CIRCUIT-SPECIFIC INHIBITION OF DOPAMINERGIC SIGNALING ASSOCIATED WITH PHANTOM GUSTATORY SENSATIONS IN DISRUPTED-IN-SCHIZOPHRENIA-1 MICE

By

Benjamin R. Fry

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Psychology—Doctor of Philosophy

2022

ABSTRACT

CIRCUIT-SPECIFIC INHIBITION OF DOPAMINERGIC SIGNALING ASSOCIATED WITH PHANTOM GUSTATORY SENSATIONS IN DISRUPTED-IN-SCHIZOPHRENIA-1 MICE

By

Benjamin R. Fry

Schizophrenia is a severe neuropsychiatric disorder characterized by a suite a symptoms occurring across three separate domains: cognitive (delayed processing, paraphasia, attentional deficits), negative (anhedonia, blunted affect, catatonia), and positive (hallucinations, delusions). While much has been learned about the neurobiological underpinnings of cognitive and negative symptoms, less is known about the positive, due in part to a lack of appropriate preclinical animal models. Treatment modalities for positive symptomology have progressed little since the discovery of dopamine receptor 2 (DRD2) antagonist antipsychotic drugs in the 1950s; side effects can be severe, and treatment adherence is a frequent issue among patients. Animal modeling of positive symptomology is an important step toward developing new treatment modalities through a deeper understanding of the neurobiology involved.

The experiments outlined in this dissertation leverage Pavlovian behavioral procedures combined with chemogenetic and transgenic manipulations to explore the role of mesocortical dopaminergic circuitry in positive-like symptomology in mice expressing a dominant-negative form of the *disrupted-in-schizophrenia-1* (DN-DISC-1) gene. Specifically, these experiments look to test the hypothesis that deficits in dopaminergic circuitry projecting from the ventral-tegmental area (VTA) to the insular cortex (IC) in the region underlie the experience of impaired reality testing (IRT) associated with phantom gustatory sensations in DISC-1 mice.

Experiments in Chapters 2 – 3 look to determine whether the VTA → IC dopamine circuit is necessary for the perception of taste, motivation to consume, and motivation to obtain

sweet tasting rewards. Chapter 4 experiments look at the ability for DISC-1 mice to learn simple Pavlovian reward discriminations as well as the effect of inactivating the VTA → IC dopamine circuit on consummatory measures directed at unflavored water when in the presence of a Pavlovian cue which previously signaled the delivery of sucrose. Chapter 5 experiments utilize quantitative real-time polymerase chain reaction (qPCR) and immunohistology to determine whether or not there are any differences in either the amount of dopamine neurons projecting from the VTA → IC or DRD1 and DRD2 expression in these regions in addition to multiple other sites along the mesocorticolimbic pathway for DISC-1 mice as compared to wild-type mice.

Overall, I found that inactivation of the VTA → IC dopamine circuit does not affect general measures of consummatory behavior or motivation to attain reward. Under conditions of Pavlovian discrimination, however, the circuit shows genotype-specific effects in both wild-type and DISC-1 mice. In the presence of a cue which was previously paired with sucrose, wild-type mice tend to show increased IRT as evidenced by enhancements in measures of licking microstructure associated with palatability as directed at unflavored water. DISC-1, however, show no alterations in their consummatory behavior, but a normalization of their otherwise aberrant approach behavior. These effects may be due to differences at the dopaminergic connections between the VTA and IC as revealed in Chapter 5. Together, these studies add to a growing body of literature concerning the behavioral and neurobiological aberrations that result from perturbations of the DISC-1 genetic locus.

This work is dedicated to all first-generation college students.
To all high school dropouts with a dream, and most especially,
To anyone who would tell them otherwise.
Be wary of those who cultivate the attitude of defeat.
Personal responsibility is the most sacred of all virtues.

AKNOWLEDGEMENTS

I would first like to thank my advisor, Professor Alex Johnson. Alex's application of firm and consistent expectations has supported me through a period of self-overcoming the likes of which I did not know was possible. Beyond intellectual challenge, Alex seemed to know exactly what I needed in terms of boundaries and accountability in order to grow. As a result, I can honestly say that I have become not only a better scholar, but a better person under his mentorship.

Next, I would like to thank all the members of my committee, including Professors Joe Lonstein, Katy Thakkar, and Michelle Mazei-Robison for their time in supporting my research as well as offering different perspectives on various empirical issues. Thank you, Katy, in particular for allowing me to gain more translational insight into schizophrenia research through collaboration with your group outside of this dissertation. I'd also like to thank Professors Lily Yan and Amy Arguello for providing me with teaching opportunities and helping to support my career goals beyond the Ph.D.

Finally, I have worked with a number of outstanding undergraduate research assistants throughout the last five years including: Andrew McLocklin, Grace Sarris, Rosalyn Schaefer, Nathan Pence, and Victoria Fex. Each of you helped me to hone my mentorship and teaching skills in a multitude of ways, in addition to providing support for my research projects, much of which would have been a far more difficult without your assistance and friendship along the way. I know you will all continue on toward great things.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

5-HT$_{2a}$	Serotonin receptor subtype 2a
BLA	Basolateral amygdala
CNO	Clozapine *N*-oxide
CS	Conditioned stimulus
CT	Cycle threshold
CTA	Conditioned taste aversion
DAT	Dopamine transporter
DISC-1	Disrupted-in-schizophrenia-1 (gene)
DN-DISC-1	Dominant-negative expression of DISC-1
DREADD	Designer receptors exclusively activated by designer drugs
DRD1	Dopamine receptor subtype 1
DRD2	Dopamine receptor subtype 2
EPSE	Extrapyramidal side-effect
HPRT	Hypoxanthine-guanine phosphoribosyltransferase
IC	Insular Cortex (gustatory cortex)
IP	Intraperitoneal
IRT	Impaired reality testing
LiCl	Lithium chloride
LIS1	Platelet-activating factor acetylhydrolase IB subunit alpha
NAc	Nucleus Accumbens
NDEL1	Nuclear distribution protein nudE-like 1

NVHL	Neonatal ventral hippocampal lesion (animal model of schizophrenia)
PB	Phosphate buffer
PBS	Phosphate buffered saline
PBP	Parabrachial pigmented nucleus of the VTA
PLCβ1-/-	Phospholipase C β1 knock-out (animal model of schizophrenia)
PrP	Prion protein promoter
PVB	Parvalbumin
qPCR	Quantitative real-time polymerase chain reaction
RPE	Reward Prediction Error
RMTA	Representation mediated taste aversion
SNR	Substantia Nigra
TH	Tyrosine-hydroxylase
US	Unconditioned stimulus
VTA	Ventral Tegmental Area

CHAPTER 1: INTRODUCTION

Schizophrenia is a severe neuropsychiatric illness associated with lifelong impairments in functioning that affects roughly 1% of the adult population at any given time (McGrath, Saha, Chant, & Welham, 2008). The disorder is characterized by a suite of symptoms occurring across three separate domains: cognitive (e.g., impaired processing, disorganized speech, difficulty maintaining attention), negative (e.g., flattened affect, anhedonia, catatonia, avolition), and positive (e.g., hallucinations and delusions). This complex admixture typically leads to lifetime impairments in functioning including chronic unemployment (Marwaha & Johnson, 2004), increased suicidality (Palmer et al., 2005), and a reduction in the average lifespan by at least 9 years (Jobe & Harrow, 2010), with some estimates ranging as high as 18.7 (Laursen, 2011). Individuals suffering from the disorder often begin to manifest symptoms in the positive and negative category during their early 20s, while cognitive impairments tend to present at a much earlier age, the degree of which frequently predicts the severity of disorder progression (Bowie & Harvey, 2006).

The potential early onset of cognitive symptoms suggests a neurodevelopmental component together with genetic risk which has been the subject of intense study in recent years. At this point, we know that heritability among identified neurophysiological and neurocognitive behavioral endophenotypes ranges from 24-55% (Greenwood et al., 2007) and that having a first-degree relative with schizophrenia increases the risk that an individual will be diagnosed with the disorder themselves 6.3-fold (Chou et al., 2017). In addition to what is known about heritability, there seem to be a number of subtle epigenetic interactions with environment (i.e., heritable alterations in phenotypes as a result of external factors that occur without changing

underlying DNA sequences), the most well-known of which involves urbanicity (Krabbendam & Van Os, 2005).

Research into the neurobiological mechanisms underlying schizophrenia began with chlorpromazine studies in the 1950s (Delay, Deniker, & Harl, 1952) and the later discovery that the drug exerted its antipsychotic effects by way of blockading dopamine D2 receptors (D2Rs; Seeman & Lee, 1975; Creese, Burt, & Snyder, 1976; Seeman, Lee, Chau-Wong, & Wong, 1976). This paved the way for an enduring hypothesis that suggested aberrations in dopaminergic signaling as a core component of the disorder (Howes & Kapur, 2009). More recent versions of the dopamine hypothesis have focused on aberrations in nigrostriatal dopamine specifically, though without entirely ruling out issues in the mesolimbic path (McCutcheon, Abi-Dargham, & Howes, 2019). The observation that ketamine and PCP also induce psychotic symptoms in humans suggests a broader network of neuropathology to include the glutamatergic system (Luby & Gottlieb, 1962; Javitt & Zukin, 1991). The glutamate hypothesis of schizophrenia, however, does not exclude the dopamine hypothesis; rather, it posits that NMDA receptor hypofunction in schizophrenia has an effect of increasing glutamate neurotransmission globally and overexciting the glutamate receptors of other classes (e.g., AMPA). This increased rate of glutamatergic firing leads to a loss of cortical synchrony and increased oxidative stress (Moghaddam & Javitt, 2012). Worth noting, cortical glutamatergic afferents terminate at the level of GABAergic medium spiny neurons (MSNs) in the striatum. These MSNs also receive dopaminergic input. Hyperactive DRD2 signaling (as proposed by aspects of the dopamine hypothesis; e.g., Adams, 2018) at the level of these MSNs leads to a feedback loop whereby signaling at NMDA receptors is decreased due to enhanced inhibition on cortical glutamatergic neurons (Laruelle, Kegeles, & Abi-Dargham, 2003). More recently, others have looked to the

hypofrontality hypothesis, a theory which posits that a loss of GABAergic interneurons in the frontal/cortical regions leads to a loss of synchrony among glutamatergic pyramidal firing and subsequent cognitive deficits associated with alterations in gamma oscillatory activity (Dienel & Lewis, 2019). More generally, functional neuroimaging has consistently shown swelling of the lateral ventricles accompanied by reductions in temporal lobe, striatum, and thalamus volume to be a hallmark of the disorder (Gaser et al., 2004).

Despite the fact that we now know schizophrenia is associated with a multitude of central nervous system changes, treatment modalities have progressed shockingly little since the famous emptying of the asylums in the 1950s (López-Muñoz et al., 2005), with D2R antagonism as the mainstay (Lieberman et al., 2005). This is problematic for a number of reasons, particularly given that treatment resistance to antipsychotic therapy is seen in 10 – 30% of patients (Safferman et al., 1991). Global blockade of D2Rs brings with it a number of unintended severe side effects for individuals with schizophrenia, including extrapyramidal side effects (EPSEs; e.g. tardive dyskinesia, parkinsonism, akathisia), cardiometabolic changes (e.g. weight gain, heart arrhythmias, myocarditis, cardiomyopathy), and a worsening of negative symptomology. While second-generation (atypical) antipsychotics (e.g., clozapine, risperidone, olanzapine) that target 5-HT_{2a} and noradrenergic receptors in addition to D2Rs are less likely to cause EPSEs, they are more likely to cause cardiometabolic events, and provide no improvement over first-generation antipsychotics (e.g. chlorpromazine, perphenazine, haloperidol) with regard to paradoxical increases in negative symptomology (Lally & MacCabe, 2015). This is likely due to the fact that the positive symptoms of the disorder are known to be related to elevated midbrain dopamine signaling, while the negative are associated with decreased frontal/cortical dopamine (Howes & Kapur, 2009; Walton et al., 2018). As such, while antipsychotics show marked

efficacy for decreasing hallucinations and delusions, they often make aspects related to depression and flattened affect worse.

Clearly, a more targeted investigation of the neurobiology underlying positive symptomology is warranted; yet, historically, such research has lagged behind other advances in neuropsychiatric treatment due to a paucity of appropriate pre-clinical models (Feifel & Schilling, 2010). Schizophrenia presents with a number of challenges related to pre-clinical modeling, primarily due to the heterogenous nature of the disorder. As such, common models (e.g., neonatal ventral hippocampal lesion [NVHL], chronic NMDA antagonism) may excel in recreating one aspect of disordered functioning (e.g., deficits in pre-pulse inhibition), while failing to encapsulate others (e.g., social withdrawal). That said, taken as a whole, animal modeling of both negative (Der-Avakian & Markou, 2012; Ferenczi et al., 2016) and cognitive (Nestler & Hyman, 2010; Johnson et al., 2013) symptomology has been relatively successful; yet, researchers have been more reticent with respect to positive symptoms due to their perception as uniquely human phenomena (Feifel & Schilling, 2010). Recently, however, behavioral procedures used to investigate associative learning have been leveraged to evoke states in both humans (Powers, Mathys, & Corlett, 2017) and animals (McDannald et al., 2011; Kim & Koh, 2015; Koh, Ahrens, & Gallagher, 2018; Fry et al., 2019; Wu, Haberman, Gallagher, & Koh, 2020) that may mimic positive-like symptomology; specifically, the experience of impaired reality testing (IRT). IRT refers to a state in which an agent comes to respond to internal representations that do not conform with external reality, a diagnostic feature of psychosis[1] (American Psychiatric Association, 2013).

[1] Psychosis can occur across a range of conditions but is most severe and disabling as manifest in the positive symptoms of schizophrenia.

Using Mediated Learning Procedures to Model Reality Testing

Drawing on the work of theorists interested in the link between mental imagery and the motivational states observed in classical conditioning (e.g., Holland, 1990), McDannald & Schoenbaum (2009) were the first to propose that Pavlovian behavioral procedures focused on mediated learning might be a valid tool for studying the neurobiology of IRT in animals. Their ideas hinged on a body of work reaching back more than a quarter of a century (Holland, 1981; Holland & Forbes, 1982; Holland, 1983; Holland, 1990; Holland, 1998; Holland, 2005) which showed that, early in the course of learning, a conditioned stimulus (CS) gains access to vivid perceptual representations of the sensory features (e.g., taste, smell) of the rewards (i.e., an unconditioned stimulus; US) they come to predict. Over time, however, this access narrows to a point in which the CS no longer evokes perceptual representations, but rather a reflexive preparatory response associated with receiving the predicted outcome (Fig. 1). Further, under certain conditions, CS evoked perceptual representations can become confused with external reality.

The most basic demonstration of this phenomenon occurs when one modifies the procedure for traditional conditioned tasted aversion (CTA). Briefly, CTA involves pairing a food reward (e.g., sucrose) with an illness inducing agent like lithium chloride (LiCl)—when presented with the food at a later time point, an animal will consume less of it, as its taste now evokes aversion by its previous association with gastric malaise. By contrast, Holland's work showed that one can evoke a taste aversion toward a food without ever pairing the food itself with illness; rather, a CS (e.g., a light or tone) previously associated with delivery of the food (i.e., the US) is paired with LiCl. Here, the idea is that because the US and the LiCl were never presented together, any changes in behavior towards the US following pairing of the CS and LiCl

must have been mediated by the ability of the CS to evoke some sort of representation (or memory) of the perceptual features of the absent US. This perceptual representation then becomes associated with illness leading to as a *representation mediated taste aversion* (RMTA; Holland, 1981) to the US. In this sense, when the animal is later presented with the food US and displays consummatory behaviors indicative of an aversion, it is acting in a manner which does not align with reality. Indeed, such behaviors have previously been discussed within a framework of IRT (e.g., McDannald & Schoenbaum, 2009). This idea is bolstered by studies showing that several animal models of schizophrenia, including the neonatal ventral hippocampal lesion model (McDannald et al., 2011), phospholipase C β1 knock-out model (Kim & Koh, 2016), and ketamine model (Koh, Ahrens, & Gallagher, 2018) show increased susceptibility to RMTA.

RMTA suggests that CSs gain access to a rich array of detailed information about the rewards they predict. Accordingly, CSs can substitute for rewards, such that animals perceive features of these rewards (e.g., taste and their flavor) without the rewards themselves being presented. This capacity of CSs to gain access to processing pathways typically activated by the US (e.g., sucrose) also endows them with the capacity to transfer perceptual experiences on to new environmental stimuli. In these *mediated performance* paradigms, the capacity of a CS to evoke representative features of a US is shown by presenting the previously trained CS following some alteration in the US. Holland (1998), for instance, paired an auditory CS with the delivery of a sucrose US; later, he devalued the sucrose US via CTA with LiCl, then tested the animals responding to the CS under conditions of extinction. This led to a reduction in responding in the presence of the CS which had previously been paired with the devalued US. By contrast, a second CS that had been paired with a non-devalued US showed no difference in responding.

Two recent studies have used mediated performance procedures to investigate impaired reality testing: one utilizing mice expressing the dominant-negative form of Disrupted-in-Schizophrenia-1 (DN-DISC-1; Fry et al., 2020), and another with mice chronically exposed to ketamine (Wu, Haberman, Gallagher, & Koh, 2020). In both instances, the mouse models used were shown to be particularly sensitive to mediated performance, an effect that was dopaminergically-dependent.

These data demonstrate that animal models which recapitulate various aspects of schizophrenia provide fruitful ground for investigating the neurobiological underpinnings of IRT. Numerous studies show that that mice with various perturbations of the DISC-1 genetic locus in particular display a variety of endophenotypes associated with neuropsychiatric illness (e.g., Hikida et al., 2007; Shen et al., 2008; Johnson et al., 2013; Jaaro-Peled et al., 2013; Niwa et al., 2013), including IRT. In the next section, I will provide an overview of what we currently know about the role of DISC-1 in the developing nervous system, its discovery, and why I have selected DISC-1 mice as the model with which to study IRT.

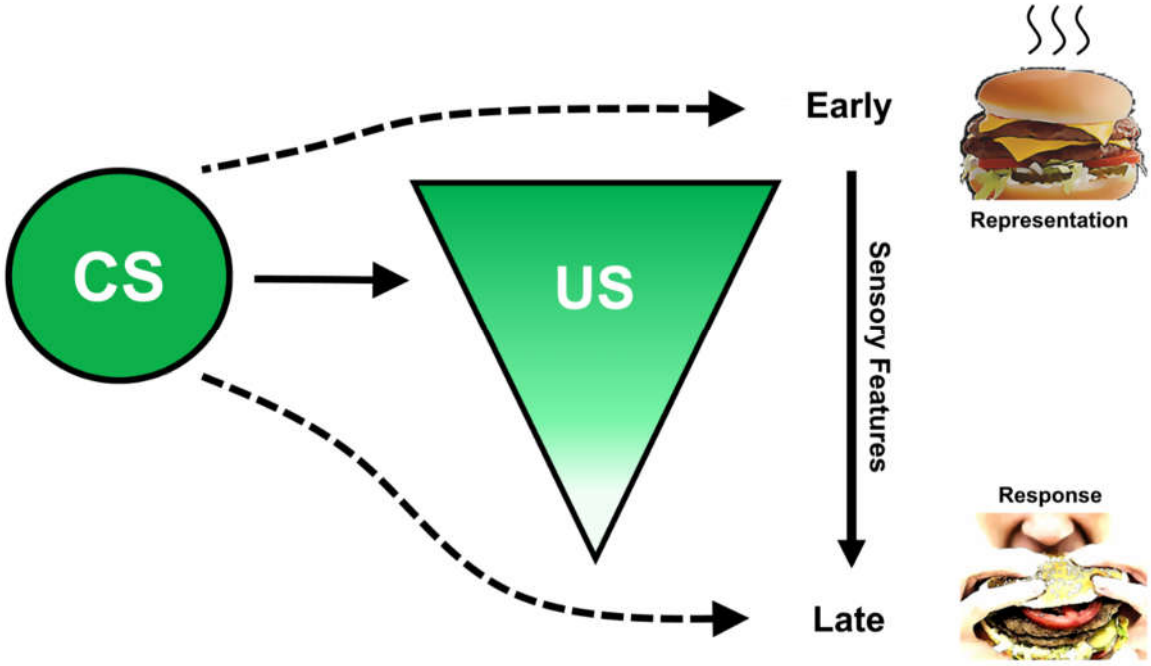


Figure 1. Event representation in Pavlovian conditioning. Early in the course of learning, cues can evoke strong perceptual representations of the sensory features (e.g., taste, smell) of rewards they later come to predict. Over time, this representational quality of the cue fades and it begins to evoke, instead, a reflexive behavioral response associated with obtaining the outcome.

Disrupted-in-Schizophrenia-1: Overview

DISC-1 is a gene which, under ordinary circumstances, codes for a protein that is involved in a number of intracellular interactions critical for early neural development. DISC-1 forms a complex with the cytoskeletal protein NDEL1 and centrosome LIS1 in a manner that affects neuronal migration via control over microtubule transport (Strat, Ramoz, & Gorwood, 2009). DISC-1 has also been shown to play a key role in neural integration and neurogenesis (Duan, et al., 2007) as well as the regulation of synapse formation (Seshadri et al., 2015). At the level of neuronal signaling, DISC-1 forms a complex with DRD2 that regulates protein kinase activity in response to activation (Su et al., 2014). Indeed, DISC-1 mice have previously been shown to have enhanced DRD2 function at the level of the striatum (Lipina et al., 2010; Jaaro-Peled et al., 2013), a critical finding given that hyperactivation of striatal DRD2 signaling has previously been implicated in the positive symptomology of schizophrenia (e.g., Adams, 2018). These are but a fraction of the known functions related to the DISC-1 interactome, though a common thread emerges even among this small sample suggesting that perturbations of the DISC-1 genetic locus may have wide-ranging implications for divergent facets of cognition, the initial clues to which began to form half a century ago.

In 1970, researchers were carrying out a cytogenetic analysis in search of chromosomal abnormalities in male offenders sentenced to a youth detention center in Scotland. During the course of their work, they identified an 18-year-old that had a balanced translocation in chromosome 1 (Jacobs, et al., 1970). At the time, the young offender had been diagnosed with a major affective disorder not specified.[2] Follow-up studies with 77 living relatives for the individual showed that 34 others carried the translocation, 16 of which met the diagnostic criteria

[2] Although some sources (e.g., Sullivan, 2013) list "conduct disorder," this was not an existing diagnosis in the Diagnostics and Statistical Manual of Mental Disorders in use at the time (DSM-II).

for some form of severe neuropsychiatric illness. These diagnoses included schizophrenia, bipolar disorder, and major depression (St Clair et al., 1990). Following the advent of improved DNA sequencing, the translocation was finally identified to disrupt two candidate genes, henceforth named DISC-1 and DISC-2, the latter of which is non-coding, but thought to regulate expression of the former (Millar et al., 2000). At the time, it was thought that DISC-1 might be identified as a specific risk gene for schizophrenia through genome-wide association studies. While these studies failed to link the DISC-1 gene itself with schizophrenia, more recent work investigating the gene sets for the suite of DISC-1 protein interactions has found a significant association with the disorder (Facal & Costas, 2019). These results suggest that while perturbations of the gene itself may not be deterministic for schizophrenia, they likely confer elevated risk dependent on other factors, the full scope of which remain unknown.

While research into the associated risk factors between perturbations of the DISC-1 genetic locus and schizophrenia in humans is ongoing, a number of mouse models of aberrant DISC-1 function have been developed. DISC-1 L100P is a mouse model created by a missense mutation in the second exon of the DISC-1 gene that leads to reduced transcription of the DISC-1 protein (Clapcote et al., 2007; Lipina et al., 2010). While L100P mice showed promise for modeling various aspects of schizophrenia (e.g., enhanced midbrain dopaminergic function and deficits in pre-pulse inhibition), the amino acids altered in the mutation (Q31 and L100) are not conserved between animals and humans, thus making it a less than ideal translational model. One potential solution to this problem was developed by way of creating the heterozygous dominant-negative mutation model of DISC-1 (DN-DISC-1). In this model, the gene is disrupted in such a way as to code for a C-truncated form of the DISC-1 protein, a mechanism which was previously theorized to occur in human perturbations of DISC-1 (Kamiya et al., 2005; Pletnikov et al.,

2007). This disturbs the functional domain of the protein while retaining the dimerization domain, thus resulting in reduced intracellular functionality when binding with the wild-type form of the protein. DN-DISC-1 has been modeled separately in a manner wherein expression is driven either by αCaMKII and thus restricted to forebrain behavioral circuits (e.g., Hikida et al., 2007; Niwa et al., 2010; Johnson et al., 2013) or the prion protein promoter (PrP), thus yielding global central nervous system expression (e.g., Niwa et al., 2013; 2016; Fry et al., 2020). DN-DISC-1 mice display a number of behavioral endophenotypes and neurophysiological aberrations associated with schizophrenia. These include deficits in social interaction, decreased motivation to attain reward, enhanced response perseveration, and the previously mentioned enhanced susceptibility to IRT (Johnson et al., 2013; Fry et al., 2020). Additionally, DN-DISC-1 mice show swelling of the lateral ventricles, decreased parvalbumin (PVB) expression associated with alterations in GABAergic signaling, increased prefrontal oxidative stress, and decreased tyrosine-hydroxylase (the rate-limiting enzyme for dopamine synthesis) expression in the frontal cortex (Hikida et al., 2007; Shen et al., 2008; Johnson et al., 2013; Niwa et al., 2013). Adding further face validity to the model, environmental stress has been shown to exacerbate many of these abnormalities (Niwa et al., 2013; 2016; Fry et al., 2020. In sum, the broadness of scale at which DN-DISC-1 mice recapitulate the heterogeneity among known behavioral and neurophysiological aberrations in schizophrenia make them a fitting candidate for deeper explorations into the neurobiology underlying positive-like symptomology.

Overview of Dissertation & Experiments

Although much is known about the frontal/cortical abnormalities associated with cognitive and negative symptomology in DN-DISC-1 mice (Hikida et al., 2007; Niwa et al., 2013; Johnson et al., 2013), the neurobiology of positive-like symptomology remains to be

explored in greater detail. The rationale for the experiments that follow builds heavily upon the findings of Fry et al. (2020), the first instance in which DN-DISC-1 mice (PrP-driven expression) were found to display behaviors indicative of positive-like symptomology. In this study, a noise CS was repeatedly paired with a liquid sucrose US over several days such that a robust association between the CS and delivery of sucrose was formed. DN-DISC-1 mice were then tested by delivering unflavored water in the presence of the previously trained CS. While wild-type mice decreased licking following the unexpected presentation of water, DN-DISC-1 mice licked at the water for longer and displayed a profile of licking typically associated with the consumption of a sweet tasting solution (i.e. increases in lick cluster size, a measure known to reflect stimulus palatability; Johnson et al., 2010; Johnson, 2018). These effects were attenuated following systemic administration of the antipsychotic dopamine-D2 antagonist haloperidol and facilitated when DN-DISC-1 mice were exposed to early adolescent social isolation. This manipulation has been shown to exaggerate the neurobiological abnormalities of the model (Niwa et al., 2013) and thought to represent a critical developmental window in facilitating risk for schizophrenia (Lewis, 1997). Remarkably, DN-DISC-1 mice also showed significantly increased cfos (a marker for neural activity) expression in the insular cortex (IC) following testing, a region of the brain containing taste reactive neurons (Bermudez-Rattoni & McGaugh, 1991; Pritchard, Macaluso, & Eslinger, 1999; Koh, Wilkins, & Bernstein, 2003). Thus, despite the sucrose being omitted during the test, these data suggest that the DN-DISC-1 brain responded as if sucrose had actually been presented.

