UNIVERSITY OF CALIFORNIA SAN DIEGO

Translational Mouse Models of Psychiatric Disorders: Genetic, Pharmacological, and Neurodevelopmental Approaches

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy

in

Neurosciences

by

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2020

The Dissertation of Molly Anne Kwiatkowski is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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DEDICATION

I dedicate this PhD to Mom, Dad, and Vin, who have always believed in and supported me; to my husband, Pat, who has been my rock since he came into my life; and to my dog, Luna, who taught me to be responsible for something other than myself.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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ABSTRACT OF THE DISSERTATION

Translational Mouse Models of Psychiatric Disorders: Genetic, Pharmacological, and Neurodevelopmental Approaches

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Psychiatric disorder pathogenesis is driven by interactions between multiple genetic and environmental risk factors. Many genes have been associated with increased risk for psychiatric disorder development. Polymorphisms in the dopamine transporter (DAT) gene have been

linked to bipolar disorder (BD), schizophrenia, and attention deficit hyperactivity disorder (ADHD), and may functionally reduce DAT expression. Assessing animals with manipulated DAT expression in cross-species translatable tasks will bridge the gap between rodent and human work and aide development of better therapeutic options for patients. An example of disease-relevant manipulation is the DAT knockdown (KD) mouse line, created to model the hyperdopaminergic state thought to drive mania symptoms in patients with BD. In the behavioral pattern monitor (BPM), which quantitatively measures locomotor activity and exploratory behaviors in both rodents and humans, patients with mania and DAT KD mice both exhibit hyperactivity and increased exploration. Using a meta-analytic approach, I showed that BPM behavioral outcomes of DAT KD mice were reproducible. Given the need for reproducible models in psychiatric research to test novel therapeutics, I assessed potential anti-mania drugs in the DAT KD/BPM model. Nicotine acetylcholine receptor agonism, via chronic nicotine administration, partially normalized hyperactivity and hyperexploration in DAT KD mice.

Given the neurodevelopmental origins of many psychiatric disorders, it is important to assess early gene x environment interactions that might drive abnormal behaviors in patients. Increased psychiatric disorder diagnoses are observed in people born in late winter/early spring. While many factors have been studied to explain this observation, altered light and activity levels during gestation/early life have been largely ignored. In adult male rodents, reduced active (dark) period (short active (SA) photoperiod) exposure induces depression-relevant behaviors and a stress response. Here, I confirmed SA photoperiod-induced stress response in adult female mice. I then examined the behavioral effects of stress-inducing gestational/early life SA photoperiod exposure in heterozygous DAT KD (DAT-HT) and wildtype (WT) littermates. SA-born WT mice exhibited behavioral changes in multiple cross-species translatable tasks, while DAT-HT mice were largely resilient. Future work will assess SA-induced changes in placental gene expression that contribute to behaviors observed following gestational SA exposure in WT mice.

Chapter 1

Introduction

The public health impact of psychiatric disorders

The 2015 National Survey on Drug Use and Health reported that 43.4 million (18%) U.S. adults (12 years or older) experienced mental illness in the past year (having any mental disorder that met Diagnostic and Statistical Manual of Mental Disorders (DSM)-IV criteria; excluding developmental disorders and substance use disorders). 9.8 million (4%) U.S. adults experienced serious mental illness, wherein the disorder negatively impacted daily functioning in one or more major life activities (Center for Behavioral Health Statistics and Quality, 2016). Globally, mental illness is the leading cause of disability-adjusted life years (DALYs), with unipolar depressive disorders alone comprising one third of mental illness DALYs (WHO, 2011). In 2010, these mental illness DALYs translated into an estimated global cost of $2.5 trillion, and was projected to increase to $6.0 trillion by 2030 (Bloom et al., 2011). Direct costs, such as hospitalizations, medication, clinic visits, are obvious contributors to the burden of mental illness. Even more costly to society, however, are the indirect costs of mental illness, such as premature mortality, incarcerations, homelessness, and reduced labor output (Insel et al., 2009). There is a clear need to better understand and treat psychiatric disorders in order to alleviate the lifetime disease burden in patients as well as decrease social and economic costs in society at large.

Environmental risk factors for psychiatric disorders

One of the difficulties of generating targeted therapeutics for patients with psychiatric disorders is that they have complex etiologies, with multiple environmental and genetic risk factors interacting to predispose toward the development of a disorder (Fig.1.1). Environmental factors play a large role in the pathophysiology of some disorders. For example, one study estimated the heritability of major depressive disorder (MDD) to be 32% (Wray and Gottesman, 2012), indicating a substantial role of environmental factors in MDD etiology. Environmental risk

factors may contribute to psychiatric disorder development as early as the perinatal period, as seen for schizophrenia (Rapoport et al., 2005; Weinberger, 1996). For example, obstetric and delivery complications and their association with schizophrenia have been extensively studied. One meta-analysis showed that gestational diabetes (OR = 7.76), placental abruption (OR = 4.02), low birth weight (<2000 g; OR = 3.89), and emergency C-section (OR = 3.24), were among the obstetric and delivery complications most strongly associated with subsequent development of schizophrenia (Cannon et al., 2002). Complications ultimately leading to or resulting from perinatal hypoxia (e.g. preeclampsia, uterine bleeding during pregnancy, small for gestational age) have been associated with risk for schizophrenia (Zornberg et al., 2000). The association between obstetric and delivery complications and affective disorders is less clear (Nosarti et al., 2012; Scott et al., 2006). Where studied, it remains clear that the perinatal period is vital for normal neurodevelopment and perturbations during this period can negatively impact psychiatric outcomes.

Stress during the prenatal and early life period is another important risk factor for psychiatric disorder development. Exposure to traumatic events during pregnancy has been associated with increased risk for psychiatric diagnoses in offspring. For example, offspring of mothers exposed to the German invasion of the Netherlands during World War II during the first and second trimesters were at increased risk for developing schizophrenia (Van Os and Selten, 1998). In mothers who experienced the death of a relative during their first trimester, offspring were at an increased risk of schizophrenia diagnosis (Khashan et al., 2008); third trimester exposure to bereavement stress increased the risk for autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD) (Class et al., 2014). Stressors during the early life period are also important contributors to risk for psychiatric disorders in adulthood. Early adverse childhood experiences, such as physical abuse, emotional abuse, and neglect, are associated with an increased risk of depressive (Macmillan et al., 2001; Shapero et al., 2014)

and psychotic symptoms (Varese et al., 2012) in adulthood. Additional stressors can have a long-lasting impact on the development of psychiatric conditions.

Other maternal conditions increase the risk for psychiatric disorder development. An increased incidence of schizophrenia and autism has been observed in offspring exposed in utero to influenza and other infections (e.g. rubella) (Brown et al., 2001, 2000; Irving et al., 2000). The associated inflammatory processes are thought to play a role in the pathophysiology of psychiatric disorders through impinging on neurodevelopment (Hagberg et al., 2012). Maternal vitamin deficiencies, particularly vitamin D deficiency, has been associated with an increased incidence of psychiatric disorders (Castrogiovanni et al., 1998; McGrath et al., 2010). This link was established following several epidemiological studies observing a higher prevalence of psychiatric diagnoses in people born in late winter/early spring (Disanto et al., 2012; Hebert et al., 2010; Schwartz, 2011; Torrey et al., 1997, 1996). In rodents, vitamin D deficiency during gestation and early life has produced variable effects on cognition, resulting in some but not all of the deficits observed in conditions such as schizophrenia (reviewed in Cope et al., 2016). While vitamin D deficiency may be one component of this season of birth effect, other factors occurring during a winter gestation, such as altered light exposure, are relatively understudied and need to be further examined.

Genetic risk factors for psychiatric disorders

The influence that any given environmental risk factor has on subsequent development of psychiatric disorders depends on, and highly interacts with an individual's genetic background risk. Psychiatric disorders are typically not the result of just one gene abnormality; rather, a combination of genes, each with a small effect size, contribute to the pathophysiology of these conditions (Sullivan et al., 2012). Numerous meta-analyses have been conducted to identify candidate genes for psychiatric disorders. Recent meta-analyses have demonstrated a

substantial overlap of genetic variation between schizophrenia, bipolar disorder (BD), major depressive disorder (MDD), ADHD, and ASD, pointing toward a shared pathophysiology of these disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; The Brainstorm Consortium, 2018). Another study showed that four regions containing single nucleotide polymorphisms (SNPs) were associated with schizophrenia, BD, MDD, ADHD, and ASD, including intronic regions on chromosomes 3 and 10, and two different SNPs associated with L-type voltage-gated calcium channels subunits (*CACNA1C* and *CACNB2*) (Smoller et al., 2013). A dysfunctional dopamine system has been implicated across these five psychiatric disorders as well (Dichter et al., 2012). The dopamine transporter (DAT) is the main driver of homeostatic control of dopamine. Numerous genome-wide association studies (GWAS), case-control, and family cohort studies have linked polymorphisms in *SLC6A3*, the gene encoding DAT, to BD (Greenwood et al., 2001, 2006; Huang et al., 2015; Pinsonneault et al., 2011), schizophrenia (Greenwood et al., 2016; Huang et al., 2010; Kennedy et al., 2016; Zheng et al., 2012), and ADHD (Akutagava-Martins et al., 2016; Bidwell et al., 2011; De Azeredo et al., 2014; Franke et al., 2010; Gizer et al., 2009; Gomez-Sanchez et al., 2016; Hawi et al., 2010). Reduced DAT levels (~40%) have been reported in unmedicated euthymic BD patients (Anand et al., 2011), and reduced DAT expression was observed in postmortem frontal cortices of BD patients (Rao et al., 2012). Additionally, DAT polymorphisms associated with psychiatric disease risk differentially modulate activity of the DAT gene promoter (Bamne et al., 2010; Talkowski et al., 2008), indicating that they have potential biological consequences such as reduced DAT expression (Horschitz et al., 2005) that influence the development of psychiatric disorders. Such consequences may have driven the decreased DAT expression in unmedicated BD patients (Anand et al., 2011). Given that many candidate genes have been identified across psychiatric disorders, it will be helpful to focus on genes, such as DAT, that are implicated across diagnostic categories in order to understand common pathophysiological mechanisms.

