-N). Total dendritic length is shown in (A), (D), (F), (H), (K), and (M). Dendritic crossings in the sholl analysis are shown in (B), (E), (G), (I), (L), and (N). Spine density is shown in (C) and (J). Results are expressed as mean ± SEM. *Significant difference from WT mice. WT: wild-type; HT: heterozygous; KO: knockout.

or spine density (Figure 4.4C). In CA1, there was no effect of genotype on apical dendritic length (Figure 4.4D) or dendritic crossings in the sholl analysis (Figure 4.4E). There was

no effect of genotype on basal dendritic length (Figure 4.4F)($F_{(2,12)}$ = .91; p = .43) or basal dendritic crossings (Figure 4.4G). In ACC, a trend level effect of genotype was found for apical dendritic length (Figure 4.4H)($F_{(2,13)}$ = 3.04; p = .08). A main effect of genotype was identified for apical dendritic crossings in the sholl analysis for ACC layer II/III pyramidal neurons (Figure 4.4I)($F_{(2,13)}$ = 3.98; p<.05. Post hoc tests did not reveal any significant contrasts. A main effect of genotype was identified for spine density in ACC (Figure 4.4J)($F_{(2,13)}$ = 4.29; p<.05). Post hoc tests revealed that *Btbd3* KO mice had greater spine density in ACC than WT or HT mice. In contrast, there was no effect of genotype on ACC basal dendritic length (Figure 4.4K) or basal dendritic crossings in the sholl analysis (Figure 4.4L). In mediodorsal thalamus, stellate neurons were assessed. There was no effect of genotype on total dendritic length in the main ANOVA (Figure 4.4M)($F_{(2,12)}$ = 2.27; p = .15). In the Kruskal-Wallis ANOVA, a trend for an effect of genotype on dendritic length was identified ($H_{(2)}$ = 4.96; p =.08). There was no effect of genotype on dendritic crossings (Figure 4.4N)($F_{(2,12)}$ = 1.96; p = .18). Spine density was not assessed in these neurons due to lack of sufficient image resolution.

4.4 Discussion

4.4.1 Summary of findings

Here, we demonstrate a critical role for the *Btbd3* gene in behavior. First, we identified impairments in putative compulsive-like behaviors in the global *Btbd3* knockout mouse (Figure 4.1). These deficits are supported by additional behavioral deficits in measures of exploration. Yet, the *Btbd3* knockout mouse does not show indiscriminant deficits across behavioral domains, as demonstrated by negative findings in several psychiatric-relevant paradigms (Figure S1) as well as grossly normal sensory and motor functioning (Figure S2). Next, we found selective prevention of barbering behavior by OCD-effective treatment in *Btbd3* WT

and HT, but not KO mice (Figure 4.2), which was not due to a general insensitivity to antidepressants (Figure S3). We next knocked down *Btbd3* expression selectively in hippocampus during early development and found that, in adulthood, these mice mirrored several behavioral deficits identified in the global *Btbd3* KO mouse (Figure 4.3) and, interestingly, had some effects stronger than those seen in the global knockout. Preliminary evidence suggests these behavioral effects may be driven by the ventral half of the hippocampus (Figure S5), and that the critical window for *Btbd3* expression is not confined to the neonatal developmental stage (Figure S6). Finally, no effects of *Btbd3* expression were identified on dendritic morphology or neuronal activity levels in hippocampus, leaving the mechanism of action of *Btbd3* in hippocampus to be determined. In sum, *Btbd3* expression in hippocampus is critical for maintaining aspects OCD-relevant behavior in mice.

4.4.2 Btbd3 knockout leads to compulsive-like behavioral deficits

Btbd3 KO mice exhibited deficits in behaviors related to compulsivity. The first aberrant behavior we identified was a robust increase in barbering behavior in *Btbd3* HT and KO mice (Figure 4.1A; 4.1B). Barbering is an abnormal behavior only found in animals in captivity, in which animals clip or pluck the fur of their peers [302]. One study showed that barbering behavior was associated with extradimensional shifting deficits in the rodent adaptation of the intradimensional/extradimensional shifting task [122]. In light of the putative connection between *BTBD3* and OCD [342], it is of interest that OCD patients also have selective deficits in extradimensional shifting on the intradimensional/extradimensional shifting task [61, 260, 265, 364, 379]. Set-shifting is a classic measure of cognitive flexibility, the capacity to flexibly update behavior based on changes in the environment [156], which is integral for goal-directed behavior [290]. 'Goal-directed' means behavior that is driven by an understanding of the association between actions and their outcomes [376]. Goal-directed behavior is impaired across compulsive disorders [131, 375], including OCD [25, 135, 132, 130], and this deficit is

thought to underlie compulsive behavior [136].

Relatedly, the aromatase knockout mouse model of compulsive behavior is characterized by barbering and excessive wheel-running [163]. We also found excessive wheel-running in the *Btbd3* KO mice (Figure 4.1C), suggesting a link between excessive wheel-running and barbering. Voluntary wheel-running is a goal-directed behavior [72] that animals are motivated to perform and find rewarding [151], suggesting that BTBD3 may modulate goal-directed behavior.

Btbd3 KO mice also had impaired goal-directed learning in the probabilistic learning task (**PLT**), which measures decision-making strategies during reinforcement learning. *Btbd3* KO mice performed worse overall, reflected by reduced accuracy (Figure 4.1D), where accuracy denotes the percentage of trials for which the animal responds at the higher reward probability nosepoke hole, the "target." To dissect the reduced accuracy found in *Btbd3* KO mice, response strategies were examined; namely, "win-stay" and "lose-shift" response patterns at the target and non-target holes. A goal-directed learning strategy takes into account the reward probability of each option to maximize reward [134], and thus manifests in responses primarily on the frequently rewarded target hole once reward contingencies are learned. Reduced accuracy on the PLT found in *Btbd3* KO mice was driven by an increased tendency to shift after losing on the target hole (Figure 4.1E), and by increased win-stay on the non-target hole (Figure 4.1F) despite its low reward probability. Thus, *Btbd3* KO mice followed a less goal-directed response strategy, resulting in reduced accuracy in the PLT. Importantly, these deficits were not due to blunted motivation for reward, as indicated by the lack of effect of genotype on breakpoint in the progressive ratio breakpoint task (Figure 4.1G). In sum, increased incidence of barbering, excessive wheel-running, and poor PLT performance converge to suggest that *Btbd3* KO mice have impaired goal-directed behavior that may constitute a compulsive-like phenotype.

4.4.3 Btbd3 knockout causes deficits in exploratory behavior

Exploration is an integral component of goal-directed behavior [74]. Thus, we sought to determine whether *Btbd3* KO mice have deficits in measures of exploration that may contribute to impairments in goal-directed behavior contributing to. The open field test is a classic test of rodent exploration [349]. *Btbd3* KO mice were hyperactive in the open field (Figure 4.1H), and this hyperactivity was confined to the first 20 minutes of testing. Both *Btbd3* HT and KO mice showed robust reductions in vertical rearing (Figure 4.1I-J), a measure of exploration [349]. Furthermore, *Btbd3* KO mice showed reduced digging in the dig test (Figure 4.1K-N), another test of exploration [5]. Corroborating findings in the dig test, *Btbd3* KO mice buried fewer marbles than WT mice (Figure 4.1O) in the marble-burying paradigm, which is thought to measure digging behavior [88, 90, 354].

The hippocampus is implicated in several of these phenotypes [89, 90]. Thus, nest-building was assessed, which is impaired in animals with adult-onset hippocampal lesions [90]. *Btbd3* KO mice had impaired nest-building behavior (Figure 4.1P), suggesting that the hippocampus may be driving behavioral deficits. Nest-building is also considered a putative measure of exploration [26] and goal-directed behavior [183]. *Btbd3* KO mice did not exhibit broad deficits in psychiatric-relevant phenotypes (Figure S1), and the behavioral deficits identified are not due to gross sensory or motor deficits in *Btbd3* KO mice (Figure S2). In sum, *Btbd3* KO mice have selective deficits in compulsive-like behaviors and exploration that could be mediated through the hippocampus.

4.4.4 Barbering is selectively responsive to OCD-effective treatment

We next sought confirmation of barbering as a compulsive-like behavior, based on its association with extradimensional shifting deficits in previous studies [122]. Chronic treatment with serotonin-reuptake inhibitors (**SRIs**) is the only effective pharmacological monotherapy for OCD, whereas other classes of antidepressants are ineffective [288]. Thus, if barbering is

compulsive-like, it may be selectively ameliorated by chronic treatment with SRIs but not other classes of antidepressants. By four weeks of treatment, SRI fluoxetine significantly reduced the onset of barbering across genotypes compared to vehicle treatment, whereas the norepinephrine reuptake inhibitor desipramine had no effect at any time point (Figure 4.2A). The selectivity of this effect to OCD-effective treatment reinforces the idea that barbering is related to compulsivity. Strikingly, fluoxetine completely prevented the onset of barbering in WT mice (Figure 4.2B), reduced barbering in *Btbd3* HT mice (Figure 4.2C), and had no effect on barbering in *Btbd3* KO mice at any time point (Figure 4.2D). This finding indicates interplay between *Btbd3* expression and fluoxetine treatment. This treatment-resistance was not due to differential fluoxetine metabolism (Supplemental Results) or to general insensitivity to antidepressant action in *Btbd3* KO mice (Figure S3), suggesting a unique relationship between *Btbd3* expression and fluoxetine treatment in the context of barbering behavior. The mechanism underlying this effect warrants further investigation. Thus, barbering behavior is selectively responsive to OCD-effective treatment, providing additional evidence that barbering may be a compulsive-like behavior.

4.4.5 Btbd3 knockdown in hippocampus recapitulates several behavioral deficits of the global knockout

Several of the behavioral deficits identified in *Btbd3* KO mice mirror impairments characteristic of hippocampal dysfunction in rodents. Mice lesioned throughout hippocampus in adulthood are hyperactive, rear less, dig less, bury fewer marbles, and build worse nests than controls [89, 90]. Cyclin D2 KO mice, which have impaired adult neurogenesis and reduced hippocampal volume, exhibit deficits in digging, marble-burying, and nest-building, and are hyperactive [182]. Furthermore, the neonatal ventral hippocampal lesion model of schizophrenia exhibits cognitive flexibility deficits [289], hyperactivity, and reductions in rearing [216]. Based on this evidence, and the robust expression of *Btbd3* in hippocampus

[219, 247], we selectively knocked down *Btbd3* expression in hippocampus of neonatal (**P2**) $Btbd3_{\text{flox}}$ mice and assessed behavior in adulthood. Knockdown (**KD**) was initiated at P2 due to behavioral overlap with the neonatal ventral hippocampal lesion model in the global *Btbd3* KO mice, and because *BTBD3* expression is low during embryonic development, but then rapidly increases early in postnatal development [234, 247].

Neonatal *Btbd3* knockdown in hippocampus recapitulated several of the behavioral deficits identified in the global knockout mouse, with some distinctions. Excessive wheel-running was not reproduced in the hippocampal knockdown mice (Figure 4.3A), but hyperactivity in the open field was (Figure 4.3B). This dissociation suggests that hippocampal *Btbd3* expression may play a specific role in novelty-induced hyperactivity. Interestingly, viral infusions in this cohort had peak expression in the ventral third of the hippocampus, and thus these habituation findings align with impaired habituation to novelty in the open field in the neonatal ventral hippocampal lesion model of schizophrenia [79]. Hippocampal *Btbd3* KD recapitulated the reductions in rearing (Figure 4.3C-D), reduced marble-burying (Figure 4.3H), and impaired nest-building (Figure 4.3I) found in the global *Btbd3* KO mouse, indicating a role for hippocampus in BTBD3-mediated exploration. Interestingly, habituation to novelty was disrupted in hippocampal KD mice across measures in the open field, which was not identified in the global *Btbd3* KO mouse. This effect is interesting in light of the blunted exploratory behaviors found in both the global KO and hippocampus KD cohorts, and suggests that *Btbd3* expression in hippocampus may play a role in exploration by regulating response to novelty. A role for hippocampal *Btbd3* expression in novelty aligns with the dissociation identified between hyperactivity on the running wheels and in the open field.

Surprisingly, some behavioral deficits were identified in hippocampal KD mice that were not found in the global knockout mouse, specifically changes in center activity and reduced spatial *d*. Low spatial *d* is indicative of perseverative hyperlocomotion [270] and describes

a rigid pattern of locomotion marked by few directional changes [270]. Interestingly, the 5-HT1BR-induced mouse model of aspects of OCD also exhibits perseverative hyperlocomotion in the open field [323, 324], not to mention hyperactivity and reduced rearing in the open field, suggesting that this pattern of behavior may be of relevance to OCD. These findings that have discrepancies from the global *Btbd3* KO mouse suggest that *Btbd3* expression in other brain regions may play a role in behavior. In sum, hippocampus plays an integral role in behavioral effects of *Btbd3* expression.

4.4.6 Behavioral effects of neonatal hippocampal Btbd3 knockdown are not primarily attributable to the dorsal hippocampus

Next, we sought to narrow down the hippocampal subregion and developmental time points requiring *Btbd3* expression for maintaining normal behavior. Ventral hippocampus is implicated in goal-directed behavior [274, 310, 311], cognitive flexibility [289], exploration [13], and response to novelty [343], making it a good candidate region for the behavioral deficits found in hippocampal *Btbd3* KD mice. However, these functions are not entirely segregated; while dorsal hippocampus is classically more important for encoding spatial information [343], it was recently implicated in goal-directed decision-making [249]. Thus, we tested whether selective neonatal *Btbd3* KD in dorsal hippocampus would be sufficient to induce the behavioral effects of full hippocampal *Btbd3* KD. Dorsal hippocampal KD did not reproduce the effects seen in the full hippocampal KD cohort (Figure S5), with the exception of robust deficits found in nest-building behavior. This result suggests that *Btbd3* expression in dorsal hippocampus plays a specific role in nest-building behavior, and that nest-building may be dissociable from the exploratory behaviors (rearing, digging, marble-burying) that were impaired in the general hippocampal KD cohort. While these findings suggest that *Btbd3* may mediate its behavioral effects primarily through ventral hippocampus, this warrants confirmation in a study knocking down *Btbd3* expression exclusively in this subregion.

4.4.7 Behavioral effects of hippocampal Btbd3 knockdown are not confined to expression loss during the neonatal developmental period

To address the critical window for effects of *Btbd3* expression on behavior, in a pilot experiment, hippocampal knockdown was performed in juvenile (P21) mice and behavior was assessed in adulthood. Female hippocampal *Btbd3* KD mice were hyperactive (Figure S6A) and spent more time in the center (Figure S6E) than control mice. These effects are reminiscent of the effects of neonatal hippocampal *Btbd3* KD (Figure 4.3), although those effects were not sex-specific. Juvenile hippocampal *Btbd3* KD robustly reduced nest-building (Figure S6G). Thus, some, but not all, effects of neonatal *Btbd3* KD in hippocampus were found with juvenile hippocampal *Btbd3* KD. These effects will require confirmation in a larger sample, but suggest that at least some behavioral effects of *Btbd3* KD in hippocampus are not specific to expression loss during the neonatal developmental stage, warranting further investigation into the critical time frame for effects of hippocampal *Btbd3* expression on behavior.

4.4.8 Behavioral effects of hippocampal Btbd3 knockdown are not attributable to cell-autonomous effects on dendritic morphology

We next sought to identify neural substrates underlying behavioral effects of *Btbd3* expression. BTBD3 is necessary for proper organization of the dendritic arbor in spiny stellate neurons of somatosensory barrel cortex in mice [234]. Thus, we hypothesized that dendritic morphology would be disrupted in *Btbd3* KO mice in key brain regions of dense *Btbd3* expression and relevance to goal-directed behavior. Surprisingly, despite robust *Btbd3* expression in hippocampus, no effects of genotype were found on dendritic morphology in either dentate granule cells or CA1 pyramidal neurons (Figure 4.4A-E). These results suggest that behavioral effects of *Btbd3* expression are likely not mediated by cell autonomous effects on

dendritic morphology in hippocampus. Interestingly, ACC layer II/III pyramidal neurons appeared to have reduced apical dendritic branching and increased spine density in *Btbd3* KO mice, although these effects were small (Figure 4.4J). Finally, a trend was identified for an effect of genotype on dendritic length in mediodorsal thalamus spiny stellate neurons (Figure 4.4M). These tentatively hypotrophic findings in ACC and mediodorsal thalamus are surprising in light of the hypertrophic phenotype found with *Btbd3* KD in barrel cortex, where spiny stellate neurons have more primary dendrites, reduced polarity, and a more complex dendritic arbor [234]. Since BTBD3 is a transcription factor, one explanation for this discrepancy is that BTBD3 may regulate different genes between brain regions that differentially affect dendritic morphology. These trend level reductions in dendritic length require confirmation in a larger sample, but suggest that these brain regions warrant further investigation as players in behavioral effects of *Btbd3* expression. Thus, dendritic remodeling is not likely to drive hippocampus-dependent behavioral effects of *Btbd3* expression.

4.4.9 Relationship of behavioral effects of Btbd3 to OCD

A SNP downstream of *BTBD3* was the first genome-wide significant hit for OCD [342], providing the first evidence that BTBD3 may be relevant for psychiatric-related phenotypes. The findings presented here indicate that BTBD3 plays a major role in OCD-relevant behaviors. *Btbd3* KO mice exhibit compulsive-like behaviors and have deficits in goal-directed behavior (Figure 4.1), much like OCD patients [25, 135, 132, 130]. Impaired goal-directed behavior is thought to underlie compulsive behavior [136], and thus may be driving compulsive-like phenotypes found in *Btbd3* KO mice, such as barbering behavior. Furthermore, exploration is a key component of goal-directed behavior [74], which is also deficient in *Btbd3* KO mice (Figure 4.1), suggesting that BTBD3 may play a role in goal-directed behavior by modulating exploratory behavior. Exploration is in part driven by novelty [320], and habituation to novelty was disrupted in neonatal hippocampal *Btbd3* KD mice (Figure 4.3).

Novelty-seeking is reduced in OCD patients [212, 227, 280], suggesting the possibility that disrupted response to novelty may contribute to deficits in goal-directed behavior.

Hippocampal *Btbd3* expression is necessary for maintaining normal exploratory behavior (Figure 4.3), in line with the known roles of ventral hippocampus in the related constructs of goal-directed behavior [274, 310, 311], cognitive flexibility [289], exploration [13], and response to novelty [343]. While historically the hippocampus has received less attention in OCD than the CSTC circuit, it was recently suggested that hippocampal dysfunction may play a primary role in OCD [299]. The hippocampus projects to medial prefrontal cortical structures [372] and ventral striatum [192], and thus may influence OCD-relevant behaviors through modulation of the limbic CSTC circuit [215]. In addition, there is evidence to suggest reduced volume [22, 40] and shape deformity [174] of the hippocampus in OCD patients, as well as aberrant hippocampal activity during performance of various tasks in neuroimaging studies [231, 244, 299]. Thus, in light of this evidence, our results suggest that closer investigation of a role for the hippocampus in OCD is warranted.

4.4.10 Conclusions

To our knowledge, this study is the first to identify a role for *Btbd3* expression in behavior. Furthermore, BTBD3 selectively regulates goal-directed behaviors and exploration, which are highly relevant to OCD. While *BTBD3* remains to be confirmed as an OCD risk gene in human GWAS, our findings reinforce the tentative hypothesis that BTBD3 may play a role in OCD etiology.

4.5 Supplemental material

4.5.1 Methods

Prepulse inhibition

Prepulse inhibition (PPI) was assessed as previously described[324]. In brief, mice were placed in startle chambers (San Diego Instruments, San Diego, CA, USA). Startle response amplitude was measured for startle alone (120 dB), prepulse trials (3, 6, or 12 dB prepulse followed by 120 dB pulse), or no stimulus. PPI was calculated as 100 * (startle - prepulse)/startle amplitude.

Light/dark

The light/dark test was performed to assess anxiety-like behavior [250]. The light/dark test was performed in the open field using dark chamber inserts that cover half the chamber without blocking infrared beams and has a central entry hole for access to the light side of the chamber (Omnitech Electronics, Inc., Columbus, OH, USA). Animals were placed in the dark side of the chamber and activity was recorded for 10 minutes. Duration in each side, proportion of distance traveled in the dark, and latency to enter the light side were used as outcome measures. Distance on each side and transitions were not used because hyperactivity in *Btbd3* KO mice would confound the results.

Olfactory dis/habituation

The olfactory dis/habituation test was used to assess olfactory sensation as previously described [406]. Mice were placed in a cage. Cotton swabs were dipped in odorant and lowered into the cage for 60 seconds, followed by a 2-minute intertrial interval. Each odorant had 3 trials in a row to habituate the animal. Then a novel odorant was introduced and repeated

for a total of 3 trials. The number of sniffs was recorded. The first odorant was always water, followed by isoamyl acetate or ethyl acetate in a counterbalanced fashion.

Olfactory memory

The olfactory memory test was used to measure the memory retention of a familiar odor as previously described [406]. Stimuli were presented in the same setup as for olfactory dis/habituation. Animals were first exposed to ethyl vanillin for a period of 4 minutes to habituate them to the stimulus. An hour later, animals were exposed to ethyl vanillin again for a second trial of 4 minutes. If the animal remembers the odorant, they are expected to sniff the stimulus less during the second trial than the first.

Whisker brushing

Whisker brushing was performed as previously described[76]. Briefly, animals were scruffed in one hand while the other brushed the distal end of the whiskers on each side of the face. Turning the face during or just after whisker brushing was considered indicative of a response.

Footprint test

The footprint test was used to assess motor coordination and balance as previously described [49]. Animals were placed in a corridor (70 cm long) lined with paper. First, animals underwent a habituation phase (2, 10 minute sessions) to train them to run the corridor. A chunk of milk chocolate was placed at the end of the corridor. The end of the corridor was covered to make it dark. Animals were scruffed and front paws were painted one color and hind paws were painted a different color. Animals were then placed at the beginning of the corridor and allowed to explore the corridor. If the animal reached the end of the corridor and the chocolate reward, they were placed back at the start with a fresh piece of

chocolate at the end. In the test phase, the corridor was lined with fresh paper and a piece of chocolate. The animal's paws were painted and then the animal was placed at the start. As soon as the animal reached the end of the corridor or turned around in the corridor, the animal was removed and the trial was over. The test phase was repeated for a total of three trials or until a clear set of footprints with the animal moving in a straight path was obtained. Output measures were stride length (distance between footfalls with the same foot), overlap (distance between the center of the plantar of the fore and hind limb on the same stride), and base width (distance between the fore or hind feet on the same stride).

Fluoxetine metabolism

Male *Btbd3* WT, HT, and KO mice were treated with fluoxetine in the drinking water at a concentration of 80 mg/L for a target dose of 10 mg/kg/day. After four weeks, animals were sacrificed and trunk blood was collected in tubes coated with EDTA. Samples were spun down and plasma layer pipetted off into new tubes. Plasma samples were sent to the Analytical Psychopharmacology Laboratories to measure fluoxetine and norfluoxetine levels using liquid chromatography with fluorescence detection (Nathan Kline Institute, Orangeburg, NY, USA) [344].

Novelty-induced hypophagia

Animals were treated continuously with fluoxetine, desipramine, or control in the drinking water (as in main methods) for four weeks. Novelty-induced hypophagia (NIH) testing was then performed as previously described [96]. Briefly, mice underwent three days of training to consume sweetened condensed milk. The following day, mice were presented with sweetened condensed milk in the home cage for 30 minutes and tested for latency to drink and consumption volume. The following day, mice were presented with sweetened condensed milk in a novel cage with no bedding and bright lighting for 30 minutes and

latency to drink and consumption volume were recorded.

Btbd3 qPCR

Btbd3$_{\text{flox}}$ mice were infused with Cre recombinase or control virus (see main methods) into hippocampus at P2. Brains were extracted and snap frozen at eight weeks. Tissue punches were taken from hippocampus and stored at -80°C until RNA purificaiton. Placement of viral infusion was verified during tissue punching by shining a UV light briefly over the tissue and visualizing fluorescence in hippocampus. RNA was purified as above. qPCR was performed using the KAPA SYBR FAST One-Step assay (KAPA Biosystems, Wilmington, MA, USA). *Btbd3* primers: (5' to 3'): CGTAAGAAGCCAGCCAACTC and CCCAACCACAAAATG-TACGTC (Integrated DNA Technologies, Inc., Coralville, IA, USA). B-actin was used as the reference gene (Integrated DNA Technologies, Inc.).