The question of what may be different neurobiologically in the DN-DISC-1 brain that predisposes the subject to IRT requires a deeper examination of the facts at hand. First, as it is known that DRD2 hyperactivation at the level of the striatum underlies many of the positive

features of schizophrenia (Adams, 2018), we might imagine that DN-DISC-1 mice share this increased signaling abnormality as well, particularly within the ventral tegmental area (VTA), a region which sends dopaminergic efferents directly to the IC (Ohara et al., 2003). The IC, in turn, contains a large population of both GABAergic interneurons and glutamatergic pyramidal cells that express both DRD1 and DRD2, though it appears as though DRD2 is predominate (Santana, Mengod, & Artigas, 2009). Activation of DRD1 generally stimulates adenylyl cyclase activity, leading to a number of downstream cascades that increase activity of the cell; conversely, DRD2 inhibits adenylyl cyclase, leading to decreases in cellular activity. This is relevant for two reasons: first, it suggests that, at least in wild-type animals, normative VTA dopamine signaling onto the IC would be more likely to activate DRD2. DRD2 signaling might lead to a decrease in the activity of GABAergic neurons, or it might decrease the activity of glutamatergic neurons synapsing onto local GABAergic neurons in the region, though the exact configuration of these connections in the IC is currently unknown. Taken together, we are left with two plausible scenarios to explain the IRT associated with phantom gustatory sensations in DN-DISC-1 mice as seen in Fry et al. (2020). The first scenario simply suggests that in DN-DISC-1 mice, increased dopaminergic tone from the VTA onto the IC leads to increased activation of DRD2 signaling in the region above baseline, thus decreasing inhibitory tone and allowing for increased reactivity of the neurons therein. The second scenario, however, does not require increased VTA dopamine signaling at all; rather, it suggests that alterations in the number and distribution of DRD1 vs DRD2 (e.g., a loss of DRD1 expression, or increased DRD2 expression) in the IC could create a scenario in which even normative dopamine signaling from the VTA onto the IC would lead to increased DRD2 activation and resultant decreases in inhibitory tone. Both of these scenarios are compatible with increased IC activity in DISC-1

mice when displaying phantom gustatory sensations, and the fact that DRD2 antagonism reduces IRT.

This aside, the experiments outlined in this dissertation look to examine whether VTA dopamine cells projecting to IC underlie the experience of IRT using both rigorous analyses of behavior and sophisticated neuropharmacological tools. In the chapters that follow, I detail the intersectional chemogenetic approach allowing for selective inactivation of dopamine cells projecting from the VTA → IC. This methodology allowed for me to examine the necessity of the VTA → IC circuit as related to specific aspects of consummatory behavior (Chapter 2), motivation to attain reward (Chapter 3), and IRT (Chapter 4). In Chapter 5, I directly address questions related to DN-DISC-1 differences in pharmacology and physiology in the VTA → IC circuit through the use of qPCR and immunohistology to quantify dopaminergic efferents, as well as differences in the amount of D1Rs and D2Rs in the regions. Collectively, these studies have the potential to, for the first time, identify a circuit for positive-like symptomology affecting a specific sensory modality (gustation), in addition to adding valuable data to the growing body of literature on the neurobiological impact of perturbations in the DISC-1 genetic locus. Such work may ultimately lead to the development of more targeted pharmacological interventions for positive symptoms in humans with schizophrenia.

CHAPTER 2: INACTIVATING VTA → IC DOPAMINE DOES NOT AFFECT MEASURES OF LICKING MICROSTRUCTURE

Introduction

One of the primary challenges for investigating positive-like symptomology in animal models of schizophrenia is identifying a suitable dependent behavioral measure. Following on the work of McDannald & Schoenbaum (2009), the field has focused largely on changes in consummatory measures that are thought to reflect IRT. This form of IRT would seem to indicate that changes in consumption are being driven by the animal's experience of a phantom gustatory sensation that is activated in the presence of a Pavlovian cue. In animal models of schizophrenia, it is clear that this internal representation of the sensory features of a substance triggered by the cue is pathologically robust, so much so that it becomes difficult for the animal to disentangle from external reality. Of the handful of studies which have examined these effects, the majority have done so through the use of RMTA procedures. RMTA involves presenting a cue which was previously paired with the delivery of sucrose in a scenario absent of sucrose—this is immediately followed-up with an injection of the gastric malaise inducing agent LiCl. Through the cue-evoked associatively activated representation of sucrose, illness now becomes associated with sucrose. In these instances, various animal models of schizophrenia have been shown to significantly reduce their consumption as compared to controls when later presented with sucrose (McDannald et al., 2011; Kim & Koh, 2016; Koh, Ahrens, & Gallagher, 2018), indicating enhanced susceptibility to IRT. However, there is a problem with these studies in that they all used a metric based on total sucrose intake to assess the presence of a phantom aversive *taste*. I emphasize the word taste here for the simple reason that overall consumption is a measure which is confounded by multiple variables, of which taste is only one. Indeed,

researchers in the field of ingestive behavior have known for many years that the individual variables which make up the act of consumption reflect discrete physiological processes (Johnson, 2018), thus making overall intake an inappropriate measure for taste alone. Fry et al. (2020), was the first study to consider more specific measures of consummatory behavior when assessing IRT. In order to do this, we eschewed the use of dry sucrose pellets, and instead delivered liquid sucrose rewards. Couple with the use of an infrared beam passed through the food cup, this allowed the recording of individual licks in time for further analysis. Using analysis of licking microstructure, we found that DN-DISC-1 mice were more susceptible to IRT and that this was indeed driven by changes in metrics which reflected alterations in taste, specifically.

Like humans, animals do not consume liquids continuously, rather, they tend to take short breaks, the duration of which are referred to as interlick-intervals (ILIs). The amount of licks contained in the 251 – 500 ms and >500 ms windows have been shown to be positively correlated with the concentration of sucrose presented to an animal (Davis & Smith, 1992; Davis & Perez, 1993; Spector et al., 1998); furthermore, these measures (referred to as burst and cluster size, respectively) are unaffected by sham feeding procedures in which the esophageal tract is made to bypass the intestines, thus indicating that they are driven primarily by pre-ingestive evaluation of the sensory features (e.g., taste) of a given substance (Davis & Campbell, 1973; Hull et al., 1951; Schneider, Gibbs & Smith, 1986; Mook et al., 1983). Conversely, the number of bouts of licking that are initiated between ILIs (burst and cluster number) do not follow the same monotonic pattern in relation to increasing concentrations of sucrose in the way that burst and cluster size do; rather, these measures tend to form an inverted u-shape function, increasing with the concentration of sucrose to a point, and then rapidly decreasing (Davis & Smith, 1992).

This is thought to reflect negative post-ingestive feedback brought on more rapidly by the increasing caloric value of sweeter solutions; thus, burst and cluster number are discrete measures reflecting physiological processes that are distinct from taste. Being a rather blunt metric, the observed pattern for overall consumption more closely mirrors the patterns seen when examining burst and cluster number, rather than size.

The experience of taste may seem to be an odd facet of phenomena to focus on with regard to animal modeling of schizophrenia, and yet, it is known that pharmacological manipulation of DRD2 signaling impacts the very measures shown to be indicative of stimulus palatability (Galistu et al., 2011; Genn et al., 2003; Hartfield et al., 2003; Liao and Ko, 1995; Schneider et al., 1990). Given that DRD2 hyperfunction is thought to underlie positive symptomology in schizophrenia (Adams, 2018), it is worth wondering how a circuit-specific manipulation of dopamine might impact measures of consumption in an animal model which recapitulates many of the behavioral endophenotypes and neurophysiological aberrations of the disorder. This in mind, the experiments outlined in this chapter utilize analysis of licking microstructure to ask whether the VTA → IC dopaminergic circuitry—herein theorized to underlie the experience of IRT—is necessary for various aspects of consumption in both wild-type and transgenic subjects under normal conditions.

Materials & Methods

Animals

PrP-DN-DISC-1 mice were originally generated and transferred from the Transgenic Core Laboratory at the Johns Hopkins University and have since been outbred with C57BL/6J mice (Jackson Laboratory, Bay Harbor, ME; strain #000664) at Michigan State University for multiple generations in the Johnson Lab. For these experiments (and those that follow), I

crossbred DN-DISC-1 positive mice with mice expressing Cre-recombinase under the control of the dopamine transporter gene (DAT-Cre, heterozygotes; Jackson Laboratory, strain #020080) in order to generate both DN-DISC-1 positive x DAT-Cre positive and wild-type x DAT-Cre positive offspring. Mice aged approximately 4 months (*n* = 19♂ and *n* = 13♀ split among *n* = 16 DN-DISC-1 positive x DAT-Cre positive, and *n* = 16 wild-type x DAT-Cre positive) were maintained at ~90% of their free-feeding body weight throughout the duration of testing using standard lab chow (Teklad 8940; Envigo, Huntingdon, UK); mice received no less than ¼ pellet per day. Mice were maintained on a 12-hour light/dark cycle with lights-out occurring at 7pm EDT. All behavioral tests for this dissertation took place during the daylight cycle between the hours of 12 – 4pm.

Genotyping

Genotyping for DN-DISC-1 (500 base pairs) and the DAT-Cre (152 base pairs) transgene was conducted via separate reactions. DNA was first isolated and amplified from individual subjects by collecting tail snips (~3 mm) from mice at approximately 4 weeks of age. Snips were placed in 200 µl of 50 mM NaOH then incubated at 75°C overnight. After incubation, 200 µl of 100 mM Tris (pH 5.0) was added to each tube prior to centrifuging for 20 seconds at 10,000 RPM. For each reaction, 2.5 µl of extracted DNA per subject was added to individual solutions containing 6 µl nuclease-free water, 10 µl of GoTaq Green 2x Mastermix (Promega, Madison, WI), and 0.3 µl of each DNA oligonucleotide primer of at 10 µM concentration (DAT gene primers, DAT-common: TGGCTGTTGGTGTAAAGTGG; DAT-wild-type: GGACAGGGACATGGTTGACT; DAT-mutant: CCAAAAGACGGCAATATGGT; DN-DISC-1 primers, P9-27 DISC1 1354-1373: GAATGGAGCCGAGGCTGTTG; PrP-AS1 CCCAGCCTAGACCACGAGAATGC). Next, solutions were loaded into a thermocycler

(Eppendorf, Hamburg, Germany) for DNA amplification via polymerase chain reaction (PCR) with the following settings. For DAT-Cre: 1) 3 minutes at 94°C; 2) 30 seconds at 94°C; 3) 1 minute at 62°C; 4) 1 minute at 72°C; 5) return to and proceed from step 2, 35x; 6) 2 minutes at 72°C; 7) hold indefinitely at 10°C. For DN-DISC-1: 1) 5 minutes at 94°C; 2) 45 seconds at 94°C; 3) 45 seconds at 58°C; 4) 1 minute at 72°C; 5) return to and proceed from step 2, 40x; 6) 7 minutes at 72°C; 7) hold indefinitely at 4°C.

Following DNA amplification, samples were analyzed visually using gel electrophoresis. Samples were run in a 2% agarose gel made with 1x TAE buffer (ThermoFisher). Ethidium bromide was added to the gel as a fluorescent marker at 0.2 µl / 100 ml gel. 20 µl of sample per subject from the previous amplification step was then added to individual wells (1 sample well). Gel was run at 200 volts/40 mAmps for a minimum of 1 hour. Gels were imaged using an Omega Lum G UV camera system (Aplegen, Pleasanton, CA).

Surgeries

Mice were anaesthetized using 5% isoflurane gas and Buprenorphine SR (0.5 mg / kg), placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) and virally infused with AAVrg-pEF1a-DIO-FLPo-WPRE-hGHpA (Plasmid #87306-AAVrg, Addgene, Watertown, MA) bilaterally at the level of IC (0.25 µl per side; AP +1.7, ML +/-2.5, DV -3.25). This cre-dependent virus has retrograde properties such that it is taken up at synaptic terminals in the IC and trafficked back to their points of origin. The virus binds with *loxP* sites at the level of DNA strands on cells which contain Cre-recombinase (i.e., cells which express DAT in our model), site-specific recombination occurs, and the cells now co-express FlpO-recombinase. Following these infusions, mice were then bilaterally injected with AAV8-DJ-hSyn-fDIO-hMD4(Gi)-mCherry (Gene Vector and Virus Core, Wu Tsai Neurosciences Institute, Stanford University;

henceforth referred to as FlpO-hM4Di) at the level of the VTA (AP -3.08, ML +/-0.6, DV -4.5). Infusion of the Flp-dependent DREADD virus within the VTA leads to the expression of inhibitory hM4Di receptors on dopaminergic cells which are selectively activated in response to the drug clozapine *N*-oxide (CNO). Specifically, CNO binds with the hM4Di receptors and activates G protein-coupled inwardly rectifying potassium channels (GIRKs), hyperpolarizing the neuron and silencing its synaptic output (Armbruster et al., 2007). This technique allowed for circuit-specific inhibition of VTA dopamine cells projecting to the IC (Fig. 2). All mice were allowed 4 weeks to recover post-surgery before the beginning behavioral experiments.

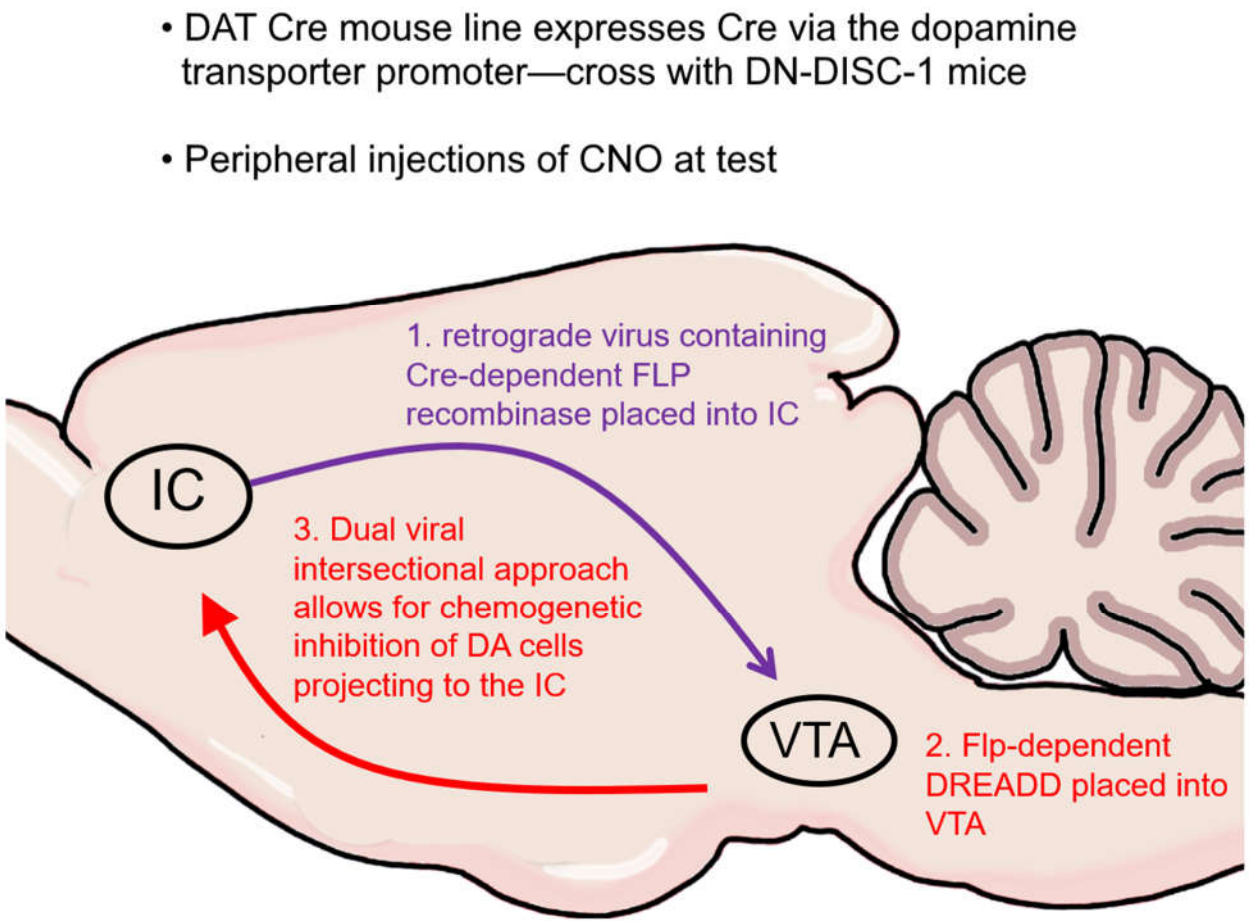


Figure 2. Dual Viral Intersectional Chemogenetic Approach. Configuration of the viral vectors used in the present studies to allow for selective inhibition of dopaminergic neurons projecting to the IC via peripheral administration of CNO. With the exception of a small population of neurons that project simultaneously to the medial NAc, septum, and ventral pallidum, VTA dopamine neurons lack collaterals and are considered to be one-to-one in their synaptic communication (Fallon, 1981; Beier et al., 2015). Thus, I can state with some confidence that the viral manipulation only affected dopaminergic signaling which originates within the VTA and terminates at the level of the IC.

Drugs

CNO was obtained from the National Institute on Drug Abuse (NIDA) and prepared as a 40x stock solution (2.4 mg / 1 ml) with 10% (2-Hydroxypropyl)-β-cyclodextrin in 0.2 M sterile PBS. Working solutions of 0.06 µg / ml were used to prepare individual doses of 0.3 mg / kg for subjects delivered at 5 ml / kg. CNO working solutions were prepared fresh daily and kept on ice

until the time of administration. For this experiment, all mice received two test sessions, one with CNO and one with vehicle (0.2 M sterile PBS administered at the same volume as CNO). Mice were injected intraperitoneally (IP) with CNO or vehicle 15 minutes prior to testing for each session.

Apparatus

For all experiments, behavioral procedures were carried out in eight identical, sound-attenuating conditioning chambers featuring steel rod floors at 0.5 cm spacing, translucent polycarbonate walls, and measuring 24 x 20 x 18 cm (Med Associates, St. Albans, VT). Within each conditioning chamber, a magazine area on the right side contained a cut-out food well capable of holding 50 µl liquid volume. For consumption testing, liquids (H_2O, 0.1 M sucrose, 0.2 M, and 1 M) were delivered at the level of the food cup via syringe pumps controlled by Med-PC software (Med Associates) on an IBM-compatible computer running Windows XP (Microsoft, Redmond, WA). H_2O was delivered via a separate pump line that was never used with sucrose. Within the food cup, fiber optic cabling was used to project an infrared beam across the meniscus such that each lick would break the beam and allow for timestamped recording of individual licks. In order to minimize any variance in lick detection, consumption testing was conducted using only one of the eight conditioning chambers. Chamber pans and floors were washed with 70% EtOH between male and female subject runs. Conditioning chambers were located in an enclosed, darkened room, illuminated only by red light.

Behavioral Procedures

Over the course of eight days, mice were placed in the conditioning chamber for a period of 10 minutes each. At the beginning of the session, the food cup filled with 50 µl of either H_2O, 0.1 M, 0.2 M, or 1 M liquid sucrose. The order of sucrose concentration was distributed via

counterbalanced Latin square design across days. Once licking began, every 10 licks initiated a 10 µl delivery of solution such that the food cup never depleted. The order of solution presentation was randomized such that no cohort received the same presentation order across days. All mice received two testing sessions with each solution: CNO session and a vehicle session. Drug order was counterbalanced in a randomized manner by subject to ensure an equal number of days in which the first experience of a solution occurred in the presence of CNO or vehicle across the experiment.

Confirmation of Viral Targeting

For all experiments in this dissertation, tissue fixation and confirmation of viral targeting took place using the following methods[3]. Following the conclusion of behavioral experiments, mice were deeply anaesthetized by way of intraperitoneal injection of sodium pentobarbital at 150 mg/kg, then sacrificed via exsanguination during transcardial perfusion with 4% paraformeldahyde (Sigma-Aldrich, St. Louis, MO). Brains were extracted and placed in a 4% paraformeldahyde solution containing 10% sucrose for 24 hours at 4° C. Afterward, brains were sliced using a freezing microtome at 30 µm. Coronal sections of VTA ranging from bregma -2.80 mm through -3.80 mm (Paxinos & Franklin, 2019) were then flow-mounted using 0.1 M PB onto microscope slides, treated with Prolong Gold Antifade Mountant with DAPI (Thermo Fisher Scientific, Waltham, MA), coverslipped, and left to cure for 24 hrs. Afterward, slides were imaged using an Olympus BX51 fluorescent microscope (Olympus Corporation, Tokyo, Japan) to determine the accuracy and spread of AAV8-DJ-hSyn-fDIO-hMD4(Gi)-mCherry infusion at the level of the VTA. At this time, $n = 2$ DN-DISC-1 mice were excluded from data analysis due to poor targeting.

[3] Immunohistological quantification of viral colocalization with dopaminergic cells in the VTA is detailed in Chapter 5.

Data Analysis

For all experiments throughout this dissertation, prior to the main experimental analyses, separate one-way ANOVAs with between-subjects variable of sex were used for each of the discussed dependent variables in order to determine whether any differences existed. Unless otherwise specified, no effect of sex was detected.

Analysis of consumption data was conducted using a repeated measures ANOVA with a between-subjects variable of genotype (DN-DISC-1 x DAT-Cre vs wild-type x DAT-Cre) and within-subjects variables of solution (H_2O, 0.1 M, 0.2 M, 1 M) and drug (CNO, vehicle). The average number of licks within a 250-500 ms pause criterion (burst size) was used as the dependent measure for palatability. Additional analyses of the average number of bouts of licking initiated prior to a 250-500 ms pause criterion (burst number), as well as overall intake within the session were conducted. Finally, as a means of ruling out any generalized alterations in motoric behaviors, ILIs under 250 ms were examined via the same process[4]. Mauchly's test of sphericity was used to examine homogeneity of variance. All data analyses were conducted using the open-source statistical package JASP (University of Amsterdam) with an α level of .05 as the criterion for significance.

Results

Analysis of consumption data showed significant changes in overall intake, burst number, and burst size relative to sweetness of the solution, regardless of genotype or drug condition (Fig. 3). Examination of ILIs under 250 ms showed a main effect of solution ($F = 66.405$; $p < .001$) with no effect of genotype or drug, thus indicating no generalized motoric differences in licking were present ($Fs < 1$). There was a main effect of solution for overall intake ($F = 85.53$, $p < .001$)

[4] This metric is thought to reflect the pre-evaluative influence of central pattern generators (Johnson, 2018).

and burst number ($F = 27.126$, $p < .001$). No effect of genotype, drug, or interactions among factors thereof was detected ($Fs < 1$). Similarly, burst size (i.e., palatability) also showed a main effect of solution ($F = 10.287$, $p < .001$) with no interaction among other factors. Given the importance of burst size as a metric for IRT throughout other experiments in this dissertation (Ch. 4), post-hoc analysis with Bonferroni correction were conducted in order to further examine this effect; these analyses showed that mean burst size with relation to 0.1 M ($p < .01$), 0.2 M ($p < .001$), and 1 M ($p < .01$) were each significantly greater than H_2O. These results confirm that all mice regardless of genotype are able to reliably detect sweetness, and do not differ in levels of post-ingestive feedback. Furthermore, inactivation of the VTA → IC dopaminergic circuitry does not appear to influence these processes in either group.

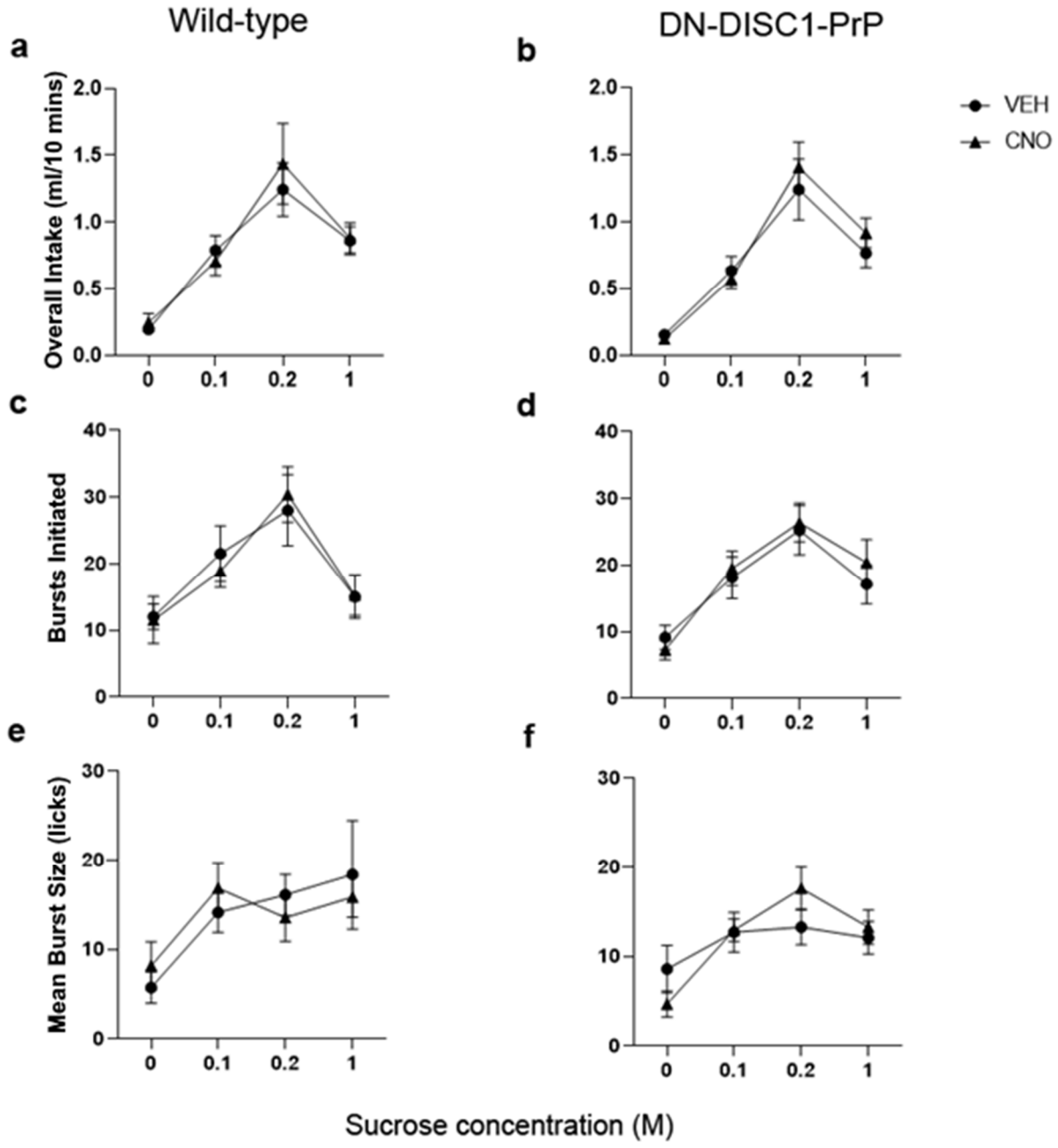


Figure 3. Analysis of licking microstructure during 10-minute consumption. DN-DISC-1 and wild-type mice showed no differences in terms of overall intake (A, B), burst number (C, D), or mean burst size (E, F) across solutions ranging from deionized water up to 1 M sucrose content. Inactivation of the VTA → IC dopaminergic circuitry at the time of test did not impact measures of licking microstructure or overall intake.