Recreating psychiatric disorder symptomatology in animal models

Given the complexities of psychiatric disorder pathogenesis, it is difficult to answer many questions related to psychiatric disorder pathogenesis in humans. There is a critical need to develop animal models that recreate the abnormalities of these disorders in a reproducible, translatable manner. Given that psychiatric diagnoses currently rely on clinical rating scales rather than objective genetic, biochemical, or imaging endpoints, it is important to create models that specifically exhibit behavioral consistency with human symptomatology to better understand these disorders and effectively test new therapeutics. Consistent with this idea, the Research Domain Criteria (RDoC) Project seeks to re-categorize psychiatric disorders "based on dimensions of observable behavior and neurobiological measures" instead of conducting research based on traditional diagnostic categories. Using this framework, cross-species translatable behavioral tasks, or tasks that have been adapted for use in both animal and human populations (Young and Markou, 2015), become an invaluable research tool to probe behavioral outputs of psychiatric disease processes in order to understand underlying neural mechanisms of symptomatology and identify therapeutic targets to ameliorate those symptoms (Fig. 1.2).

Given the diversity of genetic, pharmacological, and environmental manipulations that have been created in rodents in an effort to investigate psychiatric disorders, it is necessary to validate them using cross-species translatable tasks to improve predictive and construct validity (Kaffman and Krystal, 2012). These tasks allow for the output of brain circuits that drive underlying pathology to be measured by objective endpoints. This cross-species translation has been applied in studying the negative symptoms of schizophrenia (Young and Markou, 2015). Negative symptoms of schizophrenia can be divided into two main subclasses: 1) decreased emotional affect and spontaneous speech, and 2) avolition and anhedonia (Strauss et al.,

2013). The severity of negative symptoms has been linked to poor functional outcomes in patients with schizophrenia (Green et al., 2012; Milev et al., 2005; Rassovsky et al., 2011), so a better understanding of the neural mechanisms underlying these symptoms and development of therapies to ameliorate them is an important topic of research.

Effort-based decision-making tasks (EBDMT) are one example that has been utilized in both humans and animal models to better understand behaviors related to motivation to obtain rewards. In a rodent EBDMT, rats are required to make an active choice between a lever associated with a small reward after minimal physical effort (low-reward (LR) lever) or a lever associated with a larger reward obtained after higher physical effort (high-reward (HR) lever) (Floresco et al., 2008). In human populations, subjects are presented with a series of trials where, for example, they choose between making $1 by completing 20 button presses or making more money by completing 100 button presses (Gold et al., 2013). This task, and variations of it, has been utilized both in healthy populations and psychiatric populations. Healthy participants are able to use information related to reward magnitude and probability of receiving a reward to inform choice behavior, whereas patients with schizophrenia are impaired in their ability to utilize such information, reflected in relatively constant choice behavior across reward levels and probabilities (Gold et al., 2013; Treadway et al., 2015). Given the ability to implement this task in a cross-species manner, identifying genetic, pharmacological, or neurodevelopmental manipulations in animal models that re-create EBDMT performance deficits seen in patients with schizophrenia will help to elucidate underlying neural mechanisms driving those deficits. Furthermore, identifying pharmacological manipulations that improve performance will direct targeted therapeutic development, and testing putative therapies is made simpler due to the quantifiable behavioral outcomes and cross-species validity of the EBDMT.

Using cross-species translatable tasks to assess mania-relevant behaviors: dopamine transporter knockdown (DAT KD) mice in the behavioral pattern monitor (BPM)

Another application of utilizing cross-species translatable tasks to model psychiatry-relevant behaviors is using the behavioral pattern monitor (BPM) to model and quantify the hyperexploratory profile of BD mania patients but in mice. A hyperdopaminergic state is thought to underlie symptoms of mania (Ashok et al., 2017; Berk et al., 2007; Bunney and Garland, 1982; Cousins et al., 2009; Manji et al., 2003; van Enkhuizen et al., 2015b). Mania is the cardinal feature of bipolar disorder (BD), and recreating mania-relevant symptoms in animal models using pharmacological, environmental, and genetic methods has long been a focus of BD research (Arent et al., 2015; Fries et al., 2015; Prickaerts et al., 2006). The DAT knockdown (KD) mouse line expresses 10% of normal DAT levels, resulting in a hyperdopaminergic state (Zhuang et al., 2001a; Fig. 1.3). This mouse line was created based on several studies linking polymorphisms in *SLC6A3*, the gene encoding the DAT, to BD, as described above (Greenwood et al., 2006, 2001; Huang et al., 2015; Pinsonneault et al., 2011). From confirmation of a mania-like hyperexploratory profile in the behavioral pattern monitor (BPM; below), it was then confirmed that these mice with reduced DAT expression exhibit several aspects of mania, including increased risk-tasking behavior (van Enkhuizen et al., 2015a, 2014; Young et al., 2011) and deficits in attention (Young et al., 2020). Thus, the DAT KD line can be utilized to further understand mania-relevant behaviors and test potential therapeutics, particularly when used in combination with cross-species relevant tasks.

The BPM was originally developed for use in rats to differentiate different pharmacological agents by quantitatively measuring locomotor activity and exploratory behaviors such as rearing and hole poking (Geyer et al., 1986). The BPM was cross-species translated into mice first, then reverse translated into a human version of the task (Minassian et al., 2010; Perry et al., 2009). The human BPM was used to determine the behavioral profile of

patients with BD mania. Patients with mania exhibited abnormalities in three domains of the task when compared with healthy participants: increased locomotor activity, more object interactions (exploratory activity), and reduced spatial d (a quantification of the geometrical pattern of locomotor activity, with reduced spatial d reflecting more straight-line paths) (Henry et al., 2013; Minassian et al., 2011; Paulus and Geyer, 1993; Perry et al., 2009). In the mouse BPM, DAT KD mice exhibited a strikingly similar behavioral profile, including hyperactivity and increased exploratory behaviors, as well as reduced spatial d (Perry et al., 2009; Young et al., 2010). These similarities suggest that DAT KD mice in the BPM is a model with face, predictive, and construct validity that could be used to assess putative anti-mania drugs preclinically. Further validation of the model is observed when BD mania patients treated with standard drugs (e.g. valproate) exhibit diminished hyperactivity over time, while hyperexploration and motor activity pattern differences remain (Minassian et al., 2011). This effect is identical to that seen when DAT KD mice were treated chronically with valproate (van Enkhuizen et al., 2013), adding pharmacological predictive validity to this model.

In order to utilize this model for novel therapeutic target identification and subsequent drug testing, the consistency of BPM outcomes in DAT KD mice needed to be established. If behavioral outcomes fluctuate between experiments, changes in these endpoints following drug administration could not be used as signs of symptom remediation. Reproducibility issues exist across research fields, with >70% of researchers reporting failed attempts at replicating another scientist's experiments in a survey of 1,576 researchers conducted by *Nature* (Baker, 2016). Therefore, there is a need for improved reproducibility standards in science. In Chapter 2 of this dissertation, the reproducibility of four BPM behavioral outcomes (related to locomotor activity, exploration, and locomotor activity patterns) in DAT KD mice is assessed across experimental datasets using a meta-analytic approach.

Meta-analyses conducted on anti-mania drugs show that current therapies provide only incomplete remediation of symptoms, as only moderate effect sizes are reported, indicating a need for better therapeutic options (Cipriani et al., 2011; Yildiz et al., 2011). DAT KD mice in the BPM provide a reproducible (see Chapter 2) model with face, construct, and predictive validity in which to test potential anti-mania drug candidates. It has been postulated that mania symptoms arise from low cholinergic versus catecholaminergic activity in the brain (van Enkhuizen et al., 2015b), which is supported by the reversal of mania symptoms through cholinergic activation (Carroll et al., 1973; Dulawa and Janowsky, 2019; Janowsky et al., 1972). Therefore, drugs that either increase cholinergic activation or decrease catecholaminergic activity have the potential to reduce mania symptoms. It has also been hypothesized that purinergic system dysfunction may underlie mania symptoms, as patients with severe mania symptoms demonstrate enhanced purinergic metabolism (Machado-Vieira et al., 2002), as do people and mice with greater impulsivity (Sutin et al., 2014). Chapter 3 of this dissertation utilizes the DAT KD mouse line in the BPM to test whether drugs chosen based on the rationale of these hypotheses would remediate mania-relevant symptoms.