Western blot

Btbd3$_{\text{flox}}$ mice were infused with Cre recombinase or control virus (see main methods) into hippocampus at P2. Brains were extracted and snap frozen at eight weeks. Tissue punches were taken from hippocampus and stored at -80°C until cell lysis. Placement of viral infusion was verified during tissue punching as above. Cells were lysed and protein quantified using the BCA assay. 20 μg protein was loaded into NUPAGE 10% Bis-Tris gels (Cat#: NP0301; ThermoFisher Scientific), and run at 200 V for 50 minutes. Protein was then transferred to 0.45 μm PVDF membrane (Cat#: IPFL00010; Millipore Sigma, Billerica, MA, USA) at 30 V for 50 minutes. The membrane was washed in TBST and incubated in blocking buffer for 1 hour at room temperature. The membrane was then incubated in 1:200 rabbit anti-BTBD3 (Cat#: HPA042048; Atlas Antibodies, Bromma, Sweden) or 1:10,000 rabbit anti-GAPDH (Cat# 2118; Cell Signaling Technology, Inc., Danvers, MA, USA) primary antibody overnight at 4°C. The membrane was washed in TBST then incubated in 1:1000

HRP-conjugated anti-rabbit secondary antibody (Cat#: 7074S; Cell Signaling Technology, Inc.) for one hour at room temperature. The membrane was washed in TBST then incubated in chemiluminescent substrate (Cat#: PI34080; Fisher Scientific) for 5 minutes at room temperature before visualizing on film (Cat#: E3018; Denville Scientific Inc., Holliston, MA, USA). Film was scanned using a transparency scanner. Bands were analyzed in ImageJ [319]. Background was removed using a rolling ball radius of 200 pixels. Standard densitometry analysis was performed to normalize BTBD3 bands to GAPDH bands within each lane.

shRNA *Btbd3* knockdown pilot

Juvenile C57BL/6J mice (postnatal day 21 $\pm$ 1) received intracranial infusions of shRNA against *Btbd3*: AAV1-CAG2-tdTomato-WPRE-U6-mBTBD3-shRNA (Vector Biolabs, Malvern, PA, USA) or scrambled shRNA: AAV1-CAG2-tdTomato-WPRE-U6-scrambled-shRNA (Vector Biolabs) into hippocampus (3.28 mm posterior, $\pm$ 2.5 mm lateral, and 3.5 mm ventral of bregma) using a standard stereotaxic setup. Animals were anesthetized using isoflurane and secured in the stereotaxic. An incision was made to expose the skull and skin pulled back. Bregma was identified and the coordinates measured. The dorsal-ventral coordinate was measured at lambda as well to ensure that the head was level. Coordinates were determined relative to bregma. Holes were drilled in the skull above the target regions. Infusions were performed using a syringe pump (Harvard Apparatus) equipped with a 10 μl syringe (Hamilton Company) connected to tubing and a 28 gauge cannula (Plastics One). The cannula was lowered to the target coordinate and 1.0 μl virus infused at a rate of 0.2 μl/min. The cannula was then left in place for an additional 5 minutes to allow virus to diffuse before slowly retracting the cannula. The process was repeated for the opposite hemisphere. The scalp was stapled closed and the animal monitored closely during the post-operative period. Four weeks later, animals were assessed in the open field (30-minute test) and nest building (8-hour variation) tests.

Fos RNA quantification

Tissue punches were taken from fresh frozen brains of *Btbd3* WT, HT, and KO mice. Quantitative real time polymerase chain reaction (qPCR) was performed as previously described[165]. In brief, RNA was isolated with the RNeasy Lipid Tissue mini Kit (Qiagen, Germantown, MD). cDNA was produced by reverse transcription (Life Technologies, Grand Islands, NY). qPCR was performed using TaqMan gene expression assays for Fos (Life Technologies) and eukaryotic 18S (Life Technologies). Fold change expression values were determined using the comparative Ct method [318].

4.5.2 Results

Behavioral effects of *Btbd3* expression in mice are specific

In the PLT, a trend was identified for a main effect of genotype on the proportion of win-stay responses on the target hole (Figure 4.5A)($F_{(2,34)} = 2.93$; $p = .07$). No effect of genotype on proportion of lose-shift responses on the non-target were identified (Figure 4.5B). Animals were assessed in the Go/No-Go paradigm to measure response inhibition (n = 11-16/genotype, all male). No differences among the genotypes were identified for learning the task, as measured by d' (Figure 4.5C)($F_{(2,36)} = 1.05$; $p = .36$). Genotype did not affect false alarm rate (Figure 4.5D). However, genotype had a main effect on premature responding ($F_{(2,36)} = 3.45$; $p<.05$). Post hoc tests revealed that *Btbd3* HT and KO mice had lower instances of premature responding than WT mice (Figure 4.5E). PPI testing was performed to measure sensorimotor gating (n = 14-15/genotype/sex). Genotype did not affect PPI (Figure 4.5F)($F_{(2,80)} = .93$; $p = .39$) or startle amplitude (Figure 4.5G)($F_{(2,80)} = 1.12$; $p = .33$). Animals were assessed in the light/dark paradigm to evaluate anxiety-like behavior (n = 15/genotype/sex). No significant effects of genotype on primary outcome measures were identified. A main effect of side of the chamber was found for duration ($F_{(1,84)} = 140.97$;

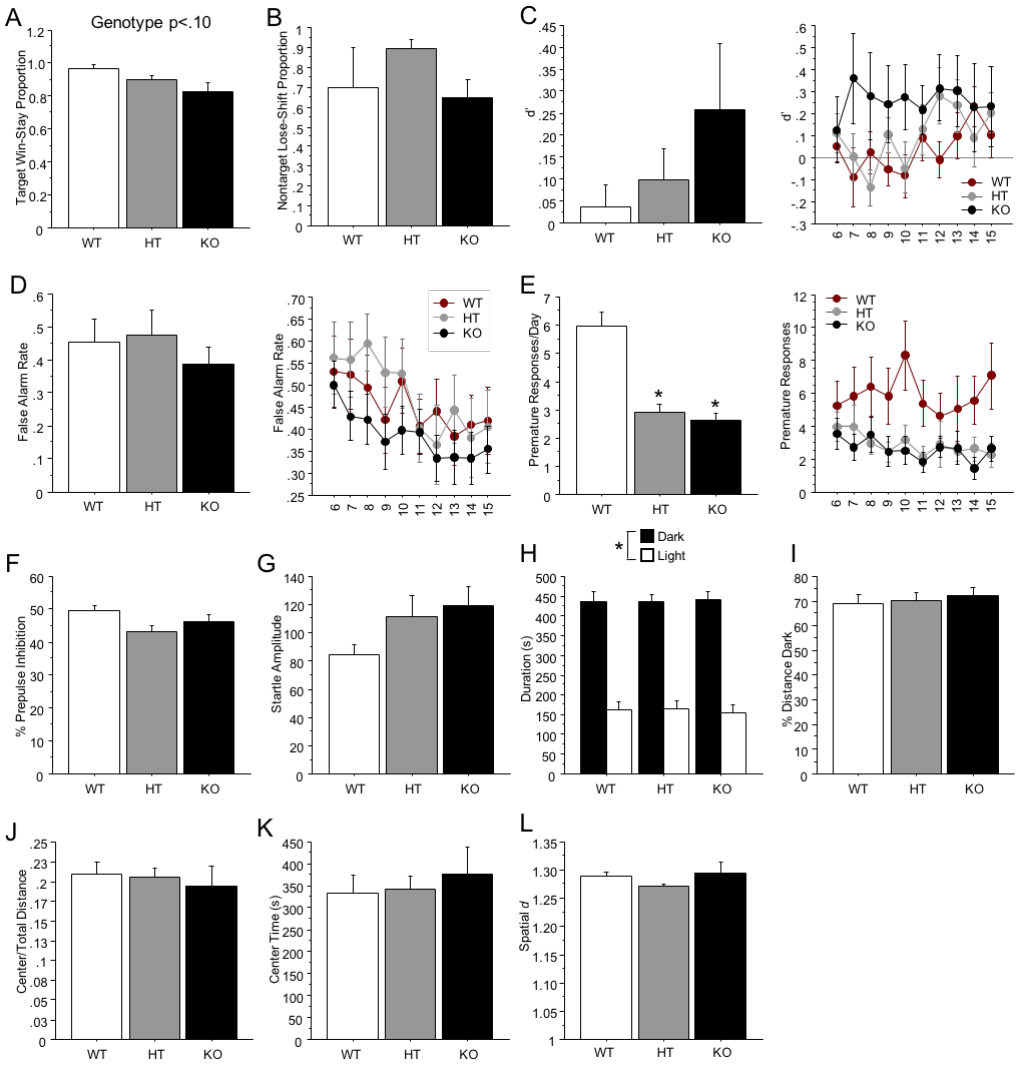

Figure 4.5: Behavioral deficits in *Btbd3* knockout mice are selective. A-B show measures in the probabilistic learning task (related to Figure 1D-F): proportion of win-stay responses on the target hole (A) and proportion of lose-shift responses on the nontarget hole (B). C-E show measures in the go/no-go task: sensitivity index d' (C), false alarm rate (D), and premature responding (E). F shows prepulse inhibition. G shows startle amplitude. H-I show the light/dark test: time spent on the dark versus light side of the box (H) and percent distance traveled on the dark side of the box (I). J-L show open field measures (related to Figure 1H-J): proportion of total distance traveled in the center (J), time spent in the center (K), and spatial d (L). Results are expressed as mean ± SEM. *Significant difference from WT mice. WT: wild-type; HT: heterozygous; KO: knockout.

p<.0001), indicating that animals spent more time on the dark side of the chamber. No genotype by side interaction for duration was found (Figure 4.5H). Percent distance in the

dark was used as a primary outcome measure to normalize to total activity, in light of the hyperactivity identified in *Btbd3* KO mice in the open field. Percent distance traveled in the dark did not differ among the genotypes (Figure 4.5I). No effect of genotype was identified on percent of total distance traveled in the dark side of the chamber. In the open field, no effect of genotype was identified for proportion of distance traveled in the center (Figure 4.5J) or time spent in the center (Figure 4.5K). There was no effect of genotype on spatial *d* (Figure 4.5L).

Btbd3 KO mice do not have major sensory or motor deficits

We next screened *Btbd3* KO mice for deficits in basic sensory and motor functioning, as *Btbd3* is robustly expressed in regions of high sensory acuity [234] and in the cerebellum [219]. Animals were assessed for olfactory sensitivity in the olfactory dis/habituation paradigm (n = 11-12/genotype/sex). A main effect of trial was identified (Figure 4.6A)($F_{(2,130)}$ = 13.72; p<.0001). No effects of genotype were found. Animals were assessed for olfactory memory in the olfactory memory paradigm (n = 11-12/genotype/sex). A main effect of genotype was identified for number of sniffs ($F_{(2,65)}$ = 7.45; p<.005). Post hoc tests revealed that *Btbd3* KO mice sniffed significantly more than HT or WT mice. However, no genotype by trial interaction was identified (Figure 4.6B)($F_{(2,65)}$ = 1.39; p = .25). Whisker brushing was performed to assess whisker reflexes (n = 15/genotype/sex). No effect of genotype was found for left (Figure 4.6C) or right (Figure 4.6D) whisker responsiveness. The footprint test was performed to assess effects of *Btbd3* expression on motor coordination (n = 12/genotype/sex). A trend for a main effect of genotype was identified for forelimb stride length (Figure 4.6E)($F_{(2,65)}$ = 2.76; p = .07). However, post hoc tests revealed that no genotypes were significantly different from each other. No effect of genotype was identified for hind-limb stride length (Figure 4.6F)($F_{(2,65)}$ = 2.35; p = .10). No effects of genotype were identified on fore- (Figure 4.6G) or hind-limb base width (Figure 4.6H). No effect of

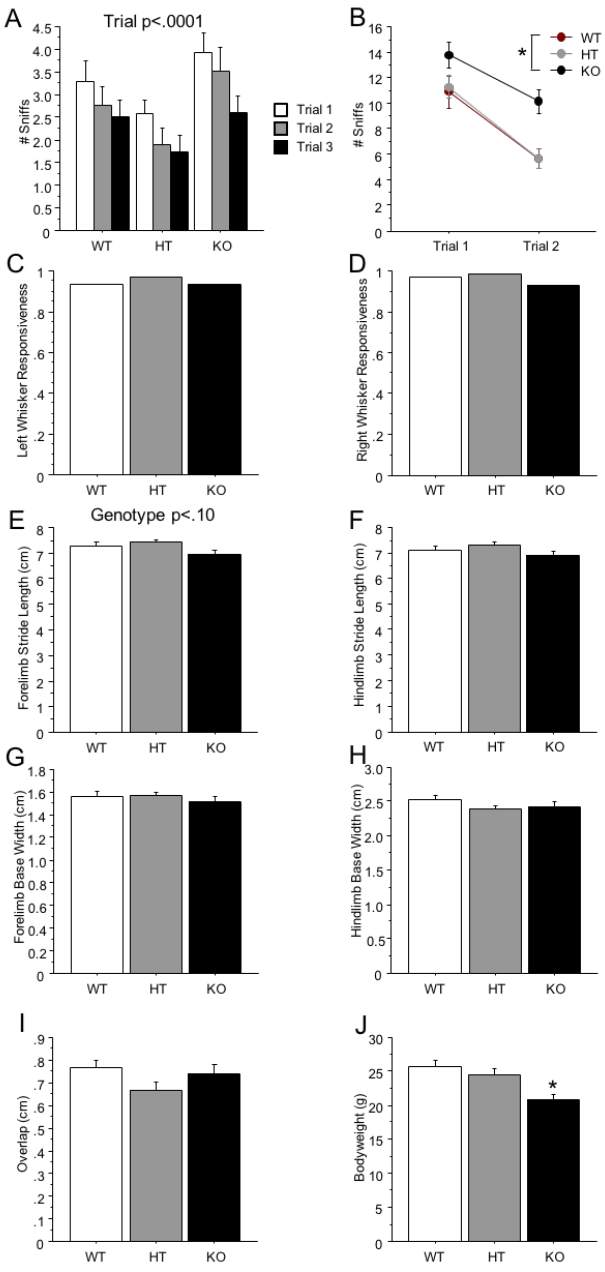

Figure 4.6: Behavioral deficits in *Btbd3* knockout mice are not due to gross impairments in sensory or motor function. A shows olfactory dis/habituation. B shows olfactory memory. C-D show whisker responsiveness. E-I show the footprint test: forelimb stride length (E), hindlimb stride length (F), forelimb base width (G), hindlimb base width (H), and overlap (I). J shows bodyweight. Results are expressed as mean ± SEM, with the exception of C-D which are categorical data and show instances. *Significant difference from WT mice. WT: wild-type; HT: heterozygous; KO: knockout.

genotype was identified for overlap (Figure 4.6I). A main effect of genotype was identified for bodyweight (Figure 4.6J)($F_{(2,66)}$ = 26.30; p<.0001). Post hoc tests revealed that *Btbd3* KO mice weighed significantly less than *Btbd3* WT or HT mice.

Effects of *Btbd3* expression on barbering responsiveness to fluoxetine are specific

Plasma fluoxetine levels were measured to determine wither genotype-specific effects of fluoxetine on barbering behavior could be due to differential fluoxetine metabolism (n = 4-6/genotype, all male). No effects of genotype were identified on plasma fluoxetine ($F_{(2,12)}$ = .62; p - .55) or norfluoxetine levels ($F_{(2,12)}$ = .21; p = .82)(data not shown). To test if *Btbd3* KO mice are generally insensitive to antidepressants, animals underwent novelty-induced hypophagia (NIH) testing after chronic treatment with fluoxetine, desipramine, or vehicle (n = 8-10/genotype/sex/treatment). For desipramine, a treatment by cage interaction was identified for latency to drink the sweetened condensed milk (Figure 4.7A)($F_{(1,98)}$ = 10.78; p<.005). Post hoc tests revealed that animals had a significantly greater latency to drink in the novel versus the home cage within both the vehicle and desipramine treatment groups. Post hoc tests also revealed that desipramine reduced latency to drink in both the home and novel cage. A treatment by cage interaction was also identified for total consumption of sweetened condensed milk (Figure 4.7B)($F_{(1,100)}$ = 6.43; p<.05). Post hoc tests revealed that animals drank less in the novel versus the home cage within both the vehicle and desipramine treatment groups. Post hoc tests also revealed that desipramine increased consumption relative to vehicle only within the novel cage condition. For fluoxetine, a main effect of treatment on latency was identified ($F_{(1,99)}$ = 5.16; p<.05), with fluoxetine decreasing latency to drink across cage conditions (Figure 4.7C). A main effect of cage was also identified ($F_{(1,99)}$ = 159.40; p<.0001), where animals had a greater latency to drink in the novel versus the home cage. For consumption, a main effect of treatment was identified

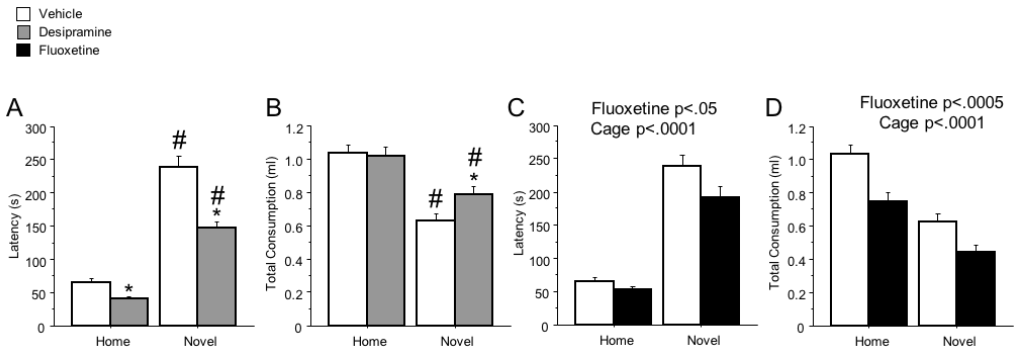

Figure 4.7: Effects of *Btbd3* expression on responsiveness of barbering to fluoxetine are not due to general insensitivity to antidepressant treatment. A-D show effects of desipramine (A-B) and fluoxetine (C-D) on novelty-induced hypophagia, measured in latency to drink sweetened condensed milk (A,C) and total consumption of sweetened condensed milk (B,D). Results are expressed as mean ± SEM. *Significant difference from vehicle within condition. #Significant difference from home cage condition within treatment condition.

($F_{(1,102)}$ = 15.23; p<.0005), with fluoxetine reducing consumption across cage conditions (Figure 4.7D). A main effect of cage was also identified ($F_{(1,102)}$ = 146.09; p<.0001), where animals consumed less sweetened condensed milk in the novel cage overall.

Cre recombinase successfully knocks down *Btbd3* expression in $Btbd3_{\text{flox}}$ mice

Because the $Btbd3_{\text{flox}}$ mouse is a custom CRISPR floxed mouse and the Cre virus used (see main methods) was custom designed, a series of experiments were performed to confirm that the Cre/loxP system was working properly to knockdown *Btbd3* expression. First, qPCR was performed to measure *Btbd3* RNA expression in hippocampus of 8-week old $Btbd3_{\text{flox}}$ mice that were infused with Cre-containing or control virus at P2 (n = 2-7/virus). A main effect of virus was identified (Figure 4.8A)($F_{(1,7)}$ = 46.39; p<.0005). A western blot was performed to determine BTBD3 protein levels in hippocampus of 8-week old mice infused with Cre-containing or control virus at P2 (n = 3/virus). A main effect of virus was identified (Figure 4.8B-C)($F_{(1,4)}$ = 22.10; p<.01). An example of tdTomato fluorescence marking the cells infected with virus in hippocampus is shown in Figure 4.8D.

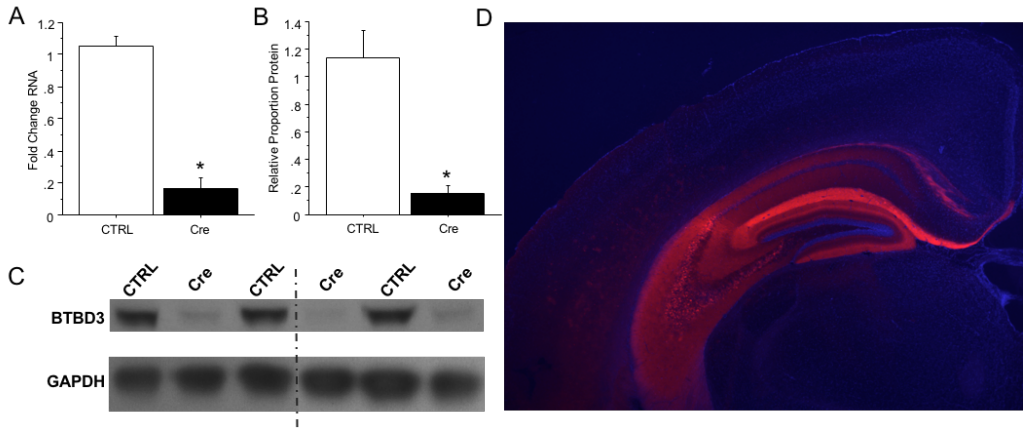

Figure 4.8: Cre virus successfully knocks down *Btbd3* expression in $Btbd3_{\text{flox}}$ mice. A shows *Btbd3* RNA. B shows BTBD3 protein. C shows BTBD3 (top) and GAPDH (bottom) bands from the western blot. D shows an example of tdTomato expression in hippocampus. Results are expressed as mean ± SEM. *Significant difference from control virus. CTRL: control.

Hippocampal *Btbd3* knockdown blunts habituation to novelty in the open field

See the main text for primary statistics for Figure 4.3. For distance traveled, post hoc ANOVAs revealed a main effect of bin within each virus condition. Thus, subsequent bins were compared within each virus condition to examine habituation to novelty (Figure 4.3B). Post hoc tests revealed a significant difference between distance traveled in bins 1 and 2, and between bins 2 and 3, within the control condition, whereas there were no differences between sequential bins within the Cre condition. Next, each bin was tested against the final, 9^{th} bin within each virus condition to further examine habituation to novelty. Within the control condition, bins 1-5 had elevated activity above that found in bin 9. In contrast, within the Cre condition, only bins 1-2 had elevated activity above that found in bin 9. For instances of vertical rearing, post hoc ANOVAs revealed a main effect of bin within each virus condition. Neither virus condition showed differences between subsequent bins. When compared to bin 9, bins 1-5 had higher instances of rearing in the control condition. In the Cre condition, bins 2-4 had higher instances of rearing than in bin 9. For time spent rearing,

post hoc ANOVAs identified a main effect of bin within each virus condition. Post hoc tests revealed no significant differences between subsequent bins within either the control or Cre virus conditions. Within the control condition, bins 1-4 had greater time spent rearing than bin 9, whereas no bins were significantly different from the final bin within the Cre virus condition. For proportion of distance traveled in the center, post hoc ANOVAs revealed a main effect of bin within each virus condition. Post hoc tests revealed a significant difference between bins 1 and 2 and between 2 and 3 within the control condition, whereas within the Cre condition only the difference between bins 1 and 2 was significant. Within the control condition, bins 1-6 had elevated proportion center distance over bin 9. In contrast, within the Cre virus condition, no bin was significantly different from bin 9. For time spent in the center, post hoc ANOVAs revealed a main effect of bin within each virus condition. Post hoc tests revealed an increase in center time between bins 1 and 2 within the Cre virus condition. No subsequent bins were significantly different in the control virus condition. Within the control condition, bins 2 and 4 had greater center time relative to bin 9. In contrast, within the Cre virus condition, no bins showed different center time from the final bin. For spatial *d*, a main effect of bin was identified within each virus condition. Post hoc tests revealed an increase in spatial d between bins 1 and 2 within both the control and Cre virus conditions. Post hoc tests also revealed an increase in spatial d between bins 1 and 9 within both the control and Cre virus conditions.