Discussion

This study replicates findings from Fry et al. (2020) which showed that DN-DISC-1 mice do not differ from wild-type controls in terms of their licking or overall consumption of sweet-tasting solutions under normal conditions. The work in this chapter extends the findings of Fry et al., however, and shows that in addition to a lack of baseline differences in licking and consumption, DN-DISC-1 mice, along with wild-type controls, are also unaffected by inactivation of the VTA → IC dopaminergic circuitry while consuming. These data are quite telling in that they bolster long-standing accounts of dopaminergic function which suggest that while this monoamine may influence motivational processes related to the willingness to engage in appetitive and consummatory behaviors (i.e., 'wanting'), it does not drive the evaluative aspects (i.e., 'liking'; Berridge & Robinson, 1998). Furthermore, we can now say that although the IC contains taste-responsive neurons (Bermudez-Rattoni & McGaugh, 1991; Pritchard, et al., 1999; Koh, et al., 2003), they appear to be unaffected by a loss of dopaminergic tone in the region, at least during normal consumption. This should be taken in the context of recent studies which have shown, for instance, that DRD1 antagonism leads to reductions in burst number and overall intake of sucrose (D'Aquila et al., 2012; Galistu & D'Aquila, 2013) whereas isolated manipulation of DRD2 signaling can influence consummatory evaluation as evinced through alterations in burst size (Galistu et al., 2011; Genn et al., 2003; Hartfield et al., 2003; Liao & Ko, 1995; Schneider et al., 1990). The fact that we did not see a reduction in overall intake following inactivation of the VTA → IC dopaminergic circuitry suggests that DRD1 is not prominent in the IC, and indeed this is in line with data showing that DRD2 makes up the majority of dopamine receptors in the region (Santana, Mengod, & Artigas, 2009). Finally, given that burst size was not altered following the circuit-specific manipulation in our study, we might conclude that

DRD2-mediated control over the sensory evaluative aspects of consummatory behavior must take place elsewhere in the brain.[5]

Given the preponderance of evidence, it seems likely that dopamine's role in learning, coupled with physiological alterations in the circuitry driven by the DISC-1 translocation, account for the effects found in Fry et al. (2020), rather than some general alteration in taste responsivity that is unique to the phenotype. Chapters 4 and 5 will explore these questions in greater detail; for the moment, however, it would seem prudent to investigate further the role of VTA → IC dopamine in more specific aspects of appetitive behavior related to motivation.

[5] Likely in the so-called 'hedonic-hotspots' of NAc shell and ventral pallidum (e.g., Mahler et al., 2007; Smith & Berridge, 2007; Castro & Berridge, 2014)

CHAPTER 3: EFFECTS OF INACTIVATING VTA → IC DOPAMINE ON MOTIVATION TO ATTAIN REWARD

Introduction

While the idea that dopamine plays a role in associative learning is without question (e.g., Schultz et al., 1997; Smith et al., 2006), it is clear too that motivational processes related to the initiation and maintenance of effortful behavior and decision-making are also within its purview. Indeed, an entire body of literature has formed alongside that of the associative learning theorists which is purely interested in how dopamine modulates incentive motivation, behavioral activation, effort, and choice (e.g., Berridge & Robinson, 1998; Floresco et al., 2006; Berridge, 2007; Grace et al., 2007; Salamone et al., 2007; Salamone et al., 2012). It is known, for instance, that pharmacological manipulations which deplete dopamine decrease the amount of effort animals are willing to put forth to gain rewards (Cousins & Salamone, 1994; Mingote et al., 2005), while drugs which stimulate dopamine release yield the opposite result (Bardgett et al., 2009; Wardle et al., 2011). The temporal nature of dopaminergic firing at the time of effortful decision-making also appears to be critical, as brief stimulation of dopamine restricted to the time of choice has a paradoxical effect on effort (Fry et al., 2021). At the level of receptors, DRD2 signaling is particularly important for the maintenance of effort (Robles & Johnson, 2017), specifically those located in the nucleus accumbens (NAc; Bryce & Floresco, 2019).

In light of these data, it seems clear that any dopaminergic manipulation of DN-DISC-1 mice carries with it a need to rule out generalized alterations in motivational processes as a cause for aberrant behavior. This in mind, the experiment outlined in this chapter was designed to investigate any potential role for the VTA → IC dopaminergic circuitry in the initial activation and sustained maintenance of effort, as well as whether any baseline differences may arise

following perturbations of the DISC-1 genetic locus. One of the classic paradigms used to investigate these aspects of motivation involves the use of a progressive ratio schedule that requires increasing amounts of effort to attain reward (e.g., Hodos, 1961); such procedures have been shown to be dopaminergically-dependent (e.g., Aberman et al., 1998; Randall et al., 2012). For this experiment, we isolated licking as the action requiring increased effort, as opposed to the more common lever-press. Though this is not the first time licking has been used in combination with progressive ratio (see Johnson et al., 2013, for example), it seemed rather salient in the course of this dissertation, particularly given that the series of studies which I sought to extend (Fry et al., 2020) centered on alterations in DN-DISC-1 mice licking, specifically. At present, this is the first and only experiment in the literature which has examined VTA → IC dopaminergic circuitry within such a context.

Materials & Methods

Animals

DN-DISC1-PrP mice were generated and crossbred with DAT-Cre mice as described in chapter 2. Mice (*n* = 9 ♂, *n* = 7♀; split among *n* = 8 DN-DISC1-PrP x DAT-Cre and *n* = 8 wild-type x DAT-Cre) were group-housed by sex up to the point of viral infusion surgeries, then singly housed thereafter. All mice were maintained on a 12-hour light/dark cycle and restricted to ~90% of their free-feeding body weight on standard lab chow (Teklad 8940) during the course of behavioral experiments.

Surgeries

Surgical procedures were identical to those described in chapter 2. All mice received bilateral infusions of AAVrg-pEF1a-DIO-FLPo-WPRE-hGHpA (Plasmid #87306-AAVrg) at the level of IC (0.25 µl per side; AP +1.7, ML +/-2.5, DV -3.25) in combination with bilateral

infusions of AAV8-DJ-hSyn-fDIO-hMD4(Gi)-mCherry at the level of the VTA (AP -3.08, ML +/-0.6, DV -4.5). Mice were given 4 weeks to recover prior to the start of behavioral testing.

Drugs

CNO was obtained and prepared for intraperitoneal injection at 0.3 mg / kg in the same manner as described in chapter 2. Sterile 0.2 M PBS was injected at the same volume as CNO on vehicle test days. Drug order on test days was counter-balanced across genotypes. All mice received one test day on CNO and one test day with vehicle injected 15 minutes prior to the beginning of the session.

Behavioral Procedures

Progressive ratio sessions lasted a maximum of 2 hours or ended if a mouse failed to respond for 10 minutes, whichever occurred first. At the beginning of the session, the magazine well filled with 50 µl of 0.2 M sucrose. Once depleted, the mouse needed to lick at the empty bowl 3 times to receive another 50 µl delivery, with each successive delivery requiring increasing numbers of licks such that the progressive ratio scheduled ran as follows: 3, 6, 9, 12, 15, 18, 21, 24, 27, 30 … 300. This schedule of reinforcement was chosen based on its successful use in previous work with DISC-1 mice (Johnson et al., 2013). All mice received two practice/training sessions before being moved on to testing.

Data Analysis

Immunofluorescence quantification of viral targeting was conducted in the same manner as detailed in chapter 2, at this time, $n = 2$ subjects was excluded from analysis of behavior due to poor targeting. For all other subjects, total licks, rewards obtained, as well as magazine entries and time were recorded for analysis. Mauchly's test of sphericity was used to ensure homogeneity of variance for all samples across all analyses. Repeated measures ANOVAs with

between-subjects variable of genotype (DN-DISC-1 x DAT-Cre vs wild-type x DAT-Cre) and within-subjects variable of drug (CNO vs vehicle) were conducted with an α level of .05 as the criterion for significance. Outliers were removed prior to inferential analysis using the interquartile range (IQR) method which sets a criterion of $x < (Q1 - 1.5 * IQR)$ or $x > (Q3 + 1.5 * IQR)$ where Q1 = the lower bound of the IQR and Q3 = the upper bound. At this time, $n = 1$ DN-DISC-1 x DAT-Cre subject was removed from further analysis of magazine entries, and $n = 2$ (split among $n = 1$ DN-DISC-1 x DAT-Cre and $n = 1$ wild-type x DAT-Cre) were removed from further analysis of time spent in magazine. Mantel-Cox test with between-subjects variable of genotype and within-subjects variable of drug was used to compare progressive ratio survival using session length as the dependent variable across groups. Given that there were no differences within viral groups at the level of drug, these data were analyzed a second time using only the between-subjects variable of genotype variable. Survival curve analysis was conducted using Prism (Graphpad Software, San Diego, CA). All other analyses were conducted using the open-source statistical package JASP (University of Amsterdam).

Results

Analysis of progressive ratio data revealed no baseline genetic differences or effect of inactivation of the VTA → IC circuit on rewards obtained or total licks (Fs < 3; Figs. 4a, 4b). Analysis of magazine entries showed no effect of genotype or drug (Fs < 3; Fig. 4c); however, when considering time spent in magazine as a percentage of total session length, a main effect of drug was observed ($F = 5.348$, $p = .04$) such that CNO decreased magazine time regardless of genotype (Fig. 4d). No effect of genotype or interactions thereof (Fs < 1) were observed for this measure. These differences in measures of approach appear to have influenced overall session survival time as well. Mantel-Cox analysis initially showed no difference between genotype or

within groups based on drug condition ($X^2 = 5.321$, $p = .15$). Given that there were no differences within groups (Fig. 4e), follow-up analyses removed drug condition as a factor. These results showed a significant difference in survival probability across the length of the progressive ratio session ($X^2 = 5.034$, $p = .02$) such that DN-DISC-1 mice, were more likely to attain greater session length as compared to wild-type (Fig. 4f). Collectively, these results suggest that inactivation of the VTA → IC circuit does not affect measures related to the maintenance of effort or motivation to attain reward; however, it does appear to affect approach behavior. Furthermore, dominant-negative expression of the DISC-1 gene seems to be associated with differences in behavior during the assay that appear to be separate from these factors.

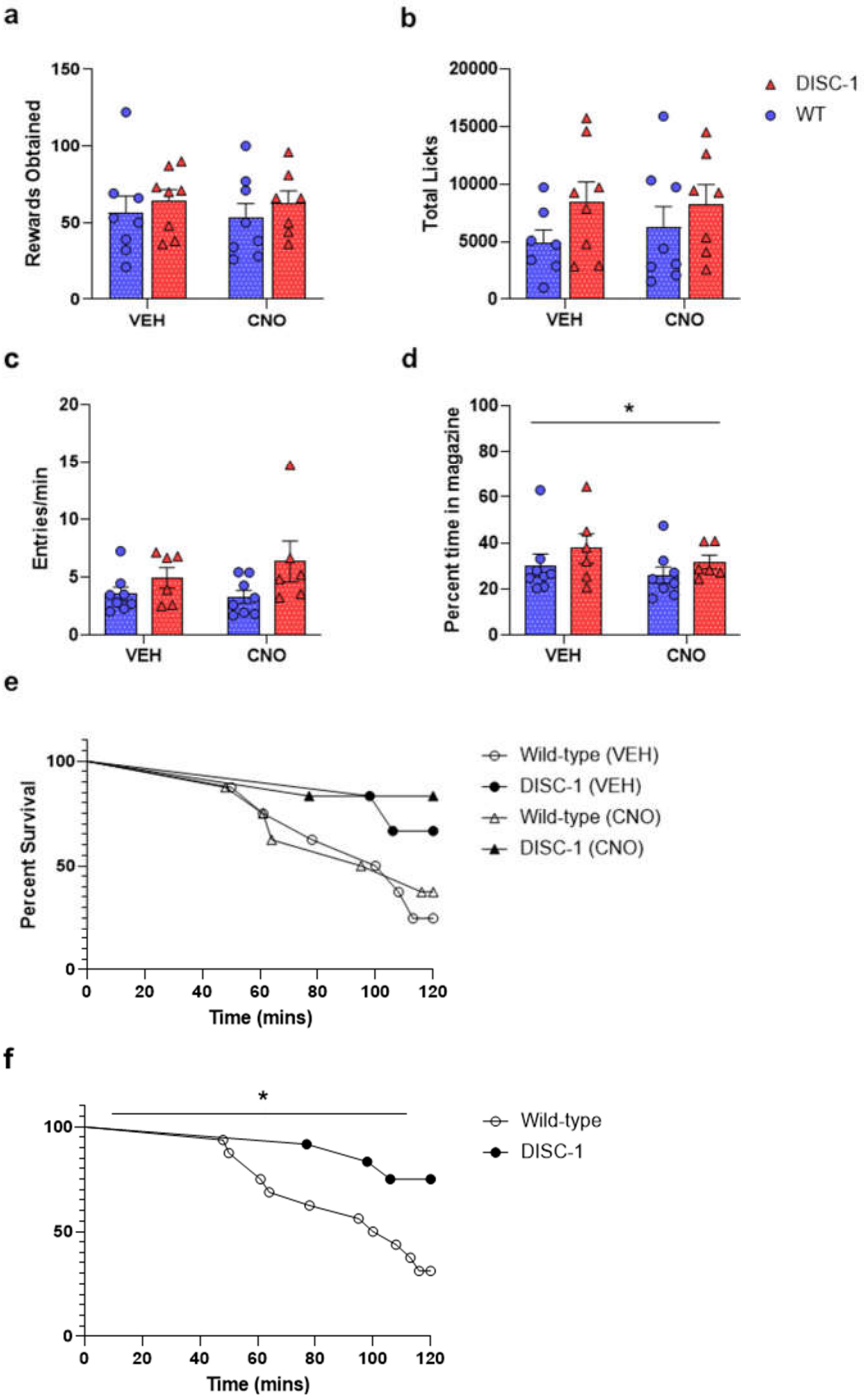


Figure 4. Analysis of progressive ratio behavior. DN-DISC-1 mice did not differ from wild-type subjects in terms of overall rewards obtained or total licks (A, B). There was a tendency for DN-DISC-1 mice to enter the magazine more frequently (C), while CNO led to a significant reduction in magazine time regardless of genotype (D). Initial examination of survival length in the progressive ratio assay showed no differences either between subjects or across drug conditions (E); however, when analysis was conducted without drug as a factor, DN-DISC-1 mice emerged as spending significantly more time in the session before timing out (F). *($^{+}p = .09$, $^{*}p < .05$)*

Discussion

In this chapter, I show that inactivating the VTA → IC dopaminergic circuitry does not impact the amount of effort an animal is willing to put forth to attain reward. This is perhaps unsurprising, given the abundance of studies which have previously shown that dopaminergic circuitry at least at the level of the NAc, specifically modulates effort (e.g., Bryce & Floresco, 2019; Nunes et al., 2013; Trifilieff et al., 2013). That said, with respect to approach behavior, we see that inactivation of the VTA → IC dopaminergic circuitry leads to a reduction in time spent in the magazine, indicating that the circuit may play a role in more nuanced aspects of preparatory behaviors (i.e., behaviors that precede a consummatory act: seeking, foraging, etc) independent of incentive motivation. Additionally, we see that DN-DISC-1 mice regardless of drug condition spend more time on the progressive ratio task. These data should be considered in light of the following caveats.

Previous work with DN-DISC-1 mice showed impairments in progressive ratio responding relative to wild type controls (Johnson et al., 2013), an effect which was not replicated here. This is mind, it is worth noting that our DN-DISC-1 mice differ from those of Johnson et al., in two fundamental ways. First, the previous study used mice in which DN-DISC-1 expression was driven by the CAMKII promoter and thus restricted dominant-negative DISC-1 expression to forebrain behavioral circuits. Conversely, in the current study, mice have global central nervous system expression driven by the PrP. Furthermore, the mice used by Johnson and colleagues carried only the DN-DISC-1 mutation, whereas the mice used for the present studies are also positive for DAT-Cre. Recent work has shown that DAT-Cre mice show sex-dependent alterations in associative learning, as well as DAT expression in the striatum. Briefly, males show resistance to extinction whereas female mice appear to show enhanced incentive

motivation. Additionally, females show increased DAT expression at the level of the NAc core, while both males and females have reduced DAT expression throughout the striatum (Costa et al., 2021). Given the dopaminergically-dependent nature of the task, it is perhaps unsurprising that our mice performed differently when taking into account the presence of the DAT-Cre knock-in gene. Additionally, as this study utilized male and female mice at a roughly even ratio, potential genetic differences accounting for replication failure cannot be ruled out[6]. That said, no differences in behavior between male and female mice in our assay were revealed, though it is certainly possible that our study was insufficiently powered to detect a sex effect.

Another unique aspect involved with interpreting these data is the nature of the response required in our assay (licks as opposed to lever press or nose poke). It is possible that analysis of magazine time and entries may be confounded by conditioned approach behaviors that develop during the process of reward learning. In a typical lever-press assay, the lever is not in the same location as the magazine, thus magazine time and entries reflect specific moments in which the animal disengages from responding in order to check for reward. Conversely, our assay requires the animal to already be in the magazine when responding. Nevertheless, that we see a drug effect which specifically impacts magazine time and that neither rewards obtained nor total licks were altered suggests the attenuation of approach behavior following inactivation of VTA-IC DA cells was independent of disruptions in incentive motivation. This is a unique finding which may reflect a discrete role for the circuit in behavioral nuances underlying responding to the context (e.g., habitual vs goal-directed).

[6] Experiments for this dissertation began in late 2019, well before anyone was aware of these issues in DAT-Cre mice. Future researchers should strongly consider restricting behavioral experiments to a single sex whenever the DAT-Cre knock-in line is involved.

These considerations aside, there is clearly something different about DN-DISC-1 mice with respect to their increased survival time during PR testing. Given that overall motivation to attain reward seems unaffected, we might consider the idea of attentional deficits arising from neurophysiological aberrations in the phenotype. Perhaps, DN-DISC-1 mice show increased survival time, not because they are more motivated to attain the reward, but simply because they have difficulty maintaining focus on the task at hand (i.e., sustained licking at the well), and thus take longer to complete the same number of ratios. DN-DISC-1 mice have previously been shown to have reduced PVB expression in frontal cortical GABAergic neurons (Hikida et al., 2007; Shen et al., 2008), an aberration which may well translate to the loss of cortical synchrony thought to underlie the cognitive and attentional deficits seen in humans with schizophrenia (Adams, 2018).

Alternatively, another interpretation of these data would eschew differences in motivation and attention altogether, instead suggesting that DN-DISC-1 mice show alterations in reward expectancy. Consider that dopamine is known to encode the relationship between expectation and outcome (e.g., Schultz et al., 1997) such that unexpected events lead to brief increases in dopaminergic signaling (i.e., a positive reward prediction error; RPE), while events that are fully expected elicit no change in dopamine. Events which are expected but fail to occur lead to decreases in dopaminergic signaling (i.e., a negative RPE). Importantly, the changes in dopaminergic firing associated with positive RPE and negative RPE are positively correlated with approach behavior. As such, it could be that aberrations in dopaminergic circuitry in DN-DISC-1 mice alter RPE in such a manner that negative RPE does not occur when expectations go unfulfilled. We might imagine, for instance, a DN-DISC-1 mouse licking at the well in an attempt to complete the current reinforcement schedule, then stopping to check for reward.

Under ordinary circumstances, a negative RPE should occur if the reward is not present, thus preventing further checking behavior; yet, in the DN-DISC-1 mouse, this does not occur. Although not significant, there is a trend toward increased magazine entries in the vehicle DN-DISC-1 condition, this coupled with the significant effect on survival time might offer support to this theory[7].

Finally, with regard to behavioral perseveration, we might consider that neither attentional deficits nor failure of negative RPE are the cause of behavioral differences for DN-DISC-1 mice in the assay. Instead, it could simply be that DN-DISC-1 mice display a pattern of appetitive behavior that is more habitual and inflexible. Theoretically, this could be described by enhanced behavioral control through stimulus-response (S-R) mechanisms that compel DN-DISC-1 mice to consistently approach the food cup throughout the duration of PR testing. S-R responding contrasts with response-outcome (R-O), which is a more goal-directed, flexible orientation. Interestingly, DN-DISC-1 mice were previously found to show deficits in R-O contingencies as evidenced by deficits in reinforcer devaluation. Specifically, the data from Johnson and colleagues (2013) show that these mice have difficulty shifting their behavior away from an established response even after the reward that response yields has been devalued. Clearly, a more thorough investigation of behavior which is capable of interfacing with such aspects related to learning (Chapter 4) as well as a neuroanatomical characterization of potential differences in the VTA → IC dopaminergic circuitry in DN-DISC-1 mice (Chapter 5) is needed.

[7] For a translational image, consider the idea of a human subject with obsessive compulsive disorder checking and re-checking the lock on their front door. They see that the door is locked, but this signal does not register and translate to the appropriate termination of goal-directed behavior.

CHAPTER 4: EFFECTS OF VTA → IC DOPAMINE INACTIVATION DURING MEDIATED PERFORMANCE

Introduction

What is the nature of a hallucination? From a phenomenological perspective, we can say that hallucinations are diverse in both content and complexity. Hallucinations are not restricted to a single sensory system but often occur across multiple domains of experience (i.e., tactile, auditory, visual, olfactory, gustatory)[8]. There is not a defining etiological source for hallucinations; yes, they occur across a range of neuropsychiatric conditions, but they also appear to be astonishingly common even among the neurotypical population (Johns et al., 2004). If there is but one facet of hallucinations that links them as a singular phenomenon, however, it is the failure to separate actual events from internal representations of absent events (Bentall et al., 1991). Given that hallucinations can occur across a range of conditions, we might imagine reality testing as a sort of sliding scale which is impacted by various factors, some of which could include emotional valence, stress, drug use, and perhaps most importantly, underlying neurobiological aberrations.

It seems only natural to focus on schizophrenia if our goal is to understand the neurobiology of impaired reality testing. No other disorder is so intrinsically tied to the idea of hallucinations, and indeed, the experience of impaired reality testing in those diagnosed. But what have we learned from the study of humans with schizophrenia over the last century or more? We know that individuals with schizophrenia see an improvement in positive symptomology when given drugs that antagonize DRD2 signaling, certainly. We know from studies of postmortem brain tissue as well as functional imaging that individuals with

[8] Although, auditory hallucinations are the most common (Mueser et al., 1990).

schizophrenia show a range of neurophysiological aberrations, including reduced frontal cortical and temporal lobe volume, as well as alterations in activity in and around these regions that are thought to reflect impairments in connectivity across regions (e.g., Camchong et al., 2009; Lynall et al., 2010; Liu et al., 2011; Hu et al., 2015). All of these observations are important, but they lack a level of mechanistic specificity which would likely hinder therapeutic innovation. Frankly, it is simply not possible to ask, for instance, how we might interrogate a given neurological circuit for hallucinations in humans with schizophrenia. It is not yet possible to ask then, how we might develop more specific pharmacological treatments that address the positive symptoms of schizophrenia without undesirable off-target effects. And so, we return to that humble species of mammal to which we owe so much of scientific progress: the rodent.

In Chapter 1, I discussed the principles underlying a specific facet of associative learning phenomena known as mediated learning. Briefly, this is the idea that early in the course of learning, Pavlovian cues come to evoke robust internal representations of the perceptual and sensory features of rewards they later predict. Furthermore, these cue-evoked representations can be used to instantiate new learning as directed toward a stimulus, even in the absence of its presentation. Mediated performance is a related form of learning, but in this case, cue-evoked representations come to elicit behaviors related to a previous stimulus that is now absent. Historically, these procedures were utilized with the goal of understanding the role of internal perception in associative learning (e.g., Holland, 1990). It wasn't until McDannald and colleagues (2011) showed that rats that received neonatal ventral hippocampal lesions (NVHL) seemed to more readily express a phantom taste aversion acquired through mediated learning (i.e., representation mediated taste aversion [RMTA]) that these procedures began to be considered as a means of investigating impaired reality testing. That the NVHL rats in the study

ceased to express the RMTA when given an antipsychotic gave further credence to the idea that this was a phenomenon which tapped into the neurobiological mechanisms underlying positive symptomology. Since then, a number of studies utilizing other animal models of schizophrenia have found similar results. Impairments in signaling pathways related to phospholipid metabolism have been found in humans with schizophrenia (du Bois et al., 2005), and sure enough, phospholipase C β1 knock-out mice (PLCβ1-/-) show enhanced RMTA (Kim & Koh, 2016). The same is true for mice sub-chronically exposed to ketamine (a procedure used to recapitulate aspects of the glutamate hypothesis of schizophrenia; Koh et al., 2018). Similar results have also been shown in studies utilizing mediated performance: both DN-DISC-1 (Fry et al., 2020) and ketamine-exposed mice (Wu et al., 2020) are more sensitive to these manipulations, the effect is DRD2-dependent, and associated with increased activity at the level of the insular cortex in both of these studies.

These data bring us to the current studies at hand. In order to extend the findings of Fry et al. (2020), it seems natural to ask what it is about DN-DISC-1 mice that underlies their enhanced sensitivity to mediated performance procedures. These mice showed enhanced lick cluster size (a measure of palatability; Johnson, 2018) as directed at unflavored water when in the presence of a Pavlovian cue that had previously been paired with sucrose. Conversely, we know that despite whatever pathologies may exist in these animals, they do not perceive sweet taste differently under ordinary circumstances (Fry et al., 2020; Ch. 2). We have two clues as to the neurobiology underlying this impairment in reality testing: enhanced activation of the IC, and the rescue of behavior following global DRD2 blockade. These in mind, I previously theorized that aberrations in a putative dopaminergic circuit running from the VTA → IC might be the answer. As such, the study outlined in this chapter seeks to test the necessity of the VTA → IC

dopaminergic circuitry for impaired reality testing associated with phantom gustatory sensations in DN-DISC-1 mice. This work has the potential to reveal new information about behavioral functions of VTA → IC projecting dopamine cells, as well as potentially identify a novel circuit for positive-like symptomology occurring along the gustatory modality.

Materials & Methods

Animals

DN-DISC-1 mice were generated and crossbred with DAT-Cre mice as described previously in order to create the two distinct lines used in this experiment: DN-DISC-1 x DAT-Cre (*n* = 29, split among *n* = 10♀ and *n* = 19♂) and wild-type x DAT-Cre (*n* = 32, split among *n* = 20♀ and *n* = 12♂). Mice were group-housed by sex until ~12 weeks of age, then singly housed following viral infusion surgeries and for the duration of behavioral experiments. All mice were maintained on a 12-hour light/dark cycle and restricted to ~90% of their baseline free-feeding weight throughout behavioral testing.

Surgeries

Targeting of the IC (0.25 µl per side; AP +1.7, ML +/-2.5, DV -3.25) and VTA (AP -3.08, ML +/-0.6, DV -4.5) for bilateral viral infusions with AAVrg-pEF1a-DIO-FLPo-WPRE-hGHpA and AAV8-DJ-hSyn-fDIO-hMD4(Gi)-mCherry, respectively, was carried out in the same manner as described previously among a cohort of *n* = 45 (split among *n* = 7♀ and *n* = 14♂ DN-DISC-1 x DAT-Cre and wild-type x DAT-Cre split among *n* = 16♀ and *n* = 8♂). A separate cohort of *n* = 16 (split among *n* = 3♀ and *n* = 5♂ DN-DISC-1 x DAT-Cre and wild-type x DAT-Cre split among *n* = 4♀ and *n* = 4♂) mice received bilateral infusions of a cre-dependent, retrograde mCherry control virus lacking the hMD4(Gi) receptor (AAVrg-hSyn-DIO-mCherry; Addgene, plasmid #50459) targeted at the IC (0.25 µl per side; AP +1.7, ML +/-2.5, DV -3.25)

in order to allow for the observation and control of any behavioral effects caused by non-specific binding of CNO in the main cohort. This method allowed for the mCherry control virus to be taken up at synaptic terminals in the IC, then trafficked backward to their point of origin and with the goal of selectively infecting Cre-expressing dopaminergic cells (presumably of the same population as in those animals that received the hM4D4[Gi] version of the virus).

Drugs

Doses of CNO and vehicle were prepared as previously detailed. CNO was injected at 0.3 mg / kg IP; sterile 0.2 M PBS was injected at the same volume as CNO on vehicle test days. Drug order on test days was randomized across genotypes. All mice received one test day on CNO and one test day with vehicle injected 15 minutes prior to the beginning of the session.

Behavioral Procedures

All mice received two food cup training sessions that began with an initial 50 µl delivery of 0.2 M sucrose present in the food magazine well. Once the animal entered the magazine, the program initiated the first of 16 trials; during each trial 50 µl delivery of 0.2 M sucrose US occurred via a 120 s random time schedule. Sessions varied between 30 – 45 min to completion with the goal of mice attaining a minimum of 10 seconds magazine time in the presence of the sucrose US before proceeding to the next phase of training. Following food cup training, mice proceeded to 8 days of individual 32-minute Pavlovian conditioning sessions. During these sessions, mice received a total of 14 pseudo-randomly distributed presentations of a 10 second duration 80 dB white noise CS paired with a 50 µl delivery of 0.2 M sucrose. After completing the Pavlovian conditioning sessions, mice underwent an individual test day (with IP injections of CNO or vehicle 15 minutes prior). Test sessions lasted 16 minutes and included 2 CS presentations (CS1, CS2) paired with the delivery of unflavored deionized water separated by 7-

minute intertrial intervals. Magazine time, entries, and licking microstructure were recorded throughout the session as well as within the discrete 10 second periods preceding the CS (pre-CS period), during the CS, and following the CS (post-CS period).