Gene x environment interactions in mouse models

While it is useful to examine adult models of psychiatric disorders to better understand underlying neural circuitry mediating abnormal behaviors, many psychiatric disorders have neurodevelopmental origins (Gardener et al., 2009; Kloiber et al., 2020; Owen et al., 2011). More research is being done to understand the complex gene x environment interactions that drive the etiology of these disorders (reviewed by Esposito et al., 2018). Therefore, examining gene x environment interactions during the perinatal period in animal models may be a more accurate way of delineating psychiatric disorder pathogenesis. One environmental risk factor that has been repeatedly associated with increased risk for psychiatric disorder development, as

previously discussed, is stress during the perinatal period. Several models have been developed to assess behavioral effects of perinatal stress, including prenatal stress manipulations in pregnant dams (Bronson and Bale, 2014; Howerton et al., 2013) and maternal separation paradigms (Houwing et al., 2019). Not all aspects of perinatal stress have received equal investigation, however.

While the behavioral effects of rodent perinatal stress models have been extensively studied (reviewed by Weinstock, 2008), fewer studies have been conducted on how perinatal and/or early life stress interacts with genetic risk factors for psychiatric disorders. People with a functional polymorphism in the serotonin transporter (SERT) gene promoter region that reduces its efficacy have an increased risk for developing depression when exposed to stressful life events (Caspi et al., 2003). A recent study examined how the interaction between reduced SERT expression and maternal separation influenced anhedonia-relevant behavior in female rats (Houwing et al., 2019). When exposed to maternal separation from postnatal day 2-15 and tested in the sucrose preference test between 10-16 weeks old, female heterozygous SERT knockout mice consumed less sucrose compared to female heterozygous mice that did not undergo maternal separation, representing an anhedonia-like state (Houwing et al., 2019). These findings may not be particularly translationally relevant, however, as patients with depression do not exhibit a reduced preference in the sweet taste test (Aguayo et al., 2012; Berlin et al., 1998; Dichter et al., 2010).

Downregulation of reelin has been observed in cortical GABAergic neurons of patients with schizophrenia, bipolar disorder with psychotic features, and autism (Fatemi, 2001). Another study examined the behavioral effects of the interaction between early maternal separation and reelin deficiency (Laviola et al., 2009). Mice with a 50% reduction in reelin expression that experienced maternal separation from postnatal day 2-6 were tested on postnatal day 9 on their ability to utilize olfaction to find the nest area in their home cage, a potential measure of social

motivation (Ognibene et al., 2007). This social motivation was unaffected in heterozygous reelin mice but was reduced in wildtype littermates, indicating that this gene x environment interaction may minimize risk for developing social amotivation at this early life timepoint, or that reduced reelin expression may imbue a *resiliency* to this stressor. While this may true, and *genetic resiliencies as well as sensitivities to the environment need to be identified*, vulnerabilities to this interaction might not appear until later in life and may affect other domains of behavioral function. More work examining these types of interactions in mouse models will be necessary to develop better early life therapeutic interventions for psychiatric disorders.

Effects of altered light exposure and activity levels on behavior in rodent models

Light exposure and activity levels during gestation/early life are potential environmental factors that are relatively understudied despite connection with the increased incidence of psychiatric disorders observed in people born late winter/early spring (Disanto et al., 2012; Hebert et al., 2010; Schwartz, 2011; Torrey et al., 1997, 1996). Behavioral and neurochemical effects of altered light exposure in adult rodent models have been studied, however (Dulcis et al., 2013; Young et al., 2018). Through increasing the length of the active (dark) phase of the light:dark (L:D) cycle from 12 hours to 19 hours in adult male rats (long active (LA) photoperiod; 5:19 L:D), Dulcis et al. (2013) reported that mania-like behaviors arose. Conversely, depression-relevant behaviors arose when the active phase of the L:D cycle was shortened from 12 hours to 5 hours (short active (SA) photoperiod; 19:5 L:D). Specifically, SA photoperiod-exposed rats exhibited higher immobility in the forced swim test (FST), while LA-exposed rats exhibited increased open arm time in the elevated plus maze (EPM), compared to rats exposed to a normal active (NA) 12:12 L:D cycle. SA-exposed rats also exhibited elevated corticotropin-releasing factor (CRF) in cerebrospinal fluid and elevated plasma corticosterone compared to NA-exposed rats, raising the possibility that SA-induced behaviors may result in part due to

stress-related changes. Young et al. (2018) recently reported cross-species validation of these photoperiod-induced behavioral effects in adult male mice exposed to LA and SA photoperiod. Hence, exposure to altered photoperiods induces behavioral changes in adult rodents, in addition to stress-related changes in the brain and periphery.

Relatively little work has been done on the impact of such photoperiod alterations on adult female rodents or on offspring during the perinatal period. However, these effects are important to investigate if we are to understand the full spectrum of environmental perturbations that contribute to neurodevelopmental disorder pathogenesis. An initial investigation of mice born and raised in a LA photoperiod (8:16 L:D) revealed that in adulthood, mice exhibited abnormal variability in molecular circadian rhythms when challenged by switching to a SA (16:8 L:D) photoperiod (Ciarleglio et al., 2011). The lasting photoperiod-induced effects on the circadian clock demonstrate the importance of environmental perturbations during the perinatal period in altering the development and affecting long-term functioning of biological systems in adulthood. Additionally, mice raised in SA photoperiod (16:8 L:D) conditions exhibited more open arm entries in an elevated zero maze and reduced FST immobility (Green et al., 2015), with long-term alterations in dorsal raphe neuron (DRN) function (e.g. firing rate, responsiveness to noradrenergic stimulation). Recently, this DRN programming by SA photoperiod was found to be prenatal, but changes in affective-like behavior required pre- and postnatal SA exposure (measured by tail suspension test; TST) (Siemann et al., 2019). It is important to note that reduced FST immobility from perinatal SA photoperiod exposure directly contrasts with the effect of adult SA photoperiod exposure; therefore, this neurodevelopmental period is likely key in altering life-long psychiatry-relevant behaviors.

More work needs to be done to characterize the behavioral profiles of SA-exposed offspring using cross-species translatable tasks, as well as on how this environmental factor may interact with genetic risk factors for psychiatric disorders. Given the link between DAT

polymorphisms and BD (Greenwood et al., 2006, 2001; Huang et al., 2015; Pinsonneault et al., 2011), schizophrenia (Greenwood et al., 2016; Huang et al., 2010; Kennedy et al., 2016; Zheng et al., 2012), and ADHD (Akutagava-Martins et al., 2016; Bidwell et al., 2011; De Azeredo et al., 2014; Franke et al., 2010; Gizer et al., 2009; Gomez-Sanchez et al., 2016; Hawi et al., 2010), reduced DAT expression in the KD mouse line can be used to determine the behavioral effects of gene x SA photoperiod interaction during the perinatal period.

In Chapter 4 of this dissertation, stress-inducing effects of SA exposure are assessed in adult female mice. Cross-species translatable tasks are used to characterize the behavioral effects of SA photoperiod exposure during gestation and early life in heterozygous DAT mice (DAT-HT) and wildtype (WT) littermates. DAT-HT mice were chosen over DAT-KD mice for these experiments given that DAT-HT mice exhibit a less extreme reduction in DAT expression (~50% of WT levels) (Young et al., 2018) compared to DAT-KD mice (~10% of WT levels) (Zhuang et al., 2001), and therefore behavioral effects are more likely to be relevant to the human condition.

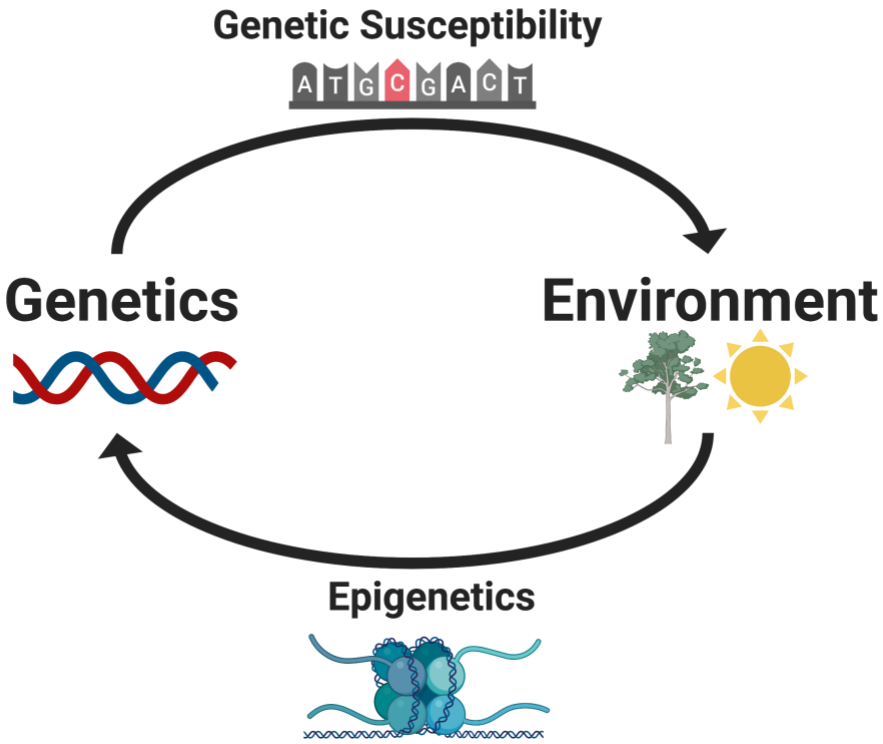

Figure 1.1. Gene x environment interactions contribute to the pathogenesis of many psychiatric disorders. Many psychiatric disorders result from a complex interplay between genetic and environmental risk factors. An individual with a given genetic background may carry alterations in their genetic code that confer susceptibility to environmental factors. In turn, environmental factors can impact genetics via epigenetic regulation of gene expression, for example. Many disorders have neurodevelopmental origins, so it is important to examine early gene x environment interactions that contribute to psychiatric disorder pathogenesis.