Dorsal hippocampal *Btbd3* knockdown does not preserve all effects seen in the general hippocampal knockdown

The first cohort of hippocampal knockdown mice (see main text, Figure 4.3) had infusions that robustly infected cells throughout the hippocampus, consistently peaking in the ventral hippocampus. A second cohort (n = 5-13/virus/sex) received infusions at more anterior coordinates (see main methods) that consistently robustly infected cells in early dorsal hip-

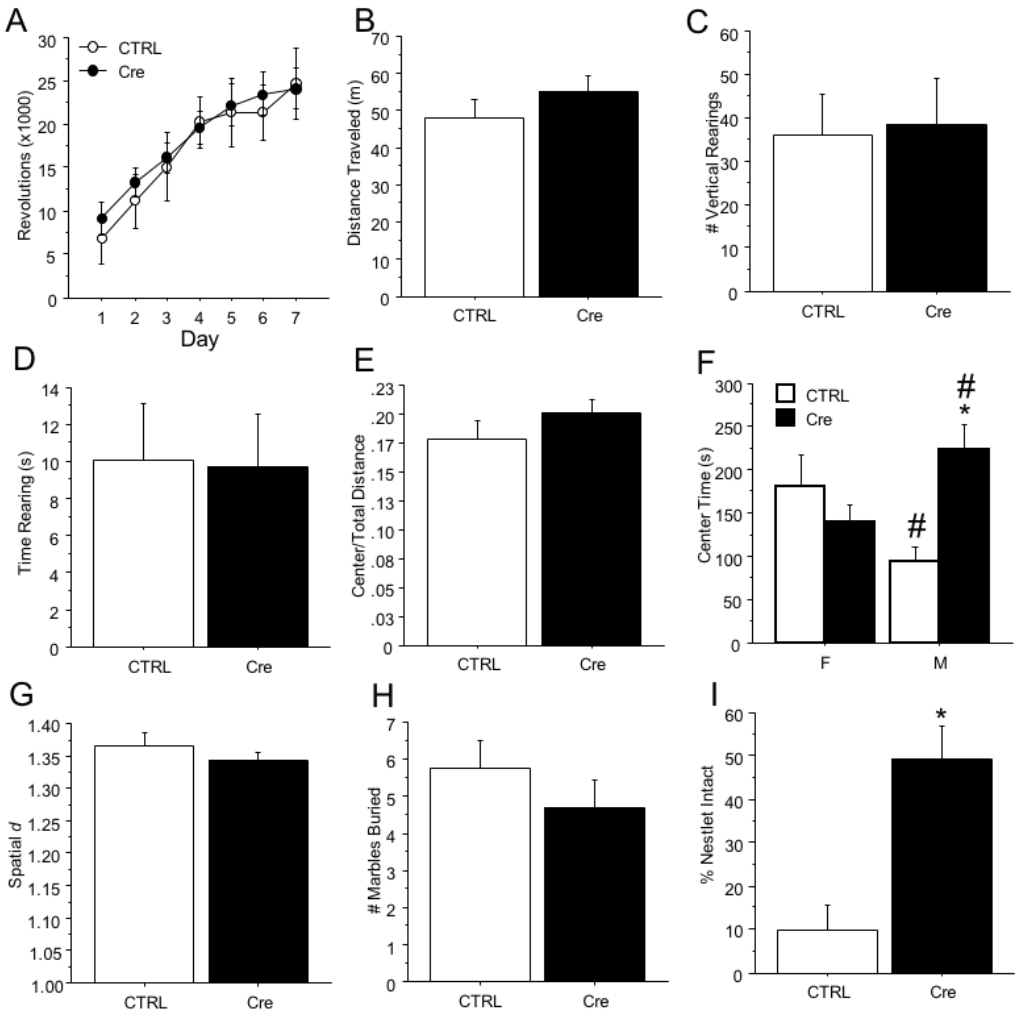

Figure 4.9: Dorsal hippocampal *Btbd3* knockdown does not recapitulate most of the behavioral deficits found in full hippocampal *Btbd3* knockdown mice. A shows wheel-running in the dark cycle. B shows distance traveled in the open field. C-D show vertical rearing in the open field. E-F show center activity in the open field. G shows spatial *d* in the open field. H shows the number of marbles buried in the marble-burying task. I shows the remaining portion of the nestlet by weight left unused for building a nest at the end of the task. Results are expressed as mean ± SEM. *Significant difference from control virus. #Significant difference from females within virus condition. CTRL: Control

pocampus and that were primarily contained within the dorsal half of the hippocampus. No effect of virus was identified on wheel-running (Figure 4.9A). In the open field, no main effect of virus was identified on total distance traveled (Figure 4.9B), instances of vertical rearing (Figure 4.9C), time spent rearing (Figure 4.9D) or proportion of distance traveled

in the center (Figure 4.9E). While there was no main effect of virus on time spent in the center (Figure 4.9F), an interaction between virus and sex was identified ($F_{(1,33)} = 8.02$; $p<.01$). Post hoc tests revealed more time spent in the center in Cre versus control animals within the males, but not females. Post hoc tests also revealed that males spent less time in the center than females within the control condition, but more time in the center within the Cre condition. No effect of virus was found for spatial *d* (Figure 4.9G). In the marble burying paradigm, no effect of virus was identified for number of marbles buried (Figure 4.9H)($F_{(1,33)} = .83$; $p = .37$). In the nest building paradigm, a main effect of virus was identified for percent nestlet remaining intact at the end of the test (Figure 4.9I)($F_{(1,33)} = 11.53$; $p<.005$).

Hippocampal *Btbd3* knockdown in adolescence recapitulates some effects of global knockout

In a pilot experiment, *Btbd3* expression was knocked down at an advanced developmental time point to assess whether the critical period for effects of *Btbd3* expression is confined to the neonatal period. Juvenile mice (P21±1 day) were infused with an shRNA virus against *Btbd3* or a scrambled virus and open field and nest building were assessed four weeks later (n = 3-4/virus/sex). In the open field, a trend was identified for a main effect of genotype on distance traveled ($F_{(1,11)} = 4.80$; $p = .051$). A virus by sex interaction was identified for distance traveled ($F_{(1,11)} = 6.75$; $p<.05$). Post hoc tests revealed that shRNA against *Btbd3* increased distance traveled relative to scrambled shRNA in females, but not males (Figure 4.10A). No effect of virus was identified for instances of vertical rearing (Figure 4.10B) or time spent rearing (Figure 4.10C). A trend for a main effect of virus on proportion of distance traveled in the center was found (Figure 4.10D)($F_{(1,11)} = 3.77$; $p = .08$). A virus by sex interaction was identified for time spent in the center ($F_{(1,11)} = 5.82$; $p<.05$). Post hoc tests revealed that *Btbd3* shRNA increased time spent in the center for females, but not males

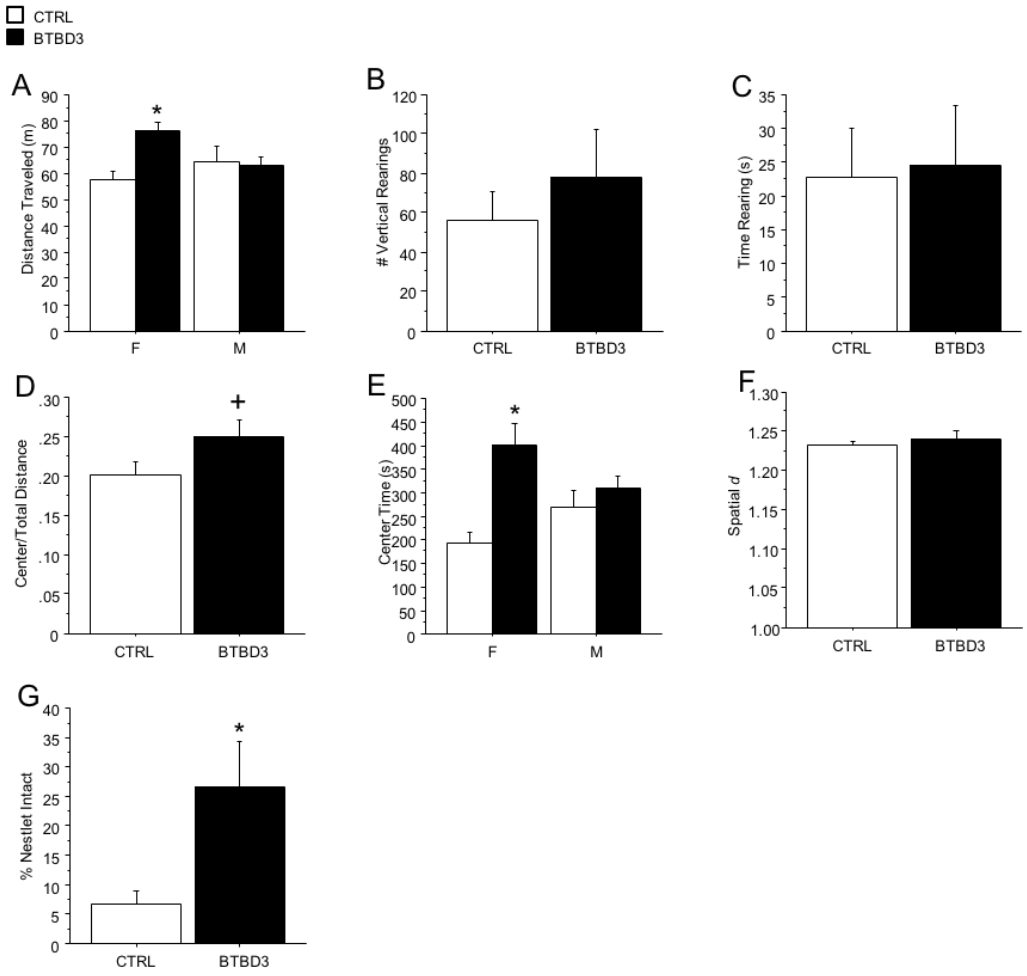

Figure 4.10: Effects of hippocampal *Btbd3* knockdown are not exclusive to the neonatal developmental window. A shows distance traveled in the open field. B-C show vertical rearing in the open field. D-E show center activity in the open field. F shows spatial d in the open field. G shows the remaining portion of the nestlet by weight left unused for building a nest at the end of the task. Results are expressed as mean ± SEM. *Significant difference from control virus. +Trend level difference from control virus. CTRL: Control

(Figure 4.10E). Virus had no effect on spatial d (Figure 4.10F). In the nest building test (8-hour variation), a main effect of virus was identified for percent nestlet remaining at the end of the test period ($F_{(1,11)} = 6.31$; $p<05$) with animals receiving shRNA against *Btbd3* leaving a greater percentage of nestlet remaining intact.

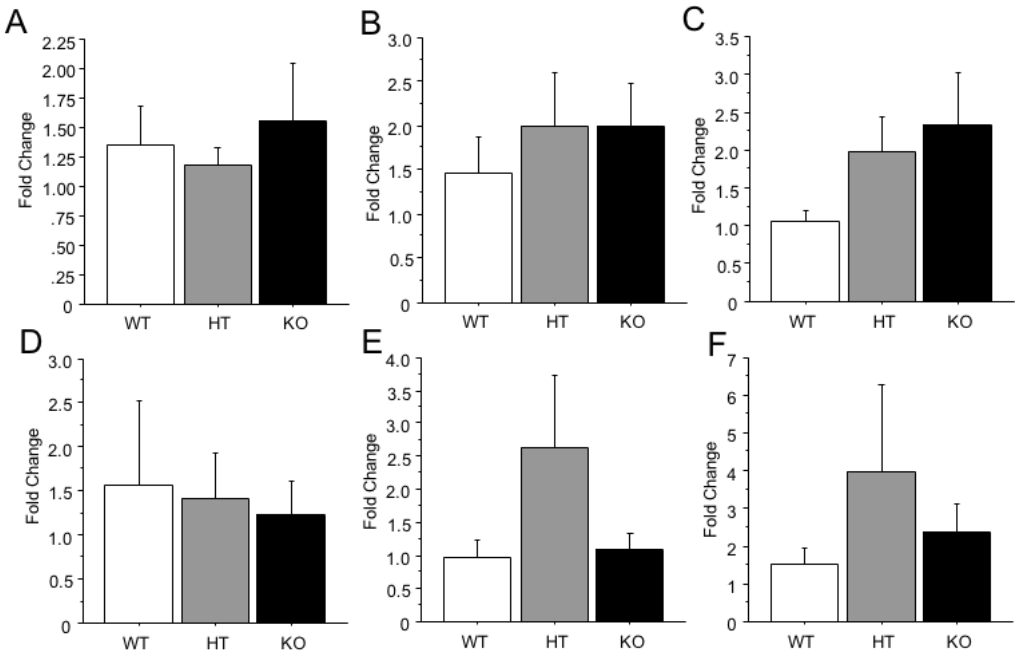

Figure 4.11: No effect of *Btbd3* genotype on Fos RNA levels. A-F show fold change of Fos RNA expression in hippocampus (A), anterior cingulate cortex (B), mediodorsal thalamus (C), caudate putamen (D), nucleus accumbens (E), and orbitofrontal cortex (F). Results are expressed as mean ± SEM. WT: wild-type; HT: heterozygous; KO: knockout.

No effects of *Btbd3* expression on regional brain activity

qPCR for Fos expression was performed to assess effects of *Btbd3* genotype on activity levels in key brain regions. In the first experiment, orbitofrontal cortex, ACC, mediodorsal thalamus, and caudate putamen were evaluated (n = 3-13/genotype, all female). No significant effects of genotype were identified for any region tested: hippocampus (Figure 4.11A), ACC (Figure 4.11B), mediodorsal thalamus (Figure 4.11C), caudate putamen (Figure 4.11D), nucleus accumbens (Figure 4.11E) or orbitofrontal cortex (Figure 4.11F). Several of these brain regions were tested in an additional cohort, again with no significant effects.

CHAPTER 5

DISCUSSION

5.1 Overview

This work focused on identifying neural underpinnings of behaviors with relevance to OCD in mouse models. The goal of this work was to contribute to our knowledge of the pathophysiology of OCD with the hope of ultimately leading to improved therapeutic options. While this work focuses on OCD, compulsivity has emerged in recent years as a transdiagnostic trait [375]. This shift in thinking is reflected in the most recent edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-5)[15], in which OCD was moved from the anxiety disorders section to a new section entitled "Obsessive-Compulsive and Related Disorders." Even still, much of the transdiagnostic work on compulsivity incorporates psychiatric disorders outside this section of DSM-5, such as binge-eating disorder and substance use disorders [375]. Thus, the applicability of work on compulsive behavior is in a state of expansion, and aspects of the present work could ideally have broader implications for compulsive behavior.

Here, in Chapter 2, we sought to dissect effects of canonical versus noncanonical signaling pathways in the 5-HT1BR-induced mouse model of aspects of OCD. In Chapter 3, we used the 5-HT1BR-induced model to assess effects of ketamine, a putative fast-acting anti-OCD treatment [39, 305], on 5-HT1BR-induced OCD-like behavior. In Chapter 4, we implicated putative OCD risk gene *BTBD3* [342] in goal-directed and exploratory behavior with an integral role for hippocampus. Thus, these studies approached OCD from several angles, from treatment, to genetics, to cell signaling pathways.

5.1.1 Chapter 2 - Summary

The goal of chapter 2 was to determine which downstream cell signaling cascades contribute to behavioral deficits in the 5-HT1BR-induced mouse model of OCD. This information would shed light on a novel aspect of the mechanism underlying 5-HT1BR-induced OCD-like behavior with the ultimate goal of refining treatment options for OCD. G-protein-coupled receptors were previously thought to signal primarily through a G-protein-initiated signaling cascade, but were recently found to signal through noncanonical pathways as well [218]. Furthermore, different ligands selectively bias signaling toward G-protein versus noncanonical pathways, opening up the possibility of developing more selective treatments [197]. We used a combined pharmacological and genetic approach to selectively block either the canonical, G-protein-coupled pathway (by way of GSK-3 inhibition), or a noncanonical, β-arrestin2-mediated pathway. We found that β-arrestin2 KO blunted 5-HT1BR-induced hyperactivity in the open field and PPI deficits. GSK-3 inhibition had no effect on 5-HT1BR-induced OCD-like behavior in the open field, but did ameliorate 5-HT1BR-induced PPI deficits. However, GSK-3 inhibition had fewer effects on 5-HT1BR-induced OCD-like behaviors than β-arrestin2 KO mice and actually worsened locomotor perseveration in the open field. Thus, we suggest that compounds biased toward the noncanonical β-arrestin2 pathway holds more promise for development of novel therapeutics for OCD.

5.1.2 Chapter 2 - Limitations and future directions

This set of experiments has some limitations. β-arrestin2 KO mice are hypoactive, making interpretation of behavioral measures in the open field difficult. Several other studies have bypassed this issue by extensively habituating animals to the open field prior to drug challenge [30, 67]. However, we believe this may simply occlude, rather than eliminate the genotype effect due to a floor effect on activity. Thus, we matched animals for baseline activity levels. However, this method has the downside of a loss in power with the substantially

reduced sample size.

We used β-arrestin2 KO mice to block the β-arrestin2-mediated noncanonical signaling pathway because no β-arrestin2 inhibitors were commercially available. However, compensatory effects of constitutive knockout cannot be ruled out, and thus our findings will need to be confirmed using a selective β-arrestin2 inhibitor once one becomes available. In the meantime, there are some improvements over using a constitutive β-arrestin2 KO. For example, a β-arrestin2 floxed mouse was recently developed [363]. Thus, future experiments could use viral-mediated gene transfer approaches to knockdown β-arrestin2 expression in adulthood followed by assessment in the 5-HT1BR-induced model. This design would resolve the issue of compensatory effects of absence of β-arrestin2 expression during development. In addition, this setup would allow for region-specific dissection of β-arrestin2-mediated OCD-like behavioral effects of RU24969 treatment. Since OCD-like behavior in the 5-HT1BR-induced model is mediated through 5-HT1BRs in the orbitofrontal cortex [324], this would be the logical target region to start with for these studies. However, since the main aim of this work was to identify signaling pathways to inform more selective treatments, these basic experiments have the tradeoff of being less translational. Thus, confirmation of effects identified in the β-arrestin2 KO using a β-arrestin2 inhibitor will still be of great utility for informing development of novel treatment options for OCD.

GSK-3 inhibitor SB216763 reduced RU24969-induced PPI deficits. However, these effects were confined to the second block of testing, were only seen with 3 mg/kg, but not 10 mg/kg RU24969 treatment, and were not strong effects. We did confirm that the negative findings for GSK-3 inhibition on RU24969-induced OCD-like behavior in the open field were not due to late onset of SB216763 effects, but did not test PPI at a later time point. Testing PPI at a later time point could serve to determine whether the block-dependent effect of GSK-3 inhibition on PPI is due to suboptimal SB216763 pretreatment timing. Furthermore, AR-A014418, a second GSK-3 inhibitor, was tested with 10 mg/kg RU24969 but not 3 mg/kg

RU24969. Thus, future studies could confirm the mitigating effects of GSK-3 inhibition on 5-HT1BR-induced PPI deficits by replicating these effects with a second GSK-3 inhibitor, AR-A014418, at the 3 mg/kg dose of RU24969.

5.1.3 Chapter 3 - Summary

In Chapter 3 we sought to determine whether ketamine would mitigate OCD-like behavior in the 5-HT1BR-induced model. Preliminary evidence suggests that ketamine works as a fast-acting anti-OCD medication [39, 305]. However, this effect has yet to be thoroughly confirmed. We found that 3 mg/kg ketamine, the lowest dose tested, was the only dose that effectively mitigated OCD-like behavior in the 5-HT1BR-induced model. On the other hand, a ten-fold higher dose of ketamine (30 mg/kg), which has had positive results in a rodent model of posttraumatic stress disorder [239], acutely exacerbated 5-HT1BR-induced behavioral deficits. Interestingly, at the 24-hour time point, this high dose caused behavioral changes in the opposite direction to RU24969, suggesting a possible delayed protective effect of high dose ketamine against OCD-like behavior. These dramatic differences between doses indicate that careful titration of ketamine dose in OCD patients may be warranted, and that doses should not be assumed equivalent across disorders.

Furthermore, we found that serotonin-reuptake inhibitor (**SRI**) fluoxetine was not acutely effective in the 5-HT1BR-induced model. We have shown previously that chronic, but not subchronic, fluoxetine treatment, administered continuously in the drinking water ameliorates OCD-like behaviors in the 5-HT1BR-induced model [323, 324], indicating that the model is sensitive to therapeutic onset. However, we had never tested effects of an acute injection of fluoxetine. Because ketamine has rapid antidepressant and anti-OCD effects in humans, it can be ambiguous whether acute effects in animal models are truly rapid therapeutic-like effects Thus, we demonstrated that fluoxetine, an OCD-effective treatment after chronic administration, was not effective acutely. This result indicates that effects

of ketamine seen at acute time points may reflect true onset. Importantly, these findings demonstrate that the 5-HT1BR-induced model is capable of identifying novel OCD treatments, as ketamine has a very different pharmacological profile from that of classic OCD treatments such as SRI fluoxetine.

5.1.4 Chapter 3 - Limitations and future directions

There are a few limitations to this work. The effects of 3 mg/kg ketamine treatment required pooling across time points to power significant effects. These studies should be replicated in larger cohorts to confirm our findings and to gain time point-specific information about ketamine efficacy in the 5-HT1BR-induced model. Once this has been established, the time point of offset for effects of ketamine should be determined by testing time points extending past 24 hours. Furthermore, because the lowest dose tested was the only effective dose identified, we do not know the optimal dose. Thus, lower doses of ketamine should be tested in order to identify the optimal dose. Finally, the delayed protective effects of high dose ketamine warrant further investigation. Since some of the effects were only trend-level, these effects should be confirmed in a larger cohort.

5.1.5 Chapter 4 - Summary

In Chapter 4 we sought to determine if putative OCD risk gene *BTBD3* plays a role in behavior, and if so, if these behaviors are relevant to OCD. The first genome-wide significant single nucleotide polymorphism for OCD is downstream of the *BTBD3* gene and is an expression quantitative trait locus for *BTBD3* [342], but has yet to be replicated. Thus, we sought additional evidence of a role for *BTBD3* in OCD by assessing effects of *Btbd3* expression on OCD-relevant behaviors in mice. This is the first characterization of a genome-wide significant hit for OCD; all published genetic models of OCD use genes from candidate studies [398] or were identified fortuitously [152, 327, 380]. *Btbd3* KO mice were found to

have deficits in measures of goal-directed behavior and exploration. Many of these deficits were found to be mediated through the hippocampus. This was determined by knocking down *Btbd3* expression in hippocampus of neonatal mouse pups, then assessing them for behavior in adulthood. Preliminary evidence suggests that these effects may be mediated primarily through ventral hippocampus, and that the critical window for these effects is likely not confined to the neonatal period. Surprisingly, no dendritic morphology changes were identified in hippocampus of *Btbd3* KO mice, but some tentative effects were found in anterior cingulate cortex (**ACC**) layer II/III pyramidal neurons and mediodorsal thalamus stellate neurons. Overall, these findings suggest that BTBD3 is of relevance to OCD and may mediate goal-directed behavior through hippocampal regulation of exploration and response to novelty.

5.1.6 Chapter 4 - Limitations and future directions

As this is the first report of behavioral implications of *Btbd3* expression, there are many future directions for this work. First, the subregion of hippocampus implicated in behavioral effects of *Btbd3* expression should be confirmed in a neonatal ventral hippocampal knockdown cohort. While negative effects in the dorsal hippocampus cohort point to this as a likelihood, it is possible that dorsal and ventral hippocampus work together to confer the OCD-relevant behavioral deficits. In addition, the dorsal hippocampus knockdown cohort had a smaller sample size than the full hippocampus knockdown cohort, rendering negative results and sex effects tentative. Next, the critical period for *Btbd3* expression in hippocampus should be identified. *Btbd3* expression dramatically increases in early postnatal development, then levels off and remains sustained throughout adulthood [247]. The preliminary results in juvenile mice suggest that *Btbd3* expression later in development is still important for maintaining normal behavior. Thus, *Btbd3* knockdown should be performed in adulthood to determine if *Btbd3* is needed constitutively to maintain normal behavior, or if

there is a developmental critical window for expression. The hippocampal *Btbd3* knockdown studies performed in Chapter 4 establish that hippocampal *Btbd3* expression is necessary for OCD-relevant behavior. Whether hippocampal *Btbd3* expression is sufficient to rescue normal behavior should be tested as well, by infusing a virus expressing *Btbd3* into hippocampus of global *Btbd3* knockout mice. The appropriate time point for these studies should be informed by the knockdown critical window experiments suggested above. Furthermore, while exploration was thoroughly measured in the hippocampal *Btbd3* KD cohorts, goal-directed behavior was not. Barbering was not practical to assess in these cohorts, as the penetrance is low and would thus require very large sample sizes. Probabilistic learning was also not tested in these cohorts. Probabilistic learning should be tested in a follow-up hippocampal *Btbd3* knockdown cohort to confirm the role of hippocampus in *Btbd3*-dependent goal-directed behavior.

The molecular mechanism underlying behavioral effects of *Btbd3* in hippocampus remains to be identified. No effect of genotype was identified for Fos expression, a marker of neuronal activation [266]. However, Fos levels were low overall, likely due to generally low levels of Fos expression in the absence of stimulation [266]. Thus, this negative finding is not stated with confidence. Fos could be measured using a more sensitive technique, such as immunohistochemistry, or a different marker of activity could be used. No dendritic morphology changes were identified in *Btbd3* KO hippocampus, although not every subregion of hippocampus was evaluated. In addition, dendritic morphology analysis was not segregated by dorsal or ventral portions of the hippocampus. If ventral hippocampus is the primary driver of effects of *Btbd3* expression on OCD-relevant behavior, changes in dendritic morphology may have been diluted by including dorsal hippocampal neurons in the analysis. The significant and trend-level effects of genotype on dendritic morphology in ACC and mediodorsal thalamus warrant further investigation as well. These borderline results suggest that this sample may have been underpowered to detect genotype effects. Thus, dendritic morphology results

should be confirmed in a larger cohort. These findings in other key brain regions also serve as a reminder that areas other than the hippocampus likely play a role in behavioral effects of *Btbd3* expression. This could be explored through knocking down *Btbd3* expression in ACC or mediodorsal thalamus and evaluating behavior in adulthood.

5.2 Conclusions

Here, we demonstrate the utility of animal models for OCD in several different applications. Chapters 2 and 3 employed the previously validated 5-HT1BR-induced model of aspects of OCD, but for distinct purposes. Chapter 2 aimed to determine pathways driving effects of RU24969, the 5-HT1BR agonist that induces OCD-like behavior, in order to move toward development of pathway-selective novel treatments; thus, leveraging neural mechanisms of an animal model to inform treatment in patients down the line. Chapter 3, on the other hand, used the 5-HT1BR-induced model as a platform for delving into preliminary evidence for a novel OCD treatment based on human studies. Similarly, Chapter 4 took preliminary genetic information from human studies, and applied it to an animal model to corroborate the human work. In Chapter 4, the animal did not serve as a model of aspects of OCD per se, but rather was a tool for investigating phenotypes of relevance to OCD. This type of work, taking preliminary human findings and confirming them using animal modeling approaches, can serve the dual purpose of 1) efficiently reinforcing tentative human findings, and thus potentially guiding further human studies, and 2) going beyond what can be studied in the human, providing mechanistic information that can again guide future human studies with the ultimate goal of improving treatment outcomes for patients.