Data Analysis

Immunohistological confirmation of viral targeting was conducted in the same manner as previously described; at this time, $n = 2$ subjects were excluded from further analysis due to poor viral expression. For training data, four-way repeated measures ANOVAs with between-subjects variables of genotype (DN-DISC-1 x DAT-Cre, wild-type x DAT-Cre) and future drug condition (CNO, vehicle), and within-subjects variables of block (sessions 1 – 2 averaged, sessions 3 – 4 averaged, sessions 5 – 6 averaged, and sessions 7 – 8 averaged) and period (pre-CS, CS) were used to compare magazine entries as a metric of Pavlovian acquisition. For test data, Two-way ANOVAs with between-subjects measures of genotype (DN-DISC-1 x DAT-Cre vs wild-type x DAT-Cre) and condition (CNO vs vehicle) were conducted for each separate test period: Pre-CS, CS and post-CS. These analyses were conducted for magazine entries and licks-per-minute. In addition, to examine whether CS presentation altered the perceived palatability of the unflavored water, mean burst size occurring between 250-500 ms, and mean lick burst number occurring between 250-500 ms was examined. Mauchly's test of sphericity was used to examine homogeneity of variance in the repeated measures analyses, while Levene's test for equality of variances was used for all others. Additionally, outliers ($n = 2$ vehicle wild-type x DAT-Cre) were identified using the IQR method in the CS period of magazine entries, specifically, and were removed from analysis. Subjects that received the mCherry control version of the virus lacking the hMD4(Gi) receptor were analyzed separately, but with identical procedures as those used above. For analysis of burst size and burst number in the hMD4(Gi) cohort, imputation with

the group condition mean was used to replace values of 0 for $n = 7$ subjects (split among $n = 3$ CNO DN-DISC-1 x DAT-Cre, $n = 1$ CNO wild-type x DAT-Cre, $n = 1$ vehicle DN-DISC-1 x DAT-Cre, and $n = 2$ vehicle wild-type x DAT-Cre). In the mCherry cohort, imputation with the group condition mean was used to replace a value of 0 for $n = 1$ CNO DN-DISC-1 x DAT-Cre subject. Additionally, $n = 2$ (split among $n = 1$ CNO wild-type x DAT-Cre and $n = 1$ vehicle wild-type x DAT-Cre) were identified as outliers in these measures using the IQR method and removed from further analysis. Significant interactions were further investigated using simple main effects analysis with post-hoc Bonferroni correction. All analyses were conducted using JASP with $\alpha = .05$ as the criterion for significance.

Results

Training

For both hMD4(Gi) and mCherry controls, analysis of Pavlovian acquisition showed that magazine entries increased during the CS period and decreased during the pre-CS period as training sessions progressed. Analysis of the mCherry cohort (Fig. 5) showed no effect of genotype or future drug condition (Fs < 1), a main effect of session block ($F = 15.834$, $p < .001$), a main effect of period ($F = 148.955$, $p < .001$), and a significant two-way interaction between these factors ($F = 69.964$, $p < .001$). There were more magazine entries during the CS as opposed to pre-CS period during training blocks 2 ($F = 90.366$, $p < .001$), 3 ($F = 98.815$, $p < .001$), and 4 ($F = 151.284$, $p < .001$), as well as significantly increased CS entries across blocks ($F = 49.741$, $p < .001$) and significantly decreased pre-CS entries across blocks ($F = 49.741$, $p < .001$).

For the hMD4(Gi) mice (Fig. 6), four-way repeated measures ANOVA revealed much of the same: no effect of genotype or future drug condition (Fs < 2), a main effect of session block ($F = 20.193$, $p < .001$), and a main effect of period ($F = 206.98$, $p < .001$). Additionally, there was a

significant two-way interaction between session and period (F = 118.226, $p < .001$), the nature of which was further investigated using simple main effects analysis. These analyses revealed that mice made significantly more magazine entries during the CS as opposed to pre-CS period during training blocks 2 (F = 137.87, $p < .001$), 3 (F = 172.357, $p < .001$), and 4 (F = 209.357, $p < .001$). Additionally, magazine entries significantly increased across blocks during the CS period (F = 73.079. $p < .001$), while magazine entries in the pre-CS period significantly decreased (F = 32.446, $p < .001$). These data indicate that all mice, regardless of viral cohort, genotype, or future drug condition were able to successfully learn in a comparable manner the Pavlovian association between CS and US prior to testing.

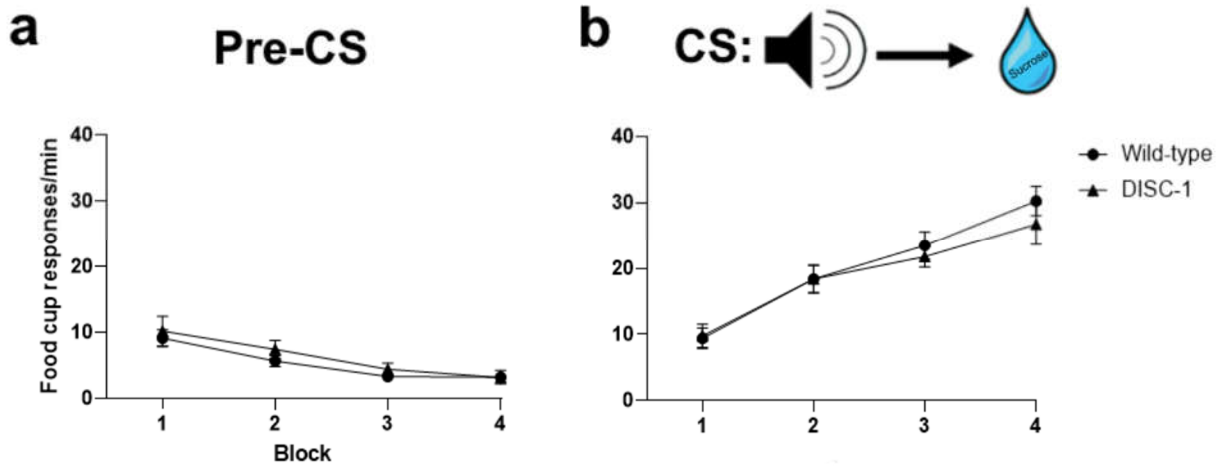


Figure 5. Pavlovian acquisition in the mCherry control cohort. All mice, regardless of genotype, successfully learned the Pavlovian association between the white noise CS and liquid sucrose US. Food cup entries decreased in the pre-CS period across days, while entries during the CS increased.

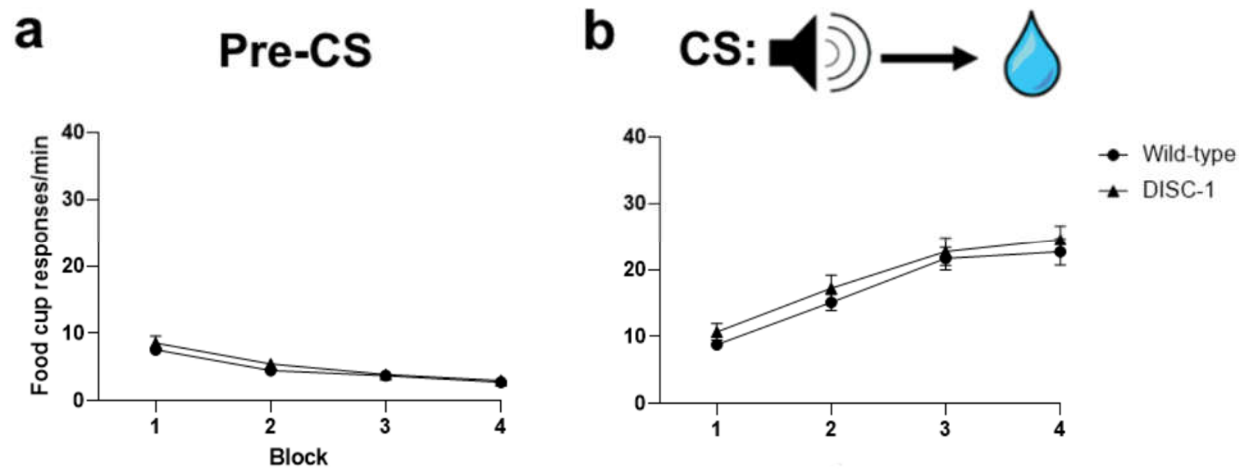


Figure 6. Pavlovian acquisition in the hMD4(Gi) cohort. All mice, regardless of genotype, successfully learned the Pavlovian association between the white noise CS and liquid sucrose US. Food cup entries decreased in the pre-CS period across days, while entries during the CS increased.

Test

Analysis of the mCherry control cohort revealed no differences between DN-DISC-1 or wild-type mice, regardless of drug condition on either measures of conditioned approach behavior (Fig. 7) or licking microstructure at test (Fig. 9). Specifically, two-way ANOVA showed no effect of genotype or drug on magazine entries or licks per minute during the pre-CS ($Fs < 1$), CS ($Fs < 2$), or post-CS period ($Fs < 1$). Similarly, there was no effect of genotype or drug on burst number or burst size ($Fs < 2$), this did not differ as a function of imputation (Figs. S.1. – S.2.).

Within the hMD4(Gi) cohort, inactivation of the VTA → IC dopaminergic circuitry decreased measures of conditioned approach behavior (Figs. 8a – c) for both wild-type x DAT-Cre and DN-DISC-1 x DAT-Cre mice, although this varied based on the test period examined (e.g., pre-CS vs CS). Two-way ANOVA revealed a significant main effect of drug ($F = 9.288$, $p = .004$) with no interaction among other factors during the pre-CS period. This suggests that

inactivation of VTA → IC dopamine, regardless of genotype, may reduce conditioned approach to the context.

During the CS period, a significant interaction between condition and genotype ($F = 4.245$, $p = .04$) was identified. Simple main effects analysis showed this interaction to be driven by DN-DISC-1 mice in the vehicle condition making significantly more entries as compared to wild-type ($F = 5.475$, $p = .02$), in addition to a tendency for CNO to reduce entries in DN-DISC-1 mice, specifically ($F = 3.773$, $p = .06$). The nature of this effect was further investigated in DN-DISC-1 subjects alone using a separate repeated measures ANOVA with within-subjects variable of period (CS1, CS2) and between-subjects variable of drug (CNO, vehicle) in order to determine whether or not magazine entries differed as a function of exposure to the US following CS1[9]. There was no effect of period ($F < 1$), but a main effect of drug ($F = 4.348$, $p = .05$) such that DN-DISC-1 mice in the CNO condition showed reduced entries relative to vehicle at both CS1 and CS2, though CS1 and CS2 entries within the CNO condition did not differ. Analyses of licks-per-minute at test (Fig. 8d – f) showed no differences regardless of genotype or drug condition during either the pre-CS, CS, or post-CS period ($Fs < 1$).

[9] During CS1, the mice are still 'naïve' to the fact that their expectations will be violated in relation to the US (i.e. receiving water instead of sucrose); thus, it would not be unexpected to find a reduction in approach behavior at CS2 relative to CS1.

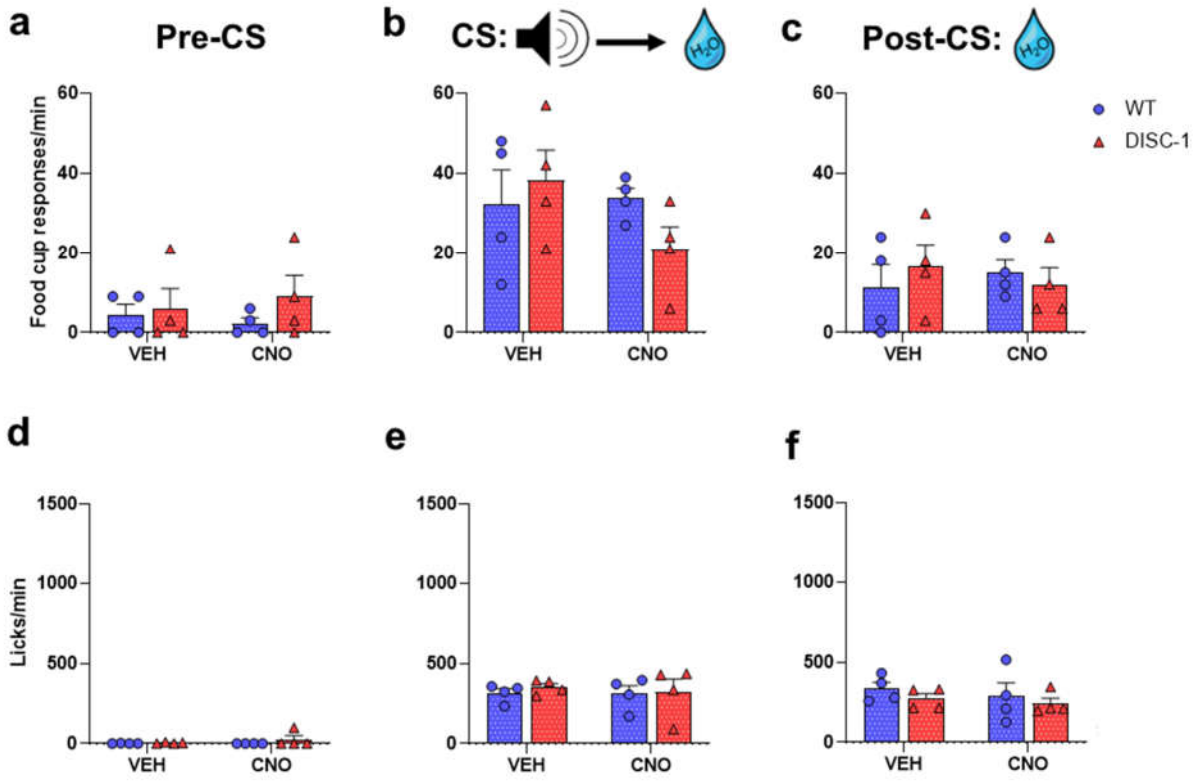


Figure 7. Analysis of conditioned approach behavior in the mCherry control cohort. There were no differences between genotypes, regardless of drug condition in terms of conditioned approach at the food magazine (A, B, C) or rate of licking for the unflavored water during either pre-CS, CS, or post-CS periods (D, E, F).

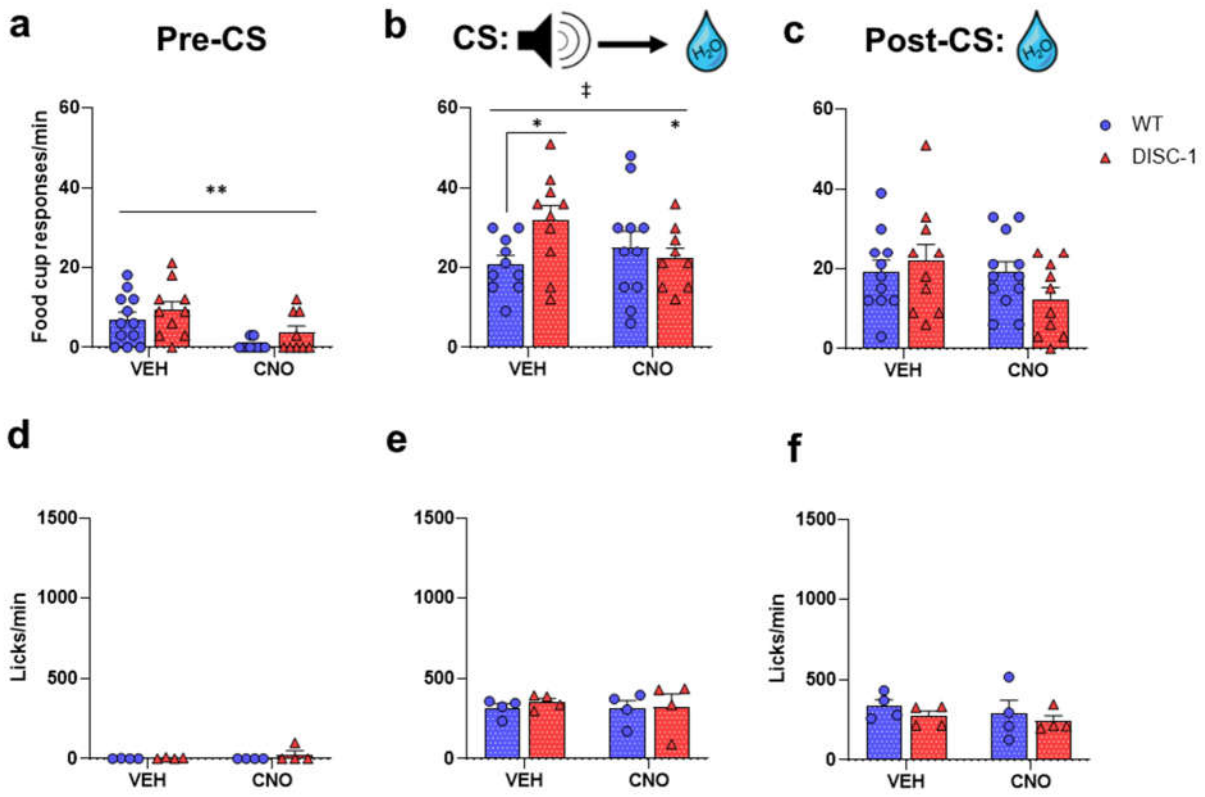


Figure 8. Analysis of conditioned approach behavior in the hMD4(Gi) cohort. Inactivation of the VTA → IC dopaminergic circuitry suppressed magazine entries during the pre-CS period (A). During the CS, DN-DISC-1 mice made significantly more food cup entries than wild-types, an effect that was suppressed following inactivation of VTA → IC dopamine (B). There were no effects of drug or genotype on magazine entries in the post-CS period (C). Rate of licking at the food cup did not differ by genotype and was not impacted by drug during any period (D, E, F). ***p < .01; ‡ two-way interaction, genotype x condition, p < .05; *p < .05*

With regard to licking microstructure, no effects were revealed at the level of burst number (Fs < 2; Fig. 10a). This did not differ based on imputation (Fig. S.1.). In terms of average burst size, however, two-way ANOVA revealed a significant interaction between genotype and condition (F = 5.486, p = .02) such that CNO increased licking in wild-type x DAT-Cre mice, specifically (Fig. 10b). Simple main effects analysis showed that CNO led to significantly increased burst size in wild-type mice relative to both DN-DISC-1 mice in the CNO condition (F = 7.644, p < .01) as well as wild-type mice that received vehicle (F = 5.827, p =.02). These effects differed slightly as a factor of imputation (Figs. S.3. – S.4.), such that, if imputed

data points and their subjects were removed, the interaction moved to trend-level ($F = 3.605$, $p = .06$), presumably due to reduced power. The direction of the effect did not change, however, with simple main effects showing the same pattern of significant elevation in burst size for wild-type subjects that received CNO relative to DN-DISC-1 that received CNO ($F = 5.079$, $p = .03$) and wild-type that received vehicle ($F = 4.311$, $p = .04$). If previously imputed subjects were allowed to remain in the dataset with their original scores of 0 for burst size maintained, the interaction was again significant ($F = 6.211$, $p = .01$), with simple main effects mirroring the previous ($F = 8.463$, $p < .01$; $F = 6.929$, $p = .01$). Taken as a whole, these results show that inactivation of the VTA → IC circuit leads to alterations in conditioned approach behavior for both wild-type and DN-DISC-1 mice as directed at the context, a normalization of aberrant approach behavior for DN-DISC-1 mice during the CS, specifically, and impaired reality testing in wild-type mice.

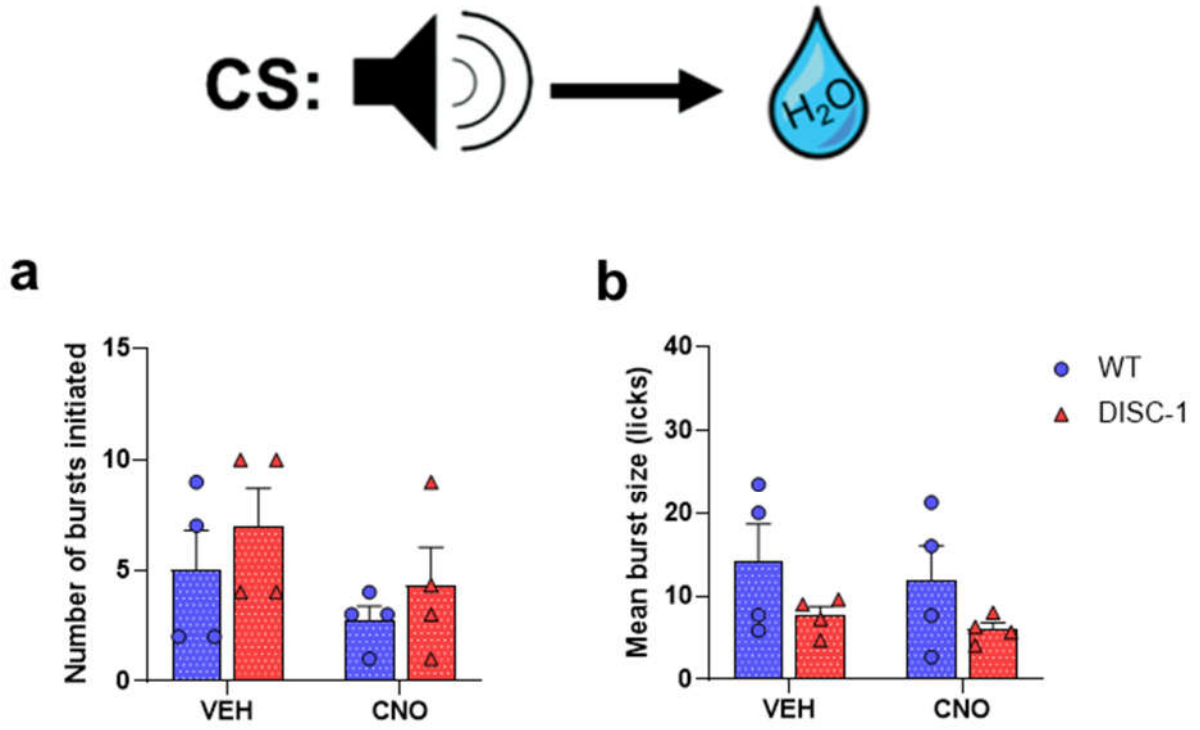


Figure 9. Analysis of licking microstructure in the mCherry control cohort. There was no effect of genotype or impact of drug on number of bursts initiated or mean burst size occurring within the 250 – 500 ms pause criterion at test (A, B).

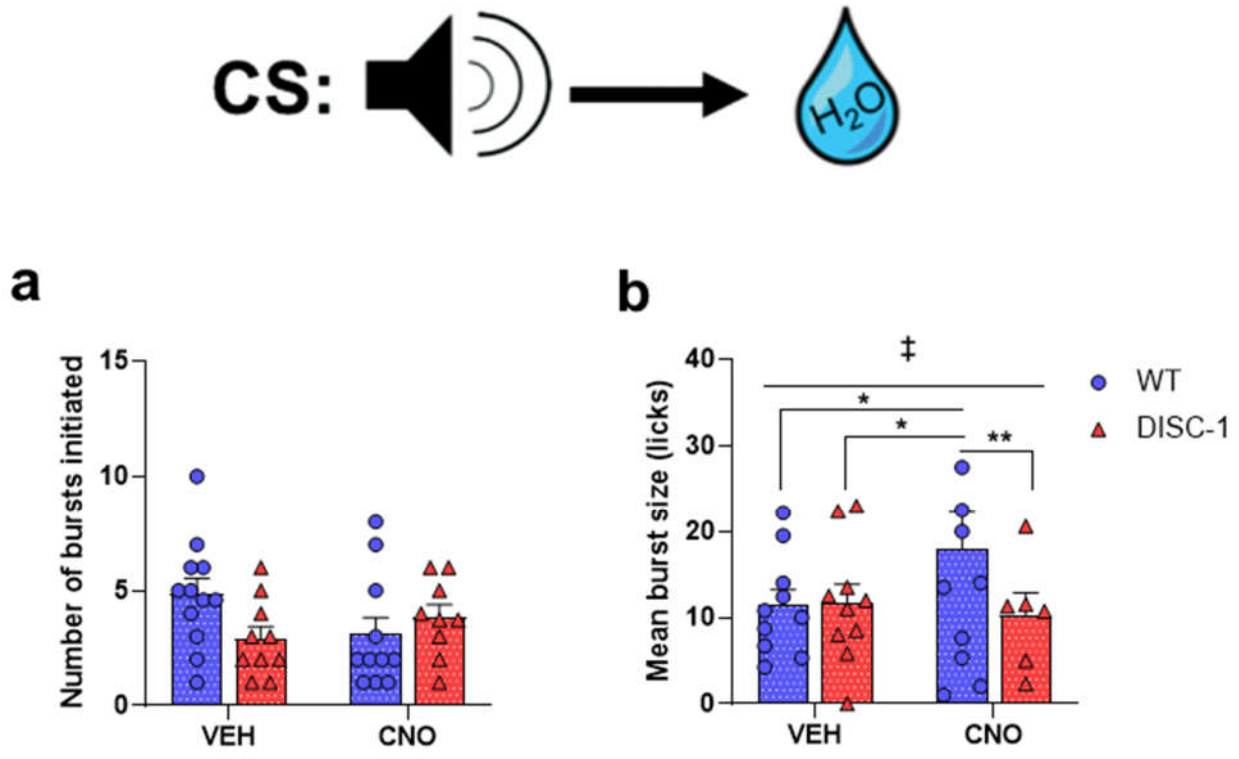


Figure 10. Analysis of licking microstructure in the hMD4(Gi) cohort. CNO did not impact the number of bursts of licking initiated, regardless of genotype (A); however, CNO led to a significant increase in mean burst size for wild-type mice, specifically (B). *‡ two-way interaction, genotype x condition, $p < .05$; **$p < .01$; *$p < .05$*

Discussion

In this study, we utilized a mediated performance assay in which DN-DISC-1 x DAT-Cre and wild-type x DAT-Cre were trained to associate a white noise CS with the delivery of a liquid sucrose US. At test, the white noise CS was paired with the delivery of unflavored water, whereupon we examined conditioned approach behavior and licking microstructure. This assay presumes animals will typically respond to the water with a pattern of approach and palatability responses that would contrast to those evoked by the original, highly valued stimulus (i.e., with decreased magazine responding and lick burst size). On the other hand, increased lick burst size toward the water, for example, would be assumed to indicate impaired reality testing as driven by the representation of sweet taste produced in proximity to the CS. Here, we show that

inactivation of the VTA → IC dopaminergic circuitry at test leads to unique alterations in behavior which are dependent upon genotype. Furthermore, given that no behavioral differences arose in training, these effects cannot be accounted for by alterations in the ability to acquire the Pavlovian association between CS and US.

Inactivation of VTA → IC DA Reduces Approach Behavior in DN-DISC-1

In the pre-CS period, we saw that CNO led to a reduction in magazine entries regardless of genotype. This suggests that the VTA → IC circuit may play a role in the capacity of the contextual environment to evoke conditioned responding, Alternatively, it could simply be related to potential off-target effects of CNO reverse-metabolizing into clozapine and leading to motoric impairments. This explanation is unlikely, however, given that no such effect was observed in the mCherry control cohort. Second, the CNO-induced reduction in magazine entries continues for DN-DISC-1 mice during the CS, but not wild-type. This is due to the fact that DN-DISC-1 mice make significantly more CS-evoked entries in the vehicle condition, an effect which is attenuated by CNO. Perhaps most tellingly, entries are reduced to the level of vehicle wild-type, a number which did not differ from those of CNO wild-type. This suggests that whatever may be occurring here with relation to conditioned approach behavior, it is specific to aberrations in the DN-DISC-1 genotype, some, but perhaps not all of which, can be accounted for differences in the VTA → IC dopaminergic circuitry. This last point will be important when considering the observed effects on measures of palatability in wild-type mice, which I will return to in the next section. For now, however, let us consider what might be driving these differences in approach behavior in DN-DISC-1 mice.