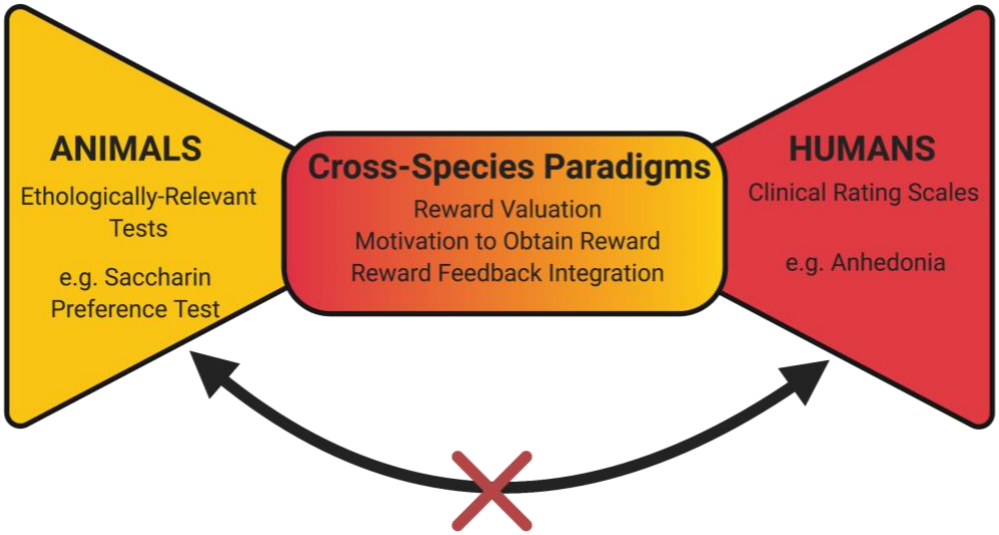

Figure 1.2. Cross-species behavioral paradigms are important to improve the translational value of preclinical research. Given the complexities of psychiatric disorder pathogenesis, and the lack of objective genetic, biochemical, or imaging endpoints, it is important to create animal models that exhibit behavioral consistency with human symptomatology and that are reproducible. Psychiatric diagnoses are made via clinical rating scales, whereas behaviors natural to animals (ethologically-relevant tests) are used in animal studies. It was thought that these behavioral measures would prove similar to symptoms measured via clinical rating scales, but that has not been the case. More emphasis is now being placed on generating cross-species paradigms that can be used in both animal and human populations to increase the chance of shared neural circuitry and improve translational value of research findings.

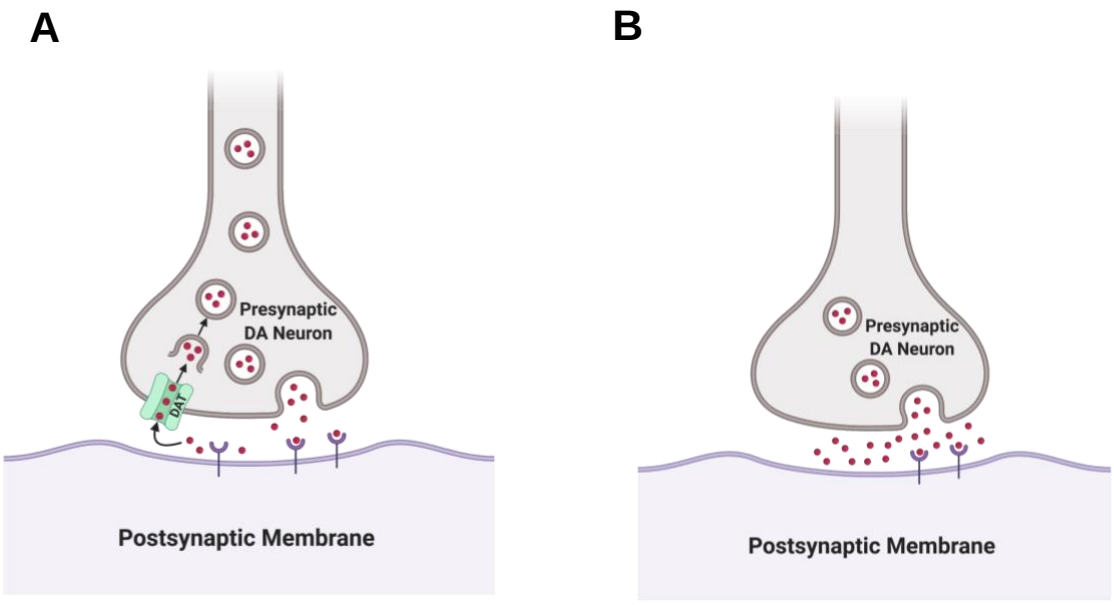

Figure 1.3. The function of the dopamine transporter (DAT) at striatal synapses and consequences of DAT knockdown (KD) in mice. (A) In wildtype mice with normal DAT expression levels, action potentials depolarize the presynaptic terminal and dopamine (DA) vesicles fuse and release DA into the synaptic cleft. DA binds DA receptors on the postsynaptic membrane. DA is taken back up by the presynaptic cell via DAT, and subsequently repackaged into vesicles. (B) In DAT KD mice, a mutation in DAT results in the reduction of DAT expression to 10% of wildtype levels. This manipulation results in a chronic hyperdopaminergic tone at striatal synapses, and a depleted DA vesicle pool due to deficient DA recycling.

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Chapter 2: Dopamine Transporter Knockdown Mice in the Behavioral Pattern Monitor: A Robust, Reproducible Model for Mania-Relevant Behaviors

Abstract

Efforts to replicate results from both basic and clinical models have highlighted problems with reproducibility in science. In psychiatry, reproducibility issues are compounded because the complex behavioral syndromes make many disorders challenging to model. We develop translatable tasks that quantitatively measure psychiatry-relevant behaviors across species. The behavioral pattern monitor (BPM) was designed to analyze exploratory behaviors, which are altered in patients with bipolar disorder (BD), especially during mania episodes. We have repeatedly assessed the behavioral effects of reduced dopamine transporter (DAT) expression in the BPM using a DAT knockdown (KD) mouse line (~10% normal expression). DAT KD mice exhibit a profile in the BPM consistent with acutely manic BD patients in the human version of the task—hyperactivity, increased exploratory behavior, and reduced spatial d (Perry et al., 2009). We collected data from multiple DAT KD BPM experiments in our laboratory to assess the reproducibility of behavioral outcomes across experiments. The four outcomes analyzed were: 1) transitions (amount of locomotor activity); 2) rearings (exploratory activity); 3) holepokes (exploratory activity); and 4) spatial d (geometrical pattern of locomotor activity). By comparing DAT KD mice to wildtype (WT) littermates in every experiment, we calculated effect sizes for each of the four outcomes and then calculated a mean effect size using a random effects model. DAT KD mice exhibited robust, reproducible changes in each of the four outcomes, including increased transitions, rearings, and holepokes, and reduced spatial d, vs. WT littermates. Our results demonstrate that the DAT KD mouse line in the BPM is a consistent, reproducible model of mania-relevant behaviors. More work must be done to assess reproducibility of behavioral outcomes across experiments in order to advance the field of psychiatry and develop more effective therapeutics for patients.

Introduction

The problem of reproducibility in science has recently been highlighted in editorials and surveys of scientists, with failures in large-scale efforts to replicate findings in the fields of cancer biology and psychology. In a survey of 1,576 researchers conducted by *Nature* (2016), >70% of researchers reported attempting and failing to reproduce another scientist's experiments (Baker, 2016). Pressure to publish and selective reporting of results were two factors that the majority of respondents (>60%) identified as always or often contributing to reproducibility problems. Determining reproducibility rates in certain scientific fields is underway, and results so far underscore the need for improved reproducibility standards. For example, the Open Science Collaboration (OSC) attempted to replicate 98 studies published in three psychology journals (Open Science Collaboration, 2015). While 97% of the original 98 studies found significant effects, only 36% of the results were successfully replicated; furthermore, effect sizes were on average half those of the original studies. The OSC is now attempting to replicate widely cited studies in the cancer biology field. Out of five studies completed so far, two have been replicated, two were inconclusive due to technical issues, and one failed to replicate (Aird et al., 2017; Horrigan et al., 2017; Horrigan and Reproducibility Project: Cancer Biology, 2017; Kandela et al., 2017; Mantis et al., 2017). Confirmation of findings through replication is ongoing and will be refined over time, but overall, currently available data emphasize the need to evaluate and validate reproducibility in both clinical and basic models.