REFERENCES

[1] M Abbruzzese, L Bellodi, S Ferri, and S Scarone. Frontal lobe dysfunction in schizophrenia and obsessive-compulsive disorder: a neuropsychological study. *Brain Cogn*, 27(2):202–212, March 1995.

[2] M Abbruzzese, S Ferri, and S Scarone. The selective breakdown of frontal functions in patients with obsessive-compulsive disorder and in patients with schizophrenia: a double dissociation experimental finding. *Neuropsychologia*, 35(6):907–912, June 1997.

[3] Amitai Abramovitch, Jonathan S. Abramowitz, and Andrew Mittelman. The neuropsychology of adult obsessive-compulsive disorder: a meta-analysis. *Clin Psychol Rev*, 33(8):1163–1171, December 2013.

[4] Jonathan S. Abramowitz and Ryan J. Jacoby. Obsessive-compulsive and related disorders: A critical review of the new diagnostic class. *Annual Review of Clinical Psychology*, 11(1):165–186, Mar 2015.

[5] E. B. Adamah-Biassi, R. L. Hudson, and M. L. Dubocovich. Genetic deletion of MT1 melatonin receptors alters spontaneous behavioral rhythms in male and female C57bl/6 mice. *Horm Behav*, 66(4):619–627, September 2014.

[6] Susanne E Ahmari, Victoria B Risbrough, Mark A Geyer, and H Blair Simpson. Impaired sensorimotor gating in unmedicated adults with obsessive-compulsive disorder. *Neuropsychopharmacology*, 37(5):1216–1223, April 2012.

[7] Susanne E. Ahmari, Timothy Spellman, Neria L. Douglass, Mazen A. Kheirbek, H. Blair Simpson, Karl Deisseroth, Joshua A. Gordon, and René Hen. Repeated cortico-striatal stimulation generates persistent ocd-like behavior. *Science*, 340(6137):1234–1239, 2013.

[8] Noa Albelda and Daphna Joel. Current animal models of obsessive compulsive disorder: An update. *Neuroscience*, 211:83–106, 09 2011.

[9] Pino Alonso, Clara López-Solà, Eva Real, Cinto Segalàs, and José Manuel Menchón. *Animal models of obsessive–compulsive disorder: Utility and limitations*, volume 11 of *Neuropsychiatric disease and treatment*. Sep 2015.

[10] Pino Alonso, José M Menchón, Susana Jiménez, Jacint Segalàs, David Mataix-Cols, Nuria Jaurrieta, Javier Labad, Julio Vallejo, Narcís Cardoner, and Jesús Pujol. Personality dimensions in obsessive-compulsive disorder: relation to clinical variables. *Psychiatry Res*, 157(1-3):159–168, January 2008.

[11] M. Altemus, J. R. Glowa, E. Galliven, Y. M. Leong, and D. L. Murphy. Effects of serotonergic agents on food-restriction-induced hyperactivity. *Pharmacol. Biochem. Behav.*, 53(1):123–131, January 1996.

[12] M. Altemus, J. R. Glowa, and D. L. Murphy. Attenuation of food-restriction-induced running by chronic fluoxetine treatment. *Psychopharmacol Bull*, 29(3):397–400, 1993.

[13] Edgardo O. Alvarez and Pablo A. Alvarez. Motivated exploratory behaviour in the rat: the role of hippocampus and the histaminergic neurotransmission. *Behav. Brain Res.*, 186(1):118–125, January 2008.

[14] S. Amar, B. C. Jones, C. Nadri, N. Kozlovsky, R. H. Belmaker, and G. Agam. Genetic correlational analysis of glycogen synthase kinase-3 beta and prepulse inhibition in inbred mice. *Genes Brain Behav.*, 3(3):178–180, June 2004.

[15] American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*. American Psychiatric Pub, May 2013.

[16] Nurith Amitai, Jared W. Young, Kerin Higa, Richard F. Sharp, Mark A. Geyer, and Susan B. Powell. Isolation rearing effects on probabilistic learning and cognitive flexibility in rats. *Cogn Affect Behav Neurosci*, 14(1):388–406, March 2014.

[17] Xiao-Lei An, Jun-Xian Zou, Rui-Yong Wu, Ying Yang, Fa-Dao Tai, Shuang-Yan Zeng, Rui Jia, Xia Zhang, En-Qi Liu, and Hugh Broders. Strain and sex differences in anxiety-like and social behaviors in C57bl/6j and BALB/cJ mice. *Exp. Anim.*, 60(2):111–123, 2011.

[18] Susan L. Andersen. Stimulants and the developing brain. *Trends Pharmacol. Sci.*, 26(5):237–243, May 2005.

[19] Susan L. Andersen, Emily A. Greene-Colozzi, and Kai C. Sonntag. A novel, multiple symptom model of obsessive-compulsive-like behaviors in animals. *Biol. Psychiatry*, 68(8):741–747, October 2010.

[20] Dane Aronsen, Jeremy Webster, and Susan Schenk. RU 24969-produced adipsia and hyperlocomotion: differential role of 5ht 1a and 5ht 1b receptor mechanisms. *Pharmacol. Biochem. Behav.*, 124:1–4, September 2014.

[21] Tushar Arora, Malay Bhowmik, Razia Khanam, and Divya Vohora. Oxcarbazepine and fluoxetine protect against mouse models of obsessive compulsive disorder through modulation of cortical serotonin and CREB pathway. *Behav. Brain Res.*, 247:146–152, June 2013.

[22] Murad Atmaca, Hanefi Yildirim, Huseyin Ozdemir, Sinan Ozler, Bilge Kara, Zuhal Ozler, Ebru Kanmaz, Osman Mermi, and Ertan Tezcan. Hippocampus and amygdalar volumes in patients with refractory obsessive-compulsive disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 32(5):1283–1286, July 2008.

[23] Anita E. Autry, Megumi Adachi, Elena Nosyreva, Elisa S. Na, Maarten F. Los, Pengfei Cheng, Ege T. Kavalali, and Lisa M. Monteggia. NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature*, 475(7354):91–95, June 2011.

[24] Kathleen R Bailey and Jacqueline N Crawley. Anxiety-related behaviors in mice. In Jerry J. Buccafusco, editor, *Methods of Behavior Analysis in Neuroscience*, Frontiers in Neuroscience, pages 77–101. CRC Press, Boca Raton (FL), 2nd edition, 2009.

[25] Paula Banca, Valerie Voon, Martin D. Vestergaard, Gregor Philipiak, Inês Almeida, Fernando Pocinho, João Relvas, and Miguel Castelo-Branco. Imbalance in habitual versus goal directed neural systems during symptom provocation in obsessive-compulsive disorder. *Brain*, 138(Pt 3):798–811, March 2015.

[26] S. A. Barnett and W. E. Hocking. Are nests built for fun? Effects of alternative activities on nest-building by wild house mice. *Behavioral and Neural Biology*, 31(1):73–81, January 1981.

[27] L C Barr, W K Goodman, A Anand, C J McDougle, and L H Price. Addition of desipramine to serotonin reuptake inhibitors in treatment-resistant obsessive-compulsive disorder. *Am J Psychiatry*, 154(9):1293–1295, September 1997.

[28] L. C. Barr, W. K. Goodman, C. J. McDougle, P. L. Delgado, G. R. Heninger, D. S. Charney, and L. H. Price. Tryptophan depletion in patients with obsessive-compulsive disorder who respond to serotonin reuptake inhibitors. *Arch. Gen. Psychiatry*, 51(4):309–317, April 1994.

[29] Jean-Martin Beaulieu, Sébastien Marion, Ramona M. Rodriguiz, Ivan O. Medvedev, Tatyana D. Sotnikova, Valentina Ghisi, William C. Wetsel, Robert J. Lefkowitz, Raul R. Gainetdinov, and Marc G. Caron. A beta-arrestin 2 signaling complex mediates lithium action on behavior. *Cell*, 132(1):125–136, January 2008.

[30] Jean-Martin Beaulieu, Tatyana D. Sotnikova, Sébastien Marion, Robert J. Lefkowitz, Raul R. Gainetdinov, and Marc G. Caron. An Akt/beta-arrestin 2/PP2a signaling complex mediates dopaminergic neurotransmission and behavior. *Cell*, 122(2):261–273, July 2005.

[31] Alexandre Beautrait, Justine S. Paradis, Brandon Zimmerman, Jenna Giubilaro, Ljiljana Nikolajev, Sylvain Armando, Hiroyuki Kobayashi, Lama Yamani, Yoon Namkung, Franziska M. Heydenreich, Etienne Khoury, Martin Audet, Philippe P. Roux, Dmitry B. Veprintsev, Stéphane A. Laporte, and Michel Bouvier. A new inhibitor of the β-arrestin/AP2 endocytic complex reveals interplay between GPCR internalization and signalling. *Nat Commun*, 8, April 2017.

[32] Catherine Belzung and Maël Lemoine. Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. *Biol Mood Anxiety Disord*, 1(1):9, 2011.

[33] A. Ben-Pazi, H. Szechtman, and D. Eilam. The morphogenesis of motor rituals in rats treated chronically with the dopamine agonist quinpirole. *Behav. Neurosci.*, 115(6):1301–1317, December 2001.

[34] R. M. Berman, A. Cappiello, A. Anand, D. A. Oren, G. R. Heninger, D. S. Charney, and J. H. Krystal. Antidepressant effects of ketamine in depressed patients. *Biol. Psychiatry*, 47(4):351–354, February 2000.

[35] Alexandre Berney, Debbie Sookman, Marco Leyton, Simon N. Young, and Chawki Benkelfat. Lack of effects on core obsessive-compulsive symptoms of tryptophan depletion during symptom provocation in remitted obsessive-compulsive disorder patients. *Biol. Psychiatry*, 59(9):853–857, May 2006.

[36] Giuseppe Bersani, Adele Quartini, Flavia Ratti, Giulio Pagliuca, and Andrea Gallo. Olfactory identification deficits and associated response inhibition in obsessive-compulsive disorder: on the scent of the orbitofronto-striatal model. *Psychiatry Res*, 210(1):208–214, November 2013.

[37] Ratan Bhat, Yafeng Xue, Stefan Berg, Sven Hellberg, Mats Ormö, Yvonne Nilsson, Ann-Cathrin Radesäter, Eva Jerning, Per-Olof Markgren, Thomas Borgegård, Martin Nylöf, Alfredo Giménez-Cassina, Félix Hernández, Jose J. Lucas, Javier Díaz-Nido, and Jesús Avila. Structural insights and biological effects of glycogen synthase kinase 3-specific inhibitor AR-A014418. *J. Biol. Chem.*, 278(46):45937–45945, November 2003.

[38] M H Bloch, J McGuire, A Landeros-Weisenberger, J F Leckman, and C Pittenger. Meta-analysis of the dose-response relationship of SSRI in obsessive-compulsive disorder. *Mol. Psychiatry*, 15(8):850–855, August 2010.

[39] Michael H. Bloch, Suzanne Wasylink, Angeli Landeros-Weisenberger, Kaitlyn E. Panza, Eileen Billingslea, James F. Leckman, John H. Krystal, Zubin Bhagwagar, Gerard Sanacora, and Christopher Pittenger. Effects of ketamine in treatment-refractory obsessive-compulsive disorder. *Biol. Psychiatry*, 72(11):964–970, December 2012.

[40] Premika S. W. Boedhoe, Lianne Schmaal, Yoshinari Abe, Stephanie H. Ameis, Paul D. Arnold, Marcelo C. Batistuzzo, Francesco Benedetti, Jan C. Beucke, Irene Bollettini, Anushree Bose, Silvia Brem, Anna Calvo, Yuqi Cheng, Kang Ik K. Cho, Sara Dallaspezia, Damiaan Denys, Kate D. Fitzgerald, Jean-Paul Fouche, Mònica Giménez, Patricia Gruner, Gregory L. Hanna, Derrek P. Hibar, Marcelo Q. Hoexter, Hao Hu, Chaim Huyser, Keisuke Ikari, Neda Jahanshad, Norbert Kathmann, Christian Kaufmann, Kathrin Koch, Jun Soo Kwon, Luisa Lazaro, Yanni Liu, Christine Lochner, Rachel Marsh, Ignacio Martínez-Zalacaín, David Mataix-Cols, José M. Menchón, Luciano Minuzzi, Takashi Nakamae, Tomohiro Nakao, Janardhanan C. Narayanaswamy, Fabrizio Piras, Federica Piras, Christopher Pittenger, Y. C. Janardhan Reddy, Joao R. Sato, H. Blair Simpson, Noam Soreni, Carles Soriano-Mas, Gianfranco Spalletta, Michael C. Stevens, Philip R. Szeszko, David F. Tolin, Ganesan Venkatasubramanian, Susanne Walitza, Zhen Wang, Guido A. van Wingen, Jian Xu, Xiufeng Xu, Je-Yeon Yun, Qing Zhao, ENIGMA OCD Working Group, Paul M. Thompson, Dan J. Stein, and Odile A. van den Heuvel. Distinct Subcortical Volume Alterations in Pediatric and Adult OCD: A Worldwide Meta- and Mega-Analysis. *Am J Psychiatry*, 174(1):60–69, 2017.

[41] Laura M. Bohn, Raul R. Gainetdinov, Tatyana D. Sotnikova, Ivan O. Medvedev, Robert J. Lefkowitz, Linda A. Dykstra, and Marc G. Caron. Enhanced rewarding properties of morphine, but not cocaine, in beta(arrestin)-2 knock-out mice. *J. Neurosci.*, 23(32):10265–10273, November 2003.

[42] M. L. Boshuisen and J. A. den Boer. Zolmitriptan (a 5-HT1b/1d receptor agonist with central action) does not increase symptoms in obsessive compulsive disorder. *Psychopharmacology (Berl.)*, 152(1):74–79, September 2000.

[43] M. Bourin, J. P. Redrobe, and G. B. Baker. Pindolol does not act only on 5-HT1a receptors in augmenting antidepressant activity in the mouse forced swimming test. *Psychopharmacology (Berl.)*, 136(3):226–234, April 1998.

[44] Laura A. Bradfield, Genevra Hart, and Bernard W. Balleine. The role of the anterior, mediodorsal, and parafascicular thalamus in instrumental conditioning. *Front Syst Neurosci*, 7:51, October 2013.

[45] D L Braff, M A Geyer, and N R Swerdlow. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl.)*, 156(2-3):234–258, July 2001.

[46] C. L. Broekkamp, H. W. Rijk, D. Joly-Gelouin, and K. L. Lloyd. Major tranquillizers can be distinguished from minor tranquillizers on the basis of effects on marble burying and swim-induced grooming in mice. *Eur. J. Pharmacol.*, 126(3):223–229, July 1986.

[47] M. L. D. Broekman, L. A. Comer, B. T. Hyman, and M. Sena-Esteves. Adeno-associated virus vectors serotyped with AAV8 capsid are more efficient than AAV-1 or -2 serotypes for widespread gene delivery to the neonatal mouse brain. *Neuroscience*, 138(2):501–510, 2006.

[48] A. Broocks, T. A. Pigott, J. L. Hill, S. Canter, T. A. Grady, F. L'Heureux, and D. L. Murphy. Acute intravenous administration of ondansetron and m-CPP, alone and in combination, in patients with obsessive-compulsive disorder (OCD): behavioral and biological results. *Psychiatry Res*, 79(1):11–20, June 1998.

[49] Simon P. Brooks, Rebecca C. Trueman, and Stephen B. Dunnett. Assessment of Motor Coordination and Balance in Mice Using the Rotarod, Elevated Bridge, and Footprint Tests. *Curr Protoc Mouse Biol*, 2(1):37–53, March 2012.

[50] Liesbeth A. Bruins Slot, Laurent Bardin, Agnès L. Auclair, Ronan Depoortere, and Adrian Newman-Tancredi. Effects of antipsychotics and reference monoaminergic ligands on marble burying behavior in mice. *Behav Pharmacol*, 19(2):145–152, March 2008.

[51] Eric Burguière, Patricia Monteiro, Luc Mallet, Guoping Feng, and Ann M Graybiel. Striatal circuits, habits, and implications for obsessive–compulsive disorder. *Current Opinion in Neurobiology*, 30:59–65, February 2015.

[52] Laure-Sophie Camilla d'Angelo, Dawn M. Eagle, Jon E. Grant, Naomi A. Fineberg, Trevor W. Robbins, and Samuel R. Chamberlain. Animal models of obsessive-compulsive spectrum disorders. *CNS Spectrums*, 19(1):28–49, 2014.

[53] B. J. Casey, Nick Craddock, Bruce N. Cuthbert, Steven E. Hyman, Francis S. Lee, and Kerry J. Ressler. DSM-5 and RDoC: progress in psychiatry research? *Nat. Rev. Neurosci.*, 14(11):810–814, November 2013.

[54] N. Castanon, K. Scearce-Levie, J. J. Lucas, B. Rocha, and R. Hen. Modulation of the effects of cocaine by 5-HT1b receptors: a comparison of knockouts and antagonists. *Pharmacol. Biochem. Behav.*, 67(3):559–566, November 2000.

[55] Lissandra Castellan Baldan, Kyle A Williams, Jean-Dominique Gallezot, Vladimir Pogorelov, Max Rapanelli, Michael Crowley, George Anderson, Erin Loring, Roxanne Gorczyca, Eileen Billingslea, Suzanne Wasylink, Kaitlyn Panza, Ad Ercan-Sencicek, Kuakarun Krusong, Bennett L Leventhal, Hiroshi Ohtsu, Michael Bloch, Zoe Hughes, John H Krystal, and Christopher Pittenger. Histidine decarboxylase deficiency causes tourette syndrome: Parallel findings in humans and mice. *Neuron*, 81:77–90, 01 2014.

[56] P. Cavedini, S. Ferri, S. Scarone, and L. Bellodi. Frontal lobe dysfunction in obsessive-compulsive disorder and major depression: a clinical-neuropsychological study. *Psychiatry Res*, 78(1-2):21–28, March 1998.

[57] João J. Cerqueira, Ricardo Taipa, Harry B. M. Uylings, Osborne F. X. Almeida, and Nuno Sousa. Specific Configuration of Dendritic Degeneration in Pyramidal Neurons of the Medial Prefrontal Cortex Induced by Differing Corticosteroid Regimens. *Cereb Cortex*, 17(9):1998–2006, September 2007.

[58] Stéphan Chabardès, Mircea Polosan, Paul Krack, Julien Bastin, Alexandre Krainik, Olivier David, Thierry Bougerol, and Alim Louis Benabid. Deep brain stimulation for obsessive-compulsive disorder: subthalamic nucleus target. *World Neurosurg*, 80(3-4):S31.e1–8, October 2013.

[59] Kathryn K. Chadman, Mu Yang, and Jacqueline N. Crawley. Criteria for validating mouse models of psychiatric diseases. *Am. J. Med. Genet. B Neuropsychiatr. Genet.*, 150B(1):1–11, January 2009.

[60] Paramita Chakrabarty, Awilda Rosario, Pedro Cruz, Zoe Siemienski, Carolina Ceballos-Diaz, Keith Crosby, Karen Jansen, David R. Borchelt, Ji-Yoen Kim, Joanna L. Jankowsky, Todd E. Golde, and Yona Levites. Capsid serotype and timing of injection determines AAV transduction in the neonatal mice brain. *PLoS ONE*, 8(6):e67680, 2013.

[61] Samuel R. Chamberlain, Naomi A. Fineberg, Andrew D. Blackwell, Trevor W. Robbins, and Barbara J. Sahakian. Motor inhibition and cognitive flexibility in obsessive-compulsive disorder and trichotillomania. *Am J Psychiatry*, 163(7):1282–1284, July 2006.

[62] Samuel R. Chamberlain, Naomi A. Fineberg, Lara A. Menzies, Andrew D. Blackwell, Edward T. Bullmore, Trevor W. Robbins, and Barbara J. Sahakian. Impaired cognitive flexibility and motor inhibition in unaffected first-degree relatives of patients with obsessive-compulsive disorder. *Am J Psychiatry*, 164(2):335–338, February 2007.

[63] Ming-Huan Chan, Pao-Hsiang Chiu, Chia-Yu Lin, and Hwei-Hsien Chen. Inhibition of glycogen synthase kinase-3 attenuates psychotomimetic effects of ketamine. *Schizophr. Res.*, 136(1-3):96–103, April 2012.

[64] Ming-Huan Chan, Pao-Hsiang Chiu, Jen-Hou Sou, and Hwei-Hsien Chen. Attenuation of ketamine-evoked behavioral responses by mGluR5 positive modulators in mice. *Psychopharmacology (Berl.)*, 198(1):141–148, May 2008.

[65] D. S. Charney, W. K. Goodman, L. H. Price, S. W. Woods, S. A. Rasmussen, and G. R. Heninger. Serotonin function in obsessive-compulsive disorder. A comparison of the effects of tryptophan and m-chlorophenylpiperazine in patients and healthy subjects. *Arch. Gen. Psychiatry*, 45(2):177–185, February 1988.

[66] H. K. Chaturvedi, Chandra Dinesh, and J. S. Bapna. Effect of NMDA receptor antagonists in forced swimming test and its modification by antidepressants. *Indian Journal of Pharmacology*, 31(2):104, March 1999.

[67] L. Chen, W. Zhou, P. C. Chen, I. Gaisina, S. Yang, and X. Li. Glycogen synthase kinase-3β is a functional modulator of serotonin-1b receptors. *Mol. Pharmacol.*, 79(6):974–986, June 2011.

[68] Ligong Chen, Gregory D. Salinas, and Xiaohua Li. Regulation of serotonin 1b receptor by glycogen synthase kinase-3. *Mol. Pharmacol.*, 76(6):1150–1161, December 2009.

[69] Shau-Kwaun Chen, Petr Tvrdik, Erik Peden, Scott Cho, Sen Wu, Gerald Spangrude, and Mario R. Capecchi. Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell*, 141(5):775–785, May 2010.

[70] G. B. Chesher and D. M. Jackson. Swim-induced grooming in mice is mediated by a dopaminergic substrate. *J. Neural Transm.*, 50(1):47–55, 1981.

[71] Jithendra Chimakurthy and Talasila Egk Murthy. Effect of curcumin on quinpirole induced compulsive checking: An approach to determine the predictive and construct validity of the model. *N Am J Med Sci*, 2(2):81–86, February 2010.

[72] Taylor Chomiak, Edward W. Block, Andrew R. Brown, G. Campbell Teskey, and Bin Hu. Development and testing of a new system for assessing wheel-running behaviour in rodents. *BMC Res Notes*, 9, May 2016.

[73] M. P. Coghlan, A. A. Culbert, D. A. Cross, S. L. Corcoran, J. W. Yates, N. J. Pearce, O. L. Rausch, G. J. Murphy, P. S. Carter, L. Roxbee Cox, D. Mills, M. J. Brown, D. Haigh, R. W. Ward, D. G. Smith, K. J. Murray, A. D. Reith, and J. C. Holder.

Selective small molecule inhibitors of glycogen synthase kinase-3 modulate glycogen metabolism and gene transcription. *Chem. Biol.*, 7(10):793–803, October 2000.

[74] Jonathan D. Cohen, Samuel M. McClure, and Angela J. Yu. Should I stay or should I go? How the human brain manages the trade-off between exploitation and exploration. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.*, 362(1481):933–942, May 2007.

[75] Jennifer A. Correll, Daniel M. Noel, A. Brianna Sheppard, Kimberly N. Thompson, Yi Li, Deling Yin, and Russell W. Brown. Nicotine sensitization and analysis of brain-derived neurotrophic factor in adolescent beta-arrestin-2 knockout mice. *Synapse*, 63(6):510–519, June 2009.

[76] Jacqueline N. Crawley. *What's wrong with my mouse?: behavioral phenotyping of transgenic and knockout mice*. Wiley-Liss, April 2000. Google-Books-ID: 78xpAAAAMAAJ.

[77] Jacqueline N Crawley. Translational animal models of autism and neurodevelopmental disorders. *Dialogues in clinical neuroscience*, 14(3):293, 2012.

[78] L. J. Cronbach and P. E. Meehl. Construct validity in psychological tests. *Psychol Bull*, 52(4):281–302, July 1955.

[79] E. W. Daenen, J. A. Van der Heyden, C. G. Kruse, G. Wolterink, and J. M. Van Ree. Adaptation and habituation to an open field and responses to various stressful events in animals with neonatal lesions in the amygdala or ventral hippocampus. *Brain Res.*, 918(1-2):153–165, November 2001.

[80] Pinhas N. Dannon, Yehuda Sasson, Shmuel Hirschmann, Iulian Iancu, Leon J. Grunhaus, and Joseph Zohar. Pindolol augmentation in treatment-resistant obsessive compulsive disorder: a double-blind placebo controlled trial. *European Neuropsychopharmacology*, 10(3):165–169, May 2000.

[81] Denis J. David, Benjamin Adam Samuels, Quentin Rainer, Jing-Wen Wang, Douglas Marsteller, Indira Mendez, Michael Drew, Douglas A. Craig, Bruno P. Guiard, Jean-Philippe Guilloux, Roman P. Artymyshyn, Alain M. Gardier, Christophe Gerald, Irina A. Antonijevic, E. David Leonardo, and René Hen. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron*, 62(4):479–493, May 2009.