The IC is known to play a role in representing both the affective and sensory experience of pain (e.g., Starr et al., 2009; Lu et al., 2016). Given that DN-DISC-1 mice have previously

been shown to display aberrant IC signaling activity (Fry et al., 2020), we might imagine that this leads to a heightened experience of pain and resultant changes in behavior in our assay following injection. It follows then, that inactivation of the VTA → IC dopamine circuitry in DN-DISC-1 mice would reduce this effect, which appears in our assay as a reduction in magazine entries to the level of controls. That pain/injection stress would only affect preparatory behaviors and leave consummatory unaffected, however, seems doubtful, particularly given that peripheral pain has been shown to enhance the suppression of consummatory behavior following incentive downshifts (Ortega et al., 2011).

As such, we're left to focus on the change in the nature of the outcome at test (i.e., from sucrose to water) and any alterations in dopaminergic function that ought to accompany it. For our animals, having received 8 days of Pavlovian conditioning in which the CS was paired with a sucrose US, we should assume that the CS has been instantiated as an expectancy marker for the delivery of sucrose. Indeed, significantly elevated magazine entries during the CS as training progressed suggests this is true. As such, we can assume that at test, when the CS is presented and the US is delivered, the animal is expecting to experience the taste, smell, and mouthfeel of sucrose—but they do not. This is a violation of expectations which, under ordinary circumstances should elicit a negative RPE and a corresponding reduction in midbrain dopamine firing leading to reduced approach behavior (e.g., Schultz et al., 1997). Perhaps, under vehicle conditions, this reduction in dopaminergic firing following a negative RPE does not occur in DN-DISC-1 mice, at least not sufficiently; thus, inactivating the VTA → IC dopaminergic circuit may remove just enough dopaminergic tone in the midbrain as to allow normal function of RPE to occur. Alternatively, it could be that the surprising aspect of tasting water when expecting sucrose leads to a reinvigoration of associability and incentive motivation as directed at the CS

(e.g., Pearce & Hall, 1980) and that this process is exaggerated in DN-DISC-1 mice. Both of these accounts make sense in light of the fact that increased midbrain dopaminergic tone is thought to underlie aspects of positive symptomology in humans with schizophrenia (Adams, 2018). We must be cautious with how far we extend these interpretations, however, given that DN-DISC-1 mice, regardless of drug condition, showed no differences in licking microstructure or licks per minute relative to wild-type mice during the CS. To be clear, if a failure and subsequent restoration of negative RPE were to occur under drug conditions, we should see this manifest within the consummatory behaviors that are actually detecting error in prediction (i.e., licking as relative to the change in taste). As such, given that these changes do not appear in licking microstructure, we must consider alternative explanations.

One final consideration, separate of negative RPE, associability, and pain, would be to return to the idea that DN-DISC-1 mice are simply more habitual as opposed to goal-directed in their behavior. As discussed in Ch. 3, this idea has previously been considered in light of deficits in response devaluation (Johnson et al., 2013), suggesting that these mice tend to perseverate in their behavior, despite alterations in the nature of the outcome. If DN-DISC-1 mice naturally display a more habitual as opposed to goal-directed phenotype evidenced by increased magazine entries sans changes in consummatory behavior, then it follows that the effect of reducing entries when inactivating the VTA → IC dopamine circuitry reduces this tendency. Furthermore, if wild-type mice do not display this exaggerated habitual phenotype, then it is perfectly reasonable that CNO altered their magazine responding during the pre-CS (i.e., prior to presentation of the reward) but not during the CS (i.e., when information about alterations in the nature of the

outcome was available[10]). Interestingly, however, wild-type mice do show alterations in consummatory behaviors at test, while DN-DISC-1 mice do not, suggesting that the VTA → IC dopamine circuitry may control independent facets of behavior, potentially dependent upon underlying genetic differences in neural signaling between the regions.

Inactivation of VTA → IC DA Impairs Reality Testing in Wild-type

The previous findings seem all the more striking when considering that inactivation of the VTA → IC dopaminergic circuitry led to significantly increased mean burst size during the CS in wild-type mice. This suggests that on receiving CNO, wild-type mice perceived the unflavored water as tasting sweeter than mice in any other condition, a phantom gustatory sensation indicative of impaired reality testing. That this effect occurred despite a comparable total number of licks between genotypes and in the absence of any alterations in conditioned approach behavior underscores the potential specificity of the VTA → IC dopaminergic circuitry for impaired reality testing occurring within the gustatory modality. This account makes sense in light of the findings in Fry et al. (2020), which showed that impaired reality testing in the mediated performance assay is both dopaminergically-dependent and associated with elevated neural activity in the IC. That said, it should be made clear that the current study failed to replicate a main finding of the previous, which is that this effect was specific to DN-DISC-1 mice. By contrast, our DN-DISC-1 mice, both in the hMD4(Gi) vehicle and mCherry cohort did not show any changes in burst size relative to wild-type vehicle mice, a result which might be accounted for by underlying differences in striatal dopaminergic circuitry (e.g., decreased DAT expression) tied to the presence of the DAT-Cre gene (Costa et al., 2021) in the current study[11].

[10] In keeping with Fry et al., (2020), this set of experiments utilized delay conditioning (as opposed to trace or simultaneous), meaning that the US was delivered during a period in which the CS was still present. Specifically, the US was delivered 5 seconds into the 10 second CS.

[11] This will be discussed in greater detail in Chapter 6.

Replication failures aside, there is clearly an effect of inactivating the VTA → IC dopaminergic circuitry in wild-type mice, which by all previous accounts in the literature (e.g., Corlett & Schoenbaum, 2021), seems to fit the profile for impaired reality testing associated with phantom gustatory sensations. So then, what might be the underlying cause of these aberrations? To start, we know that the IC contains a large population of GABAergic interneurons and glutamatergic pyramidals modulated by both DRD1 and DRD2 signaling. Further, these neurons are thought to receive synaptic input from a distinct subpopulation of dopamine neurons in the VTA (Ohara et al., 2013). Could it be that inactivation of the VTA → IC dopaminergic circuitry leads to a loss of computational specificity in the region that results in unchecked neural firing in response to stimuli? Such activity could, theoretically, lead to the generation of abnormally strong internal perceptions of absent external stimuli. Lack of precision, at least with regard to sensory *input* (e.g., taste) has previously been considered as a causal factor underlying the generation and maintenance of hallucinations in humans (Adams, 2018; Benrimoh et al., 2018); perhaps then, this account is not far off with respect to our mice.

Conclusion

The data at hand shed new light on the dopaminergically-dependent impairments in reality testing associated with aberrant IC activity observed by Fry et al. (2020) in DN-DISC-1 mice. Here, we show that a loss VTA → IC dopaminergic tone leads to alterations in conditioned approach behavior that may be due to differences in response to outcome expectations or attentional factors underlying learning in DN-DISC-1 mice, specifically. Strikingly, we also see that inactivating VTA → IC dopamine leads to the generation of phantom gustatory sensations in wild-type mice, while DN-DISC-1 mice remain unaffected. These results underscore the

importance of conducting a thorough neuroanatomical characterization of the circuit as reported upon in Chapter 5.

CHAPTER 5: CHARACTERIZATION OF VTA → IC DOPAMINERGIC CIRCUITRY IN DN-DISC-1 & WILD-TYPE MICE

Introduction

As of recent analyses (e.g., Kim et al., 2019), we know that the VTA contains roughly 70% dopamine neurons. Countless studies have confirmed that these neurons play a role in a variety of facets underlying goal-directed behavior including learning (e.g., Schultz & Montague, 1997), reward (e.g., Berridge, 2007), effort (e.g., Salamone et al., 2007), and decision-making (e.g., Floresco & Magyar, 2006). Furthermore, we know that these neurons and their receptors have been implicated in the positive symptoms of schizophrenia. For instance, humans with schizophrenia show increased presynaptic dopamine synthesis at the level of the striatum (e.g., Lindström et al., 1999; Meyer-Lindberg et al., 2002), this leads to increased binding of DRD2s which is thought to be a primary mechanism for the ability of antipsychotic drugs to reduce positive symptoms (e.g., Howes & Kapur, 2009; Adams, 2018). At this point, there has been a great deal of work involved in the characterizing of major VTA dopaminergic efferents to areas like the NAc, basolateral amygdala (BLA), and prefrontal cortex due to their role in the behaviors previously mentioned. Comparatively less is known, however, about dopamine neurons that project from the VTA to the IC. We do not know yet, for instance, whether dopamine neurons projecting to the IC originate from a specific subregion of the VTA, nor whether the distribution of these neurons and their receptors in the circuit might be affected by translocation of the DISC-1 gene. The studies outlined in this chapter will address these questions directly through the use of virally-mediated tracing in combination with both immunohistochemistry and qPCR. In addition to a thorough characterization of the VTA → IC dopaminergic circuitry, we will also compare the expression of DRD1 and DRD2 in other major

regions of the brain that receive major dopaminergic innervation (e.g., NAc, BLA, and dorsal striatum [DS]). Together, this work has the potential to yield new information related to the functional anatomical consequences of DISC-1 translocation.

EXP 1: Characterization of VTA → IC Projecting Dopamine Neurons

Materials & Methods

Animals

DN-DISC-1 mice were crossbred with DAT-Cre mice as previously described. Mice (n = 8, split among n = 4 DN-DISC-1 x DAT-Cre and n = 4 wild-type x DAT-Cre) used in this study had previously undergone Pavlovian conditioning and were assayed for mediated performance as described in Chapter 4.

Surgeries

All mice in this study underwent bilateral infusion with AAVrg-pEF1a-DIO-FLPo-WPRE-hGHpA at the level of the IC (0.25 µl per side; AP +1.7, ML +/-2.5, DV -3.25) and AAV8-DJ-hSyn-fDIO-hMD4(Gi)-mCherry at the level of the VTA (0.25 µl per side; AP -3.08, ML +/-0.6, DV -4.5) as previously described. Seven weeks elapsed between surgery to sacrifice.

Immunohistology

Mice were sacrificed by way of transcardial perfusion with 4% paraformeldahyde as previously described. Brains were extracted and stored as previously described, then sliced at 30 µm using a freezing microtome. Coronal sections of VTA ranging from bregma -2.80 mm through -3.88 mm (Paxinos & Franklin, 2019) were moved through six, 8-minute washes in 0.1 M PBS. Next, slices were placed in a solution consisting of 3% normal donkey serum (NDS; Catalog# 017-000-121; Jackson Immunoresearch, West Grove, PA) and 10% Triton-x (MilliporeSigma, Burlington, MA) in PBS for 1 hour. Finally, sections of VTA were placed in a

solution of 3% NDS, 10% Triton-x, and mouse-anti-TH primary (Catalog# MAB318; MilliporeSigma) at 1:1000 concentration in 0.1 M PBS for 24 hours. The next day, slices were moved through six washes in 0.1 M PBS for 8 minutes each, then placed in a solution consisting of 3% NDS, 10% Triton-x, and Alexa-Fluor donkey-anti-mouse 488 secondary antibody (Catalog# A32766; ThermoFisher Scientific) at 1:500 concentration for 2 hours. Afterward, sections were move through two 8-minute washes in 0.1 M PBS before being flow-mounted using 0.1 M PB onto microscope slides. Slides were treated with Prolong Gold Antifade Mountant with DAPI before being coverslipped and left to cure for 24 hours. Slides were then imaged using an Olympus BX51 fluorescent microscope (Olympus Corporation).

Data Analysis

Images of the VTA were first examined qualitatively in order to ascertain the extent and spread of the hMD4(Gi)-mCherry virus. During this time, it was determined that mCherry-positive cells were restricted to regions of the VTA ranging from -2.92 through -3.16 mm (Fig. 11). Afterward, two separate raters blind to genotype counted the number of TH-positive cells, mCherry positive cells, and cells in which mCherry and TH were colocalized. Interrater-reliability was assessed by calculating the percent difference between counts for the two raters and determined to be $\geq$ 92% across regions before proceeding to statistical analysis. Two-way repeated measures ANOVAs with between-subjects variable of genotype (DN-DISC-1, wild-type) and within-subjects variables of depth (-2.92 mm, -3.08 mm, -3.16 mm) were used to assess whether any differences in the number of TH, mCherry, or colocalized cells were present throughout the VTA. Viral efficiency was calculated by comparing the number of colocalized cells to TH positive in a one-way ANOVA with between-subjects variable of genotype—this analysis was restricted only to regions in which colocalized cells were present. Analysis of viral

specificity was carried out in a similar manner by comparing the number of mCherry cells to TH positive cells.

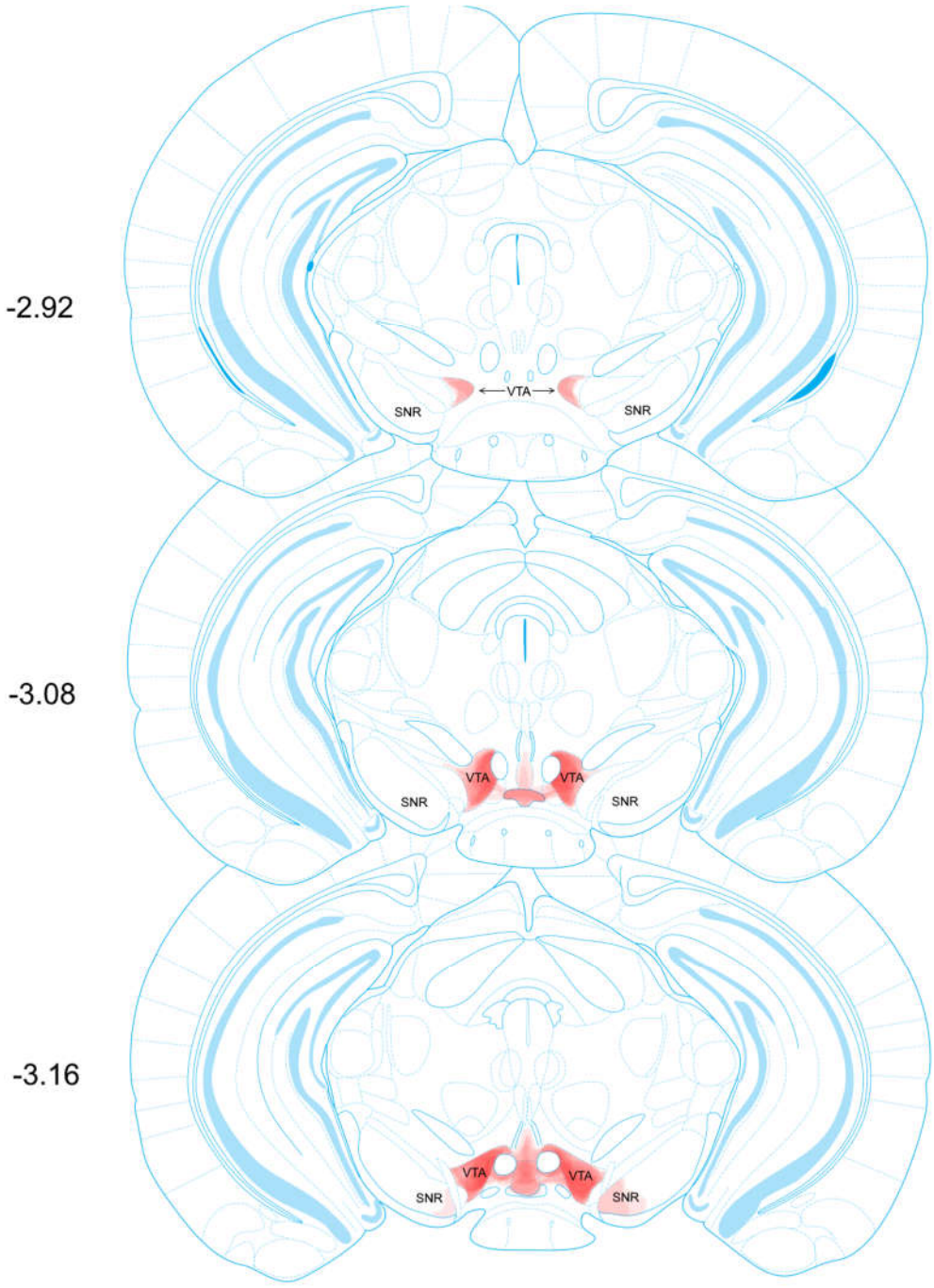


Figure 11. AAV8-DJ-hSyn-fDIO-hMD4(Gi)-mCherry viral transfection. Confirmed expression of the Flp-dependent inhibitory DREADD virus ranging from anterior to medial VTA. Darker shading represents increased viral expression relative to lighter. Viral spread was highly specific to VTA with minimal expression in off-target regions, including substantia nigra (SNR).

Results

Analysis of TH immunoreactivity (Fig. 12a) showed no effect of genotype ($F < 1$) and an expected significant main effect of depth ($F = 13.483$, $p < .001$), such that TH cell counts increased moving from rostral to medial VTA. Analysis of cells expressing the viral mCherry reporter (Fig. 12b) showed no effect of depth ($F < 2$), but a significant main effect of genotype ($F = 7.809$, $p = .03$) such that DN-DISC-1 subjects had less positive mCherry-expressing cells. Colocalization counts (Fig. 12c) confirmed this pattern, once again showing no effect of depth, and a significant main effect of genotype ($F = 32.604$, $p < .001$) in the same direction as the previous, indicating DN-DISC-1 mice had less virally-transfected TH immuno-reactive cells in the VTA. These data were echoed in the analyses of viral efficiency and specificity which showed that the virus was significantly less efficient in DN-DISC-1 mice ($F = 19.979$; $p = .004$) while there were no differences between genotypes in terms of specificity ($F < 1$). These data suggest that DN-DISC-1 mice have less dopamine cells which project from the VTA to the IC (Fig. 13).

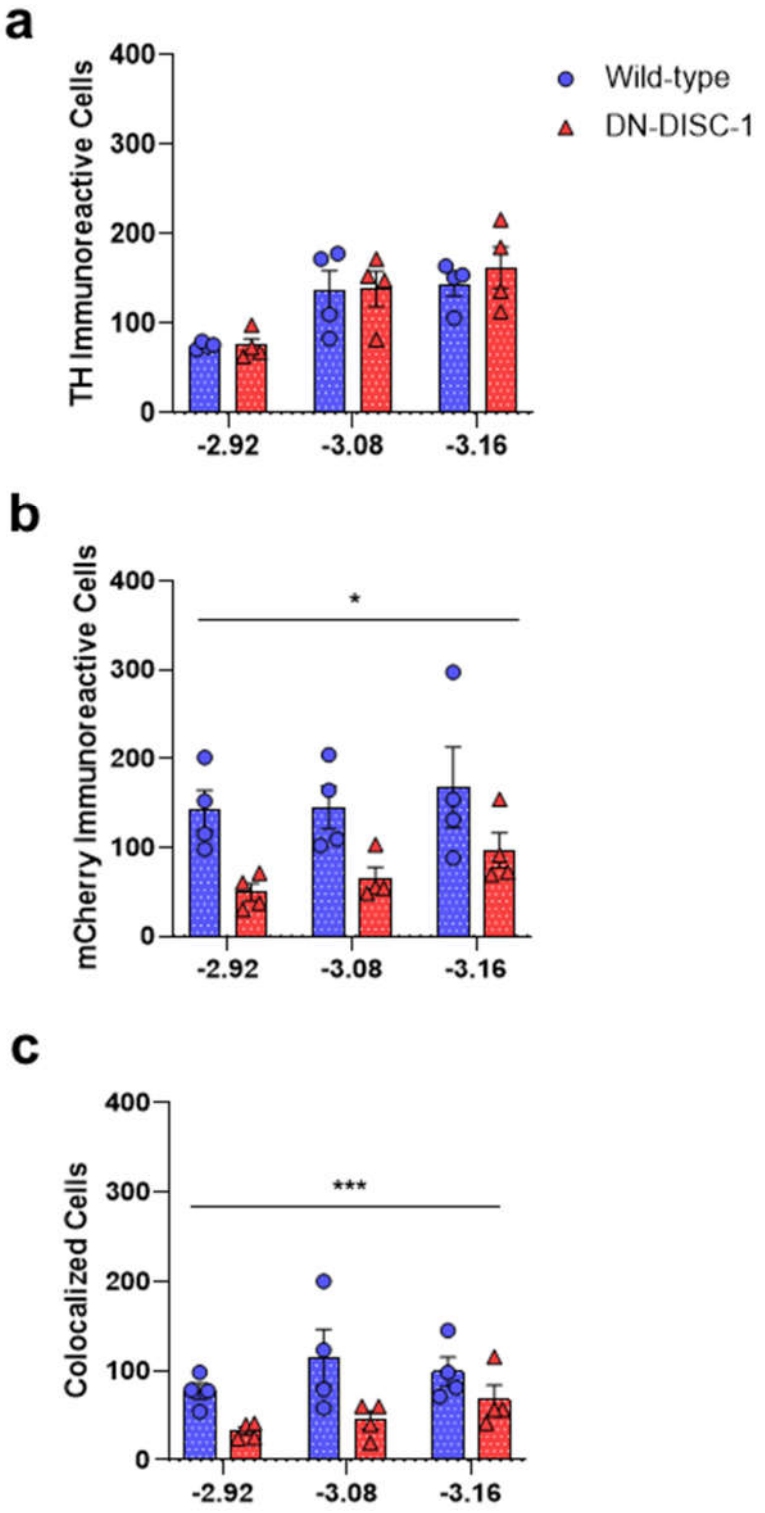


Figure 12. Quantification of TH+, mCherry+, and colocalized cells. (A) Analysis of TH-immunoreactive cells in the VTA showed no differences between genotypes, the number of TH cells increased proportionally to the size of the VTA at each depth (shown in mm relative to Bregma), as would be expected. Cells counts for both mCherry and cells expressing both mCherry and TH differed significantly as a factor of genotype (B, C).

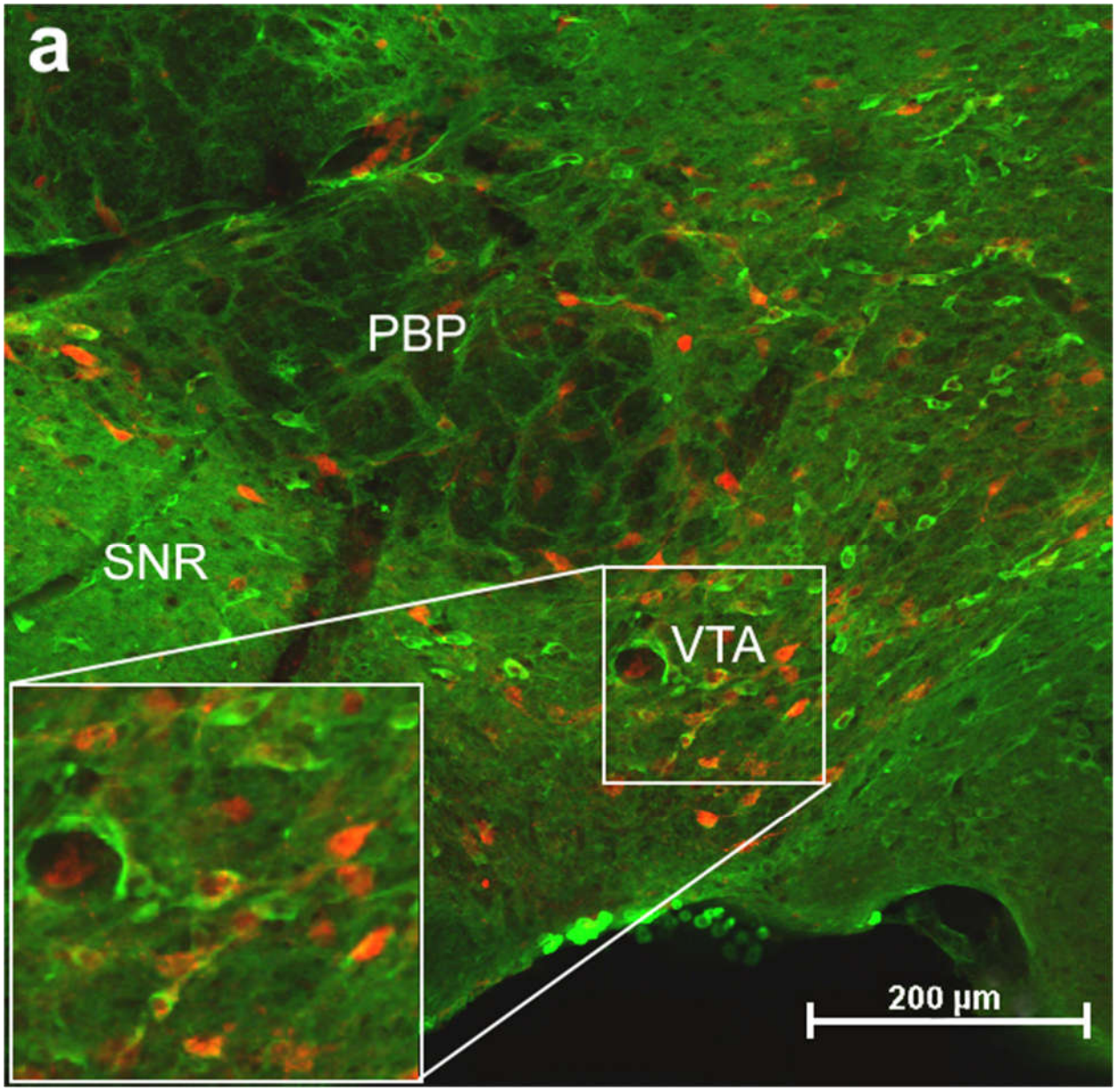
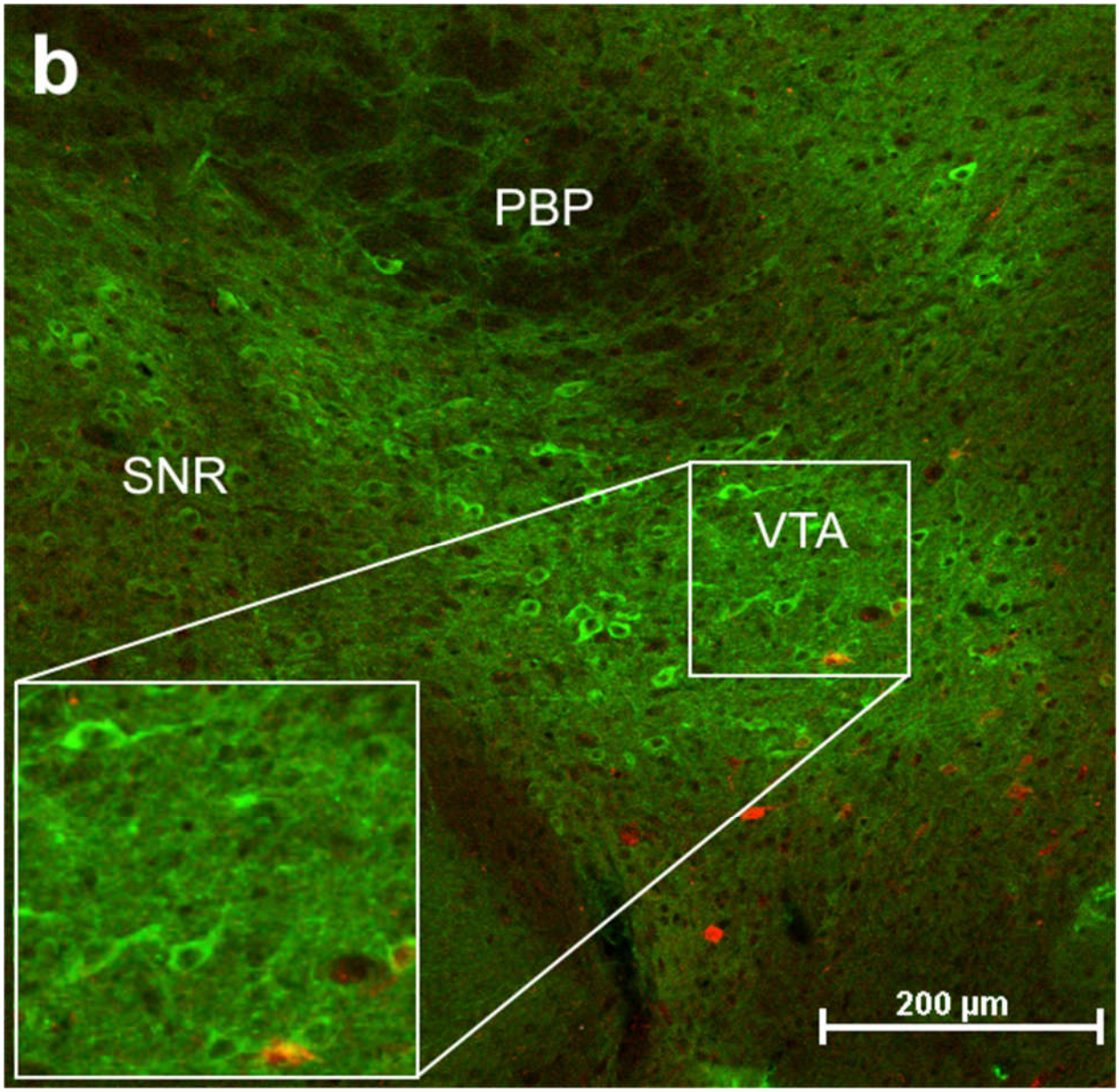


Figure 13. VTA → IC dopaminergic projections in wild-type and DN-DISC-1 mice. Representative images of the VTA at 10x magnification -2.92 mm relative to Bregma in both wild-type (A) and DN-DISC-1 (B) mice. TH-immunoreactivity is shown in green with the flp-dependent mCherry virus in red. Cells exhibiting both green and red signatures are presumed to express dopamine and project to the IC.