Most of the efforts addressing reproducibility have been aimed at assessing inter-laboratory reproducibility (i.e. conducting the same experiment across multiple different laboratories). Intra-laboratory reproducibility (i.e. repeating an experiment in the same laboratory multiple times) is also important to examine, particularly in the field of psychiatry where it has been problematic to generate reproducible basic models. These difficulties arise

for two main reasons: 1) psychiatric disorders are comprised of diagnostic categories based on mainly subjective heterogeneous symptoms, and 2) the general knowledge of underlying etiology and pathophysiology of these disorders remains limited. Some intra-laboratory reproducibility studies have been conducted; e.g., isolation rearing-induced deficits in sensorimotor gating as measured by prepulse inhibition (PPI; Geyer et al., 1993), a paradigm commonly used to measure schizophrenia-relevant behavioral changes (Braff and Geyer, 1990; Geyer and Braff, 1987; Swerdlow et al., 2017), has been assessed. Largely reproducible isolation rearing-induced PPI deficits were reported, although only in 14 out of 18 cohorts (Cilia et al., 2005). Other laboratories have not reproduced isolation rearing-induced PPI deficits, however (Weiss et al., 1999). Methodological differences make it difficult to compare results across laboratories, and further complicate reproducibility issues in the field. Furthermore, many psychiatric disorders are complex and challenging to model, adding to reproducibility issues.

Bipolar disorder (BD) is a complex disease to model in rodents due to its genetic variability and heterogeneous symptoms (Seifuddin et al., 2013) that are often cyclical, including switching between extreme states (mania and depression). These alternating episodes of mania and depression in particular have been challenging to recreate in rodent models (Gould and Einat, 2007; Young and Dulcis, 2015). Modeling BD has focused on recreating specific mania-like symptoms in rodents, given that mania is the cardinal feature of BD, using pharmacological (Fries et al., 2015), environmental (Arent et al., 2015), and genetic methods (Prickaerts et al., 2006). We have reported that knockdown (KD) of the dopamine transporter (DAT) in mice (to 10% of normal levels) results in mania-like symptoms, including abnormal exploration (Perry et al., 2009; Young et al., 2010a) and increased risk-taking behavior (van Enkhuizen et al., 2014b, 2015; Young et al., 2011) seen in BD mania patients. The model was based on genetic linkage studies showing an association between DAT polymorphisms and BD

(Greenwood et al., 2001, 2006) which may functionally reduce DAT expression, with confirmation of reduced DAT expression in postmortem frontal cortices of BD patients (Rao et al., 2012) and in unmedicated euthymic patients using PET (Anand et al., 2011). Hence, reduced DAT expression recreates several aspects of BD mania and has construct validity (Young et al., 2011).

In terms of reproducibility of this animal model of BD, we have repeatedly assessed the effect of reduced DAT expression on exploration in the behavioral pattern monitor (BPM). This exploratory chamber – originally developed for use in rats (Geyer et al., 1986) – is also available for human testing (Minassian et al., 2010), and has been used to show that BD mania patients exhibit abnormalities in three core exploratory domains: amount of motor activity, exploration of novel stimuli, and motor activity patterns (Henry et al., 2013; Minassian et al., 2011; Paulus and Geyer, 1993; Perry et al., 2009). These three aspects of exploratory behavior are similarly altered in DAT KD mice compared with respective controls in the BPM paradigm (Perry et al., 2009; Young et al., 2010a). Further validation is seen when BD mania patients treated with standard treatment (e.g. valproate, among others) exhibit diminished hyperactivity over time, while object interactions (hyperexploration) and motor activity pattern differences remain (Minassian et al., 2011). This effect is identical to that seen when DAT KD mice were treated chronically with valproate (van Enkhuizen et al., 2013), supporting the predictive validity of this model. Therefore, our previous research indicates that DAT KD mice in the BPM accurately model mania behavior of BD patients.

To our knowledge, we are the only laboratory conducting experiments in the BPM using the DAT KD mouse line, making our aggregate dataset collected across different cohorts unique. Given the cross-species relevance of the BPM and the reproducibility issues present in psychiatric research, we determined whether the mania-like behavioral profile of DAT KD mice in the BPM paradigm was reproducible across different experiments, as well as background

strains, in our laboratory. Such reproducibility would increase the internal validity of using DAT KD mice as a model for mania-like behavior and contribute to current global efforts addressing issues of reproducibility in psychiatry research.

In the current analysis, we collated the results of studies performed in our laboratory (from 2004-2015) on DAT KD vs. WT mice using the BPM. Cohen's *d* effect sizes were calculated for four core dimensions of the mania-like behavioral profile. These effect sizes were compared using a meta-analysis across studies and between behavioral dimensions to assess the reproducibility of the DAT KD mouse line in the BPM as a model for BD mania.

Methods

Study Identification

To examine the reproducibility of the DAT KD mouse model of mania in our BPM paradigm, we identified studies conducted in our laboratory between 2004-2015 using DAT KD vs. WT mice in the BPM (see section 2.1.2 for task description). The DAT KD mouse line has been maintained on a 129Sv/J (129) and C57/BL6J (C57) genetic background over this period. Overall analyses included DAT KD and WT littermates from both genetic backgrounds; separate sub-analyses were conducted on each strain to examine inter-background differences in behavioral outcomes. We included both published and unpublished results in this analysis; findings that have been previously published have been cited and identified accordingly. Data from drug treatment groups in three published studies were also collected in order to assess effects on BPM outcomes in this model.

Behavioral Pattern Monitor (BPM)

Locomotor and exploratory behavior was analyzed in BPM chambers (San Diego Instruments, San Diego, CA) as described previously by our group (e.g. Risbrough et al., 2006;

van Enkhuizen et al., 2013). Briefly, each Plexiglas chamber (30.5x61x38 cm) contains eight wall holes (1.25 cm diameter, 1.9 cm above floor) and three floor holes (Fig. 2.1). Each hole contains an infrared beam to detect holepoking. A grid of 12x24 infrared photobeams located 1 cm above the floor records mouse location every 0.1 sec., allowing calculation of transitions (i.e. locomotor activity) from one of nine defined regions to another. A second set of 16 infrared photobeams located 2.5 cm above the floor keeps track of number of mouse rearings. Each session lasts 40-60 minutes, and mice are free to explore the BPM during this time. Each chamber is enclosed such that external light and noise is minimized, and sessions are performed with an internal white light (350 lux in the center, 92 lux in the four chamber corners).

The four BPM outcome measures assessed in the current meta-analysis were: 1) number of transitions (locomotor activity); 2) number of rearings (exploratory behavior); 3) number of holepokes (exploratory behavior); and 4) spatial d (locomotor pattern). Spatial d (ranging from 1-2) quantifies the dimensionality of the pattern of locomotor activity, where d=1 describes a straight-line path and d=2 describes small, circumscribed movements (Paulus and Geyer, 1991).

Effect Size Calculation

Sample sizes, means, and associated standard deviations for each BPM outcome were collected from previous analyses in order to calculate Cohen's *d* effect sizes in DAT KD vs. WT mice. Cohen's *d* was computed using the following equation:

$$d = \frac{mean_{DATKD} - mean_{WT}}{\sqrt{\frac{(N_{WT} - 1)SD_{WT}^2 + (N_{DATKD} - 1)SD_{DATKD}^2}{N_{WT} + N_{DATKD} - 2}}}$$

Positive *d* values reflected a larger mean in the DAT KD vs. WT group, while negative *d* values reflected the opposite. An effect size was calculated for each of the four BPM outcome measures per experiment (transitions, rearing, holepokes, and spatial d). In experiments where

treatment dosing occurred, effect sizes for drug data were calculated by comparing DAT KD drug data to WT drug data (Fig. 2-5, purple data points). Comparing drug effect sizes to effect sizes calculated from DAT KD vs. WT vehicle treatment data enabled drug effect assessment on BPM outcomes in DAT KD mice.

Statistical Methods for Meta-Analysis

Effect sizes obtained from individual experiments were used to calculate a mean effect size for each BPM outcome measure. First, each effect size was weighted by inverse variance to correct for bias due to differences in sample size; this process gave greater weight to effect sizes that were more reliably estimated. After weighting each effect size by inverse variance, a Q statistic was calculated using a meta-analysis macro in SPSS 24.0 (IBM Corp., Armonk, NY) to assess for homogeneity of effect sizes within each BPM outcome measure (meta-analysis macros for SPSS were retrieved June 7, 2017, from http://mason.gmu.edu/~dwilsonb/ma.html). To aid interpretation of the degree of heterogeneity, an I_2 statistic was calculated from the Q statistic and associated df: $I^2 = \frac{Q-df}{Q} * 100$. I_2 values of 25, 50, and 75 represent low, medium, and high heterogeneity, respectively (Higgins and Thompson, 2002). Negative I_2 values were rounded up to zero.

The same meta-analysis macro (Wilson, DB 2006) was used to calculate mean effect sizes for each BPM outcome using a random effects model. The random effects model assumes there are factors changing across experiments (e.g. experimenter experience, animal age, time of day during testing) that might influence observed effect sizes, making it the most appropriate model for the current analyses. Drug data were not included in the meta-analyses. Forest plots were generated using GraphPad Prism 7 (GraphPad Software, La Jolla, CA).