[82] Brittany A. Davis and Anthony R. Isles. Modelling the genetic contribution to mental illness: a timely end for the psychiatric rodent? *Eur. J. Neurosci.*, 39(11):1933–1942, June 2014.

[83] Lea K. Davis, Dongmei Yu, Clare L. Keenan, Eric R. Gamazon, Anuar I. Konkashbaev, Eske M. Derks, Benjamin M. Neale, Jian Yang, S. Hong Lee, Patrick Evans, Cathy L. Barr, Laura Bellodi, Fortu Benarroch, Gabriel Bedoya Berrio, Oscar J. Bienvenu, Michael H. Bloch, Rianne M. Blom, Ruth D. Bruun, Cathy L. Budman, Beatriz

Camarena, Desmond Campbell, Carolina Cappi, Julio C. Cardona Silgado, Danielle C. Cath, Maria C. Cavallini, Denise A. Chavira, Sylvain Chouinard, David V. Conti, Edwin H. Cook, Vladimir Coric, Bernadette A. Cullen, Dieter Deforce, Richard Delorme, Yves Dion, Christopher K. Edlund, Karin Egberts, Peter Falkai, Thomas V. Fernandez, Patience J. Gallagher, Helena Garrido, Daniel Geller, Simon L. Girard, Hans J. Grabe, Marco A. Grados, Benjamin D. Greenberg, Varda Gross-Tsur, Stephen Haddad, Gary A. Heiman, Sian M. J. Hemmings, Ana G. Hounie, Cornelia Illmann, Joseph Jankovic, Michael A. Jenike, James L. Kennedy, Robert A. King, Barbara Kremeyer, Roger Kurlan, Nuria Lanzagorta, Marion Leboyer, James F. Leckman, Leonhard Lennertz, Chunyu Liu, Christine Lochner, Thomas L. Lowe, Fabio Macciardi, James T. McCracken, Lauren M. McGrath, Sandra C. Mesa Restrepo, Rainald Moessner, Jubel Morgan, Heike Muller, Dennis L. Murphy, Allan L. Naarden, William Cornejo Ochoa, Roel A. Ophoff, Lisa Osiecki, Andrew J. Pakstis, Michele T. Pato, Carlos N. Pato, John Piacentini, Christopher Pittenger, Yehuda Pollak, Scott L. Rauch, Tobias J. Renner, Victor I. Reus, Margaret A. Richter, Mark A. Riddle, Mary M. Robertson, Roxana Romero, Maria C. Rosàrio, David Rosenberg, Guy A. Rouleau, Stephan Ruhrmann, Andres Ruiz-Linares, Aline S. Sampaio, Jack Samuels, Paul Sandor, Brooke Sheppard, Harvey S. Singer, Jan H. Smit, Dan J. Stein, E. Strengman, Jay A. Tischfield, Ana V. Valencia Duarte, Homero Vallada, Filip Van Nieuwerburgh, Jeremy Veenstra-Vanderweele, Susanne Walitza, Ying Wang, Jens R. Wendland, Herman G. M. Westenberg, Yin Yao Shugart, Euripedes C. Miguel, William McMahon, Michael Wagner, Humberto Nicolini, Danielle Posthuma, Gregory L. Hanna, Peter Heutink, Damiaan Denys, Paul D. Arnold, Ben A. Oostra, Gerald Nestadt, Nelson B. Freimer, David L. Pauls, Naomi R. Wray, S. Evelyn Stewart, Carol A. Mathews, James A. Knowles, Nancy J. Cox, and Jeremiah M. Scharf. Partitioning the heritability of Tourette syndrome and obsessive compulsive disorder reveals differences in genetic architecture. *PLoS Genet.*, 9(10):e1003864, October 2013.

[84] M. Davis. The role of the amygdala in fear and anxiety. *Annual Review of Neuroscience*, 15(1):353–375, Mar 1992.

[85] L. A. Dawson and H. Q. Nguyen. The role of 5-HT(1a) and 5-HT(1b/1d) receptors on the modulation of acute fluoxetine-induced changes in extracellular 5-HT: the mechanism of action of (+/-)pindolol. *Neuropharmacology*, 39(6):1044–1052, April 2000.

[86] Aart S. de Leeuw and Herman G. M. Westenberg. Hypersensitivity of 5-HT2 receptors in OCD patients. An increased prolactin response after a challenge with metachlorophenylpiperazine and pre-treatment with ritanserin and placebo. *J Psychiatr Res*, 42(11):894–901, September 2008.

[87] Robert M. J. Deacon. Assessing nest building in mice. *Nat Protoc*, 1(3):1117–1119, 2006.

[88] Robert M. J. Deacon. Digging and marble burying in mice: simple methods for in vivo identification of biological impacts. *Nat Protoc*, 1(1):122–124, 2006.

[89] Robert M. J. Deacon, Adam Croucher, and J. Nicholas P. Rawlins. Hippocampal cytotoxic lesion effects on species-typical behaviours in mice. *Behav. Brain Res.*, 132(2):203–213, May 2002.

[90] Robert M. J. Deacon and J. Nicholas P. Rawlins. Hippocampal lesions, species-typical behaviours and anxiety in mice. *Behav. Brain Res.*, 156(2):241–249, January 2005.

[91] W. N. Dember and H. Fowler. Spontaneous alternation behavior. *Psychol Bull*, 55(6):412–428, November 1958.

[92] M. Didriksen and A. V. Christensen. The attenuation of schedule-induced polydipsia by dopamine blockers is not an expression of extrapyramidal side effect liability. *Behav Pharmacol*, 4(5):517–522, October 1993.

[93] Markus Dold, Martin Aigner, Rupert Lanzenberger, and Siegfried Kasper. Antipsychotic Augmentation of Serotonin Reuptake Inhibitors in Treatment-Resistant Obsessive-Compulsive Disorder: An Update Meta-Analysis of Double-Blind, Randomized, Placebo-Controlled Trials. *Int. J. Neuropsychopharmacol.*, May 2015.

[94] T. N. Douma, M. J. Millan, D. Boulay, G. Griebel, P. M. Verdouw, K. G. Westphal, B. Olivier, and L. Groenink. CRF1 receptor antagonists do not reverse pharmacological disruption of prepulse inhibition in rodents. *Psychopharmacology (Berl.)*, 231(7):1289–1303, April 2014.

[95] Paul A. Dudchenko. An overview of the tasks used to test working memory in rodents. *Neurosci Biobehav Rev*, 28(7):699–709, November 2004.

[96] Stephanie C. Dulawa and Rene Hen. Recent advances in animal models of chronic antidepressant effects: the novelty-induced hypophagia test. *Neurosci Biobehav Rev*, 29(4-5):771–783, 2005.

[97] Stephanie C Dulawa, Kerri A Holick, Brigitta Gundersen, and Rene Hen. Effects of chronic fluoxetine in animal models of anxiety and depression. *Neuropsychopharmacology*, 29(7):1321–1330, July 2004.

[98] Sevil Duvarci and Denis Pare. Amygdala microcircuits controlling learned fear. *Neuron*, 82(5):966–980, 2014.

[99] Susanne E Ahmari. Using mice to model obsessive compulsive disorder: From genes to circuits. *Neuroscience*, 321, 11 2015.

[100] Dawn M. Eagle, Andrea Bari, and Trevor W. Robbins. The neuropsychopharmacology of action inhibition: cross-species translation of the stop-signal and go/no-go tasks. *Psychopharmacology (Berl.)*, 199(3):439–456, August 2008.

[101] Willi Ecker and Sascha Gönner. Incompleteness and harm avoidance in OCD symptom dimensions. *Behav Res Ther*, 46(8):895–904, August 2008.

[102] David Eilam and Henry Szechtman. Psychostimulant-induced behavior as an animal model of obsessive-compulsive disorder: an ethological approach to the form of compulsive rituals. *CNS Spectr*, 10(3):191–202, March 2005.

[103] David Eilam, Rama Zor, Naomi Fineberg, and Haggai Hermesh. Animal behavior as a conceptual framework for the study of obsessive-compulsive disorder (OCD). *Behav. Brain Res.*, 231(2):289–296, June 2012.

[104] David Eilam, Rama Zor, Henry Szechtman, and Haggai Hermesh. Rituals, stereotypy and compulsive behavior in animals and humans. *Neuroscience & Biobehavioral Reviews*, 30(4):456–471, 2006.

[105] Ahmed El-Kordi, Daniela Winkler, Kurt Hammerschmidt, Anne Kästner, Dilja Krueger, Anja Ronnenberg, Caroline Ritter, Jasmin Jatho, Konstantin Radyushkin, Thomas Bourgeron, Julia Fischer, Nils Brose, and Hannelore Ehrenreich. Development of an autism severity score for mice using Nlgn4 null mutants as a construct-valid model of heritable monogenic autism. *Behavioural Brain Research*, 251:41–49, August 2013.

[106] Tanja Endrass, Lisa Kloft, Christian Kaufmann, and Norbert Kathmann. Approach and avoidance learning in obsessive-compulsive disorder. *Depress Anxiety*, 28(2):166–172, February 2011.

[107] Nicole M. Enman and Ellen M. Unterwald. Inhibition of GSK3 attenuates amphetamine-induced hyperactivity and sensitization in the mouse. *Behav. Brain Res.*, 231(1):217–225, May 2012.

[108] S. Erzegovesi, L. Martucci, M. Henin, and L. Bellodi. Low versus standard dose mCPP challenge in obsessive-compulsive patients. *Neuropsychopharmacology*, 24(1):31–36, January 2001.

[109] Mark R. Etherton, Cory A. Blaiss, Craig M. Powell, and Thomas C. Südhof. Mouse neurexin-1alpha deletion causes correlated electrophysiological and behavioral changes consistent with cognitive impairments. *Proc. Natl. Acad. Sci. U.S.A.*, 106(42):17998–18003, October 2009.

[110] Susan Ettelt, Hans Joergen Grabe, Stephan Ruhrmann, Friederike Buhtz, Andrea Hochrein, Susanne Kraft, Ralf Pukrop, Joachim Klosterkötter, Peter Falkai, Wolfgang Maier, Ulrich John, Harald J. Freyberger, and Michael Wagner. Harm avoidance in subjects with obsessive-compulsive disorder and their families. *J Affect Disord*, 107(1-3):265–269, April 2008.

[111] J. L. Falk. Production of polydipsia in normal rats by an intermittent food schedule. *Science*, 133(3447):195–196, January 1961.

[112] Adriana Feder, Michael K. Parides, James W. Murrough, Andrew M. Perez, Julia E. Morgan, Shireen Saxena, Katherine Kirkwood, Marije Aan Het Rot, Kyle A. B. Lapidus, Le-Ben Wan, Dan Iosifescu, and Dennis S. Charney. Efficacy of intravenous

ketamine for treatment of chronic posttraumatic stress disorder: a randomized clinical trial. *JAMA Psychiatry*, 71(6):681–688, June 2014.

[113] A. Fernández-Guasti, R. E. Ulloa, and H. Nicolini. Age differences in the sensitivity to clomipramine in an animal model of obsessive-compulsive disorder. *Psychopharmacology (Berl.)*, 166(3):195–201, March 2003.

[114] Jamie D. Feusner, Emily Hembacher, and Katharine A. Phillips. The mouse who couldn't stop washing: Pathologic grooming in animals and humans. *CNS Spectrums*, 14(9):503–513, 2009.

[115] Naomi A. Fineberg, Angus Brown, Samar Reghunandanan, and Ilenia Pampaloni. Evidence-based pharmacotherapy of obsessive-compulsive disorder. *Int. J. Neuropsychopharmacol.*, 15(8):1173–1191, September 2012.

[116] Naomi A. Fineberg, Samar Reghunandanan, Angus Brown, and Ilenia Pampaloni. Pharmacotherapy of obsessive-compulsive disorder: evidence-based treatment and beyond. *Aust N Z J Psychiatry*, 47(2):121–141, February 2013.

[117] Naomi A. Fineberg, Samar Reghunandanan, Helen B. Simpson, Katharine A. Phillips, Margaret A. Richter, Keith Matthews, Dan J. Stein, Jitender Sareen, Angus Brown, and Debbie Sookman. Obsessive–compulsive disorder (OCD): Practical strategies for pharmacological and somatic treatment in adults. *Psychiatry Research*, 227(1):114–125, May 2015.

[118] Nadja Freund, Britta S. Thompson, Kevin J. Norman, Patrick Einhorn, and Susan L. Andersen. Developmental emergence of an obsessive-compulsive phenotype and binge behavior in rats. *Psychopharmacology (Berl.)*, May 2015.

[119] Randy O. Frost, Gail Steketee, and David F. Tolin. Diagnosis and assessment of hoarding disorder. *Annual Review of Clinical Psychology*, 8(1):219–242, Apr 2012.

[120] Alvaro Frías-Ibáñez, Carol Palma-Sevillano, Francisco Barón-Fernández, Inma Bernáldez-Fernández, and Elena Aluco-Sánchez. Nosological status of compulsive hoarding: obsessive-compulsive disorder subtype or independent clinical entity. *Actas Esp Psiquiatr*, 42(3):116–124, June 2014.

[121] E. A. Gaffan and Jeffrey Davies. The role of exploration in win-shift and win-stay performance on a radial maze. *Learning and Motivation*, 12(3):282–299, August 1981.

[122] Joseph P. Garner, Collette M. Thogerson, Brett D. Dufour, Hanno Würbel, James D. Murray, and Joy A. Mench. Reverse-translational biomarker validation of Abnormal Repetitive Behaviors in mice: an illustration of the 4p's modeling approach. *Behav. Brain Res.*, 219(2):189–196, June 2011.

[123] Brianna N. Gaskill, Alicia Z. Karas, Joseph P. Garner, and Kathleen R. Pritchett-Corning. Nest building as an indicator of health and welfare in laboratory mice. *J Vis Exp*, (82):51012, Dec 2013.

[124] Daniel H. Geschwind and Jonathan Flint. Genetics and genomics of psychiatric disease. *Science*, 349(6255):1489–1494, September 2015.

[125] Mark A Geyer and Athina Markou. The Role of Preclinical Models in the Development of Psychotropic Drugs. In Kenneth L Davis, Dennis Charney, Joseph T Coyle, and Charles Nemeroff, editors, *Neuropsychopharmacology: The Fifth Generation of Progress : an Official Publication of the American College of Neuropsychopharmacology*. Lippincott, Williams, & Wilkins, Philadelphia, Pennsylvania, 2002.

[126] Geyer, MA and Markou, A. Animal models of psychiatric disorders. In Floyd E Bloom and David J Kupfer, editors, *The American College of Neuropsychopharmacology: The fourth generation of progress online*. Raven Press, New York, New York, 1995.

[127] M. Ghasemi, M. Raza, and A. R. Dehpour. NMDA receptor antagonists augment antidepressant-like effects of lithium in the mouse forced swimming test. *J. Psychopharmacol. (Oxford)*, 24(4):585–594, April 2010.

[128] Erinn S. Gideons, Ege T. Kavalali, and Lisa M. Monteggia. Mechanisms underlying differential effectiveness of memantine and ketamine in rapid antidepressant responses. *Proc. Natl. Acad. Sci. U.S.A.*, 111(23):8649–8654, June 2014.

[129] Heather Giles, S.j. Lansdell, M.-L. Bolofo, H.l. Wilson, and G.r. Martin. Characterization of a 5-HT1b receptor on CHO cells: functional responses in the absence of radioligand binding. *British Journal of Pharmacology*, 117(6):1119–1126, March 1996.

[130] Claire M. Gillan, Annemieke M. Apergis-Schoute, Sharon Morein-Zamir, Gonzalo P. Urcelay, Akeem Sule, Naomi A. Fineberg, Barbara J. Sahakian, and Trevor W. Robbins. Functional neuroimaging of avoidance habits in obsessive-compulsive disorder. *Am J Psychiatry*, 172(3):284–293, March 2015.

[131] Claire M. Gillan, Michal Kosinski, Robert Whelan, Elizabeth A. Phelps, and Nathaniel D. Daw. Characterizing a psychiatric symptom dimension related to deficits in goal-directed control. *Elife*, 5, March 2016.

[132] Claire M. Gillan, Sharon Morein-Zamir, Muzaffer Kaser, Naomi A. Fineberg, Akeem Sule, Barbara J. Sahakian, Rudolf N. Cardinal, and Trevor W. Robbins. Counterfactual processing of economic action-outcome alternatives in obsessive-compulsive disorder: further evidence of impaired goal-directed behavior. *Biol. Psychiatry*, 75(8):639–646, April 2014.

[133] Claire M. Gillan, Sharon Morein-Zamir, Gonzalo P. Urcelay, Akeem Sule, Valerie Voon, Annemieke M. Apergis-Schoute, Naomi A. Fineberg, Barbara J. Sahakian, and Trevor W. Robbins. Enhanced Avoidance Habits in Obsessive-Compulsive Disorder. *Biological Psychiatry*, 75(8):631–638, April 2014.

[134] Claire M. Gillan, A. Ross Otto, Elizabeth A. Phelps, and Nathaniel D. Daw. Model-based learning protects against forming habits. *Cogn Affect Behav Neurosci*, 15(3):523–536, September 2015.

[135] Claire M. Gillan, Martina Papmeyer, Sharon Morein-Zamir, Barbara J. Sahakian, Naomi A. Fineberg, Trevor W. Robbins, and Sanne de Wit. Disruption in the balance between goal-directed behavior and habit learning in obsessive-compulsive disorder. *Am J Psychiatry*, 168(7):718–726, July 2011.

[136] Claire M. Gillan and Trevor W. Robbins. Goal-directed learning and obsessive-compulsive disorder. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.*, 369(1655), November 2014.

[137] E. M. Glaser and H. Van der Loos. Analysis of thick brain sections by obverse-reverse computer microscopy: application of a new, high clarity Golgi-Nissl stain. *J. Neurosci. Methods*, 4(2):117–125, August 1981.

[138] W. K. Goodman, C. J. McDougle, L. H. Price, L. C. Barr, O. F. Hills, J. F. Caplik, D. S. Charney, and G. R. Heninger. m-Chlorophenylpiperazine in patients with obsessive-compulsive disorder: absence of symptom exacerbation. *Biol. Psychiatry*, 38(3):138–149, August 1995.

[139] W K Goodman, L H Price, P L Delgado, J Palumbo, J H Krystal, L M Nagy, S A Rasmussen, G R Heninger, and D S Charney. Specificity of serotonin reuptake inhibitors in the treatment of obsessive-compulsive disorder. Comparison of fluvoxamine and desipramine. *Arch. Gen. Psychiatry*, 47(6):577–585, June 1990.

[140] C. T. Gordon, R. C. State, J. E. Nelson, S. D. Hamburger, and J. L. Rapoport. A double-blind comparison of clomipramine, desipramine, and placebo in the treatment of autistic disorder. *Arch. Gen. Psychiatry*, 50(6):441–447, June 1993.

[141] J. M. Gorman, M. R. Liebowitz, A. J. Fyer, D. Dillon, S. O. Davies, J. Stein, and D. F. Klein. Lactate infusions in obsessive-compulsive disorder. *Am J Psychiatry*, 142(7):864–866, July 1985.

[142] M. Göthert. Presynaptic serotonin receptors in the central nervous system. *Ann. N. Y. Acad. Sci.*, 604:102–112, 1990.

[143] Irving I. Gottesman and Todd D. Gould. The endophenotype concept in psychiatry: Etymology and strategic intentions. *American Journal of Psychiatry*, 160(4):636–645, Apr 2003.

[144] Jurgen Gotz and Lars M. Ittner. Animal models of alzheimer's disease and frontotemporal dementia. *Nat Rev Neurosci*, 9(7):532–544, Jul 2008.

[145] Marco A. Grados, John Walkup, and Samuel Walford. Genetics of obsessive-compulsive disorders: new findings and challenges. *Brain Dev.*, 25 Suppl 1:S55–61, December 2003.

[146] F K Graham. Presidential Address, 1974. The more or less startling effects of weak prestimulation. *Psychophysiology*, 12(3):238–248, May 1975.

[147] Jon E. Grant. Clinical practice: Obsessive-compulsive disorder. *N. Engl. J. Med.*, 371(7):646–653, August 2014.

[148] Jacob Gratten, Naomi R. Wray, Matthew C. Keller, and Peter M. Visscher. Large-scale genomics unveils the genetic architecture of psychiatric disorders. *Nat. Neurosci.*, 17(6):782–790, June 2014.

[149] B. D. Greenberg, L. A. Gabriels, D. A. Malone, A. R. Rezai, G. M. Friehs, M. S. Okun, N. A. Shapira, K. D. Foote, P. R. Cosyns, C. S. Kubu, P. F. Malloy, S. P. Salloway, J. E. Giftakis, M. T. Rise, A. G. Machado, K. B. Baker, P. H. Stypulkowski, W. K. Goodman, S. A. Rasmussen, and B. J. Nuttin. Deep brain stimulation of the ventral internal capsule/ventral striatum for obsessive-compulsive disorder: worldwide experience. *Mol. Psychiatry*, 15(1):64–79, January 2010.

[150] D. M. Greene-Schloesser, E. A. Van der Zee, D. K. Sheppard, M. R. Castillo, K. A. Gregg, T. Burrow, H. Foltz, M. Slater, and A. Bult-Ito. Predictive validity of a non-induced mouse model of compulsive-like behavior. *Behav. Brain Res.*, 221(1):55–62, August 2011.

[151] Benjamin N. Greenwood, Teresa E. Foley, Tony V. Le, Paul V. Strong, Alice B. Loughridge, Heidi E. W. Day, and Monika Fleshner. Long-term voluntary wheel running is rewarding and produces plasticity in the mesolimbic reward pathway. *Behavioural Brain Research*, 217(2):354–362, March 2011.

[152] Joy M Greer and Mario R Capecchi. Hoxb8 is required for normal grooming behavior in mice. *Neuron*, 33(1):23–34, January 2002.

[153] J. H. Greist, J. W. Jefferson, K. A. Kobak, D. J. Katzelnick, and R. C. Serlin. Efficacy and tolerability of serotonin transport inhibitors in obsessive-compulsive disorder. A meta-analysis. *Arch. Gen. Psychiatry*, 52(1):53–60, January 1995.

[154] R. Gross-Isseroff, Y. Sasson, H. Voet, T. Hendler, K. Luca-Haimovici, H. Kandel-Sussman, and J. Zohar. Alternation learning in obsessive-compulsive disorder. *Biol. Psychiatry*, 39(8):733–738, April 1996.

[155] Ruth Gross-Isseroff, Rivka Cohen, Yehuda Sasson, Hillary Voet, and Joseph Zohar. Serotonergic dissection of obsessive compulsive symptoms: a challenge study with m-chlorophenylpiperazine and sumatriptan. *Neuropsychobiology*, 50(3):200–205, 2004.

[156] Patricia Gruner and Christopher Pittenger. Cognitive inflexibility in Obsessive-Compulsive Disorder. *Neuroscience*, 345:243–255, March 2017.

[157] Eugenia V. Gurevich, John J. G. Tesmer, Arcady Mushegian, and Vsevolod V. Gurevich. G protein-coupled receptor kinases: more than just kinases and not only for GPCRs. *Pharmacol. Ther.*, 133(1):40–69, January 2012.

[158] T. B. Halene, R. S. Ehrlichman, Y. Liang, E. P. Christian, G. J. Jonak, T. L. Gur, J. A. Blendy, H. C. Dow, E. S. Brodkin, F. Schneider, R. C. Gur, and S. J. Siegel. Assessment of NMDA receptor NR1 subunit hypofunction in mice as a model for schizophrenia. *Genes Brain Behav.*, 8(7):661–675, October 2009.

[159] J. C. Halford and J. E. Blundell. The 5-HT1b receptor agonist CP-94,253 reduces food intake and preserves the behavioural satiety sequence. *Physiol. Behav.*, 60(3):933–939, September 1996.

[160] J. F. Hall and P. V. Hanford. Activity as a function of a restricted feeding schedule. *J Comp Physiol Psychol*, 47(5):362–363, October 1954.

[161] P. R. Hartig, D. Hoyer, P. P. Humphrey, and G. R. Martin. Alignment of receptor nomenclature with the human genome: classification of 5-HT1b and 5-HT1d receptor subtypes. *Trends Pharmacol. Sci.*, 17(3):103–105, March 1996.

[162] Athanasios Hassoulas, Louise McHugh, and Phil Reed. Avoidance and behavioural flexibility in obsessive compulsive disorder. *J Anxiety Disord*, 28(2):148–153, March 2014.

[163] Rachel A. Hill, Kerry J. McInnes, Emily C. H. Gong, Margaret E. E. Jones, Evan R. Simpson, and Wah Chin Boon. Estrogen deficient male mice develop compulsive behavior. *Biol. Psychiatry*, 61(3):359–366, February 2007.

[164] Kazufumi Hirano, Ryohei Kimura, Yumi Sugimoto, Jun Yamada, Shinya Uchida, Yasuhiro Kato, Hisakuni Hashimoto, and Shizuo Yamada. Relationship between brain serotonin transporter binding, plasma concentration and behavioural effect of selective serotonin reuptake inhibitors. *Br. J. Pharmacol.*, 144(5):695–702, March 2005.