Figure 13 (cont'd)



EXP 2: Comparing Dopamine Receptor Subtype Expression

Materials & Methods

Animals

This experiment utilized DN-DISC-1 x DAT-Cre (n = 5) and wild-type x DAT-Cre (n = 3) mice, bred and maintained as previously described. Subjects were restricted to males as a means of ruling out any potential confounding effects on dopamine receptors due to the presence of DAT-Cre (see Costa et al., 2021). Mice ranged from 8 – 10 months of age.

Real Time qPCR

Mice were briefly anaesthetized using isoflurane then decapitated for brain extraction. Once extracted, brains were rapidly frozen by submersing in 2-methylbutane, then stored at -80 °C. Brains were coronally sectioned at 150 µm using a cryostat and mounted onto slides for bilateral punching of target regions. Tissue punches (1.0 mm; WellTech Labs, Taichung, Taiwan) from the IC (~1.7 – 0.26 mm relative to Bregma), NAc (~1.94 – 0.86 mm), DS (1.7 - .02 mm), BLA ([-0.07] – [-1.46] mm), and VTA (~[-2.92] – [-3.16] mm) were then homogenized via 25% amplitude pulsed sonication over the course of 30 seconds in a buffer of RLT plus (74134, Qiagen, Valencia, CA) and β-mercaptoethanol at 1:100 concentration. mRNA was extracted from the homogenized samples using the RNeasy Plus Mini Kit (Qiagen) and quantified using a Qubit Flex Fluorometer and Qubit RNA high sensitivity assay kit (Thermo Fisher). Samples were normalized to a concentration of 2.5 ng mRNA / µl, then converted to cDNA using a high-capacity cDNA reverse transcriptase kit (#4368814; Thermo Fisher) and stored at -20 °C prior to qPCR analysis.

DRD1 (forward primer: CTCGTTGAGTCCAGGGGTTTT; reverse primer: AATGCCCTTGCTTTAGAGTCAC) and DRD2 (forward primer:

CCGAGCTTTCAGAGCCAACC; reverse primer: GGGTACAGTTGCCCTTGAGT) mRNA were quantified for all regions of interest with hypoxanthine-guanine phosphoribosyltransferase (HPRT; forward primer: GAAATGTCTGTTGCTGCGTCC; reverse primer: GCCTACAGGCTCATAGTGCAA) used as a control given its role as a housekeeping gene with ubiquitous expression throughout cell types (Tan et al., 2012). Samples were run in triplicate with a single receptor per well containing 150 ng of cDNA, 5 µl PowerUP SYBR green mastermix (#A25741; Thermo Fisher), and 1 µl each of the forward and reverse primers (made in a 2.5 µM concentration). Five, 96-well plates were used with each plate being dedicated to a single brain region (e.g., VTA) and containing all (n = 8) subjects with their respective DRD1, DRD2, and HPRT receptor primers. For analysis, a QuantStudio5 Real-Time PCR System (Thermo Fisher) was used with the following settings: 1) 2 minutes at 50°C; 2) 2 minutes at 95°C; 3) 1 second at 95°C; 4) 30 seconds at 60 °C; 5) repeat steps 3 – 4 for 40 cycles.

Data Analysis

Individual cycle threshold (CT) values for each subject were converted to relative mRNA expression using the ΔΔCT method (Schmittgen and Livak, 2008) as a means of calculating fold change between genotypes (DN-DISC-1, wild-type) within the regions of interest (IC, NAc, DS, BLA, VTA). Separate two-way repeated measures ANOVAs with between-subjects variables of genotype (DN-DISC-1, wild-type) and within-subjects variables of region (IC, NAc, DS, BLA, VTA) were used to compare relative mRNA expression of the DRD1 and DRD2 receptors for between genotypes.

Results

Analysis of DRD1 and DRD2 expression (Fig. 14) showed no effect of genotype or region, nor interaction between factors thereof (Fs < 1).

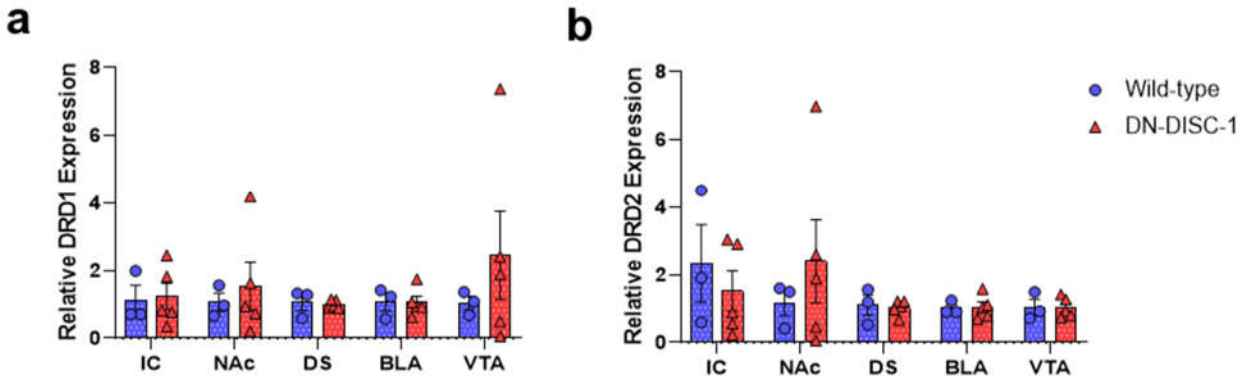


Figure 14. DRD1 and DRD2 receptor expression in DN-DISC-1 and wild-type mice. Relative mRNA expression for DRD1 (A) and DRD2 (B) across various brain regions was quantified, no differences between genotypes were found.

Discussion

Translocation of the DISC-1 gene is associated with increased risk of neuropsychiatric illness (Jacobs, et al., 1970; Millar et al., 2000), including schizophrenia (Facal & Costas, 2019). Studies from animal models have shown that mice with perturbations of the DISC-1 genetic locus display a variety of neurophysiological and behavioral deficits (Hikida et al., 2007; Shen et al., 2008; Johnson et al., 2013; Niwa et al., 2013), in addition to enhanced susceptibility to impaired reality testing (Fry et al., 2020). Despite associations with schizophrenia, and the knowledge that aberrations in midbrain dopamine are thought to underlie the positive features of this illness (e.g., Howes & Kapur, 2009; Adams, 2018), little was known about any potential differences in this circuitry present in DN-DISC-1 mice. Here, we show that DN-DISC-1 mice do not differ from wild-type mice in terms of DRD1 and DRD2 expression in either the VTA, NAc, BLA, DS, or IC. Furthermore, these mice seem to have comparable levels of cells expressing dopaminergic precursors within the VTA. Strikingly, however, we find that DN-DISC-1 have significantly less VTA dopamine cells that project to the IC. This finding has wide-

ranging implications with regard to dopaminergic modulation of the IC, as well as potential behavioral consequences in the model.

That dominant-negative expression of the DISC-1 gene leads to alterations in the number of dopamine neurons projecting to the IC is not entirely unsurprising given the role of DISC-1 in early neuronal development. We know for instance that the DISC-1 protein forms a complex with cytoskeletal proteins that exert control over microtubule transport and neuronal migration (Strat, Ramoz, & Gorwood, 2009); additionally, DISC-1 has also been shown to play a role in regulating synapse formation via inhibitory interactions with neuregulin-1 (NRG1) and its receptor ErbB4 (Seshadri et al., 2015). Taken together, it is not difficult to imagine how perturbations of the DISC-1 genetic locus might negatively impact connectivity between brain regions. The current data in mind, we might imagine the consequences for the IC, broadly speaking, as a loss of computational precision in the region. This could occur through several different mechanisms, however, given that previous work has shown that DRD2 antagonism decreases impaired reality testing in DN-DISC-1 mice (Fry et al., 2020), it seems that whatever account we land on should focus on overactivity of DRD2 in the region, specifically[12]. Perhaps, this loss of VTA → IC dopamine leads to decreased DRD1 activation in the region, with what remains of the projections activating DRD2 at a level above what would be found in the wild-type condition. This would make sense given that DRD2s are known to predominate in the IC (Santana, Mengod, & Artigas, 2009).

With regard to dopamine receptor subtypes, it is worth considering the null finding in terms of differences in expression between DN-DISC-1 and wild-type. I had previously theorized (Ch. 1) that aberrant activity in the IC of DN-DISC-1 mice was due to a skewed

[12] We must be careful with speculation here regarding these DN-DISC-1 x DAT-Cre mice specifically, however, given the failure to replicate IRT as discussed in the previous chapter.

distribution among these receptors on either glutamatergic of GABAergic neurons in the region; yet, the finding that there are simply less dopamine neurons that project to the IC seemingly accounts for these effects without any need to invoke alterations in receptor expression. Still, we might also note that previous work with DISC-1 has shown alterations in DRD2 binding, at least at the level of the striatum (Lipina et al., 2010; Jaaro-Peled et al., 2013). However, the mice used by Lipina and colleagues differ critically from ours in that theirs were generated using a missense mutation in the second exon (L100P) of the gene (see Clapcote et al., 2007), whereas ours express the dominant-negative form of the gene in a manner driven by the PrP. Additionally, the previously mentioned studies did not assess RNA expression of DRD2, but rather receptor occupancy, potentially accounting for this difference in findings. This said, we must also acknowledge that the current study of receptor expression may simply have been underpowered to detect any differences.

In sum, these data yield new insights with regard to both translocations of the DISC-1 gene and the connectivity of the VTA to the IC. We first show that VTA dopamine neurons that project to the IC form a unique population running from anterior to medial VTA; second, that the projections of the dopamine neurons to the IC in this population are significantly decreased in DN-DISC-1 mice, and finally, that despite these differences in connectivity, there appear to be no genotypic differences in either the amount of cells which express dopaminergic precursors in the VTA, nor in the level of expression of DRD1 or DRD2 in various regions of interest throughout the mesolimbocortical reward path. Given the role of DISC-1 in neuronal migration and synapse formation during development, future studies should look to determine whether VTA dopaminergic projections to other regions of interest (e.g., NAc, BLA, mPFC, etc) are altered in these mice. Previous research using fMRI has shown decreased functional connectivity

between various regions of the brain in humans with schizophrenia (e.g., Lynall et al., 2010; Camchong et al., 2011; Liu et al., 2011); the current data and suggested future studies have the potential to add marked detail to this body of work.

CHAPTER 6: GENERAL DISCUSSION

I began this work with a discussion of schizophrenia noting that surprisingly little progress has been made in the way of pharmacological treatment given the last half century or more of research. I suggest that this is due to an existing knowledge gap with regard to the precise neurobiological mechanisms underlying various aspects of positive symptomology, and furthermore, I contend that such a gap exists due to a lack of appropriate pre-clinical animal models. These dissertation studies, then, had the goal of identifying, for the first time, a putative circuit controlling a facet of positive symptomology known as impaired reality testing (IRT). To do so, I utilized mice with global central nervous system expression of a dominant-negative form of the Disrupted-in-schizophrenia-1 (DN-DISC-1) gene. These mice were selected based on known associations with perturbations of the DISC-1 genetic locus and incidence of neuropsychiatric illnesses that include schizophrenia. Moreover, previous work I was involved with in the Johnson lab showed that DN-DISC-1 mice are more susceptible to IRT as evidenced by Pavlovian conditioning procedures which elicit a false percept of sweet taste as directed at unflavored water (Fry et al., 2020). Furthermore, these results showed that IRT in DN-DISC-1 mice was dopaminergically-dependent and associated with elevated activity in the insular cortex (IC). Given these two critical points of data and based on an understanding of positive symptomology in schizophrenia as being at least partly generated by aberrant midbrain dopamine signaling, I proposed to investigate the necessity of dopamine neurons which originate in the ventral tegmental area (VTA) and project specifically to the IC for IRT associated with phantom gustatory sensations. To do this, I utilized an intersectional chemogenetic approach which allowed for inactivation of VTA → IC projecting dopamine neurons during various behavioral experiments.

In Chapter 2, I examined the effect of inactivating VTA → IC dopamine on measures of licking microstructure as directed at liquid solutions of varying levels of sweetness ranging from 0 – 1 M sucrose content. I replicated previous findings which showed that DN-DISC-1 mice do not differ from wild-types in their ability to detect sweetness (Fry et al., 2020) and, importantly, I showed that inactivation of VTA → IC dopamine does not influence the motivation to consume or palatability of the sucrose solution. We know from previous work that the IC plays a critical role in the encoding of the taste features of stimuli in humans and animals. For instance, both lesioning the IC (Bermudez-Rattoni & McGaugh, 1991) and blocking glutamatergic signaling by way of NMDA antagonism in the region (Gutierrez et al., 1999) prevents the acquisition of conditioned taste aversion. Animals with lesions of the IC are also insensitive to sensory-specific satiety (i.e., sating an animal with a particular taste generally leads to a reduction in that animal's responding when placed in a scenario in which they have the opportunity to obtain more of the same reward; Balleine & Dickinson, 2000), and humans with lesions of the IC show an inability to discern between different tastes (Pritchard et al., 1999). In relation to these studies, my findings advance this body of literature by showing, for the first time, that dopamine from the VTA is not necessary for the IC to maintain its role in detecting a sweet taste. Furthermore, these data allowed for continued behavioral investigation with the knowledge that manipulation of the circuit would not be confounded by baseline disruptions in the dependent measures I planned to use as a means of examining reality testing.

Chapter 3 served as another important investigation of potentially confounding factors that might arise in the course of inactivating VTA → IC dopamine while examining reality testing. Given the known role of midbrain dopamine in activation, maintenance of effort, and particularly incentive motivation (e.g., Berridge & Robinson, 1998; Floresco et al., 2006;

Berridge, 2007; Grace et al., 2007; Salamone et al., 2007; Salamone et al., 2012), it seemed critical to determine whether the circuit might influence these aspects of behavior. To do this, I used a progressive ratio task in which mice had to respond a set number of times in order to receive a liquid sucrose reward. Each successive reward required an increased number of responses in order to obtain. Here, I showed that DN-DISC-1 mice do not differ from wild-types in terms of the number of rewards they are able to obtain during the task, however, they do spend more time completing the task than wild-types. This may be due to differences in factors related to DN-DISC-1 mice and their capacity to maintain attention on the task at hand, or they might reflect a more habit as opposed to goal-driven nature when seeking out rewards which reflects differences at the level of striatal dopaminergic circuitry (e.g., Horvitz, 2009; Stalnaker et al., 2010). Interestingly, these results contrast with previous studies which have shown that DN-DISC-1 mice show impairments in incentive motivation during both food seeking tasks (Hikida et al., 2007) and progressive ratio (Johnson et al., 2013). Worth noting, previous studies which have examined DN-DISC-1 incentive motivation have used all used a model in which DISC-1 expression is driven by αCaMKII, and thus restricted to forebrain behavioral circuits, whereas the mice used in these studies have global central nervous system expression driven by the PrP. This, coupled with the presence of the DAT-Cre gene and its effect of potentially decreasing dopamine reuptake in the striatum (Costa et al., 2021) may account for this discrepancy.

Beyond these baseline genetic differences, however, inactivation of VTA → IC dopamine had differing effects in the progressive ratio task. While there was no effect on overall rewards obtained, the loss of VTA → IC dopaminergic tone led to a reduction in the amount of time mice spent in the magazine, regardless of genotype. That inactivation of the VTA → IC dopaminergic circuitry reduced magazine time without affecting rewards obtained suggests that the circuit may

have influence over aspects of behavior related to learned contextual features of the reward environment (e.g., location of reward) that are separate from incentive motivation per-se. This separation of effects between aspects of seeking and responding is not without precedent and fits perhaps with what we know about differences in sign and goal tracking behavior—the idea that underlying differences in dopaminergic circuitry leads some animals to fixate more so on the signs (e.g., levers, cues, etc) that predict reward, while others fixate on the goal area where the reward will appear (e.g., Kuhn et al., 2018). Neither sign nor goal-trackers show impairments in motivation relative to one another, yet they differ in this seeking-related dimension. Perhaps then, a loss of VTA → IC dopamine may shift animals toward more of a goal-tracker state, an idea which would extend the scope of recent findings which showed that lesions of the IC reduce sign-tracking (Pribut et al., 2022).

The observed effects on aspects of learning which are separate from incentive motivation in Chapter 3 make the findings from Chapter 4, in which I employed Pavlovian conditioning as a means of examining the necessity of VTA → IC dopamine for IRT, all the more interesting. Here, I trained mice to associate a white noise cue (i.e., the CS) with the delivery of a liquid sucrose reward (i.e., the US). Once the CS had been instantiated as an expectancy marker for the US, the mice progressed to a test period in which the CS was presented in combination with the delivery of unflavored water. At test, inactivation of VTA → IC dopamine reduced the number of entries mice made during the pre-CS period, regardless of genotype. This would seem to suggest a generalized reduction in incentive motivation as directed at the context, however, given that no effects on related processes (e.g., rewards obtained) in the progressive ratio task were observed, we may well assume once more that this is related to a potential role for VTA → IC dopamine in modulating sign vs goal-tracking behavior. During the CS, inactivation of the VTA

→ IC dopaminergic circuitry continued to reduce magazine entries, but only for DN-DISC-1 mice, this implies that a different process may be at work. Coupled with the fact that DN-DISC-1 mice also made significantly more entries during the CS than wild-type in the vehicle condition, this suggests aberrations in this circuitry which are specific to the DN-DISC-1 mice may underlie alterations in CS-evoked approach behavior. Given that no such effect on entries occurred during training, however, we can surmise that these DN-DISC-1 specific alterations are specific to extinction. This fits with what we saw with regard to differences in approach behavior for DN-DISC-1 mice in the progressive ratio task, suggesting again that DN-DISC-1 animals may simply be more habitual as opposed to goal-driven in their responding, and that perhaps, inactivating VTA → IC dopamine reduces this tendency. Such an idea would not be out of context with studies which have shown A) that DN-DISC-1 mice perseverate (Johnson et al., 2013) and B) implicate the insula in pathologies wherein habit is taken to an extreme (i.e., addiction; Naqvi et al., 2014; Naqvi & Bechara, 2017).

Most strikingly of all, however, while lick burst size in DN-DISC-1 mice was unaffected by inactivating the VTA → IC dopaminergic circuitry, wild-type mice responded in a manner suggesting that the loss of dopaminergic tone caused them to experience a phantom gustatory sensation indicative of IRT. These results suggest that, at least in the wild-type condition, VTA → IC dopamine signaling may play a role in encoding information related to the sensory-specific features (e.g., taste) of an outcome, the loss of which results in abnormally strong internal perception of these features which can be triggered in the presence of previously associated cues. Given the known role of the insula in encoding incoming information related to taste (Bermudez-Rattoni & McGaugh, 1991; Gutierrez et al., 1999; Pritchard et al., 1999; Balleine & Dickinson, 2000; Koh et al., 2003), it seems quite probably that a loss dopaminergic tone in the region might

impair the processing of incoming sensory information in the wild-type condition. Taken a step further, if a loss of VTA → IC dopamine also predisposes the IC to increased firing in response to reward-predictive cues, this implicates the region in a form of IRT akin to what has previously been theorized to underlie the generation and maintenance of hallucinations in humans with schizophrenia (i.e., aberrant internal perception generated by an overreliance on prior knowledge at the expense of incoming sensory experience, e.g., Powers et al., 2017; Adams, 2018; Corlett et al., 2019).

Finally, in Chapter 5, I conducted a thorough anatomical investigation of the VTA → IC dopaminergic circuitry, to determine what, if any differences may exist between DN-DISC-1 and wild-type mice. While initially, I had hypothesized that there might be differences in the expression of DRD1 and/or DRD2 between DN-DISC-1 and wild-type mice, I found no such differences in any of the regions I investigated, including VTA and IC. While it is possible that the study may not have been sufficiently powered to detect such a difference, if this is not the case, then it is worth considering in the context of previous work which has shown that both DISC-1 L100P mice (Lipina et al., 2010) and αCaMKII DN-DISC-1 mice (Jaaro-Peled et al., 2013) show increased DRD2 binding in the striatum. At first glance, this would seem to be a contradiction, however, it must be stated that both of these prior studies used autoradiographic binding assays as opposed to qPCR for assessing DRD2. Briefly, the former assay measures the amount of receptor binding which is capable of occurring in a region while the latter quantifies genetic expression of the receptor itself. This is a crucial difference which means that previous DISC-1 studies do not necessarily imply increased DRD2 expression, rather, it could be that these animals have reduced extracellular dopamine and thus more unoccupied receptors for the radioactive ligand to bind, a caveat which the authors themselves acknowledge. If this is the

case, the data from my study would mark a critical juncture in our understanding of striatal dopaminergic aberrations in the model.

Beyond quantification of dopamine receptors, I was also able to confirm that the IC receives dopamine projections from a specific region of the VTA ranging from the rostral to medial sections. This fits with previous findings that have shown GABAergic neurons in the rostral agranular insular cortex to receive dopaminergic input from the VTA (Ohara et al., 2003). Strikingly, while the regional distribution of these IC projecting VTA dopamine neurons was conserved in both wild-type and DN-DISC-1 mice, I found that the latter had significantly less dopaminergic projections to the IC, despite showing no differences in the amount of VTA cells which express dopaminergic precursors. Functional outcomes which may result from this loss of connectivity could include decreased modulatory control by way of decreased activation for all dopamine receptor subtypes in the region, or a biasing of activation toward one specific receptor subject based on its prevalence relative to others[13]. These results seem to underscore the importance of DISC-1 protein interactions with various factors involved in cytoskeletal development, neural migration, and synapse formation, ultimately suggesting that perturbations of the DISC-1 genetic locus can lead to a loss of connectivity between brain regions. These data may hold relevance for the role of the DISC-1 interactome in conferring genetic risk for neuropsychiatric illnesses which have been associated with decreased functional connectivity between regions (e.g., schizophrenia), however, it makes the null finding with relation to DN-DISC-1 mice and IRT from the previous chapter all the more puzzling.

[13] While it is without question that the IC contains both neurons which express DRD1 and DRD2, conflicting studies have each claimed either increased DRD1 (Hurd et al., 2001) or DRD2 (Santana et al., 2009) relative to the other, while my own work shows no difference. This may reflect differences in the location and configuration of the IC across species as Hurd et al. used human brain tissue without distinguishing between regions of the IC, while Santana et al. looked specifically at the rostral agranular aspect of the IC in rats. My own studies examined rostral to caudal IC as a whole in mice.

In sum, the studies I have conducted throughout this work reveal new information about the role of VTA dopamine neurons that project to the IC in both wild-type and DN-DISC-1 mice. Along the way, certain commonalities have emerged, as well differences which appear to be genotype specific (Table 1). With regard to commonalities, we now know that VTA → IC dopamine does not appear to play a role in consummatory behaviors which include hedonic evaluation and overall intake, nor does it affect incentive motivation or motoric output. Inactivation of the circuit appears to reduce context-driven approach behavior in a manner that is clearly distinct from incentive motivation, suggesting it may play a role in linking a given environment to outcome expectation in the absence of more discrete stimuli (i.e., the CS), or perhaps modulating sign vs goal-tracking behavior. In terms of differences, we see that inactivation of the circuit normalizes excessive approach behavior in the DN-DISC-1 phenotype that occurs in the presence of the cue. Coupled with the tendency for these mice to perseverate when the circuit is active, we might suggest that perturbations of the DISC-1 gene lead to a unique VTA → IC phenotype which lends itself to a more habitual as opposed to goal-driven nature, an idea which is underscored by the observation that DN-DISC-1 mice have significantly less dopaminergic projections to the IC as compared to wild-types. This genotype specific alteration in the VTA → IC dopaminergic circuit may well explain why inactivation of the circuit leads to IRT in wild-type mice, but not DN-DISC-1. Perhaps, DN-DISC-1 mice, already with decreased connectivity between the VTA and IC adopt a more habit-driven, rigid approach as a means of compensating for their lack of reliability with regard to incoming sensory information[14]. Such an account would fit with contemporary theories of schizophrenia which suggest that the behavior of affected individuals is driven more so by prior beliefs as opposed to

[14] Previously theorized to underlie the experience of IRT in the wild-type condition following inactivation of the circuit.

current experiences (e.g., Powers et al., 2017; Adams, 2018; Corlett et al., 2019). While such a theory seems quite plausible for explaining how it is a loss of dopaminergic tone in the IC might shift the behavior of wild-types to a state which is more prone to IRT, we must remember that the lack of IRT in the DN-DISC-1 vehicle condition (as compared to Fry et al., 2020) necessitates a tempering of enthusiasm.

Baseline Differences		**Wild-type**	**DN-DISC-1**
Increased survival time during progressive ratio			✓
Increased magazine entries during the CS			✓
Decreased dopaminergic projections from VTA → IC			✓
Effects of Inactivating VTA → IC DA		**Wild-type**	**DN-DISC-1**
Decreased magazine time during progressive ratio		✓	✓
Decreased magazine entries during pre-CS		✓	✓
Decreased magazine entries during CS			✓
Impaired Reality testing		✓	

Table 1. Summary of observed effects. Wild-type and DN-DISC-1 mice differed at several critical points prior to and after inactivating VTA → IC dopamine.

Limitations

What difference between the current series of studies and Fry et al. (2020) might explain the lack of IRT in our DN-DISC-1 mice? As I have alluded to throughout this work, the behavioral studies conducted, while yielding some surprising findings, are confounded by the presence of the DAT-Cre knock-in gene. This gene has been linked to a number of sex-dependent differences in behavior, as well as alterations in expression of the dopamine transporter (Costa et al., 2021). Specifically, both male and female DAT-Cre mice show

locomotor hyperactivity when compared to wild-types, males show deficits in extinction learning, and females show enhanced responding during associative learning. Furthermore, both males and females show reduced DAT expression throughout the striatum compared to wild-types, while females show a paradoxical increase in DAT expression at the level of the NAc, specifically. This overall decrease in DAT expression may explain, in part, why the results of my progressive ratio study differed from previous investigations of incentive motivation in DN-DISC-1 vs wild-type mice (e.g., Johnson et al., 2013). Furthermore, the fact that these mice show reduced DAT expression at the level of the striatum brings to mind questions about the efficacy of our viral manipulation, given that it was targeted to DAT-expressing neurons in the ventral tegmental area, many of which project to the ventromedial striatum. And yet, given that I did find effects of inactivating the circuit on distinct components of behavior, if anything, we might imagine that using a different mouse line (e.g., one in which Cre expression is driven by the tyrosine-hydroxylase promoter; [TH-Cre]) would show similar effects, but perhaps more strongly.