Statistical Comparison of DAT KD Effect Sizes in C57 vs. 129 Genetic Backgrounds

To assess whether DAT KD mutant behavioral profiles differed from WT mice across the two genetic backgrounds studied, DAT KD vs. WT littermate effect sizes were calculated separately for each genetic background (C57 and 129). For each BPM outcome measure, the difference in effect size between C57 and 129 mice was calculated as a z-score: $z = -|d_{C57} - d_{129}| \Big/ \sqrt{(SE_{C57}^2 + SE_{129}^2)}$

A two-tailed p-value was determined from the z-score using a standard normal distribution (van de Lagemaat et al., 2017).

Results

For each BPM outcome measure, 28 effect sizes were obtained for the differences between DAT KD and WT mice. Initially, DAT KD mice were maintained on a 129 background. Subsequently, DAT KD mice were crossed over to the C57 background strain. Data for both 129 and C57 background strains were obtained and utilized in the mean effect size analyses. For holepokes, only 26 effect sizes were obtained given that two experiments did not record any holepokes during the session. All mean effect sizes were calculated using a random effects model.

BPM Outcome Measure: Transitions

Testing for homogeneity of effect sizes for transitions resulted in Q(27)=71.0 ($p<0.0001$; $I^2=62.0$). The resulting mean effect size was 1.49 (95% CI=1.26, 1.71; Z=13.0; $p<0.0001$; Fig. 2.2), indicating that DAT KD mice exhibited a large, reproducible increase in number of transitions in the BPM vs. WT littermates. This hyperactivity of DAT KD mice compared to WT littermates is consistent with modeling BD mania.

To address the heterogeneity seen in effect sizes for transitions, a sub-analysis was performed to determine whether background strain contributed to the observed heterogeneity. DAT KD vs. WT mice on a C57 background were analyzed for homogeneity of effect sizes. $Q(14)=18.0$ ($p>0.05$; $I_2=22.2$), and the mean effect size was 1.80 (95% CI=1.57, 2.02; $Z=15.6$; $p<0.0001$). In contrast, analyses using mice on a 129 background resulted in $Q(12) = 27.0$ ($p<0.01$; $I_2=55.6$) and a mean effect size of 1.15 (95% CI = 0.87, 1.44; $Z = 7.9$; $p<0.0001$). Taken together, effect sizes observed from DAT KD vs. WT littermates on a 129 background were the main driver of heterogeneity in the overall transitions analysis, as effect sizes from DAT KD vs. WT mice on a C57 background displayed a low level of heterogeneity.

Another sub-analysis was performed to address the remaining heterogeneity of effect sizes seen in DAT KD mice on a 129 background. The overall mean effect size analysis included cohorts with repeated exposure to the BPM paradigm, and it was hypothesized that these exposures might introduce variability to the analysis. Experiments on DAT KD mice (129 background) with repeated exposure to the BPM were excluded in this sub-analysis to test this hypothesis. This "first exposure" analysis yielded $Q(5)=13.3$ ($p<0.05$; $I_2=62.4$), indicating that heterogeneity of effect sizes still persisted in this sub-group. This analysis was not conducted in mice on a C57 background given the consistency in effect sizes observed in the strain-specific analysis.

Beyond homogeneity across studies, the mean effect size of DAT KD vs. WT mice on a C57 background was larger than the 129 background, indicative of a larger difference in transitions between DAT KD and WT mice on a C57 vs. 129 background. This difference in effect size was confirmed by computing a z-score and deriving a two-tailed p-value from a standard normal distribution, resulting in a z-score of -3.50 ($p<0.001$ at $\alpha=0.05$). Therefore, compared to mice on a 129 background, DAT KD mice on a C57 background provided a significantly more robust model of hyperactivity in the BPM.

BPM Outcome Measure: Rearing

The test for homogeneity of effect sizes of DAT KD vs. WT mice for rearing resulted in Q(27)=62.0 ($p<0.01$; I_2=56.5) and a mean effect size of 0.56 was subsequently calculated (95% CI=0.37, 0.75; Z=5.8; $p<0.0001$; Fig. 2.3). The mean effect size for rearing was smaller in magnitude compared to the mean effect size for transitions, reflecting a moderate, reproducible increase in exploratory activity for DAT KD vs. WT littermates in the BPM across experiments.

Given the heterogeneity of effect sizes for rearing, follow-up sub-analyses were performed to determine sources of heterogeneity. Consistent with transitions (above), heterogeneity analyses of C57 and 129 background strains were conducted separately. For the C57 background, Q(14)=19.0 ($p>0.05$; I_2=26.3) and the mean effect size was 0.89 (95% CI=0.69, 1.10; Z=8.5; $p<0.0001$). For mice on a 129 background, Q(12)=14.2 ($p>0.05$; I_2=15.5) and the mean effect size was 0.24 (95% CI=0.05, 0.43; Z=2.5; $p<0.05$). Therefore, both background strains exhibited internal homogeneity of effect sizes, indicating that the combination of background strains in the overall analysis contributed to the observed heterogeneity.

Similar to transitions, DAT KD mice on a C57 background exhibited a more robust increase in number of rearings vs. WT littermates compared to DAT KD mice on a 129 background (mean effect size of 0.89 for C57 mice vs. 0.24 for 129 mice). The computed z-score for the C57 and 129 background effect sizes was -4.54 ($p<0.00001$). Again, the DAT KD mice on a C57 background were a significantly more robust model for observing rearing differences vs. WT littermates in the BPM compared to mice on the 129 background.

BPM Outcome Measure: Holepokes

For the overall holepokes effect size analysis, Q(25) = 25.4 ($p>0.05$; I_2=1.6), and meta-analysis resulted in a mean effect size of 0.41 (95% CI = 0.29, 0.54; Z = 6.5; $p<0.0001$; Fig.

2.4). This value reflected a moderate, reproducible increase in holepokes in DAT KD vs. WT littermates, corroborating the increase in exploratory activity seen with rearing. Sub-analysis by background strain confirmed homogeneity of effect sizes in both C57 and 129 background strain groups (C57: $Q(14)=12.6$, $p>0.05$, $I_2=0.0$; mean effect size=0.45, 95% CI=0.28, 0.61, $Z=5.4$, $p<0.0001$; 129: $Q(10)=12.4$, $p>0.05$, $I_2=19.4$; mean effect size=0.37, 95% CI=0.15, 0.58, $Z=3.4$, $p<0.001$).

The calculated z-score between the C57 and 129 sub-groups was -0.58 ($p>0.05$), indicating that the increased effect size magnitude in the C57 sub-group was not significantly different from the effect size observed in the 129 sub-group. Therefore, for exploratory activity measured by holepokes in the BPM, genetic background strain did not influence behavioral profiles.

BPM Outcome Measure: Spatial d

Spatial d, a calculated measure of the geometrical pattern of locomotor activity in the BPM chamber, was the fourth BPM outcome measure analyzed. Spatial d values range between 1 and 2, with a value closer to 1 indicating a straight-line path and values closer to 2 indicating local, circumscribed movements (closer to a filled plane). For the overall analysis, $Q(27)=54.4$ ($p<0.01$; $I_2=50.4$), and the mean effect size was -0.53 (95% CI= -0.71, -0.36; $Z=-5.9$; $p<0.0001$; Fig. 2.5), reflecting a moderate, reproducible reduction of spatial d in DAT KD vs. WT littermates across BPM experiments.

Sub-analyses revealed that effect sizes in both the C57 and 129 backgrounds were homogenous (C57: $Q(14)=12.1$, $p>0.05$, $I_2=0.0$; 129: $Q(12)=16.9$, $p>0.05$, $I_2=29.0$). The mean effect size for DAT KD mice on a C57 background was -0.83 (95% CI= -1.00, -0.66, $Z=-9.7$, $p<0.0001$), whereas the mean effect size for DAT KD mice on a 129 background was -0.21 (95% CI= -0.41, 0.00, $Z=-1.9$, $p>0.05$). The p-value for the mean effect size in the 129 sub-analysis failed to reach significance, indicating that DAT KD mice on a 129 background do not

exhibit a significant reduction in spatial d in the BPM compared to WT littermates. Accordingly, DAT KD mice on a C57 background exhibited a significantly larger mean effect size magnitude vs. WT littermates compared to DAT KD vs. WT mice on a 129 background (z-score= -4.52, $p<0.00001$).

Drug Effects on BPM Outcomes in DAT KD Mice

For three published studies, data from drug treatment groups were collected in order to compute effect sizes comparing DAT KD mice vs. WT littermates treated with various drugs. Drug effect sizes were qualitatively compared to effect sizes generated from vehicle treatment groups in the same study (DAT KD vs. WT littermates treated with vehicle) to determine how behavioral outcomes in DAT KD mice were affected by treatment. All drug treatments were conducted with DAT KD and WT littermate mice on a C57 background.

When DAT KD mice were treated over 28 days with 15 mg/kg valproate chow, they exhibited a reduction in number of transitions and spatial d, an increase in rearing, and no change in holepokes, vs. DAT KD mice treated with normal chow (Fig. 2.2-2.5, data points labeled “van Enkhuizen (2013)”). When mice were treated with a tyrosine hydroxylase inhibitor alpha-methyl-p-tyrosine (AMPT; reduces the synthesis of dopamine), DAT KD mice exhibited reduced transitions, rearing, and spatial d, and increased holepokes (Fig. 2.2-2.5, data points labeled “van Enkhuizen (2014a)”). Finally, treatment with the $D_{2/3}$ antagonist brexpiprazole increased the number of transitions, rearings, and holepokes, with no change in spatial d, in DAT KD mice (Fig. 2.2-2.5, data points labeled “Milienne-Petiot (2017)”).