[165] Emily V. Ho, Summer L. Thompson, William R. Katzka, Mitra F. Sharifi, James A. Knowles, and Stephanie C. Dulawa. Clinically effective OCD treatment prevents 5-HT1b receptor-induced repetitive behavior and striatal activation. *Psychopharmacology (Berl.)*, 233(1):57–70, January 2016.

[166] W. Hodos. Progressive ratio as a measure of reward strength. *Science*, 134(3483):943–944, September 1961.

[167] Klaus Hoenig, Andrea Hochrein, Boris B. Quednow, Wolfgang Maier, and Michael Wagner. Impaired Prepulse Inhibition of Acoustic Startle in Obsessive-Compulsive Disorder. *Biological Psychiatry*, 57(10):1153–1158, May 2005.

[168] Sandy Hogg and Ashutosh Dalvi. Acceleration of onset of action in schedule-induced polydipsia: combinations of SSRI and 5-HT1a and 5-HT1b receptor antagonists. *Pharmacol. Biochem. Behav.*, 77(1):69–75, January 2004.

[169] E. Hollander, C. DeCaria, R. Gully, A. Nitescu, R. F. Suckow, J. M. Gorman, D. F. Klein, and M. R. Liebowitz. Effects of chronic fluoxetine treatment on behavioral

and neuroendocrine responses to meta-chlorophenylpiperazine in obsessive-compulsive disorder. *Psychiatry Res*, 36(1):1–17, January 1991.

[170] E. Hollander, C. M. DeCaria, A. Nitescu, R. Gully, R. F. Suckow, T. B. Cooper, J. M. Gorman, D. F. Klein, and M. R. Liebowitz. Serotonergic function in obsessive-compulsive disorder. Behavioral and neuroendocrine responses to oral m-chlorophenylpiperazine and fenfluramine in patients and healthy volunteers. *Arch. Gen. Psychiatry*, 49(1):21–28, January 1992.

[171] E. Hollander, M. Fay, B. Cohen, R. Campeas, J. M. Gorman, and M. R. Liebowitz. Serotonergic and noradrenergic sensitivity in obsessive-compulsive disorder: behavioral findings. *Am J Psychiatry*, 145(8):1015–1017, August 1988.

[172] E. Hollander, S. Novotny, A. Allen, B. Aronowitz, C. Cartwright, and C. DeCaria. The relationship between repetitive behaviors and growth hormone response to sumatriptan challenge in adult autistic disorder. *Neuropsychopharmacology*, 22(2):163–167, February 2000.

[173] Eric Hollander and Stefano Pallanti. Current and experimental therapeutics of OCD. In Kenneth L. Davis, Dennis S. Charney, Joseph T. Coyle, and Charles Nemeroff, editors, *Neuropsychopharmacology: The Fifth Generation of Progress : an Official Publication of the American College of Neuropsychopharmacology*, pages 1647–1664. Lippincott Williams & Wilkins, 2002.

[174] Soon Beom Hong, Yong-Wook Shin, Sun Hyung Kim, So Young Yoo, Jong-Min Lee, In Young Kim, Sun I. Kim, and Jun Soo Kwon. Hippocampal shape deformity analysis in obsessive-compulsive disorder. *Eur Arch Psychiatry Clin Neurosci*, 257(4):185–190, June 2007.

[175] Thomas Insel, Bruce Cuthbert, Marjorie Garvey, Robert Heinssen, Daniel S. Pine, Kevin Quinn, Charles Sanislow, and Philip Wang. Research domain criteria (rdoc): Toward a new classification framework for research on mental disorders. *American Journal of Psychiatry*, 167(7):748–751, Jul 2010.

[176] Thomas R Insel and Joseph Zohar. Psychopharmacologic Approaches to Obsessive-Compulsive Disorder. In Herbert Y Meltzer, editor, *Psychopharmacology: The Third Generation of Progress*. Raven Press, New York, New York, 1987.

[177] International Obsessive Compulsive Disorder Foundation Genetics Collaborative (IOCDF-GC) and OCD Collaborative Genetics Association Studies (OCGAS). Revealing the complex genetic architecture of obsessive-compulsive disorder using meta-analysis. *Mol. Psychiatry*, August 2017.

[178] Lucrezia Islam, Angelo Franzini, Giuseppe Messina, Silvio Scarone, and Orsola Gambini. Deep brain stimulation of the nucleus accumbens and bed nucleus of stria terminalis for obsessive-compulsive disorder: a case series. *World Neurosurg*, 83(4):657–663, April 2015.

[179] Yasmin Issaria, Ewgeni Jakubovski, Christine A. Bartley, Christopher Pittenger, and Michael H. Bloch. Early onset of response with selective serotonin reuptake inhibitors in obsessive-compulsive disorder: a meta-analysis. *J Clin Psychiatry*, 77(5):e605–611, May 2016.

[180] Stacey A. W. Jackson, Nicole K. Horst, Andrew Pears, Trevor W. Robbins, and Angela C. Roberts. Role of the Perigenual Anterior Cingulate and Orbitofrontal Cortex in Contingency Learning in the Marmoset. *Cereb. Cortex*, 26(7):3273–3284, July 2016.

[181] Patricia H. Janak and Kay M. Tye. From circuits to behaviour in the amygdala. *Nature*, 517(7534):284–292, Jan 2015. 25592533[pmid].

[182] Paulina Jedynak, Piotr Jaholkowski, Grazyna Wozniak, Carmen Sandi, Leszek Kaczmarek, and Robert K. Filipkowski. Lack of cyclin D2 impairing adult brain neurogenesis alters hippocampal-dependent behavioral tasks without reducing learning ability. *Behav. Brain Res.*, 227(1):159–166, February 2012.

[183] Paulin Jirkof. Burrowing and nest building behavior as indicators of well-being in mice. *J. Neurosci. Methods*, 234:139–146, August 2014.

[184] Rapoport JL, Ryland DH, and Kriete M. Drug treatment of canine acral lick: An animal model of obsessive-compulsive disorder. *Archives of General Psychiatry*, 49(7):517–521, Jul 1992.

[185] D. Joel and A. Avisar. Excessive lever pressing following post-training signal attenuation in rats: a possible animal model of obsessive compulsive disorder? *Behav. Brain Res.*, 123(1):77–87, August 2001.

[186] D. Joel, E. Ben-Amir, J. Doljansky, and S. Flaisher. 'Compulsive' lever-pressing in rats is attenuated by the serotonin re-uptake inhibitors paroxetine and fluvoxamine but not by the tricyclic antidepressant desipramine or the anxiolytic diazepam. *Behav Pharmacol*, 15(3):241–252, May 2004.

[187] D. Joel, J. Doljansky, N. Roz, and M. Rehavi. Role of the orbital cortex and of the serotonergic system in a rat model of obsessive compulsive disorder. *Neuroscience*, 130(1):25–36, 2005.

[188] Daphna Joel and Julia Doljansky. Selective alleviation of compulsive lever-pressing in rats by D1, but not D2, blockade: possible implications for the involvement of D1 receptors in obsessive-compulsive disorder. *Neuropsychopharmacology*, 28(1):77–85, January 2003.

[189] Daphna Joel, Julia Doljansky, and Daniela Schiller. 'Compulsive' lever pressing in rats is enhanced following lesions to the orbital cortex, but not to the basolateral nucleus of the amygdala or to the dorsal medial prefrontal cortex. *Eur. J. Neurosci.*, 21(8):2252–2262, April 2005.

[190] Daphna Joel and Oded Klavir. The effects of temporary inactivation of the orbital cortex in the signal attenuation rat model of obsessive compulsive disorder. *Behav. Neurosci.*, 120(4):976–983, August 2006.

[191] Mikhail Kalinichev and Lee A. Dawson. Evidence for antimanic efficacy of glycogen synthase kinase-3 (GSK3) inhibitors in a strain-specific model of acute mania. *Int. J. Neuropsychopharmacol.*, 14(8):1051–1067, September 2011.

[192] Benjamin C. Kalivas and Peter W. Kalivas. Corticostriatal circuitry in regulating diseases characterized by intrusive thinking. *Dialogues Clin Neurosci*, 18(1):65–76, March 2016.

[193] Olli Kampman, Merja Viikki, Kaija Järventausta, and Esa Leinonen. Meta-analysis of anxiety disorders and temperament. *Neuropsychobiology*, 69(3):175–186, 2014.

[194] David Kapfhamer, Karen H. Berger, F. Woodward Hopf, Taban Seif, Viktor Kharazia, Antonello Bonci, and Ulrike Heberlein. Protein Phosphatase 2a and glycogen synthase kinase 3 signaling modulate prepulse inhibition of the acoustic startle response by altering cortical M-Type potassium channel activity. *J. Neurosci.*, 30(26):8830–8840, June 2010.

[195] Himani Kashyap, J. Keshav Kumar, Thennarasu Kandavel, and Y. C. Janardhan Reddy. Neuropsychological functioning in obsessive-compulsive disorder: are executive functions the key deficit? *Compr Psychiatry*, 54(5):533–540, July 2013.

[196] Anne Katrin Kuelz, Dieter Riemann, Roland Zahn, and Ulrich Voderholzer. Object alternation test–is it sensitive enough to detect cognitive dysfunction in obsessive-compulsive disorder? *Eur. Psychiatry*, 19(7):441–443, November 2004.

[197] Terry Kenakin. Functional selectivity and biased receptor signaling. *J. Pharmacol. Exp. Ther.*, 336(2):296–302, February 2011.

[198] Kenneth S. Kendler. What psychiatric genetics has taught us about the nature of psychiatric illness and what is left to learn. *Mol Psychiatry*, 18(10):1058–1066, October 2013.

[199] S. Khanna, J. P. John, and L. P. Reddy. Neuroendocrine and behavioral responses to mCPP in Obsessive-Compulsive Disorder. *Psychoneuroendocrinology*, 26(2):209–223, February 2001.

[200] Jin Hee Kim, Sang-Rok Lee, Li-Hua Li, Hye-Jeong Park, Jeong-Hoh Park, Kwang Youl Lee, Myeong-Kyu Kim, Boo Ahn Shin, and Seok-Yong Choi. High cleavage efficiency of a 2a peptide derived from porcine teschovirus-1 in human cell lines, zebrafish and mice. *PLoS ONE*, 6(4):e18556, 2011.

[201] Oded Klavir, Shira Flash, Christine Winter, and Daphna Joel. High frequency stimulation and pharmacological inactivation of the subthalamic nucleus reduces 'compulsive' lever-pressing in rats. *Exp. Neurol.*, 215(1):101–109, January 2009.

[202] Oded Klavir, Christine Winter, and Daphna Joel. High but not low frequency stimulation of both the globus pallidus and the entopeduncular nucleus reduces 'compulsive' lever-pressing in rats. *Behav. Brain Res.*, 216(1):84–93, January 2011.

[203] Stephanie J. Klenotich, Mariel P. Seiglie, Matthew S. McMurray, Jamie D. Roitman, Daniel Le Grange, Priya Dugad, and Stephanie C. Dulawa. Olanzapine, but not fluoxetine, treatment increases survival in activity-based anorexia in mice. *Neuropsychopharmacology*, 37(7):1620–1631, June 2012.

[204] S. Kohl, K. Heekeren, J. Klosterkötter, and J. Kuhn. Prepulse inhibition in psychiatric disorders – Apart from schizophrenia. *Journal of Psychiatric Research*, 47(4):445–452, April 2013.

[205] GF Koob, SC Heinrichs, and K Britton. Animal models of anxiety disorders. In AF Schatzberg and CB Nemeroff, editors, *The American Psychiatric Press Textbook of Psychopharmacology*, pages 133–144. American Psychiatric Press, Washington DC-London, 2 edition, 1998.

[206] L. M. Koran, S. Pallanti, and L. Quercioli. Sumatriptan, 5-HT(1d) receptors and obsessive-compulsive disorder. *Eur Neuropsychopharmacol*, 11(2):169–172, April 2001.

[207] Lorrin M. Koran, Elias Aboujaoude, Kim D. Bullock, Bettina Franz, Nona Gamel, and Michael Elliott. Double-Blind Treatment With Oral Morphine in Treatment-Resistant Obsessive-Compulsive Disorder. *J Clin Psychiatry*, 66(3):353–359, March 2005.

[208] Lorrin M Koran, Gregory L Hanna, Eric Hollander, Gerald Nestadt, and Helen Blair Simpson. Practice guideline for the treatment of patients with obsessive-compulsive disorder. *The American journal of psychiatry*, 164(7):1, 2007.

[209] Schaun Korff, Dan J. Stein, and Brian H. Harvey. Stereotypic behaviour in the deer mouse: pharmacological validation and relevance for obsessive compulsive disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 32(2):348–355, February 2008.

[210] Schaun Korff, Dan J. Stein, and Brian H. Harvey. Cortico-striatal cyclic AMP-phosphodiesterase-4 signalling and stereotypy in the deer mouse: attenuation after chronic fluoxetine treatment. *Pharmacol. Biochem. Behav.*, 92(3):514–520, May 2009.

[211] Deborah S. Kreiss, Catherine F. Coffman, Nicholas R. Fiacco, Jason C. Granger, Bernadette M. Helton, Jennifer C. Jackson, Leonid V. Kim, Rishi S. Mistry, Tammie M. Mizer, Lolita V. Palmer, Jay A. Vacca, Stuart S. Winkler, and Benjamin A. Zimmer. Ritualistic chewing behavior induced by mCPP in the rat is an animal model of obsessive compulsive disorder. *Pharmacol. Biochem. Behav.*, 104:119–124, March 2013.

[212] K Kusunoki, T Sato, C Taga, T Yoshida, K Komori, T Narita, S Hirano, N Iwata, and N Ozaki. Low novelty-seeking differentiates obsessive-compulsive disorder from major depression. *Acta Psychiatr Scand*, 101(5):403–405, May 2000.

[213] Anne Katrin Külz, Sandra Meinzer, Marta Kopasz, and Ulrich Voderholzer. Effects of tryptophan depletion on cognitive functioning, obsessive-compulsive symptoms and mood in obsessive-compulsive disorder: preliminary results. *Neuropsychobiology*, 56(2-3):127–131, 2007.

[214] Robert Lalonde. The neurobiological basis of spontaneous alternation. *Neurosci Biobehav Rev*, 26(1):91–104, January 2002.

[215] Kyle A. B. Lapidus, Emily R. Stern, Heather A. Berlin, and Wayne K. Goodman. Neuromodulation for ObsessiveCompulsive Disorder. *Neurotherapeutics*, 11(3):485–495, July 2014.

[216] L. Lecourtier, M. C. Antal, B. Cosquer, A. Schumacher, B. Samama, M. J. Angst, A. Ferrandon, E. Koning, J. C. Cassel, and A. Nehlig. Intact neurobehavioral development and dramatic impairments of procedural-like memory following neonatal ventral hippocampal lesion in rats. *Neuroscience*, 207:110–123, April 2012.

[217] Joseph E LeDoux. *The emotional brain: the mysterious underpinnings of emotional life.* New York, NY, US: Simon & Schuster, 1996.

[218] Robert J. Lefkowitz and Sudha K. Shenoy. Transduction of receptor signals by beta-arrestins. *Science*, 308(5721):512–517, April 2005.

[219] Ed S. Lein, Michael J. Hawrylycz, Nancy Ao, Mikael Ayres, Amy Bensinger, Amy Bernard, Andrew F. Boe, Mark S. Boguski, Kevin S. Brockway, Emi J. Byrnes, Lin Chen, Li Chen, Tsuey-Ming Chen, Mei Chi Chin, Jimmy Chong, Brian E. Crook, Aneta Czaplinska, Chinh N. Dang, Suvro Datta, Nick R. Dee, Aimee L. Desaki, Tsega Desta, Ellen Diep, Tim A. Dolbeare, Matthew J. Donelan, Hong-Wei Dong, Jennifer G. Dougherty, Ben J. Duncan, Amanda J. Ebbert, Gregor Eichele, Lili K. Estin, Casey Faber, Benjamin A. Facer, Rick Fields, Shanna R. Fischer, Tim P. Fliss, Cliff Frensley, Sabrina N. Gates, Katie J. Glattfelder, Kevin R. Halverson, Matthew R. Hart, John G. Hohmann, Maureen P. Howell, Darren P. Jeung, Rebecca A. Johnson, Patrick T. Karr, Reena Kawal, Jolene M. Kidney, Rachel H. Knapik, Chihchau L. Kuan, James H. Lake, Annabel R. Laramee, Kirk D. Larsen, Christopher Lau, Tracy A. Lemon, Agnes J. Liang, Ying Liu, Lon T. Luong, Jesse Michaels, Judith J. Morgan, Rebecca J. Morgan, Marty T. Mortrud, Nerick F. Mosqueda, Lydia L. Ng, Randy Ng, Geralyn J. Orta, Caroline C. Overly, Tu H. Pak, Sheana E. Parry, Sayan D. Pathak, Owen C. Pearson, Ralph B. Puchalski, Zackery L. Riley, Hannah R. Rockett, Stephen A. Rowland, Joshua J. Royall, Marcos J. Ruiz, Nadia R. Sarno, Katherine Schaffnit, Nadiya V. Shapovalova, Taz Sivisay, Clifford R. Slaughterbeck, Simon C. Smith, Kimberly A. Smith, Bryan I. Smith, Andy J. Sodt, Nick N. Stewart, Kenda-Ruth Stumpf, Susan M. Sunkin, Madhavi Sutram, Angelene Tam, Carey D. Teemer, Christina Thaller, Carol L. Thompson, Lee R. Varnam, Axel Visel, Ray M. Whitlock, Paul E. Wohnoutka, Crissa K. Wolkey, Victoria Y. Wong, Matthew Wood, Murat B. Yaylaoglu, Rob C. Young, Brian L. Youngstrom, Xu Feng Yuan, Bin Zhang, Theresa A.

Zwingman, and Allan R. Jones. Genome-wide atlas of gene expression in the adult mouse brain. *Nature*, 445(7124):168–176, January 2007.

[220] H. L. Leonard, S. E. Swedo, M. C. Lenane, D. C. Rettew, D. L. Cheslow, S. D. Hamburger, and J. L. Rapoport. A double-blind desipramine substitution during long-term clomipramine treatment in children and adolescents with obsessive-compulsive disorder. *Arch. Gen. Psychiatry*, 48(10):922–927, October 1991.

[221] Colin Lever, Stephen Burton, and John O'Keefe. Rearing on hind legs, environmental novelty, and the hippocampal formation. *Rev Neurosci*, 17(1-2):111–133, 2006.

[222] Nanxin Li, Rong-Jian Liu, Jason M. Dwyer, Mounira Banasr, Boyoung Lee, Hyeon Son, Xiao-Yuan Li, George Aghajanian, and Ronald S. Duman. Glutamate N-methyl-D-aspartate receptor antagonists rapidly reverse behavioral and synaptic deficits caused by chronic stress exposure. *Biol. Psychiatry*, 69(8):754–761, April 2011.

[223] Xia Li, Denise Morrow, and Jeffrey M. Witkin. Decreases in nestlet shredding of mice by serotonin uptake inhibitors: comparison with marble burying. *Life Sci.*, 78(17):1933–1939, March 2006.

[224] Stanley L. Lin, Shilpy Setya, Nadine N. Johnson-Farley, and Daniel S. Cowen. Differential coupling of 5-HT(1) receptors to G proteins of the G(i) family. *Br. J. Pharmacol.*, 136(7):1072–1078, August 2002.

[225] Christine Lochner, Soraya Seedat, Pieter L. du Toit, Daniel G. Nel, Dana J. H. Niehaus, Robin Sandler, and Dan J. Stein. Obsessive-compulsive disorder and trichotillomania: a phenomenological comparison. *BMC Psychiatry*, 5:2, 2005.

[226] L. Luyten, S. Hendrickx, S. Raymaekers, L. Gabriëls, and B. Nuttin. Electrical stimulation in the bed nucleus of the stria terminalis alleviates severe obsessive-compulsive disorder. *Mol. Psychiatry*, August 2015.

[227] I K Lyoo, D W Lee, Y S Kim, S W Kong, and J S Kwon. Patterns of temperament and character in subjects with obsessive-compulsive disorder. *J Clin Psychiatry*, 62(8):637–641, August 2001.

[228] Jennifer M Chou-Green, Todd D Holscher, Mary Dallman, and Susan Akana. *Compulsive behavior in the 5-HT2C receptor knockout mouse*, volume 78 of *Physiology & behavior*, pages 641–9. May 2003.

[229] Sungho Maeng, Carlos A. Zarate, Jing Du, Robert J. Schloesser, Joseph McCammon, Guang Chen, and Husseini K. Manji. Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol. Psychiatry*, 63(4):349–352, February 2008.

[230] A. Markou, F. Weiss, L. H. Gold, S. B. Caine, G. Schulteis, and G. F. Koob. Animal models of drug craving. *Psychopharmacology (Berl.)*, 112(2-3):163–182, 1993.

[231] Rachel Marsh, Gregory Z. Tau, Zhishun Wang, Yuankai Huo, Ge Liu, Xuejun Hao, Mark G. Packard, Bradley S. Peterson, and H. Blair Simpson. Reward-based spatial learning in unmedicated adults with obsessive-compulsive disorder. *Am J Psychiatry*, 172(4):383–392, April 2015.

[232] James R. Martin, Theresa M. Ballard, and Guy A. Higgins. Influence of the 5-HT2c receptor antagonist, SB-242084, in tests of anxiety. *Pharmacol. Biochem. Behav.*, 71(4):615–625, April 2002.

[233] Riccardo Maria Martoni, Giulia Salgari, Elisa Galimberti, Maria Cristina Cavallini, and Joseph O'Neill. Effects of gender and executive function on visuospatial working memory in adult obsessive-compulsive disorder. *Eur Arch Psychiatry Clin Neurosci*, May 2015.

[234] Asuka Matsui, May Tran, Aya C Yoshida, Satomi S Kikuchi, Mami U, Masaharu Ogawa, and Tomomi Shimogori. BTBD3 controls dendrite orientation toward active axons in mammalian neocortex. *Science*, 342(6162):1114–1118, November 2013.

[235] M. Mattheisen, J. F. Samuels, Y. Wang, B. D. Greenberg, A. J. Fyer, J. T. McCracken, D. A. Geller, D. L. Murphy, J. A. Knowles, M. A. Grados, M. A. Riddle, S. A. Rasmussen, N. C. McLaughlin, E. L. Nurmi, K. D. Askland, H.-D. Qin, B. A. Cullen, J. Piacentini, D. L. Pauls, O. J. Bienvenu, S. E. Stewart, K.-Y. Liang, F. S. Goes, B. Maher, A. E. Pulver, Y. Y. Shugart, D. Valle, C. Lange, and G. Nestadt. Genome-wide association study in obsessive-compulsive disorder: results from the OCGAS. *Mol. Psychiatry*, 20(3):337–344, March 2015.

[236] Thomas J. McCown, Xiao Xiao, Juan Li, George R. Breese, and R. Jude Samulski. Differential and persistent expression patterns of CNS gene transfer by an adeno-associated virus (AAV) vector. *Brain Research*, 713(1):99–107, March 1996.

[237] M. P. McDonald, R. Wong, G. Goldstein, B. Weintraub, S. Y. Cheng, and J. N. Crawley. Hyperactivity and learning deficits in transgenic mice bearing a human mutant thyroid hormone beta1 receptor gene. *Learn. Mem.*, 5(4-5):289–301, October 1998.

[238] C. J. McDougle, L. C. Barr, W. K. Goodman, G. H. Pelton, S. C. Aronson, A. Anand, and L. H. Price. Lack of efficacy of clozapine monotherapy in refractory obsessive-compulsive disorder. *Am J Psychiatry*, 152(12):1812–1814, December 1995.

[239] Josephine C. McGowan, Christina T. LaGamma, Sean C. Lim, Melina Tsitsiklis, Yuval Neria, Rebecca A. Brachman, and Christine A. Denny. Prophylactic Ketamine Attenuates Learned Fear. *Neuropsychopharmacology*, 42(8):1577–1589, July 2017.

[240] Joseph F. McGuire, Eric A. Storch, Adam B. Lewin, Lawrence H. Price, Steven A. Rasmussen, and Wayne K. Goodman. The role of avoidance in the phenomenology of obsessive-compulsive disorder. *Compr Psychiatry*, 53(2):187–194, February 2012.

[241] W. T. McKinney and W. E. Bunney. Animal model of depression. I. Review of evidence: implications for research. *Arch. Gen. Psychiatry*, 21(2):240–248, August 1969.

[242] William T. McKinney. *Models of Mental Disorders.* Springer Science & Business Media, 1988.

[243] Lara Menzies, Samuel R. Chamberlain, Angela R. Laird, Sarah M. Thelen, Barbara J. Sahakian, and Ed T. Bullmore. Integrating evidence from neuroimaging and neuropsychological studies of obsessive-compulsive disorder: the orbitofronto-striatal model revisited. *Neurosci Biobehav Rev*, 32(3):525–549, 2008.