Next, with regard to the lack of IRT in our DN-DISC-1 x DAT-Cre mice, given that Fry et al. (2020) showed this to be dopaminergically-dependent, it seems obvious that a reduction in striatal DAT expression could disrupt this effect. Interestingly, however, the unique mixture of underlying neurophysiological aberrations that can be attributed to dominant-negative expression of DISC-1 combined with this reduction in striatal DAT expression seems to have conferred some sort of protection against the deleterious effects of inactivating the circuit on reality testing as shown in wild-type x DAT-Cre mice. One potential explanation for this involves interactions with the DAT-Cre gene and pre-existing genotypic differences in dopaminergic signaling (Fig. 15). Perturbations of the DISC-1 genetic locus have previously been shown to result in hyper

dopaminergic function at the level of the striatum as well as increased DAT expression (Lipina et al., 2010). The dopamine transporter serves as a critical mediator of dopaminergic tone given its role in reuptake at the synapse. Given that the DAT-Cre knock-in gene reduces expression of the DAT, however, we might imagine that this reduces striatal dopaminergic function to normative levels in the DN-DISC-1 condition. Conversely, wild-type mice might wind up with hypo striatal dopaminergic function in the presence of the transgene, given that their baseline level is already lower than that of DN-DISC-1 mice. Perhaps, inactivating the VTA → IC circuit in the presence of already reduced striatal dopaminergic function then leads to differences at the level of receptor activation in the IC[15], and a resultant increased propensity for IRT. This is in mind, other baseline genetic differences that arose in the vehicle condition of these studies (e.g., with regard to survival time in the progressive ratio task, and increased magazine entries during the CS in the mediated performance task), while clearly reflective of neurological differences, should be interpreted cautiously. To be clear, I cannot state with certainty that these effects are solely due to dominant-negative expression of DISC-1.

[15] Using the findings of Santana et al., (2009) as an example, if DRD2 is more prevalent in the IC than DRD1, reduced dopaminergic signaling as sent to the region may well bias the area towards DRD2 activation. That this leads to IRT is supported by the findings of Fry et al. (2020) which showed that DRD2 antagonism eliminates IRT.

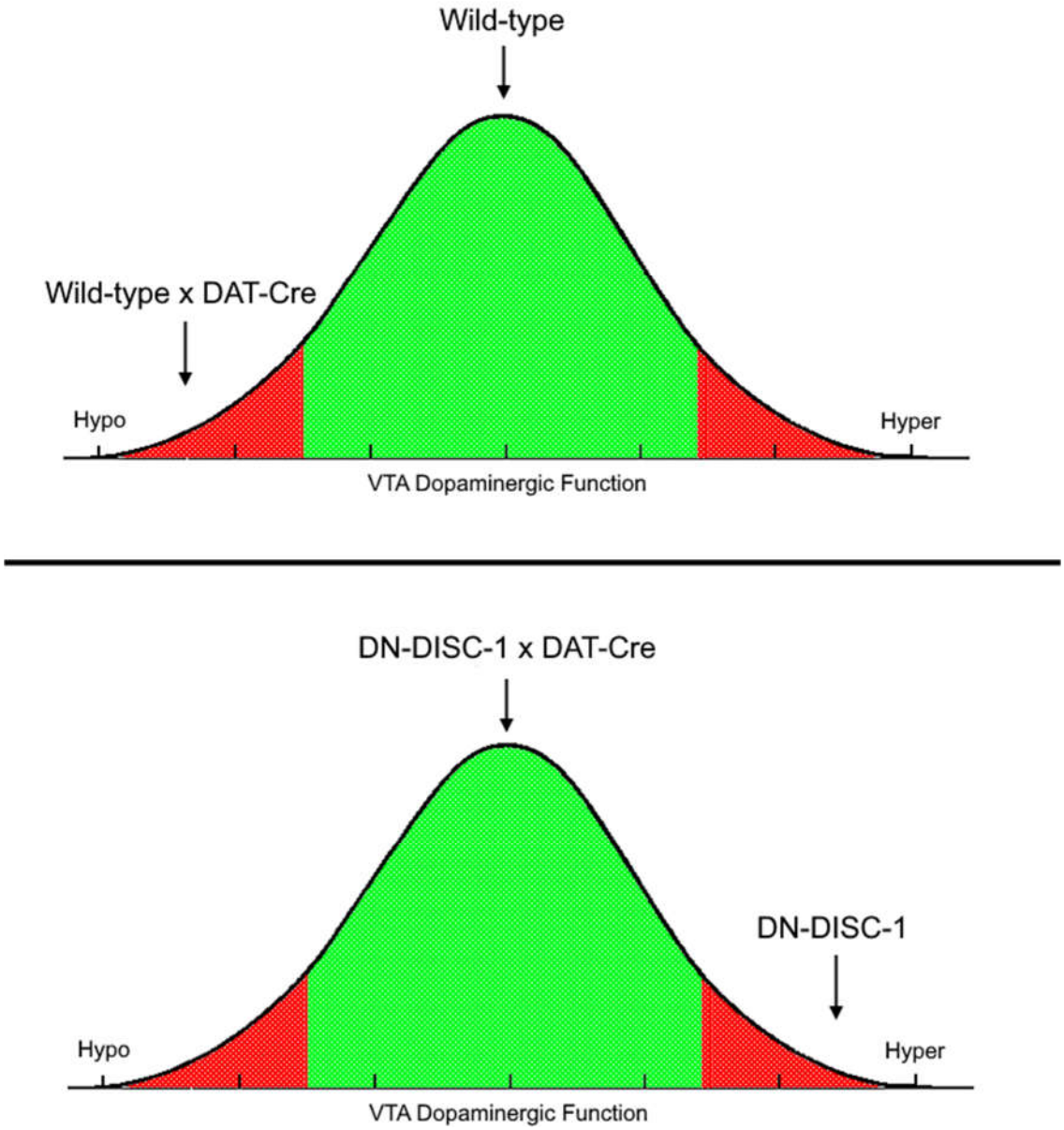


Figure 15. Theoretical distribution of VTA dopaminergic function across genotypes. Perturbations of the DISC-1 gene are known to result in enhanced midbrain dopaminergic function. The DAT-Cre knock-in gene has the unintended consequence of reducing dopaminergic function by way of knocking down expression of the dopamine transporter. The combination of these factors may lead to different outcomes prior to and following inactivation of the VTA → IC dopaminergic circuitry for wild-type x DAT-Cre and DN-DISC-1 x DAT-Cre mice when compared to previous studies (e.g., Johnson et al., 2013; Fry et al., 2020).

One aspect of these studies that appears potentially unmarred by the DAT-Cre gene is the finding that DN-DISC-1 mice have significantly less dopaminergic projections from the VTA → IC. Given that DN-DISC-1 mice (absent of the DAT-Cre gene) have not previously been shown to have reduced DAT expression in the VTA, and further, that we showed no difference in the number of cells which express dopaminergic precursors in the region, we can assume with some degree of confidence that any reduction in DAT expression within the region caused by the knock-in gene should have been equivalent in the wild-type condition. This in mind, it seems likely that the difference in dopaminergic projections which was uncovered is likely to be due to perturbations of the DISC-1 gene, not the inclusion of DAT-Cre. An attempt to confirm the veracity of these findings could be made by infusing a Cre-dependent retrograde tracer virus at the level of the IC in DN-DISC-1 x TH-Cre mice, however, the TH-Cre line is not without its own problems (e.g., decreased specificity for targeting midbrain dopamine neurons; Lammel et al., 2015). Another, perhaps better option, might simply be to infuse a retrograde mCherry reporter virus into the IC of DN-DISC-1 and wild-type mice lacking the DAT-Cre gene. This would lead to the expression of mCherry in all cells which project to the IC (dopaminergic or not), you could then follow-up by staining for a dopamine-specific marker (e.g., tyrosine-hydroxylase or DAT) in the VTA and quantifying the number of colocalized cells.

Future Directions & Conclusion

Overall, while certain aspects of these studies appear to be confounded by the presence of the DAT-Cre transgene, others have revealed fruitful ground for further research. The finding that inactivation of the VTA → IC dopaminergic circuitry leads to IRT in wild-type mice should be followed-up with studies which could more accurately determine whether or not the insula can be implicated in IRT more broadly. For instance, if as I theorized in Chapter 4, a loss of

dopaminergic precision in the VTA → IC circuit leads to unchecked firing in the IC in response to stimuli (thus generating abnormally strong internal perceptions which become confused with external reality—i.e., IRT), it might be worth examining whether simply stimulating the IC (i.e., via non cell-type specific use of ChR2 or hM3Dq[16]) at test in the mediated performance task would be sufficient to reproduce our results in the wild-type condition. Furthermore, given that the insula has also been implicated in auditory conditioned hallucinations occurring in humans (Powers, Mathys, & Corlett, 2017), it would be worth knowing whether inactivation of VTA → IC dopaminergic circuitry could yield the experience of absent percepts along other sensory modalities (e.g., olfactory, auditory) in our mice. Such studies could provide important clues with regard to how information processing might go awry and lead to hallucinations in individuals experiencing psychotic states, as well as confirm the IC as a critical region for hallucinations independent of modality. Finally, with regard to schizophrenia in particular, studies with DN-DISC-1 mice should move forward with an emphasis on furthering our understanding of disruptions in neural connectivity that may arise as a result of perturbations of the gene. Given what I discovered with regard to the loss of connectivity between the VTA and IC, it seems quite probable that other areas may be similarly affected. These studies would add to the growing body of literature on deficits in functional connectivity seen in humans with schizophrenia, and combined with the previous, may even offer some novel targets for pharmacological treatment of symptoms.

[16] *ChR2* is shorthand for an optogenetic adenovirus vector which causes cells to express an ion channel ("channel rhodopsin") that allows Na^+ influx, and resultant action potentials, in response to 473 nm wavelengths of light. *hM3Dq* refers to an excitatory DREADD, which, when activated by CNO leads to increased intracellular Ca^{2+} release, and resultant increases in excitability.

APPENDIX

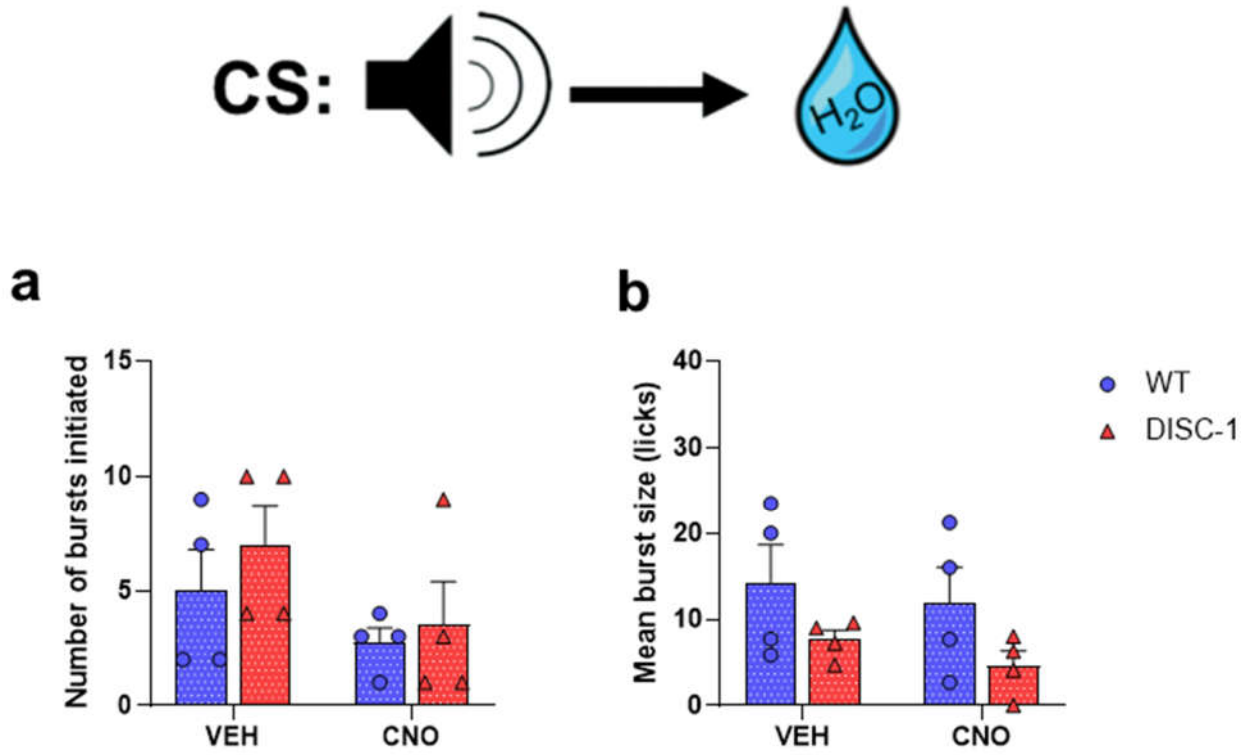


Figure S.1. Analysis of licking microstructure (mCherry); no imputation, zero value contained. There was no effect of genotype or impact of drug on number of bursts initiated or mean burst size occurring within the 250 – 500 ms pause criterion at test (A, B).

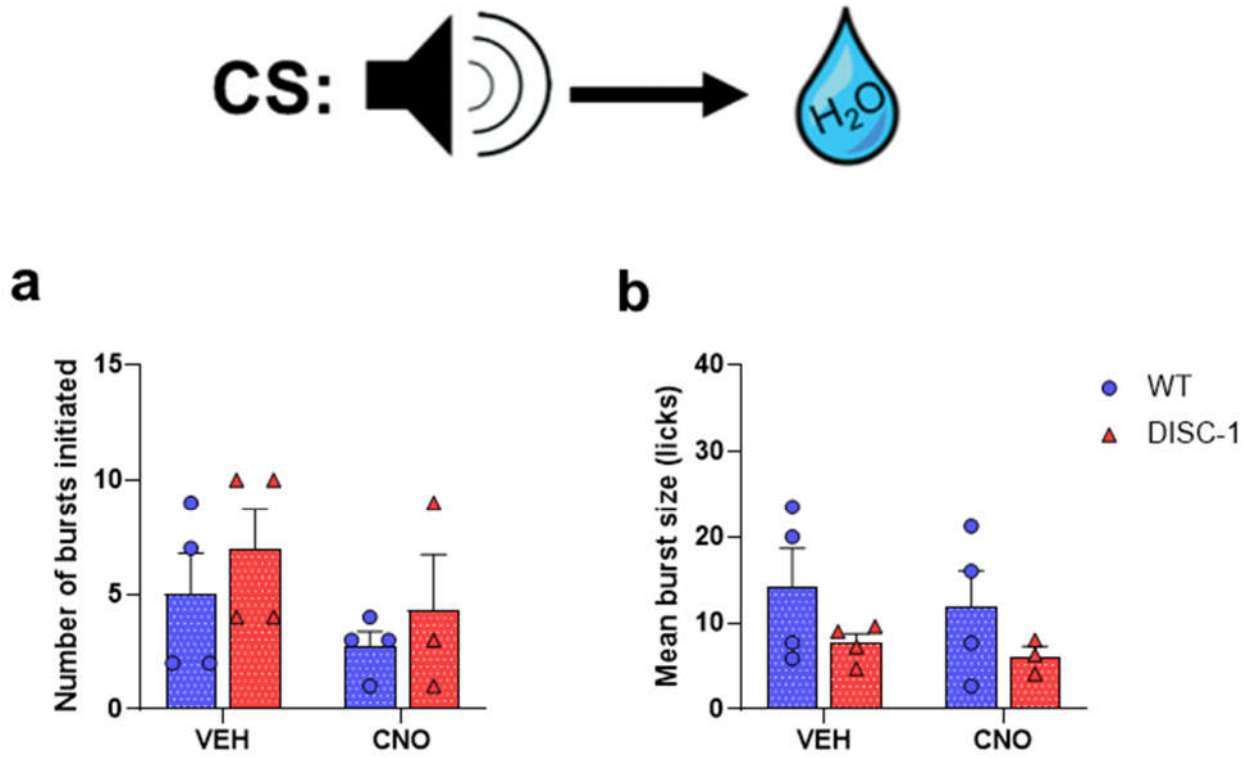


Figure S.2. Analysis of licking microstructure (mCherry); no imputation, zero value removed. There was no effect of genotype or impact of drug on number of bursts initiated or mean burst size occurring within the 250 – 500 ms pause criterion at test (A, B).

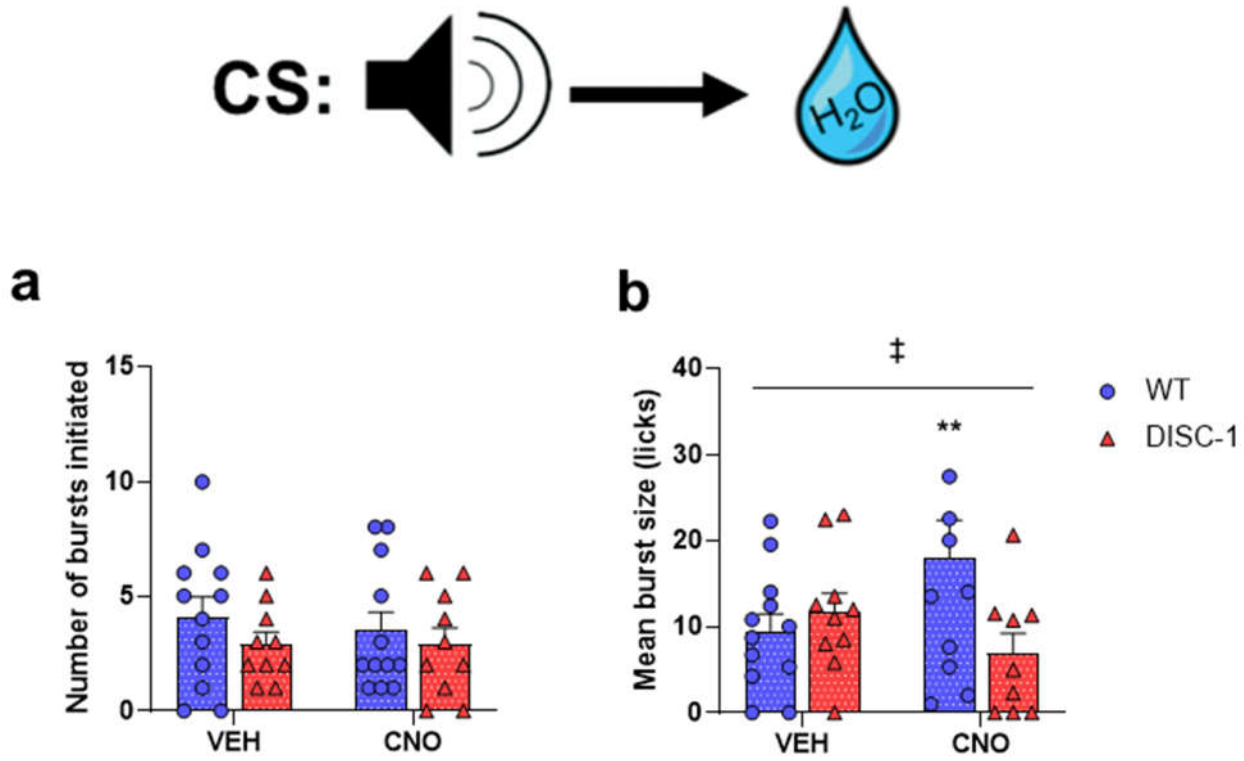


Figure S.3. Analysis of licking microstructure; no imputation, zero values contained. CNO did not impact the number of bursts of licking initiated, regardless of genotype (A); however, CNO led to a significant increase in mean burst size for wild-type mice, specifically (B). + *two-way interaction, genotype x condition, ‡ p = .01; **p < .01*

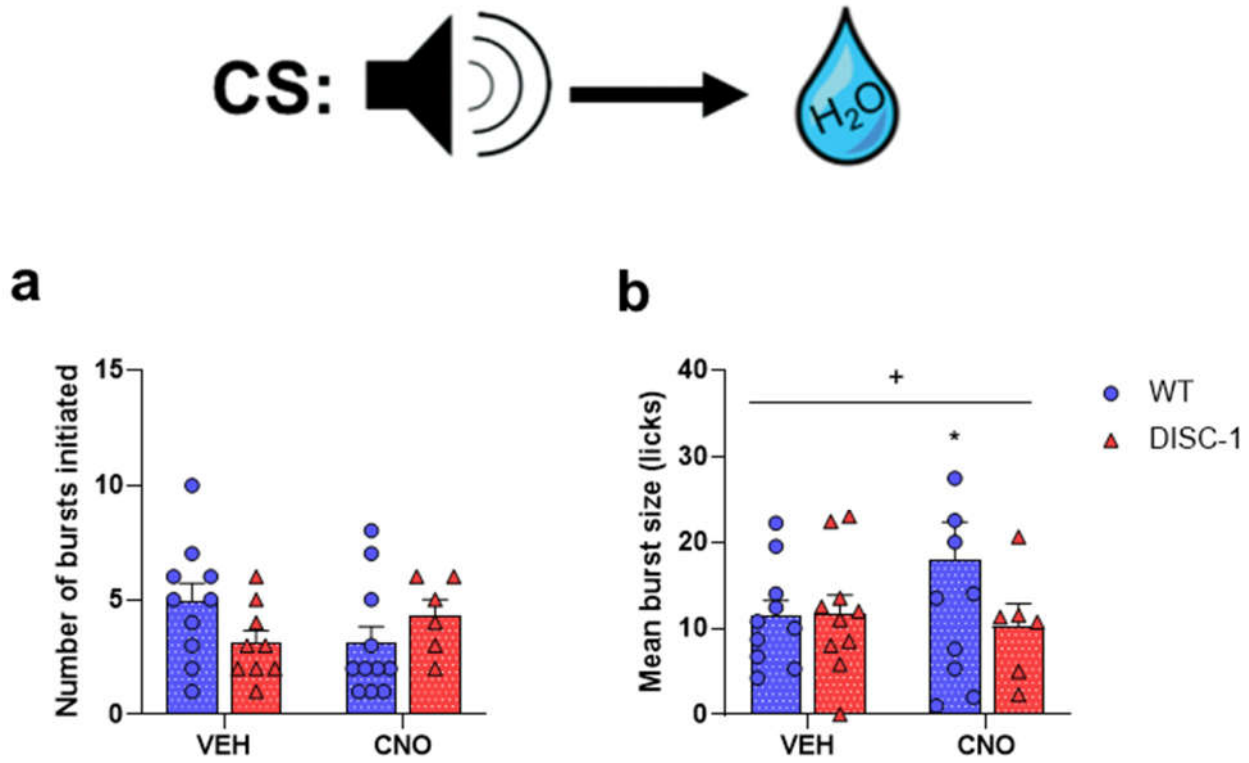


Figure S.4. Analysis of licking microstructure; no imputation, zero values removed. CNO did not impact the number of bursts of licking initiated, regardless of genotype (A); however, CNO led to a significant increase in mean burst size for wild-type mice, specifically (B). + *two-way interaction, genotype x condition, p = .06; *p < .05*

REFERENCES

REFERENCES

Aberman, J. E., Ward, S. J., & Salamone, J. D. (1998). Effects of dopamine antagonists and accumbens dopamine depletions on time-constrained progressive-ratio performance. Pharmacology Biochemistry and Behavior, 61(4), 341-348.

Adams, R. A. (2018). Bayesian Inference, predictive coding, and computational models of psychosis. In Computational psychiatry (pp. 175-195). Academic Press.

Armbruster, B. N., Li, X., Pausch, M. H., Herlitze, S., & Roth, B. L. (2007). Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. Proceedings of the National Academy of Sciences, 104(12), 5163-5168.

Bai, Y., Peng, Y., Liang, J., & Zheng, X. (2012). Differential patterns of neuronal activation in rostral versus caudal ventral tegmental area involved in behavioral sensitization induced by an escalating-dose morphine administration paradigm. European journal of pharmacology, 689(1-3), 118-124.

Balleine, B. W., & Dickinson, A. (2000). The effect of lesions of the insular cortex on instrumental conditioning: evidence for a role in incentive memory. Journal of Neuroscience, 20(23), 8954-8964.

Bardgett, M. E., Depenbrock, M., Downs, N., Points, M., & Green, L. (2009). Dopamine modulates effort-based decision making in rats. Behavioral neuroscience, 123(2), 242.

Beier, K. T., Steinberg, E. E., DeLoach, K. E., Xie, S., Miyamichi, K., Schwarz, L., ... & Luo, L. (2015). Circuit architecture of VTA dopamine neurons revealed by systematic input-output mapping. Cell, 162(3), 622-634.

Benrimoh, D. A., Parr, T., Vincent, P., Adams, R. A., & Friston, K. (2018). Active Inference and Auditory Hallucinations. Computational Psychiatry, 2(0), 183. https://doi.org/10.1162/cpsy_a_00022

Bentall, R. P., Baker, G. A., & Havers, S. (1991). Reality monitoring and psychotic hallucinations. British journal of clinical psychology, 30(3), 213-222.

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?. Brain research reviews, 28(3), 309-369.

Berridge, K. C. (2007). The debate over dopamine's role in reward: the case for incentive salience. Psychopharmacology, 191(3), 391-431.

Bermudez-Rattoni, F., & McGaugh, J. L. (1991). Insular cortex and amygdala lesions differentially affect acquisition on inhibitory avoidance and conditioned taste aversion.

Brain research, 549(1), 165-170.

du Bois, T. M., Deng, C., & Huang, X. F. (2005). Membrane phospholipid composition, alterations in neurotransmitter systems and schizophrenia. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 29(6), 878-888.

Bowie, C. R., & Harvey, P. D. (2006). Cognitive deficits and functional outcome in schizophrenia. Neuropsychiatric disease and treatment, 2(4), 531.

Bryce, C. A., & Floresco, S. B. (2019). Alterations in effort-related decision-making induced by stimulation of dopamine D1, D2, D3, and corticotropin-releasing factor receptors in nucleus accumbens subregions. Psychopharmacology, 236(9), 2699-2712.

Camchong, J., MacDonald III, A. W., Bell, C., Mueller, B. A., & Lim, K. O. (2011). Altered functional and anatomical connectivity in schizophrenia. Schizophrenia bulletin, 37(3), 640-650.

Castro, D. C., & Berridge, K. C. (2014). Opioid hedonic hotspot in nucleus accumbens shell: mu, delta, and kappa maps for enhancement of sweetness "liking" and "wanting". Journal of Neuroscience, 34(12), 4239-4250.

Chou, I. J., Kuo, C. F., Huang, Y. S., Grainge, M. J., Valdes, A. M., See, L. C., ... & Zhang, W. (2017). Familial aggregation and heritability of schizophrenia and co-aggregation of psychiatric illnesses in affected families. Schizophrenia bulletin, 43(5), 1070-1078.

Clapcote, S. J., Lipina, T. V., Millar, J. K., Mackie, S., Christie, S., Ogawa, F., ... & Roder, J. C. (2007). Behavioral phenotypes of Disc1 missense mutations in mice. Neuron, 54(3), 387-402.

Collins, A. G., Brown, J. K., Gold, J. M., Waltz, J. A., & Frank, M. J. (2014). Working memory contributions to reinforcement learning impairments in schizophrenia. Journal of Neuroscience, 34(41), 13747-13756.

Corlett, P. R., Horga, G., Fletcher, P. C., Alderson-Day, B., Schmack, K., & Powers III, A. R. (2019). Hallucinations and strong priors. Trends in cognitive sciences, 23(2), 114-127.

Corlett, P. R., & Schoenbaum, G. (2021). Leveraging Basic Science for the Clinic—From Bench to Bedside. JAMA psychiatry, 78(3), 331-334.

Costa, K. M., Schenkel, D., & Roeper, J. (2021). Sex-dependent alterations in behavior, drug responses and dopamine transporter expression in heterozygous DAT-Cre mice. Scientific Reports, 11(1), 1-11.

Cousins, M. S., & Salamone, J. D. (1994). Nucleus accumbens dopamine depletions in rats affect relative response allocation in a novel cost/benefit procedure. Pharmacology

Biochemistry and Behavior, 49(1), 85-91.