Discussion

Across cohorts and experiments, DAT KD mice exhibited a reproducible increase in number of transitions, rearing, and holepokes, plus reduced spatial d, vs. WT littermates in the

BPM (see Fig. 2.6 for a summary of overall mean effect sizes calculated for the four BPM outcome measures discussed). The reproducibility of this model was observed irrespective of background strain as DAT KD vs. WT littermate mice exhibited this profile on both C57 and 129 background strains (with the exception of spatial d—DAT KD mice on a 129 background did not exhibit a significant reduction in spatial d vs. WT littermates). However, DAT KD mice on a C57 background were a more robust model for these outcomes compared to mice on a 129 background for three out of the four BPM outcomes (transitions, rearing, and spatial d). The reproducible changes in the exploratory profile of these mice in the BPM, in addition to their face, construct, and predictive validity, support the use of this model for BD mania research. Drug-induced changes in DAT KD BPM locomotor activity, exploratory activity, and spatial d support use of the DAT KD mouse line as a translatable model of bipolar mania in humans. Furthermore, drug-induced changes in DAT KD BPM profiles can be used to screen the effects of novel therapeutics on various behavioral aspects of mania.

A dysregulated dopamine system has been implicated in multiple psychiatric disorders, including schizophrenia, attention-deficit hyperactivity disorder, bipolar disorder, and autism spectrum disorders (Dichter et al., 2012). It is, therefore, essential to fully understand the contributions of altered dopamine homeostasis on these disorders. The DAT KD mouse line was created for this purpose, in addition to circumventing the growth retardation phenotype seen with dopamine transporter knockout lines (Zhuang et al., 2001). Numerous studies have been conducted with the DAT KD mouse line since its creation, yielding valuable insights into the influences of altered dopamine signaling on psychiatry-relevant behavioral profiles (Berridge et al., 2005; Cagniard et al., 2006; Milienne-Petiot et al., 2017b; Peciña et al., 2003; Perry et al., 2009; Tilley et al., 2007; van Enkhuizen et al., 2014b, 2014a; Young et al., 2010a, 2011; Zhuang et al., 2001). This work is the first to demonstrate reproducible findings—across >20 cohorts of studies spanning >13 years of research—that these mice exhibit reliable BD mania-relevant

behavior in the BPM (BD mania patients exhibit increased locomotor activity, more object interactions (exploratory activity), and reduced spatial d (Perry et al., 2009) when compared with healthy participants in the BPM). Therefore, the DAT KD model in our BPM paradigm is reliable, reproducible, and also translatable to human populations.

Given the vast number of studies utilizing genetically engineered mutant mouse lines to address research questions, surprisingly little work has been done to assess the reproducibility of experimental findings across mouse background strains and cohorts. Recently, van de Lagemaat et al. (2017) assessed the similarity of mutant behavioral phenotypes across different genetic background strains using three mutations crossed onto two strains. They analyzed the behavioral impact of each genetic mutation on both background strains using 16 behavioral variables, calculating mutant vs. WT effect sizes for each variable. Importantly, it was observed that 85% of the mutant phenotypes exhibited similar effect sizes across C57 and 129S5 strains. The current research adds to these findings that behavioral consistency of mutant strains can be observed across multiple backgrounds.

Some minor differences between robustness of signal and background strain were observed in the current dataset, however. For example, DAT KD mice on a 129 background did not exhibit a significant reduction in spatial d in the BPM vs. WT littermates. Accordingly, DAT KD mice on a C57 background were a significantly more robust model for spatial d in the BPM, as well as for transitions and rearing outcomes. Importantly, however, while effect sizes were larger in magnitude for C57 mice for transitions and rearing outcomes, the direction of change was the same across strains (increased number of transitions and rearings). Effect sizes for holepokes did not differ significantly between the two background strains. This similarity was reflected by the fact that the overall pool of effect sizes used to compute the holepoke mean effect size was homogeneous. Hence, despite minor differences, the effect of the mutation drove the same changes in behavior in the same direction of effect.

Similar changes in effect sizes were observed in the original study using 129 background DAT KD mice (Zhuang et al., 2001). Although not tested in the BPM, overall activity and rearing was measured using an open-field test. In terms of activity, the KD vs. WT effect size was 1.12, almost identical to the 1.15 effect size in our BPM 129 background strain studies. These data support the inter-laboratory reproducibility in addition to the intra-laboratory reproducibility described above. Although other activity studies in DAT KD mice have been conducted and report similar directionality of effect (hyperactivity in DAT KD vs. WT littermates), insufficient data were presented to calculate effect sizes for comparison (Cagniard et al., 2014; Tilley et al., 2007). Increased rearing in DAT KD vs. WT littermates was also observed (Zhuang et al., 2001), with a larger effect size (1.07) compared with our reports (0.24). Although not similar in magnitude, they are consistent in direction. Magnitude differences were likely due to inter-laboratory variations (e.g. BPM which includes holepokes vs. open-field test without, testing in the dark vs. light phase, etc.). The inter-laboratory consistency supports the strong intra-laboratory reproducibility described here.

In our intra-laboratory analysis, heterogeneity of effect sizes observed with transitions, rearing, and spatial d prompted sub-analyses by strain for these three BPM outcomes. When considered separately, homogeneity of effect sizes was observed within each background strain with the exception of transitions, as while the C57 sub-analysis was homogeneous, the 129 sub-analysis remained heterogeneous. We hypothesized that DAT KD mice on a 129 background might be more sensitive to subsequent exposure to the BPM chamber, and performed an additional sub-analysis that included only first-time exposures to the BPM. Effect sizes for this sub-analysis remained heterogeneous, suggesting that factors other than repeated exposure drove heterogeneity of transitions in the 129 background strain in the BPM. DAT KD mice on a 129 background may be more susceptible to subtle changes in experimental conditions, such as light levels, noise levels, or room temperature, thereby driving effect size variation.

Alternatively, the 129 background strain in particular contains numerous sub-strains that cross four distinct genetic lineages (Kiselycznyk and Holmes, 2011). The sub-strain used for the generation of the DAT KD line, 129Sv/J, was created by combining the 129Sv strain with another unknown strain (Threadgill et al., 1997), perhaps introducing intra-genetic background variability and driving the heterogeneous effect sizes seen with transitions in the current analysis. The consistency of effect sizes of DAT KD vs. WT mice on the C57 background supports its continued use in research on BD mania-related behaviors and their treatment (Milienne-Petiot et al., 2017b; van Enkhuizen et al., 2013, 2014a, 2014b; Young et al., 2011).

Utilizing the C57 background strain of mutation, we have conducted several drug treatment studies. Chronic valproate exposure resulted in a reduction in locomotor activity, an increase or no change in exploratory behavior, and a worsening of locomotor patterns (van Enkhuizen et al., 2013). Chronic valproate (and other) treatment-induced reduction in locomotor effect sizes was also observed in mania patients repeatedly tested over time in the human BPM, where no change on specific exploration or locomotor pattern effect sizes was observed (Minassian et al., 2011). These data highlight the need for therapeutics that address multiple aberrant behaviors in mania, rather than drugs restricted to certain behavioral dimensions (e.g. hyperactivity). Furthermore, given that valproate and lithium exhibit equivalent response rates when used to treat mania episodes in patients with bipolar disorder (Yildiz et al., 2011), the inclusion of this valproate study further demonstrates the validity of the DAT KD mouse line in modeling mania-like behaviors. Future studies should test the response to other anti-mania treatments (e.g. lithium) in order to further assess the validity of this model.

With administration of AMPT, which depleted tyrosine hydroxylase levels, improvements in 3 out of 4 BPM outcomes were observed (excluding holepokes). These improvements in the DAT KD BPM profile lend support to the hypothesis that reduced DAT expression, and resultant hyperdopaminergia, drove the aberrant behavioral profile observed at baseline in DAT KD mice

(van Enkhuizen et al., 2014a). Finally, brexpiprazole treatment worsened or exerted no effect on all 4 BPM outcomes in DAT KD mice (Milienne-Petiot et al., 2017a), potentially indicating that this drug did not specifically target the mechanism by which DAT KD mice exhibit mania-like symptoms. Overall, these data demonstrate that testing the DAT KD mouse line in the BPM provides a strong model for psychiatric therapeutic development and testing.