[244] Mohammed R Milad, Sharon C Furtak, Jennifer L Greenberg, Aparna Keshaviah, Jooyeon J Im, Martha J Falkenstein, Michael Jenike, Scott L Rauch, and Sabine Wilhelm. Deficits in conditioned fear extinction in obsessive-compulsive disorder and neurobiological changes in the fear circuit. *JAMA Psychiatry*, 70(6):608–618, June 2013.

[245] Mohammed R Milad and Scott L Rauch. Obsessive-compulsive disorder: beyond segregated cortico-striatal pathways. *Trends Cogn. Sci. (Regul. Ed.)*, 16(1):43–51, January 2012.

[246] Morgane Milienne-Petiot, James P. Kesby, Mary Graves, Jordy van Enkhuizen, Svetlana Semenova, Arpi Minassian, Athina Markou, Mark A. Geyer, and Jared W. Young. The effects of reduced dopamine transporter function and chronic lithium on motivation, probabilistic learning, and neurochemistry in mice: Modeling bipolar mania. *Neuropharmacology*, 113, Part A:260–270, February 2017.

[247] Jeremy A. Miller, Song-Lin Ding, Susan M. Sunkin, Kimberly A. Smith, Lydia Ng, Aaron Szafer, Amanda Ebbert, Zackery L. Riley, Joshua J. Royall, Kaylynn Aiona, James M. Arnold, Crissa Bennet, Darren Bertagnolli, Krissy Brouner, Stephanie Butler, Shiella Caldejon, Anita Carey, Christine Cuhaciyan, Rachel A. Dalley, Nick Dee, Tim A. Dolbeare, Benjamin A. C. Facer, David Feng, Tim P. Fliss, Garrett Gee, Jeff Goldy, Lindsey Gourley, Benjamin W. Gregor, Guangyu Gu, Robert E. Howard, Jayson M. Jochim, Chihchau L. Kuan, Christopher Lau, Chang-Kyu Lee, Felix Lee, Tracy A. Lemon, Phil Lesnar, Bergen McMurray, Naveed Mastan, Nerick Mosqueda, Theresa Naluai-Cecchini, Nhan-Kiet Ngo, Julie Nyhus, Aaron Oldre, Eric Olson, Jody Parente, Patrick D. Parker, Sheana E. Parry, Allison Stevens, Mihovil Pletikos, Melissa Reding, Kate Roll, David Sandman, Melaine Sarreal, Sheila Shapouri, Nadiya V. Shapovalova, Elaine H. Shen, Nathan Sjoquist, Clifford R. Slaughterbeck, Michael Smith, Andy J. Sodt, Derric Williams, Lilla Zöllei, Bruce Fischl, Mark B. Gerstein, Daniel H. Geschwind, Ian A. Glass, Michael J. Hawrylycz, Robert F. Hevner, Hao Huang, Allan R. Jones, James A. Knowles, Pat Levitt, John W. Phillips, Nenad estan, Paul Wohnoutka, Chinh Dang, Amy Bernard, John G. Hohmann, and Ed S. Lein. Transcriptional landscape of the prenatal human brain. *Nature*, 508(7495):199–206, April 2014.

[248] Jonathan S. Miller, Ronald J. Tallarida, and Ellen M. Unterwald. Inhibition of GSK3 attenuates dopamine D1 receptor agonist-induced hyperactivity in mice. *Brain Res. Bull.*, 82(3-4):184–187, May 2010.

[249] Kevin J. Miller, Matthew M. Botvinick, and Carlos D. Brody. Dorsal hippocampus contributes to model-based planning. *Nat. Neurosci.*, July 2017.

[250] R. Misslin, C. Belzung, and E. Vogel. Behavioural validation of a light/dark choice procedure for testing anti-anxiety agents. *Behav. Processes*, 18(1-3):119–132, 1989.

[251] Patricia Monteiro and Guoping Feng. Learning from animal models of obsessive-compulsive disorder. *Biological Psychiatry*, 79(1):7–16, May 2015.

[252] S. A. Montgomery and A. Manceaux. Fluvoxamine in the treatment of obsessive compulsive disorder. *International Clinical Psychopharmacology*, pages 5–10, June 1992.

[253] S Moritz, S Fricke, M Wagner, and I Hand. Further evidence for delayed alternation deficits in obsessive-compulsive disorder. *J. Nerv. Ment. Dis.*, 189(8):562–564, August 2001.

[254] Steffen Moritz, Birgit Hottenrott, Sarah Randjbar, Ruth Klinge, Francesca Vitzthum Von Eckstaedt, Tania M Lincoln, and Lena Jelinek. Perseveration and not strategic deficits underlie delayed alternation impairment in obsessive-compulsive disorder (OCD). *Psychiatry Res*, 170(1):66–69, November 2009.

[255] Steffen Moritz, Martin Kloss, Dirk Jacobsen, Susanne Fricke, Carrie Cutler, Stefanie Brassen, and Iver Hand. Neurocognitive impairment does not predict treatment outcome in obsessive-compulsive disorder. *Behav Res Ther*, 43(6):811–819, June 2005.

[256] Charles I. Mosier. A critical examination of the concepts of face validity. *Educational and Psychological Measurement*, 7:191–205, 1947.

[257] Adrian Mundt, Julia Klein, Daphna Joel, Andreas Heinz, Anais Djodari-Irani, Daniel Harnack, Andreas Kupsch, Helmut Orawa, Georg Juckel, Rudolf Morgenstern, and Christine Winter. High-frequency stimulation of the nucleus accumbens core and shell reduces quinpirole-induced compulsive checking in rats. *Eur. J. Neurosci.*, 29(12):2401–2412, June 2009.

[258] Sadia Najmi, Jennie M. Kuckertz, and Nader Amir. Automatic avoidance tendencies in individuals with contamination-related obsessive-compulsive symptoms. *Behav Res Ther*, 48(10):1058–1062, October 2010.

[259] National Collaborating Centre for Mental Health (UK). *Obsessive-Compulsive Disorder: Core Interventions in the Treatment of Obsessive-Compulsive Disorder and Body Dysmorphic Disorder*. National Institute for Health and Clinical Excellence: Guidance. British Psychological Society, Leicester (UK), 2006.

[260] Maja Nedeljkovic, Michael Kyrios, Richard Moulding, Guy Doron, Kylie Wainwright, Chris Pantelis, Rosemary Purcell, and Paul Maruff. Differences in neuropsychological performance between subtypes of obsessive-compulsive disorder. *Aust N Z J Psychiatry*, 43(3):216–226, March 2009.

[261] Eric J. Nestler and Steven E. Hyman. Animal models of neuropsychiatric disorders. *Nat Neurosci*, 13(10):1161–1169, Oct 2010.

[262] K. Njung'e and S. L. Handley. Effects of 5-HT uptake inhibitors, agonists and antagonists on the burying of harmless objects by mice; a putative test for anxiolytic agents. *Br. J. Pharmacol.*, 104(1):105–112, September 1991.

[263] Y. Noda, Y. Ochi, E. Shimada, and M. Oka. Involvement of central cholinergic mechanism in RU-24969-induced behavioral deficits. *Pharmacol. Biochem. Behav.*, 38(2):441–446, February 1991.

[264] EJ Nordstrom and FH Burton. A transgenic model of comorbid tourette's syndrome and obsessive-compulsive disorder circuitry. *Molecular psychiatry*, 7(6):617, 2002.

[265] R. D. Oades. Stimulus dimension shifts in patients with schizophrenia, with and without paranoid hallucinatory symptoms, or obsessive compulsive disorder: strategies, blocking and monoamine status. *Behav. Brain Res.*, 88(1):115–131, October 1997.

[266] Hiroyuki Okuno. Regulation and function of immediate-early genes in the brain: beyond neuronal activity markers. *Neurosci. Res.*, 69(3):175–186, March 2011.

[267] David S. Olton and Robert J. Samuelson. Remembrance of places passed: Spatial memory in rats. *Journal of Experimental Psychology: Animal Behavior Processes*, 2(2):97–116, April 1976.

[268] Sébastien Parnaudeau, Kathleen Taylor, Scott S. Bolkan, Ryan D. Ward, Peter D. Balsam, and Christoph Kellendonk. Mediodorsal thalamus hypofunction impairs flexible goal-directed behavior. *Biol. Psychiatry*, 77(5):445–453, March 2015.

[269] M P Paulus and M A Geyer. A temporal and spatial scaling hypothesis for the behavioral effects of psychostimulants. *Psychopharmacology (Berl.)*, 104(1):6–16, 1991.

[270] M. P. Paulus and M. A. Geyer. Quantitative assessment of the microstructure of rat behavior: I, f(d), the extension of the scaling hypothesis. *Psychopharmacology (Berl.)*, 113(2):177–186, 1993.

[271] R. Paylor and J. N. Crawley. Inbred strain differences in prepulse inhibition of the mouse startle response. *Psychopharmacology (Berl.)*, 132(2):169–180, July 1997.

[272] João Peça, Cátia Feliciano, Jonathan T Ting, Wenting Wang, Michael F Wells, Talaignair N Venkatraman, Christopher D Lascola, Zhanyan Fu, and Guoping Feng. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature*, 472(7344):437, 2011.

[273] S. Pellow, P. Chopin, S. E. File, and M. Briley. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J. Neurosci. Methods*, 14(3):149–167, August 1985.

[274] C. M. A. Pennartz, R. Ito, P. F. M. J. Verschure, F. P. Battaglia, and T. W. Robbins. The hippocampal-striatal axis in learning, prediction and goal-directed behavior. *Trends Neurosci.*, 34(10):548–559, October 2011.

[275] Roberto Perez-Torrado, Daisuke Yamada, and Pierre-Antoine Defossez. Born to bind: the BTB protein-protein interaction domain. *Bioessays*, 28(12):1194–1202, December 2006.

[276] William Perry, Arpi Minassian, Brian Lopez, Leeza Maron, and Alan Lincoln. Sensorimotor Gating Deficits in Adults with Autism. *Biological Psychiatry*, 61(4):482–486, February 2007.

[277] William Perry, Arpi Minassian, Martin P. Paulus, Jared W. Young, Meegin J. Kincaid, Eliza J. Ferguson, Brook L. Henry, Xiaoxi Zhuang, Virginia L. Masten, Richard F. Sharp, and Mark A. Geyer. A reverse-translational study of dysfunctional exploration in psychiatric disorders: from mice to men. *Arch. Gen. Psychiatry*, 66(10):1072–1080, October 2009.

[278] Alberto Pertusa, Miguel A. Fullana, Satwant Singh, Pino Alonso, José M. Menchón, and David Mataix-Cols. Compulsive Hoarding: OCD Symptom, Distinct Clinical Syndrome, or Both? *AJP*, 165(10):1289–1298, October 2008.

[279] Benoit Petit-Demouliere, Franck Chenu, and Michel Bourin. Forced swimming test in mice: a review of antidepressant activity. *Psychopharmacology (Berl.)*, 177(3):245–255, January 2005.

[280] B Pfohl, D Black, R Noyes, Jr, M Kelley, and N Blum. A test of the tridimensional personality theory: association with diagnosis and platelet imipramine binding in obsessive-compulsive disorder. *Biol. Psychiatry*, 28(1):41–46, July 1990.

[281] K. L. Pian, H. G. Westenberg, H. J. van Megen, and J. A. den Boer. Sumatriptan (5-HT1d receptor agonist) does not exacerbate symptoms in obsessive compulsive disorder. *Psychopharmacology (Berl.)*, 140(3):365–370, December 1998.

[282] Ashley S. Pietrefesa and Meredith E. Coles. Moving beyond an exclusive focus on harm avoidance in obsessive-compulsive disorder: behavioral validation for the separability of harm avoidance and incompleteness. *Behav Ther*, 40(3):251–259, September 2009.

[283] T. A. Pigott, J. L. Hill, T. A. Grady, F. L'Heureux, S. Bernstein, C. S. Rubenstein, and D. L. Murphy. A comparison of the behavioral effects of oral versus intravenous mCPP administration in OCD patients and the effect of metergoline prior to i.v. mCPP. *Biol. Psychiatry*, 33(1):3–14, January 1993.

[284] T. A. Pigott, J. Zohar, J. L. Hill, S. E. Bernstein, G. N. Grover, R. C. Zohar-Kadouch, and D. L. Murphy. Metergoline blocks the behavioral and neuroendocrine effects of orally administered m-chlorophenylpiperazine in patients with obsessive-compulsive disorder. *Biol. Psychiatry*, 29(5):418–426, March 1991.

[285] Christopher Pittenger. Pathophysiological Modeling of Obsessive-Compulsive Disorder: Challenges, and Progress. *Biological Psychiatry*, 70(11):1002–1003, December 2011.

[286] Christopher Pittenger. *Chapter 47 - Animal Models of Tourette Syndrome and Obsessive-Compulsive Disorder A2 - LeDoux, Mark S.*, pages 747–764. Academic Press, Boston, 2015.

[287] Christopher Pittenger, Thomas G. Adams, Jean-Dominique Gallezot, Michael J. Crowley, Nabeel Nabulsi, James Ropchan, Hong Gao, Stephen A. Kichuk, Ryan Simpson, Eileen Billingslea, Jonas Hannestad, Michael Bloch, Linda Mayes, Zubin Bhagwagar, and Richard E. Carson. OCD is associated with an altered association between sensorimotor gating and cortical and subcortical 5-HT1b receptor binding. *J Affect Disord*, 196:87–96, May 2016.

[288] Mariana Pizarro, Leonardo F. Fontenelle, Daniel C. Paravidino, Murat Yücel, Eurípedes C. Miguel, and Gabriela B. de Menezes. An updated review of antidepressants with marked serotonergic effects in obsessive-compulsive disorder. *Expert Opin Pharmacother*, 15(10):1391–1401, July 2014.

[289] Katerina Placek, William C. Dippel, Shelby Jones, and Anne Marie Brady. Impairments in set-shifting but not reversal learning in the neonatal ventral hippocampal lesion model of schizophrenia: further evidence for medial prefrontal deficits. *Behav. Brain Res.*, 256:405–413, November 2013.

[290] Franziska Plessow, Andrea Kiesel, and Clemens Kirschbaum. The stressed prefrontal cortex and goal-directed behaviour: acute psychosocial stress impairs the flexible implementation of task goals. *Exp Brain Res*, 216(3):397–408, February 2012.

[291] Piotr Popik, Tomasz Kos, Magdalena Sowa-Kućma, and Gabriel Nowak. Lack of persistent effects of ketamine in rodent models of depression. *Psychopharmacology (Berl.)*, 198(3):421–430, June 2008.

[292] S. B. Powell, H. A. Newman, T. A. McDonald, P. Bugenhagen, and M. H. Lewis. Development of spontaneous stereotyped behavior in deer mice: effects of early and late exposure to a more complex environment. *Dev Psychobiol*, 37(2):100–108, September 2000.

[293] S. B. Powell, H. A. Newman, J. F. Pendergast, and M. H. Lewis. A rodent model of spontaneous stereotypy: initial characterization of developmental, environmental, and neurobiological factors. *Physiol. Behav.*, 66(2):355–363, April 1999.

[294] R. Purcell, P. Maruff, M. Kyrios, and C. Pantelis. Cognitive deficits in obsessive-compulsive disorder on tests of frontal-striatal function. *Biol. Psychiatry*, 43(5):348–357, March 1998.

[295] R. Purcell, P. Maruff, M. Kyrios, and C. Pantelis. Neuropsychological deficits in obsessive-compulsive disorder: a comparison with unipolar depression, panic disorder, and normal controls. *Arch. Gen. Psychiatry*, 55(5):415–423, May 1998.

[296] Stanley J. Rachman and Ray J. Hodgson. *Obsessions and compulsions*. Prentice Hall, 1980.

[297] Naren P. Rao, Y. C. Janardhan Reddy, Keshav J. Kumar, Thennarasu Kandavel, and C. R. Chandrashekar. Are neuropsychological deficits trait markers in OCD? *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 32(6):1574–1579, August 2008.

[298] S. A. Rasmussen, W. K. Goodman, S. W. Woods, G. R. Heninger, and D. S. Charney. Effects of yohimbine in obsessive compulsive disorder. *Psychopharmacology (Berl.)*, 93(3):308–313, 1987.

[299] Scott L. Rauch, Michelle M. Wedig, Christopher I. Wright, Brian Martis, Katherine G. McMullin, Lisa M. Shin, Paul A. Cannistraro, and Sabine Wilhelm. Functional magnetic resonance imaging study of regional brain activation during implicit sequence learning in obsessive-compulsive disorder. *Biol. Psychiatry*, 61(3):330–336, February 2007.

[300] S. Raymaekers, K. Vansteelandt, L. Luyten, C. Bervoets, K. Demyttenaere, L. Gabriëls, and B. Nuttin. Long-term electrical stimulation of bed nucleus of stria terminalis for obsessive-compulsive disorder. *Mol. Psychiatry*, August 2016.

[301] Louise K. Refsgaard, Darryl S. Pickering, and Jesper T. Andreasen. Investigation of antidepressant-like and anxiolytic-like actions and cognitive and motor side effects of four N-methyl-D-aspartate receptor antagonists in mice. *Behav Pharmacol*, 28(1):37–47, February 2017.

[302] Viktor Reinhardt. Hair pulling: a review. *Lab Anim*, 39(4):361–369, October 2005.

[303] Angelika Richter and Wolfgang Löscher. *Pathophysiology of idiopathic dystonia: Findings from genetic animal models*, volume 54 of *Progress in neurobiology*. May 1998.

[304] M A Richter, L J Summerfeldt, R T Joffe, and R P Swinson. The Tridimensional Personality Questionnaire in obsessive-compulsive disorder. *Psychiatry Res*, 65(3):185–188, December 1996.

[305] Carolyn I. Rodriguez, Lawrence S. Kegeles, Amanda Levinson, Tianshu Feng, Sue M. Marcus, Donna Vermes, Pamela Flood, and Helen B. Simpson. Randomized controlled crossover trial of ketamine in obsessive-compulsive disorder: proof-of-concept. *Neuropsychopharmacology*, 38(12):2475–2483, November 2013.

[306] Carolyn I. Rodriguez, Michael Wheaton, Jordana Zwerling, Shari A. Steinman, Danae Sonnenfeld, Hanga Galfalvy, and Helen Blair Simpson. Can exposure-based CBT extend the effects of intravenous ketamine in obsessive-compulsive disorder? an open-label trial. *J Clin Psychiatry*, 77(3):408–409, March 2016.

[307] David Rodríguez, José Brea, María Isabel Loza, and Jens Carlsson. Structure-based discovery of selective serotonin 5-HT(1b) receptor ligands. *Structure*, 22(8):1140–1151, August 2014.

[308] Bangalore N. Roopesh, Y. C. Janardhan Reddy, and C. R. Mukundan. Neuropsychological deficits in drug naïve, non-depressed obsessive-compulsive disorder patients. *Asian J Psychiatr*, 6(2):162–170, April 2013.

[309] Angelo O. Rosa, Manuella P. Kaster, Ricardo W. Binfaré, Susana Morales, Ester Martín-Aparicio, Maria Luisa Navarro-Rico, Ana Martinez, Miguel Medina, Antonio G. García, Manuela G. López, and Ana Lúcia S. Rodrigues. Antidepressant-like effect of the novel thiadiazolidinone NP031115 in mice. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 32(6):1549–1556, August 2008.

[310] Sébastien Royer, Anton Sirota, Jagdish Patel, and György Buzsáki. Distinct representations and theta dynamics in dorsal and ventral hippocampus. *J. Neurosci.*, 30(5):1777–1787, February 2010.

[311] Sarah Ruediger, Dominique Spirig, Flavio Donato, and Pico Caroni. Goal-oriented searching mediated by ventral hippocampus early in trial-and-error learning. *Nat Neurosci*, 15(11):1563–1571, November 2012.

[312] A M Ruscio, D J Stein, W T Chiu, and R C Kessler. The epidemiology of obsessive-compulsive disorder in the National Comorbidity Survey Replication. *Mol. Psychiatry*, 15(1):53–63, January 2010.

[313] Kinga Sałat, Agata Siwek, Gabriela Starowicz, Tadeusz Librowski, Gabriel Nowak, Urszula Drabik, Ryszard Gajdosz, and Piotr Popik. Antidepressant-like effects of ketamine, norketamine and dehydronorketamine in forced swim test: Role of activity at NMDA receptor. *Neuropharmacology*, 99:301–307, December 2015.

[314] Rodney C. Samaco, John D. Fryer, Jun Ren, Sharyl Fyffe, Hsiao-Tuan Chao, Yaling Sun, John J. Greer, Huda Y. Zoghbi, and Jeffrey L. Neul. A partial loss of function allele of methyl-CpG-binding protein 2 predicts a human neurodevelopmental syndrome. *Hum. Mol. Genet.*, 17(12):1718–1727, June 2008.

[315] S Saxena and S L Rauch. Functional neuroimaging and the neuroanatomy of obsessive-compulsive disorder. *Psychiatr. Clin. North Am.*, 23(3):563–586, September 2000.

[316] U Schall, A Schön, D Zerbin, C Eggers, and R D Oades. Event-related potentials during an auditory discrimination with prepulse inhibition in patients with schizophrenia, obsessive-compulsive disorder and healthy subjects. *Int. J. Neurosci.*, 84(1-4):15–33, February 1996.

[317] Eduardo A. Schilman, Oded Klavir, Christine Winter, Reinhard Sohr, and Daphna Joel. The role of the striatum in compulsive behavior in intact and orbitofrontal-cortex-lesioned rats: possible involvement of the serotonergic system. *Neuropsychopharmacology*, 35(4):1026–1039, March 2010.

[318] Thomas D. Schmittgen and Kenneth J. Livak. Analyzing real-time PCR data by the comparative CT method. *Nat. Protocols*, 3(6):1101–1108, June 2008.

[319] Caroline A. Schneider, Wayne S. Rasband, and Kevin W. Eliceiri. Nih image to imagej: 25 years of image analysis. *Nat Methods*, 9(7):671–675, Jul 2012. 22930834[pmid].

[320] Philipp Schwartenbeck, Thomas FitzGerald, Raymond J. Dolan, and Karl Friston. Exploration, novelty, surprise, and free energy minimization. *Front Psychol*, 4, October 2013.

[321] Robert M Sears, Hillary C Schiff, and Joseph E LeDoux. Molecular mechanisms of threat learning in the lateral nucleus of the amygdala. *Prog Mol Biol Transl Sci*, 122(1):263–304, 2014.

[322] M.-L. Selenica, H. S. Jensen, A. K. Larsen, M. L. Pedersen, L. Helboe, M. Leist, and J. Lotharius. Efficacy of small-molecule glycogen synthase kinase-3 inhibitors in the postnatal rat model of tau hyperphosphorylation. *Br. J. Pharmacol.*, 152(6):959–979, November 2007.

[323] Nancy A Shanahan, Kerri A Holick Pierz, Virginia L Masten, Christian Waeber, Mark Ansorge, Jay A Gingrich, Mark A Geyer, Rene Hen, and Stephanie C Dulawa. Chronic reductions in serotonin transporter function prevent 5-HT1b-induced behavioral effects in mice. *Biol. Psychiatry*, 65(5):401–408, March 2009.

[324] Nancy A Shanahan, Lady P Velez, Virginia L Masten, and Stephanie C Dulawa. Essential role for orbitofrontal serotonin 1b receptors in obsessive-compulsive disorder-like behavior and serotonin reuptake inhibitor response in mice. *Biol. Psychiatry*, 70(11):1039–1048, December 2011.

[325] Nathan C. Shaner, Paul A. Steinbach, and Roger Y. Tsien. A guide to choosing fluorescent proteins. *Nat. Methods*, 2(12):905–909, December 2005.

[326] Morgan Sheng and Michael E. Greenberg. The regulation and function of c-fos and other immediate early genes in the nervous system. *Neuron*, 4(4):477–485, April 1990.

[327] Sergey V Shmelkov, Adília Hormigo, Deqiang Jing, Catia C Proenca, Kevin G Bath, Till Milde, Evgeny Shmelkov, Jared S Kushner, Muhamed Baljevic, Iva Dincheva, Andrew J Murphy, David M Valenzuela, Nicholas W Gale, George D Yancopoulos, Ipe Ninan, Francis S Lee, and Shahin Rafii. Slitrk5 deficiency impairs corticostriatal circuitry and leads to obsessive-compulsive-like behaviors in mice. *Nat. Med.*, 16(5):598–602, 1p following 602, May 2010.

[328] Steven J Siegel, John C Talpos, and Mark A Geyer. Animal models and measures of perceptual processing in schizophrenia. *Neuroscience & Biobehavioral Reviews*, 37(9):2092–2098, 2013.

[329] Owen M Siggs and Bruce Beutler. The BTB-ZF transcription factors. *Cell Cycle*, 11(18):3358–3369, September 2012.

[330] E. Smeraldi, G. Diaferia, S. Erzegovesi, A. Lucca, L. Bellodi, and E. A. Moja. Tryptophan depletion in obsessive-compulsive patients. *Biol. Psychiatry*, 40(5):398–402, September 1996.

[331] Hannah R. Snyder, Roselinde H. Kaiser, Stacie L. Warren, and Wendy Heller. Obsessive-compulsive disorder is associated with broad impairments in executive function: A meta-analysis. *Clin Psychol Sci*, 3(2):301–330, March 2015.