Creese, I., Burt, D. R., & Snyder, S. H. (1976). Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science, 192(4238), 481-483.

Culler, E. A. (1938). Recent advances in some concepts of conditioning. Psychological Review, 45(2), 134.

Davis, J. D., & Campbell, C. S. (1973). Peripheral control of meal size in the rat: Effect of sham feeding on meal size and drinking rate. Journal of comparative and physiological psychology, 83(3), 379.

Davis, J. D., & Perez, M. C. (1993). Food deprivation-and palatability-induced microstructural changes in ingestive behavior. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 264(1), R97-R103.

Davis, J. D., & Smith, G. P. (1992). Analysis of the microstructure of the rhythmic tongue movements of rats ingesting maltose and sucrose solutions. Behavioral neuroscience, 106(1), 217.

Delay, J., Deniker, P., & Harl, J. M. (1952, June). Therapeutic use in psychiatry of phenothiazine of central elective action (4560 RP). In Annales medico-psychologiques (Vol. 110, No. 2 1, p. 112).

Der-Avakian, A., & Markou, A. (2012). The neurobiology of anhedonia and other reward-related deficits. Trends in neurosciences, 35(1), 68-77.

Dienel, S. J., & Lewis, D. A. (2019). Alterations in cortical interneurons and cognitive function in schizophrenia. Neurobiology of disease, 131, 104208.

Duan, X., Chang, J. H., Ge, S., Faulkner, R. L., Kim, J. Y., Kitabatake, Y., ... & Song, H. (2007). Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. Cell, 130(6), 1146-1158

Dwyer, D. M., & Killcross, S. (2006). Lesions of the basolateral amygdala disrupt conditioning based on the retrieved representations of motivationally significant events. Journal of Neuroscience, 26(32), 8305-8309..

Edition, F. (2013). Diagnostic and statistical manual of mental disorders. Am Psychiatric Assoc.

Fallon, J. H. (1981). Collateralization of monoamine neurons: mesotelencephalic dopamine projections to caudate, septum, and frontal cortex. Journal of Neuroscience, 1(12), 1361-1368.

Feifel, D., & Shilling, P. D. (2010). Promise and pitfalls of animal models of schizophrenia. Current psychiatry reports, 12(4), 327-334.

Ferenczi, E. A., Zalocusky, K. A., Liston, C., Grosenick, L., Warden, M. R., Amatya, D., ... & Kalanithi, P. (2016). Prefrontal cortical regulation of brainwide circuit dynamics and reward-related behavior. Science, 351(6268).

Floresco, S. B., & Magyar, O. (2006). Mesocortical dopamine modulation of executive functions: beyond working memory. Psychopharmacology, 188(4), 567-585.

Fry, B. R., Russell, N., Gifford, R., Robles, C. F., Manning, C. E., Sawa, A., ... & Johnson, A. W. (2020). Assessing Reality Testing in Mice Through Dopamine-Dependent Associatively Evoked Processing of Absent Gustatory Stimuli. Schizophrenia bulletin, 46(1), 54-67.

Fry, B. R., Pence, N. T., McLocklin, A., & Johnson, A. W. (2021). Disruptions in effort-based decision-making following acute optogenetic stimulation of ventral tegmental area dopamine cells. Learning & Memory, 28(4), 104-108.

Galistu, A., Modde, C., Pireddu, M. C., Franconi, F., Serra, G., & D'Aquila, P. S. (2011). Clozapine increases reward evaluation but not overall ingestive behaviour in rats licking for sucrose. Psychopharmacology, 216(3), 411-420.

Galistu, A., & D'Aquila, P. S. (2013). Dopamine on D2-like receptors "reboosts" dopamine D1-like receptor-mediated behavioural activation in rats licking for a isotonic NaCl solution. Psychopharmacology, 229(2), 357-366.

Gardner, M. P., Schoenbaum, G., & Gershman, S. J. (2018). Rethinking dopamine as generalized prediction error. Proceedings of the Royal Society B, 285(1891), 20181645.

Gaser, C., Nenadic, I., Buchsbaum, B. R., Hazlett, E. A., & Buchsbaum, M. S. (2004). Ventricular enlargement in schizophrenia related to volume reduction of the thalamus, striatum, and superior temporal cortex. American Journal of Psychiatry, 161(1), 154-156.

Genn, R. F., Higgs, S., & Cooper, S. J. (2003). The effects of 7-OH-DPAT, quinpirole and raclopride on licking for sucrose solutions in the non-deprived rat. Behavioural Pharmacology, 14(8), 609-617.

Grace, A. A., Floresco, S. B., Goto, Y., & Lodge, D. J. (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. Trends in neurosciences, 30(5), 220-227.

Greenwood, T. A., Braff, D. L., Light, G. A., Cadenhead, K. S., Calkins, M. E., Dobie, D. J., ... & Mintz, J. (2007). Initial heritability analyses of endophenotypic measures for schizophrenia: the consortium on the genetics of schizophrenia. Archives of general

psychiatry, 64(11), 1242-1250.

Gutierrez, H., Hernandez-Echeagaray, E., Ramırez-Amaya, V., & Bermudez-Rattoni, F. (1999). Blockade of N-methyl-D-aspartate receptors in the insular cortex disrupts taste aversion and spatial memory formation. Neuroscience, 89(3), 751-758.

Hartfield, A. W., Moore, N. A., & Clifton, P. G. (2003). Effects of clozapine, olanzapine and haloperidol on the microstructure of ingestive behaviour in the rat. Psychopharmacology, 167(2), 115-122.

Hikida, T., Jaaro-Peled, H., Seshadri, S., Oishi, K., Hookway, C., Kong, S., ... & Mori, S. (2007). Dominant-negative DISC1 transgenic mice display schizophrenia-associated phenotypes detected by measures translatable to humans. Proceedings of the National Academy of Sciences, 104(36), 14501-14506.

Hodos, W. (1961). Progressive ratio as a measure of reward strength. Science, 134(3483), 943-944.

Holland, P. C. (1981). Acquisition of representation-mediated conditioned food aversions. Learning and Motivation, 12(1), 1-18.

Holland, P. C., & Forbes, D. T. (1982). Representation-mediated extinction of conditioned flavor aversions. Learning and Motivation, 13(4), 454-471.

Holland, P. C. (1983). Representation-mediated overshadowing and potentiation of conditioned aversions. Journal of Experimental Psychology: Animal Behavior Processes, 9(1), 1.

Holland, P. C. (1990). Event representation in Pavlovian conditioning: Image and action. Cognition, 37(1-2), 105-131.

Holland, P. (1998). Amount of training affects associatively-activated event representation. Neuropharmacology, 37(4-5), 461-469.

Holland, P. C. (2005). Amount of training effects in representationmediated food aversion learning: No evidence of a role for associability changes. Learning & behavior, 33(4), 464-478.

Horvitz, J. C. (2009). Stimulus–response and response–outcome learning mechanisms in the striatum. Behavioural brain research, 199(1), 129-140.

Howes, O. D., & Kapur, S. (2009). The dopamine hypothesis of schizophrenia: version III—the final common pathway. Schizophrenia bulletin, 35(3), 549-562.

Hu, W., MacDonald, M. L., Elswick, D. E., & Sweet, R. A. (2015). The glutamate hypothesis of schizophrenia: evidence from human brain tissue studies. Annals of the New York

Academy of Sciences, 1338(1), 38-57.

Hull, C. L., Livingston, J. R., Rouse, R. O., & Barker, A. N. (1951). True, sham, and esophageal feeding as reinforcements. Journal of comparative and physiological psychology, 44(3), 236.

Hurd, Y. L., Suzuki, M., & Sedvall, G. C. (2001). D1 and D2 dopamine receptor mRNA expression in whole hemisphere sections of the human brain. Journal of chemical neuroanatomy, 22(1-2), 127-137.

Ikemoto, S., Murphy, J. M., & Mcbride, W. J. (1998). Regional differences within the rat ventral tegmental area for muscimol self-infusions. Pharmacology Biochemistry and Behavior, 61(1), 87-92.

Jaaro-Peled, H., Niwa, M., Foss, C. A., Murai, R., de Los Reyes, S., Kamiya, A., ... & Sawa, A. (2013). Subcortical dopaminergic deficits in a DISC1 mutant model: a study in direct reference to human molecular brain imaging. Human molecular genetics, 22(8), 1574-1580.

Javitt, D. C., & Zukin, S. R. (1991). Recent advances in the phencyclidine model of schizophrenia. The American journal of psychiatry.

Jobe, T. H., & Harrow, M. (2010). Schizophrenia course, long-term outcome, recovery, and prognosis. Current Directions in Psychological Science, 19(4), 220-225.

Johns, L. C., Cannon, M., Singleton, N., Murray, R. M., Farrell, M., Brugha, T., ... & Meltzer, H. (2004). Prevalence and correlates of self-reported psychotic symptoms in the British population. The British Journal of Psychiatry, 185(4), 298-305.

Johnson, A. W., Gallagher, M., & Holland, P. C. (2009). The basolateral amygdala is critical to the expression of pavlovian and instrumental outcome-specific reinforcer devaluation effects. Journal of Neuroscience, 29(3), 696-704.

Johnson, A. W., Sherwood, A., Smith, D. R., Wosiski-Kuhn, M., Gallagher, M., & Holland, P. C. (2010). An analysis of licking microstructure in three strains of mice. Appetite, 54(2), 320-330.

Johnson, A. W., Jaaro-Peled, H., Shahani, N., Sedlak, T. W., Zoubovsky, S., Burruss, D., ... & Gallagher, M. (2013). Cognitive and motivational deficits together with prefrontal oxidative stress in a mouse model for neuropsychiatric illness. Proceedings of the National Academy of Sciences, 110(30), 12462-12467.

Johnson, A. W. (2018). Characterizing ingestive behavior through licking microstructure: Underlying neurobiology and its use in the study of obesity in animal models. International Journal of Developmental Neuroscience, 64, 38-47.

Kakava-Georgiadou, N., Zwartkruis, M. M., Bullich-Vilarrubias, C., Luijendijk, M., Garner, K. M., van der Plasse, G., & Adan, R. A. (2019). An intersectional approach to target neural circuits with cell-and projection-type specificity: validation in the mesolimbic dopamine system. Frontiers in molecular neuroscience, 12, 49.

Kamiya, A., Kubo, K. I., Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., ... & Sawa, A. (2005). A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. Nature cell biology, 7(12), 1167-1178.

Kim, H. J., & Koh, H. Y. (2016). Impaired reality testing in mice lacking phospholipase Cβ1: observed by persistent representation-mediated taste aversion. PLoS One, 11(1), e0146376.

Kim, H. J., Kim, M., Kang, B., Yun, S., Ryeo, S. E., Hwang, D., & Kim, J. H. (2019). Systematic analysis of expression signatures of neuronal subpopulations in the VTA. Molecular Brain, 12(1), 1-9.

Koh, M. T., Wilkins, E. E., & Bernstein, I. L. (2003). Novel tastes elevate c-fos expression in the central amygdala and insular cortex: implication for taste aversion learning. Behavioral neuroscience, 117(6), 1416.

Koh, M. T., Ahrens, P. S., & Gallagher, M. (2018). A greater tendency for representation mediated learning in a ketamine mouse model of schizophrenia. Behavioral neuroscience, 132(2), 106.

Krabbendam, L., & Van Os, J. (2005). Schizophrenia and urbanicity: a major environmental influence—conditional on genetic risk. Schizophrenia bulletin, 31(4), 795-799.

Kuhn, B. N., Campus, P., & Flagel, S. B. (2018). The neurobiological mechanisms underlying sign-tracking behavior. Sign-tracking and drug addiction.

Lally, J., & MacCabe, J. H. (2015). Antipsychotic medication in schizophrenia: a review. British medical bulletin, 114(1), 169-179.

Lammel, S., Steinberg, E. E., Földy, C., Wall, N. R., Beier, K., Luo, L., & Malenka, R. C. (2015). Diversity of transgenic mouse models for selective targeting of midbrain dopamine neurons. Neuron, 85(2), 429-438.

Langdon, A. J., Sharpe, M. J., Schoenbaum, G., & Niv, Y. (2018). Model-based predictions for dopamine. Current opinion in neurobiology, 49, 1-7.

Laruelle, M., Kegeles, L. S., & ABI-DARGHAM, A. N. I. S. S. A. (2003). Glutamate, dopamine, and schizophrenia: from pathophysiology to treatment. Annals of the New York Academy of Sciences, 1003(1), 138-158.

Laursen, T. M. (2011). Life expectancy among persons with schizophrenia or bipolar affective disorder. Schizophrenia research, 131(1-3), 101-104. & H

Lewis, D. A. (1997). Development of the prefrontal cortex during adolescence: insights into vulnerable neural circuits in schizophrenia. Neuropsychopharmacology, 16(6), 385-398.

Le Strat, Y., Ramoz, N., & Gorwood, P. (2009). The role of genes involved in neuroplasticity and neurogenesis in the observation of a gene-environment interaction (GxE) in schizophrenia. Current molecular medicine, 9(4), 506-518.

Liao, R. M., & Ko, M. C. (1995). Chronic effects of haloperidol and SCH23390 on operant and licking behaviors in the rat. Chinese Journal of Physiology, 38, 65-74.

Lieberman, J. A., Stroup, T. S., McEvoy, J. P., Swartz, M. S., Rosenheck, R. A., Perkins, D. O., ... & Severe, J. (2005). Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. New England journal of medicine, 353(12), 1209-1223.

Lindström, L. H., Gefvert, O., Hagberg, G., Lundberg, T., Bergström, M., Hartvig, P., & Långström, B. (1999). Increased dopamine synthesis rate in medial prefrontal cortex and striatum in schizophrenia indicated by L-(β-11C) DOPA and PET. Biological psychiatry, 46(5), 681-688.

Lipina, T. V., Niwa, M., Jaaro-Peled, H., Fletcher, P. J., Seeman, P., Sawa, A., & Roder, J. C. (2010). Enhanced dopamine function in DISC1-L100P mutant mice: implications for schizophrenia. Genes, Brain and Behavior, 9(7), 777-789.

Liu, H., Fan, G., Xu, K., & Wang, F. (2011). Changes in cerebellar functional connectivity and anatomical connectivity in schizophrenia: a combined resting-state functional MRI and diffusion tensor imaging study. Journal of Magnetic Resonance Imaging, 34(6), 1430-1438.

López-Muñoz, F., Alamo, C., Cuenca, E., Shen, W. W., Clervoy, P., & Rubio, G. (2005). History of the discovery and clinical introduction of chlorpromazine. Annals of Clinical Psychiatry, 17(3), 113-135.

Lu, C., Yang, T., Zhao, H., Zhang, M., Meng, F., Fu, H., ... & Xu, H. (2016). Insular cortex is critical for the perception, modulation, and chronification of pain. Neuroscience bulletin, 32(2), 191-201.

Luby, E. D., Gottlieb, J. S., Cohen, B. D., Rosenbaum, G., & Domino, E. F. (1962). Model psychoses and schizophrenia. American Journal of Psychiatry, 119(1), 61-67.

Lynall, M. E., Bassett, D. S., Kerwin, R., McKenna, P. J., Kitzbichler, M., Muller, U., & Bullmore, E. (2010). Functional connectivity and brain networks in schizophrenia. Journal of Neuroscience, 30(28), 9477-9487.

Mahler, S. V., Smith, K. S., & Berridge, K. C. (2007). Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking'of a sweet reward. Neuropsychopharmacology, 32(11), 2267-2278.

Marwaha, S., & Johnson, S. (2004). Schizophrenia and employment. Social psychiatry and psychiatric epidemiology, 39(5), 337-349.

McCutcheon, R. A., Abi-Dargham, A., & Howes, O. D. (2019). Schizophrenia, dopamine and the striatum: from biology to symptoms. Trends in neurosciences, 42(3), 205-220.

McDannald, M., & Schoenbaum, G. (2009). Toward a model of impaired reality testing in rats. Schizophrenia bulletin, 35(4), 664-667.

McDannald, M. A., Whitt, J. P., Calhoon, G. G., Piantadosi, P. T., Karlsson, R. M., O'Donnell, P., & Schoenbaum, G. (2011). Impaired reality testing in an animal model of schizophrenia. Biological psychiatry, 70(12), 1122-1126.

McGrath, J., Saha, S., Chant, D., & Welham, J. (2008). Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiologic reviews, 30(1), 67-76.

Meyer-Lindenberg, A., Miletich, R. S., Kohn, P. D., Esposito, G., Carson, R. E., Quarantelli, M., ... & Berman, K. F. (2002). Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia. Nature neuroscience, 5(3), 267-271.

Millar, J. K., Wilson-Annan, J. C., Anderson, S., Christie, S., Taylor, M. S., Semple, C. A., ... & Porteous, D. J. (2000). Disruption of two novel genes by a translocation co-segregating with schizophrenia. Human molecular genetics, 9(9), 1415-1423.

Mingote, S., Weber, S. M., Ishiwari, K., Correa, M., & Salamone, J. D. (2005). Ratio and time requirements on operant schedules: effort-related effects of nucleus accumbens dopamine depletions. European Journal of Neuroscience, 21(6), 1749-1757.

Moghaddam, B., & Javitt, D. (2012). From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology, 37(1), 4-15.

Mook, D. G., Culberson, R., Gelbart, R. J., & McDonald, K. (1983). Oropharyngeal control of ingestion in rats: acquisition of sham-drinking patterns. Behavioral neuroscience, 97(4), 574.

Mueser, K. T., Bellack, A. S., & Brady, E. U. (1990). Hallucinations in schizophrenia. Acta Psychiatrica Scandinavica, 82(1), 26-29.

Naqvi, N. H., Gaznick, N., Tranel, D., & Bechara, A. (2014). The insula: a critical neural substrate for craving and drug seeking under conflict and risk. Annals of the New York Academy of Sciences, 1316(1), 53-70.

Naqvi, N. H., & Bechara, A. (2017). The role of the insula in goal-directed drug seeking and choice in addiction.

Nestler, E. J., & Hyman, S. E. (2010). Animal models of neuropsychiatric disorders. Nature neuroscience, 13(10), 1161.

Niwa, M., Jaaro-Peled, H., Tankou, S., Seshadri, S., Hikida, T., Matsumoto, Y., ... & Sawa, A. (2013). Adolescent stress–induced epigenetic control of dopaminergic neurons via glucocorticoids. Science, 339(6117), 335-339.

Nunes, E. J., Randall, P. A., Podurgiel, S., Correa, M., & Salamone, J. D. (2013). Nucleus accumbens neurotransmission and effort-related choice behavior in food motivation: effects of drugs acting on dopamine, adenosine, and muscarinic acetylcholine receptors. Neuroscience & Biobehavioral Reviews, 37(9), 2015-2025.

Ohara, P. T., Granato, A., Moallem, T. M., Wang, B. R., Tillet, Y., & Jasmin, L. (2003). Dopaminergic input to GABAergic neurons in the rostral agranular insular cortex of the rat. Journal of neurocytology, 32(2), 131-141.

Ortega, L. A., Daniel, A. M., Davis, J. B., Fuchs, P. N., & Papini, M. R. (2011). Peripheral pain enhances the effects of incentive downshifts. Learning and Motivation, 42(3), 203-209.

Palmer, B. A., Pankratz, V. S., & Bostwick, J. M. (2005). The lifetime risk of suicide in schizophrenia: a reexamination. Archives of general psychiatry, 62(3), 247-253.

Paolo, S. D., Rossi, R., Rizzi, A., & Galistu, A. (2012). Possible role of dopamine D1-like and D2-like receptors in behavioural activation and “contingent” reward evaluation in sodium-replete and sodium-depleted rats licking for NaCl solutions. Pharmacology Biochemistry and Behavior, 101(1), 99-106.

Paxinos, G., & Franklin, K. B. (2019). Paxinos and Franklin's the mouse brain in stereotaxic coordinates. Academic press.

Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: variations in the effectiveness of conditioned but not of unconditioned stimuli. Psychological review, 87(6), 532.

Pletnikov, M. V., Xu, Y., Ovanesov, M. V., Kamiya, A., Sawa, A., & Ross, C. A. (2007). PC12 cell model of inducible expression of mutant DISC1: new evidence for a dominant-negative mechanism of abnormal neuronal differentiation. Neuroscience research, 58(3), 234-244.

Powers, A. R., Mathys, C., & Corlett, P. R. (2017). Pavlovian conditioning–induced hallucinations result from overweighting of perceptual priors. Science, 357(6351), 596-600.

Pribut, H. J., Sciarillo, X. A., & Roesch, M. R. (2022). Insula lesions reduce stimulus-driven control of behavior during odor-guided decision-making and autoshaping. Brain Research, 1785, 147885.

Pritchard, T. C., Macaluso, D. A., & Eslinger, P. J. (1999). Taste perception in patients with insular cortex lesions. Behavioral neuroscience, 113(4), 663.

Randall, P. A., Pardo, M., Nunes, E. J., López Cruz, L., Vemuri, V. K., Makriyannis, A., ... & Salamone, J. D. (2012). Dopaminergic modulation of effort-related choice behavior as assessed by a progressive ratio chow feeding choice task: pharmacological studies and the role of individual differences.

Robles, C. F., & Johnson, A. W. (2017). Disruptions in effort-based decision-making and consummatory behavior following antagonism of the dopamine D2 receptor. Behavioural brain research, 320, 431-439.

Safferman, A., Lieberman, J. A., Kane, J. M., Szymanski, S., & Kinon, B. (1991). Update on the clinical efficacy and side effects of clozapine. Schizophrenia bulletin, 17(2), 247-261.

Salamone, J. D., Correa, M., Farrar, A., & Mingote, S. M. (2007). Effort-related functions of nucleus accumbens dopamine and associated forebrain circuits. Psychopharmacology, 191(3), 461-482.

Salamone, J. D., Correa, M., Nunes, E. J., Randall, P. A., & Pardo, M. (2012). The behavioral pharmacology of effort-related choice behavior: dopamine, adenosine and beyond. Journal of the experimental analysis of behavior, 97(1), 125-146.

Santana, N., Mengod, G., & Artigas, F. (2009). Quantitative analysis of the expression of dopamine D1 and D2 receptors in pyramidal and GABAergic neurons of the rat prefrontal cortex. Cerebral Cortex, 19(4), 849-860.

Saunders, B. T., Richard, J. M., Margolis, E. B., & Janak, P. H. (2018). Dopamine neurons create Pavlovian conditioned stimuli with circuit-defined motivational properties. Nature neuroscience, 21(8), 1072-1083.

Schlagenhauf, F., Huys, Q. J., Deserno, L., Rapp, M. A., Beck, A., Heinze, H. J., ... & Heinz, A. (2014). Striatal dysfunction during reversal learning in unmedicated schizophrenia patients. Neuroimage, 89, 171-180.

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. Nature protocols, 3(6), 1101-1108.

Schneider, L. H., Davis, J. D., Watson, C. A., & Smith, G. P. (1990). Similar effect of raclopride and reduced sucrose concentration on the microstructure of sucrose sham feeding. European journal of pharmacology, 186(1), 61-70.

Schneider, L. H., Gibbs, J., & Smith, G. P. (1986). D-2 selective receptor antagonists suppress sucrose sham feeding in the rat. Brain research bulletin, 17(4), 605-611.

Schultz, W., Dayan, P., & Montague, P. R. (1997). A neural substrate of prediction and reward. Science, 275(5306), 1593-1599.

Seshadri, S., Faust, T., Ishizuka, K., Delevich, K., Chung, Y., Kim, S. H., ... & Sawa, A. (2015). Interneuronal DISC1 regulates NRG1-ErbB4 signalling and excitatory–inhibitory synapse formation in the mature cortex. Nature communications, 6(1), 1-12.

Sharpe, M. J., Chang, C. Y., Liu, M. A., Batchelor, H. M., Mueller, L. E., Jones, J. L., ... & Schoenbaum, G. (2017). Dopamine transients are sufficient and necessary for acquisition of model-based associations. Nature Neuroscience, 20(5), 735-742.

Shen, S., Lang, B., Nakamoto, C., Zhang, F., Pu, J., Kuan, S. L., ... & St Clair, D. (2008). Schizophrenia-related neural and behavioral phenotypes in transgenic mice expressing truncated Disc1. Journal of Neuroscience, 28(43), 10893-10904.

Seeman, P., & Lee, T. (1975). Antipsychotic drugs: direct correlation between clinical potency and presynaptic action on dopamine neurons. Science, 188(4194), 1217-1219.

Seeman, P., Lee, T., Chau-Wong, M., & Wong, K. (1976). Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature, 261(5562), 717-719.

Smith, A., Li, M., Becker, S., & Kapur, S. (2006). Dopamine, prediction error and associative learning: a model-based account. Network: Computation in Neural Systems, 17(1), 61-84.

Smith, K. S., & Berridge, K. C. (2007). Opioid limbic circuit for reward: interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. Journal of neuroscience, 27(7), 1594-1605.

Spector, A. C., Klumpp, P. A., & Kaplan, J. M. (1998). Analytical issues in the evaluation of food deprivation and sucrose concentration effects on the microstructure of licking behavior in the rat. Behavioral neuroscience, 112(3), 678.

St Clair, D., Blackwood, D., Muir, W., Walker, M., Carothers, A., Spowart, G., ... & Evans, H. J. (1990). Association within a family of a balanced autosomal translocation with major mental illness. The Lancet, 336(8706), 13-16.

Stalnaker, T. A., Calhoon, G. G., Ogawa, M., Roesch, M. R., & Schoenbaum, G. (2010). Neural correlates of stimulus-response and response-outcome associations in dorsolateral versus dorsomedial striatum. Frontiers in integrative neuroscience, 4, 12.

Starr, C. J., Sawaki, L., Wittenberg, G. F., Burdette, J. H., Oshiro, Y., Quevedo, A. S., & Coghill, R. C. (2009). Roles of the insular cortex in the modulation of pain: insights from brain

lesions. Journal of Neuroscience, 29(9), 2684-2694.

Su, P., Li, S., Chen, S., Lipina, T. V., Wang, M., Lai, T. K., ... & Liu, F. (2014). A dopamine D2 receptor-DISC1 protein complex may contribute to antipsychotic-like effects. Neuron, 84(6), 1302-1316.

Sullivan, P. F. (2013). Questions about DISC1 as a genetic risk factor for schizophrenia. Molecular psychiatry, 18(10), 1050-1052.

Takahashi, Y. K., Batchelor, H. M., Liu, B., Khanna, A., Morales, M., & Schoenbaum, G. (2017). Dopamine neurons respond to errors in the prediction of sensory features of expected rewards. Neuron, 95(6), 1395-1405.

Tan, S. C., Carr, C. A., Yeoh, K. K., Schofield, C. J., Davies, K. E., & Clarke, K. (2012). Identification of valid housekeeping genes for quantitative RT-PCR analysis of cardiosphere-derived cells preconditioned under hypoxia or with prolyl-4-hydroxylase inhibitors. Molecular biology reports, 39(4), 4857-4867.

Trifilieff, P., Feng, B., Urizar, E., Winiger, V., Ward, R. D., Taylor, K. M., ... & Javitch, J. (2013). Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation. Molecular psychiatry, 18(9), 1025-1033.

Walton, E., Hibar, D. P., Van Erp, T. G., Potkin, S. G., Roiz-Santianez, R., Crespo-Facorro, B., ... & Cahn, W. (2018). Prefrontal cortical thinning links to negative symptoms in schizophrenia via the ENIGMA consortium. Psychological medicine, 48(1), 82-94.

Wardle, M. C., Treadway, M. T., Mayo, L. M., Zald, D. H., & de Wit, H. (2011). Amping up effort: effects of d-amphetamine on human effort-based decision-making. Journal of Neuroscience, 31(46), 16597-16602.

Wu, J. L., Haberman, R. P., Gallagher, M., & Koh, M. T. (2020). Probing for Conditioned Hallucinations Through Neural Activation in a Ketamine Mouse Model of Schizophrenia. Neuroscience Bulletin, 1-5.

Zhu, H., & Roth, B. L. (2014). Silencing synapses with DREADDs. Neuron, 82(4), 723-725.