[332] G M Soomro, D Altman, S Rajagopal, and M Oakley-Browne. Selective serotonin re-uptake inhibitors (SSRIs) versus placebo for obsessive compulsive disorder (OCD). *Cochrane Database Syst Rev*, (1):CD001765, 2008.

[333] G. Mustafa Soomro. Obsessive compulsive disorder. *BMJ Clin Evid*, 2012, 2012.

[334] Latha Soorya, Jessica Kiarashi, and Eric Hollander. Psychopharmacologic interventions for repetitive behaviors in autism spectrum disorders. *Child Adolesc Psychiatr Clin N Am*, 17(4):753–771, viii, October 2008.

[335] Amelie Soumier, Rayna M. Carter, Timothy J. Schoenfeld, and Heather A. Cameron. New Hippocampal Neurons Mature Rapidly in Response to Ketamine But Are Not Required for Its Acute Antidepressant Effects on Neophagia in Rats. *eNeuro*, 3(2), April 2016.

[336] Vladan Starcevic, David Berle, Vlasios Brakoulias, Peter Sammut, Karen Moses, Denise Milicevic, and Anthony Hannan. The nature and correlates of avoidance in obsessive-compulsive disorder. *Aust N Z J Psychiatry*, 45(10):871–879, October 2011.

[337] D. J. Stein, E. Hollander, L. S. Mullen, C. M. Decaria, and M. R. Liebowitz. Comparison of clomipramine, alprazolam and placebo in the treatment of obsessive—compulsive disorder. *Hum. Psychopharmacol. Clin. Exp.*, 7(6):389–395, November 1992.

[338] D. J. Stein, B. Van Heerden, C. J. Wessels, J. Van Kradenburg, J. Warwick, and H. J. Wasserman. Single photon emission computed tomography of the brain with Tc-99m HMPAO during sumatriptan challenge in obsessive-compulsive disorder: investigating the functional role of the serotonin auto-receptor. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 23(6):1079–1099, August 1999.

[339] Dan Stein, Nicholas Dodman, and Alice Moon-Fanelli. Cats and obsessive-compulsive disorder. *S Afr Med J*, 86:1614–5, 01 1997.

[340] Shari A. Steinman, Susanne E. Ahmari, Tse Choo, Marcia B. Kimeldorf, Rachel Feit, Sarah Loh, Victoria Risbrough, Mark A. Geyer, Joanna E. Steinglass, Melanie Wall, Franklin R. Schneier, Abby J. Fyer, and H. Blair Simpson. PREPULSE INHIBITION DEFICITS ONLY IN FEMALES WITH OBSESSIVE-COMPULSIVE DISORDER. *Depress Anxiety*, February 2016.

[341] Emily R. Stern and Stephan F. Taylor. Cognitive neuroscience of obsessive-compulsive disorder. *Psychiatr. Clin. North Am.*, 37(3):337–352, September 2014.

[342] S E Stewart, D Yu, J M Scharf, B M Neale, J A Fagerness, C A Mathews, P D Arnold, P D Evans, E R Gamazon, L K Davis, L Osiecki, L McGrath, S Haddad, J Crane, D Hezel, C Illman, C Mayerfeld, A Konkashbaev, C Liu, A Pluzhnikov, A Tikhomirov, C K Edlund, S L Rauch, R Moessner, P Falkai, W Maier, S Ruhrmann, H-J Grabe, L Lennertz, M Wagner, L Bellodi, M C Cavallini, M A Richter, E H Cook, Jr, J L Kennedy, D Rosenberg, D J Stein, S M J Hemmings, C Lochner, A Azzam, D A Chavira, E Fournier, H Garrido, B Sheppard, P Umaña, D L Murphy, J R Wendland, J Veenstra-VanderWeele, D Denys, R Blom, D Deforce, F Van Nieuwerburgh, H G M Westenberg, S Walitza, K Egberts, T Renner, E C Miguel, C Cappi, A G Hounie, M Conceição do Rosário, A S Sampaio, H Vallada, H Nicolini, N Lanzagorta, B Camarena, R Delorme, M Leboyer, C N Pato, M T Pato, E Voyiaziakis, P Heutink, D C Cath, D Posthuma, J H Smit, J Samuels, O J Bienvenu, B Cullen, A J Fyer, M A Grados, B D Greenberg, J T McCracken, M A Riddle, Y Wang, V Coric, J F Leckman, M Bloch, C Pittenger, V Eapen, D W Black, R A Ophoff, E Strengman, D Cusi, M Turiel, F Frau, F Macciardi, J R Gibbs, M R Cookson, A Singleton, North American Brain Expression Consortium, J Hardy, UK Brain Expression Database, A T Crenshaw, M A Parkin, D B Mirel, D V Conti, S Purcell, G Nestadt, G L Hanna, M A Jenike, J A Knowles, N Cox, and D L Pauls. Genome-wide association study of obsessive-compulsive disorder. *Mol. Psychiatry*, 18(7):788–798, July 2013.

[343] Bryan A. Strange, Menno P. Witter, Ed S. Lein, and Edvard I. Moser. Functional organization of the hippocampal longitudinal axis. *Nat. Rev. Neurosci.*, 15(10):655–669, October 2014.

[344] R. F. Suckow, M. F. Zhang, and T. B. Cooper. Sensitive and selective liquid-chromatographic assay of fluoxetine and norfluoxetine in plasma with fluorescence detection after precolumn derivatization. *Clinical Chemistry*, 38(9):1756–1761, September 1992.

[345] N R Swerdlow, C H Benbow, S Zisook, M A Geyer, and D L Braff. A preliminary assessment of sensorimotor gating in patients with obsessive compulsive disorder. *Biol. Psychiatry*, 33(4):298–301, February 1993.

[346] H. Szechtman, M. J. Eckert, W. S. Tse, J. T. Boersma, C. A. Bonura, J. Z. McClelland, K. E. Culver, and D. Eilam. Compulsive checking behavior of quinpirole-sensitized rats as an animal model of Obsessive-Compulsive Disorder(OCD): form and control. *BMC Neurosci*, 2:4, 2001.

[347] H. Szechtman, W. Sulis, and D. Eilam. Quinpirole induces compulsive checking behavior in rats: a potential animal model of obsessive-compulsive disorder (OCD). *Behav. Neurosci.*, 112(6):1475–1485, December 1998.

[348] Keizo Takao, Katsunori Kobayashi, Hideo Hagihara, Koji Ohira, Hirotaka Shoji, Satoko Hattori, Hisatsugu Koshimizu, Juzoh Umemori, Keiko Toyama, Hironori K. Nakamura, Mahomi Kuroiwa, Jun Maeda, Kimie Atsuzawa, Kayoko Esaki, Shun Yamaguchi, Shigeki Furuya, Tsuyoshi Takagi, Noah M. Walton, Nobuhiro Hayashi, Hidenori Suzuki, Makoto Higuchi, Nobuteru Usuda, Tetsuya Suhara, Akinori Nishi, Mitsuyuki Matsumoto, Shunsuke Ishii, and Tsuyoshi Miyakawa. Deficiency of schnurri-2, an MHC enhancer binding protein, induces mild chronic inflammation in the brain and confers molecular, neuronal, and behavioral phenotypes related to schizophrenia. *Neuropsychopharmacology*, 38(8):1409–1425, July 2013.

[349] Shoji Tanaka, Jared W Young, Adam L Halberstadt, Virginia L Masten, and Mark A Geyer. Four factors underlying mouse behavior in an open field. *Behav. Brain Res.*, 233(1):55–61, July 2012.

[350] Matthew J. Taylor, Nick Freemantle, John R. Geddes, and Zubin Bhagwagar. Early onset of selective serotonin reuptake inhibitor antidepressant action: systematic review and meta-analysis. *Arch. Gen. Psychiatry*, 63(11):1217–1223, November 2006.

[351] S Taylor. Molecular genetics of obsessive-compulsive disorder: a comprehensive meta-analysis of genetic association studies. *Mol. Psychiatry*, 18(7):799–805, July 2013.

[352] TECHNICAL recommendations for psychological tests and diagnostic techniques. *Psychol Bull*, 51(2:2):1–38, March 1954.

[353] L. Tenenbaum, F. Jurysta, A. Stathopoulos, Z. Puschban, C. Melas, W. T. Hermens, J. Verhaagen, B. Pichon, T. Velu, and M. Levivier. Tropism of AAV-2 vectors for neurons of the globus pallidus. *Neuroreport*, 11(10):2277–2283, July 2000.

[354] Alexia Thomas, April Burant, Nghiem Bui, Deanna Graham, Lisa A. Yuva-Paylor, and Richard Paylor. Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology (Berl.)*, 204(2):361–373, June 2009.

[355] P. Thorén, M. Asberg, B. Cronholm, L. Jörnestedt, and L. Träskman. Clomipramine treatment of obsessive-compulsive disorder. I. A controlled clinical trial. *Arch. Gen. Psychiatry*, 37(11):1281–1285, November 1980.

[356] L.L. Thurstone. *The reliability and validity of tests*. Edwards, Oxford, England, 1932.

[357] D. Treit, J. P. Pinel, and H. C. Fibiger. Conditioned defensive burying: a new paradigm for the study of anxiolytic agents. *Pharmacol. Biochem. Behav.*, 15(4):619–626, October 1981.

[358] J. K. Trivedi, M. Dhyani, D. Goel, S. Sharma, A. P. Singh, P. K. Sinha, and R. Tandon. Neurocognitive dysfunction in patients with obsessive compulsive disorder. *Afr J Psychiatry (Johannesbg)*, 11(3):204–209, August 2008.

[359] Eleftheria Tsaltas, Dimitris Kontis, Sofia Chrysikakou, Haralambos Giannou, Angeliki Biba, Stella Pallidi, Angeliki Christodoulou, Antonis Maillis, and Andreas Rabavilas. Reinforced spatial alternation as an animal model of obsessive-compulsive disorder (OCD): investigation of 5-HT2c and 5-HT1d receptor involvement in OCD pathophysiology. *Biol. Psychiatry*, 57(10):1176–1185, May 2005.

[360] Mark C. Tucci, Anna Dvorkin-Gheva, Dawn Graham, Sean Amodeo, Paul Cheon, Ashley Kirk, John Peel, Leena Taji, and Henry Szechtman. Effects of the serotonergic agonist mCPP on male rats in the quinpirole sensitization model of obsessive-compulsive disorder (OCD). *Psychopharmacology (Berl.)*, 227(2):277–285, May 2013.

[361] Cortney A. Turner and Mark H. Lewis. Environmental enrichment: effects on stereotyped behavior and neurotrophin levels. *Physiol. Behav.*, 80(2-3):259–266, November 2003.

[362] Cortney A. Turner, Mark H. Lewis, and Michael A. King. Environmental enrichment: effects on stereotyped behavior and dendritic morphology. *Dev Psychobiol*, 43(1):20–27, July 2003.

[363] Nikhil M. Urs, Steven M. Gee, Thomas F. Pack, John D. McCorvy, Tama Evron, Joshua C. Snyder, Xiaobao Yang, Ramona M. Rodriguiz, Emiliana Borrelli, William C. Wetsel, Jian Jin, Bryan L. Roth, Patricio O'Donnell, and Marc G. Caron. Distinct cortical and striatal actions of a β-arrestin-biased dopamine D2 receptor ligand reveal unique antipsychotic-like properties. *Proc. Natl. Acad. Sci. U.S.A.*, 113(50):E8178–E8186, December 2016.

[364] Matilde M. Vaghi, Petra E. Vértes, Manfred G. Kitzbichler, Annemieke M. Apergis-Schoute, Febe E. van der Flier, Naomi A. Fineberg, Akeem Sule, Rashid Zaman, Valerie Voon, Prantik Kundu, Edward T. Bullmore, and Trevor W. Robbins. Specific Frontostriatal Circuits for Impaired Cognitive Flexibility and Goal-Directed Planning in Obsessive-Compulsive Disorder: Evidence From Resting-State Functional Connectivity. *Biol. Psychiatry*, 81(8):708–717, April 2017.

[365] Matthijs van der Meer, Zeb Kurth-Nelson, and A. David Redish. Information processing in decision-making systems. *Neuroscientist*, 18(4):342–359, August 2012.

[366] Nic J. A. van der Wee, Nick F. Ramsey, Johan M. Jansma, Damiaan A. Denys, Harold J. G. M. van Megen, Herman M. G. Westenberg, and René S. Kahn. Spatial working memory deficits in obsessive compulsive disorder are associated with excessive engagement of the medial frontal cortex. *Neuroimage*, 20(4):2271–2280, December 2003.

[367] Nic J. A. van der Wee, Nick F. Ramsey, Harold J. G. M. van Megen, Damiaan Denys, Herman G. M. Westenberg, and René S. Kahn. Spatial working memory in obsessive-compulsive disorder improves with clinical response: A functional MRI study. *Eur Neuropsychopharmacol*, 17(1):16–23, January 2007.

[368] Daniël S van Grootheest, Daniëlle C Cath, Aartjan T Beekman, and Dorret I Boomsma. Twin studies on obsessive-compulsive disorder: a review. *Twin Res Hum Genet*, 8(5):450–458, October 2005.

[369] Kris van Kuyck, Katrien Brak, John Das, Dimitris Rizopoulos, and Bart Nuttin. Comparative study of the effects of electrical stimulation in the nucleus accumbens, the mediodorsal thalamic nucleus and the bed nucleus of the stria terminalis in rats with schedule-induced polydipsia. *Brain Res.*, 1201:93–99, March 2008.

[370] Kris van Kuyck, Hilde Demeulemeester, Hilde Feys, Willy De Weerdt, Maarten Dewil, Thomas Tousseyn, Paul De Sutter, Jan Gybels, Kris Bogaerts, Rene Dom, and Bart Nuttin. Effects of electrical stimulation or lesion in nucleus accumbens on the behaviour of rats in a T-maze after administration of 8-OH-DPAT or vehicle. *Behav. Brain Res.*, 140(1-2):165–173, March 2003.

[371] David Veale, Sarah Miles, Nicola Smallcombe, Haben Ghezai, Ben Goldacre, and John Hodsoll. Atypical antipsychotic augmentation in SSRI treatment refractory obsessive-compulsive disorder: a systematic review and meta-analysis. *BMC Psychiatry*, 14:317, 2014.

[372] Robert P. Vertes. Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. *Neuroscience*, 142(1):1–20, September 2006.

[373] Biju Viswanath, Y. C. Janardhan Reddy, Keshav J. Kumar, Thennarasu Kandavel, and C. R. Chandrashekar. Cognitive endophenotypes in OCD: a study of unaffected siblings of probands with familial OCD. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 33(4):610–615, June 2009.

[374] G. Vogel, D. Neill, M. Hagler, and D. Kors. A new animal model of endogenous depression: a summary of present findings. *Neurosci Biobehav Rev*, 14(1):85–91, 1990.

[375] V. Voon, K. Derbyshire, C. Rück, M. A. Irvine, Y. Worbe, J. Enander, L. R. N. Schreiber, C. Gillan, N. A. Fineberg, B. J. Sahakian, T. W. Robbins, N. A. Harrison, J. Wood, N. D. Daw, P. Dayan, J. E. Grant, and E. T. Bullmore. Disorders of compulsivity: a common bias towards learning habits. *Mol. Psychiatry*, 20(3):345–352, March 2015.

[376] Valerie Voon, Andrea Reiter, Miriam Sebold, and Stephanie Groman. Model-Based Control in Dimensional Psychiatry. *Biol. Psychiatry*, 82(6):391–400, September 2017.

[377] Peter De Vries, Carlos M. Villalón, and Pramod R. Saxena. Pharmacology of triptans. *Emerging Drugs*, 4(1):107–125, January 1999.

[378] Lizheng Wang, Zixuan Wang, Fangfang Zhang, Rui Zhu, Jinpeng Bi, Jiaxin Wu, Haihong Zhang, Hui Wu, Wei Kong, Bin Yu, and Xianghui Yu. Enhancing Transgene Expression from Recombinant AAV8 Vectors in Different Tissues Using Woodchuck Hepatitis Virus Post-Transcriptional Regulatory Element. *Int J Med Sci*, 13(4):286–291, 2016.

[379] Laura H. Watkins, Barbara J. Sahakian, Mary M. Robertson, David M. Veale, Robert D. Rogers, Kathryn M. Pickard, Michael R. F. Aitken, and Trevor W. Robbins. Executive function in Tourette's syndrome and obsessive-compulsive disorder. *Psychol Med*, 35(4):571–582, April 2005.

[380] Jeffrey M. Welch, Jing Lu, Ramona M. Rodriguiz, Nicholas C. Trotta, Joao Peca, Jin-Dong Ding, Catia Feliciano, Meng Chen, J. Paige Adams, Jianhong Luo, Serena M. Dudek, Richard J. Weinberg, Nicole Calakos, William C. Wetsel, and Guoping Feng. Cortico-striatal synaptic defects and OCD-like behaviors in SAPAP3 mutant mice. *Nature*, 448(7156):894–900, August 2007.

[381] Vanessa C. Wheeler, Wojtek Auerbach, Jacqueline K. White, Jayalakshmi Srinidhi, Anna Auerbach, Angela Ryan, Mabel P. Duyao, Vladimir Vrbanac, Meredith Weaver, James F. Gusella, Alexandra L. Joyner, and Marcy E. MacDonald. Length-dependent gametic cag repeat instability in the huntington's disease knock-in mouse. *Human Molecular Genetics*, 8(1):115–122, Jan 1999.

[382] P. Willner. The validity of animal models of depression. *Psychopharmacology (Berl.)*, 83(1):1–16, 1984.

[383] P. Willner. Validation criteria for animal models of human mental disorders: learned helplessness as a paradigm case. *Prog. Neuropsychopharmacol. Biol. Psychiatry*, 10(6):677–690, 1986.

[384] P. Willner. Animal models of depression: validity and applications. *Adv. Biochem. Psychopharmacol.*, 49:19–41, 1995.

[385] Christine Winter, Adrian Mundt, Rafed Jalali, Daphna Joel, Daniel Harnack, R. Morgenstern, Georg Juckel, and A. Kupsch. High frequency stimulation and temporary inactivation of the subthalamic nucleus reduce quinpirole-induced compulsive checking behavior in rats. *Exp. Neurol.*, 210(1):217–228, March 2008.

[386] Nancy S Woehrle, Stephanie J Klenotich, Naseem Jamnia, Emily V Ho, and Stephanie C Dulawa. Effects of chronic fluoxetine treatment on serotonin 1b receptor-induced deficits in delayed alternation. *Psychopharmacology (Berl.)*, 227(3):545–551, June 2013.

[387] De Wet Wolmarans, Linda Brand, Dan J. Stein, and Brian H. Harvey. Reappraisal of spontaneous stereotypy in the deer mouse as an animal model of obsessive-compulsive disorder (OCD): response to escitalopram treatment and basal serotonin transporter (SERT) density. *Behav. Brain Res.*, 256:545–553, November 2013.

[388] A. Woods, C. Smith, M. Szewczak, R. W. Dunn, M. Cornfeldt, and R. Corbett. Selective serotonin re-uptake inhibitors decrease schedule-induced polydipsia in rats: a potential model for obsessive compulsive disorder. *Psychopharmacology (Berl.)*, 112(2-3):195–198, 1993.

[389] Meiyu Xu, Andrew Kobets, Jung-Chieh Du, Jessica Lennington, Lina Li, Mounira Banasr, Ronald S Duman, Flora M Vaccarino, Ralph J DiLeone, and Christopher Pittenger. Targeted ablation of cholinergic interneurons in the dorsolateral striatum produces behavioral manifestations of tourette syndrome. *Proceedings of the National Academy of Sciences*, 112(3):893–898, 2015.

[390] Meiyu Xu, Lina Li, Hiroshi Ohtsu, and Christopher Pittenger. Histidine decarboxylase knockout mice, a genetic model of tourette syndrome, show repetitive grooming after induced fear. *Neuroscience letters*, 595:50–53, 2015.

[391] E. Yadin, E. Friedman, and W. H. Bridger. Spontaneous alternation behavior: an animal model for obsessive-compulsive disorder? *Pharmacol. Biochem. Behav.*, 40(2):311–315, October 1991.

[392] Jared W. Young, Arpi Minassian, Martin P. Paulus, Mark A. Geyer, and William Perry. A reverse-translational approach to bipolar disorder: rodent and human studies in the Behavioral Pattern Monitor. *Neurosci Biobehav Rev*, 31(6):882–896, 2007.

[393] Pazit Zadicario, Sharon Ronen, and David Eilam. Modulation of quinpirole-induced compulsive-like behavior in rats by environmental changes: implications for OCD rituals and for exploration and navigation. *BMC Neurosci*, 8:23, 2007.

[394] Panos Zanos, Mackenzie E. Nelson, Jaclyn N. Highland, Samuel R. Krimmel, Polymnia Georgiou, Todd D. Gould, and Scott M. Thompson. A Negative Allosteric Modulator for $\alpha 5$ Subunit-Containing GABA Receptors Exerts a Rapid and Persistent Antidepressant-Like Action without the Side Effects of the NMDA Receptor Antagonist Ketamine in Mice. *eNeuro*, 4(1), February 2017.

[395] Panos Zanos, Sean C. Piantadosi, Hui-Qiu Wu, Heather J. Pribut, Matthew J. Dell, Adem Can, H. Ralph Snodgrass, Carlos A. Zarate, Robert Schwarcz, and Todd D. Gould. The Prodrug 4-Chlorokynurenine Causes Ketamine-Like Antidepressant Effects, but Not Side Effects, by NMDA/GlycineB-Site Inhibition. *J Pharmacol Exp Ther*, 355(1):76–85, October 2015.

[396] Carlos A. Zarate, Jaskaran B. Singh, Paul J. Carlson, Nancy E. Brutsche, Rezvan Ameli, David A. Luckenbaugh, Dennis S. Charney, and Husseini K. Manji. A random-

ized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. *Arch. Gen. Psychiatry*, 63(8):856–864, August 2006.

[397] Wenjun Zhou, Ligong Chen, Jodi Paul, Sufen Yang, Fuzeng Li, Karen Sampson, Jim R. Woodgett, Jean Martin Beaulieu, Karen L. Gamble, and Xiaohua Li. The effects of glycogen synthase kinase-3beta in serotonin neurons. *PLoS ONE*, 7(8):e43262, 2012.

[398] Isaac D. Zike, Muhammad O. Chohan, Jared M. Kopelman, Emily N. Krasnow, Daniel Flicker, Katherine M. Nautiyal, Michael Bubser, Christoph Kellendonk, Carrie K. Jones, Gregg Stanwood, Kenji Fransis Tanaka, Holly Moore, Susanne E. Ahmari, and Jeremy Veenstra-VanderWeele. Ocd candidate gene slc1a1/eaat3 impacts basal ganglia-mediated activity and stereotypic behavior. *Proceedings of the National Academy of Sciences*, 114(22):5719–5724, 2017.

[399] J. Zohar, T. R. Insel, R. C. Zohar-Kadouch, J. L. Hill, and D. L. Murphy. Serotonergic responsivity in obsessive-compulsive disorder. Effects of chronic clomipramine treatment. *Arch. Gen. Psychiatry*, 45(2):167–172, February 1988.

[400] J. Zohar, E. A. Mueller, T. R. Insel, R. C. Zohar-Kadouch, and D. L. Murphy. Serotonergic responsivity in obsessive-compulsive disorder. Comparison of patients and healthy controls. *Arch. Gen. Psychiatry*, 44(11):946–951, November 1987.

[401] Joseph Zohar, James L. Kennedy, Eric Hollander, and Lorrin M. Koran. Serotonin-1d hypothesis of obsessive-compulsive disorder: an update. *J Clin Psychiatry*, 65 Suppl 14:18–21, 2004.

[402] R. Zor, H. Keren, H. Hermesh, H. Szechtman, J. Mort, and D. Eilam. Obsessive-compulsive disorder: a disorder of pessimal (non-functional) motor behavior. *Acta Psychiatr Scand*, 120(4):288–298, October 2009.

[403] Rama Zor, Naomi Fineberg, David Eilam, and Haggai Hermesh. Video telemetry and behavioral analysis discriminate between compulsive cleaning and compulsive checking in obsessive-compulsive disorder. *Eur Neuropsychopharmacol*, 21(11):814–824, November 2011.

[404] Rama Zor, Haggai Hermesh, Henry Szechtman, and David Eilam. Turning order into chaos through repetition and addition of elementary acts in obsessive-compulsive disorder (OCD). *World J. Biol. Psychiatry*, 10(4 Pt 2):480–487, 2009.

[405] Rama Zor, Henry Szechtman, Haggai Hermesh, Naomi A. Fineberg, and David Eilam. Manifestation of incompleteness in obsessive-compulsive disorder (OCD) as reduced functionality and extended activity beyond task completion. *PLoS ONE*, 6(9):e25217, 2011.

[406] Junhui Zou, Wenbin Wang, Yung-Wei Pan, Song Lu, and Zhengui Xia. Methods to measure olfactory behavior in mice. *Curr Protoc Toxicol*, 63:11.18.1–21, February 2015.

[407] Lars-Göran Öst, Audun Havnen, Bjarne Hansen, and Gerd Kvale. Cognitive behavioral treatments of obsessive-compulsive disorder. A systematic review and meta-analysis of studies published 1993-2014. *Clin Psychol Rev*, 40:156–169, June 2015.