When developing animal models of human disorders, it is important to also assess response to negative control treatments (Young et al., 2010c). Previous studies have reported an amphetamine-induced reduction in locomotor activity in DAT KD mice (Zhuang et al., 2001), which is seemingly contradictory to its use as a model of mania-like behavior given that amphetamine can induce mania episodes in patients and is used for modeling mania in normal rodents (Arban et al., 2005; Cosgrove et al., 2016; Valvassori et al., 2008). This reduction in locomotor activity, however, could reflect a hypersensitivity to amphetamine (as was observed with another DAT inhibitor, GBR 12909; Young et al., 2010b). Amphetamine at 1 mg/kg induced hyperactivity in WT mice, yet did not affect baseline hyperactivity in DAT KD mice (Zhuang et al., 2001); WT mice continued to exhibit a dose-dependent increase in locomotor activity at 3 mg/kg, whereas DAT KD mice exhibited a reduction in locomotion activity at this dose. A bimodal effect of amphetamine on normal rodent locomotor behaviors was previously reported, where low doses (<2 mg/kg) induced heightened locomotor activity and higher doses reduced locomotor activity via increased stereotypic behaviors (Minassian et al., 2016; Yates et al., 2007). Hence, it is likely that DAT KD mice exhibit a leftward shift in this bimodal pattern, whereby lower amphetamine doses that induce hyperactivity in normal rodents now functionally act like higher doses to suppress locomotor activity – consistent with hypersensitivity of DAT KD mice to GBR12909. Therefore, the results reported by Zhuang et al. (2001) may reflect a hypersensitivity to amphetamine, consistent with the hypersensitivity to amphetamine seen in

patients with bipolar disorder (Wingo and Ghaemi, 2008), and requires lower doses to be assessed for confirmation.

In conclusion, the DAT KD mouse line in the BPM paradigm is a robust, reproducible model for quantitatively analyzing mania-like behaviors, as well as for testing potential therapeutics on such behaviors. A general effort must be made to characterize and understand the consistency of behavioral profiles across experiments, as well as to measure the impact of genetic background strains on those profiles (Bespalov et al., 2016). Particularly in the field of psychiatry, where disorders mainly manifest as alterations in behavior, ensuring reproducibility in behavioral outcomes from animal models is critical to advancing the field and developing more effective treatments to improve patient care.

Acknowledgements

Chapter 2 contains material as a reprint of the following paper: Kwiatkowski MA, Hellemann G, Sugar CA, Cope ZA, Minassian A, Perry W, Geyer MA, Young JW. Dopamine transporter knockdown mice in the behavioral pattern monitor: a robust, reproducible model for mania-relevant behaviors. *Pharmacology, Biochemistry, and Behavior.* 2019;178:42-50. The dissertation author was the primary investigator and author of this material.

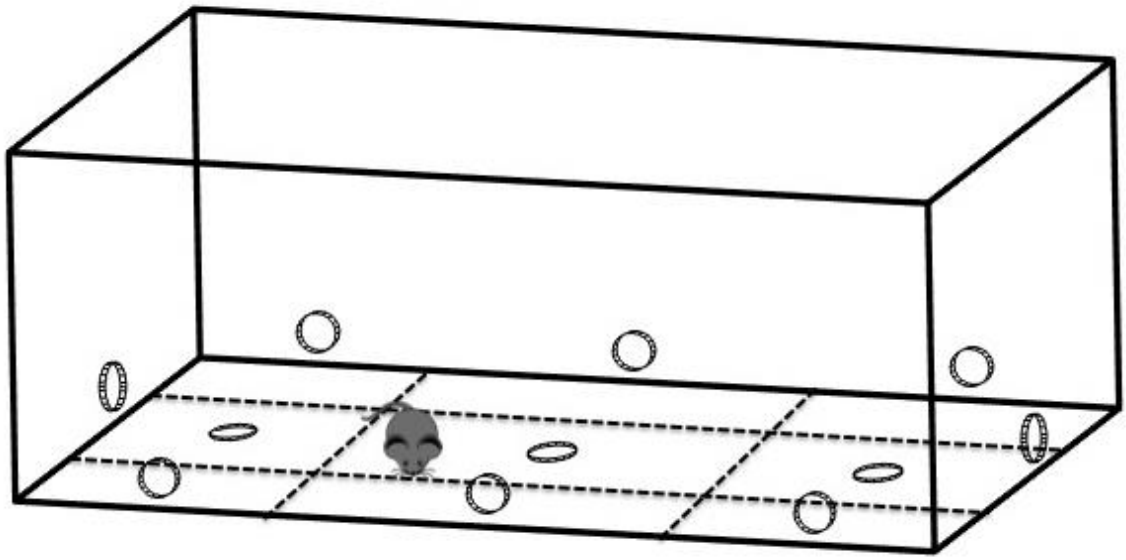

Figure 2.1. Schematic of the mouse behavioral pattern monitor (BPM). The BPM is a Plexiglas chamber (30.5x61x38 cm) containing 8 wall holes (1.25 cm diameter, 1.9 cm above floor) and 3 floor holes. Each hole contains an infrared beam to detect holepoking. A 12x24 grid of infrared photobeams located 1 cm above the floor records mouse location every 0.1 sec allowing for calculation of number of transitions from 1 of 9 defined regions to another (dashed lines illustrate the 9 regions). The mouse location using this grid is also used to quantify locomotor patterns such as spatial d. Another grid of 16 infrared photobeams located 2.5 cm above the floor tracks the number of rearings. Mice are free to explore the chamber during each test session, which can last 45-180 minutes. Each chamber is enclosed to minimize external light and noise, and sessions are performed with an internal white light (350 lux in the center, 92 lux in the 4 chamber corners).

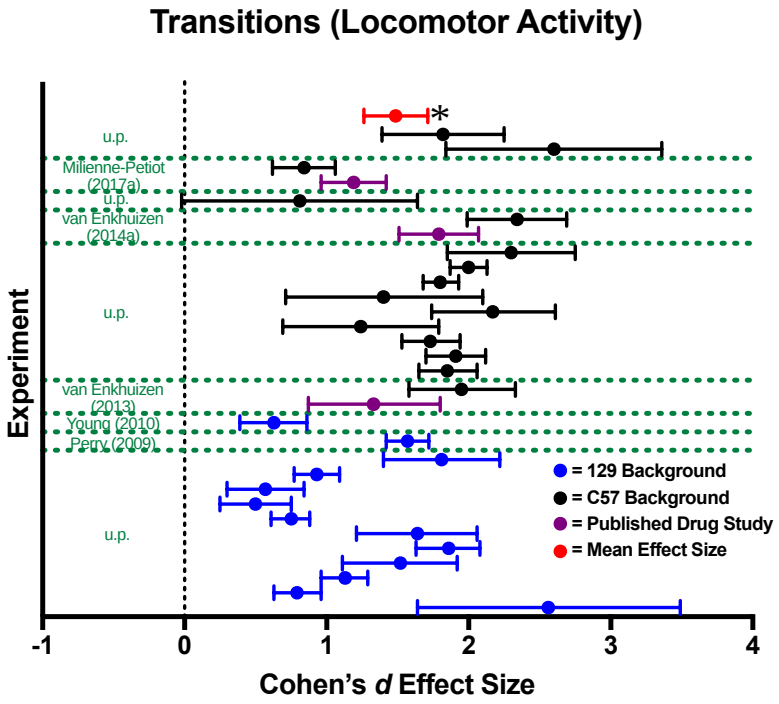

Figure 2.2. DAT KD mice exhibit a significant, reproducible increase in locomotor activity, as measured by transitions in the BPM, compared with WT littermates across experiments. Each row represents a different experiment, and each point is the calculated effect size for that experiment. Blue points: data from DAT KD mice on a 129/SJ (129) background; black points: data from DAT KD mice on a C57/BL6J (C57) background. The dashed black vertical line denotes a Cohen's *d* effect size of zero. All data points are located to the right of the dashed line, reflecting an increase in transitions in DAT KD mice vs. WT littermates for each experiment. Homogeneity of effect sizes was tested using a Q statistic prior to mean effect size calculation. $Q(27)=71.0$ ($p<0.0001$; $I^2=62.0$), indicating heterogeneity of effect sizes in the overall effect size pool. Using a random effects model, the calculated mean effect size was 1.49 (95% CI=1.26, 1.71, $Z=13.0$, $^*p<0.0001$; red data point), reflecting a substantial, reproducible increase in transitions in DAT KD mice vs. WT littermates across all experiments. When analyzed separately by background strain, the effect sizes from mice on a C57 background were homogeneous ($Q(14)=18.0$, $p>0.05$; $I^2=22.2$), and resulted in a mean effect size of 1.80 (95% CI=1.57, 2.02, $Z=15.6$, $p<0.0001$). Effect sizes from mice on a 129 background remained heterogeneous ($Q(12)=27.0$, $p<0.01$; $I^2=55.6$), and a mean effect size of 1.15 was calculated (95% CI=0.87, 1.44, $Z=7.9$, $p<0.0001$). Purple data points represent effect sizes calculated from published studies that included a drug treatment group (DAT KD on drug vs. WT littermates on drug). DAT KD mice treated with 15 mg/kg valproate chow for 28 days exhibited a reduction in transitions vs. DAT KD mice treated with normal chow (black (control) and purple (drug) data points labeled "van Enkhuizen (2013)"). A reduction in transitions was also observed in DAT KD mice treated with 30 mg/kg of a tyrosine hydroxylase inhibitor alpha-methyl-p-tyrosine (AMPT) for 4 days vs. DAT KD mice treated with vehicle (black (control) and purple (drug) data points labeled "van Enkhuizen (2014a)"). In contrast, treatment of DAT KD mice with 0.3 mg/kg of the $D_{2/3}$ antagonist brexpiprazole resulted in an increase in transitions vs. DAT KD mice treated with vehicle (black (control) and purple (drug) data points labeled "Milienne-Petiot (2017)"). Data points are organized in ascending (bottom to top) chronological order based on date of data acquisition. Published data are denoted by: first author last name (year of publication); "u.p." = unpublished data.

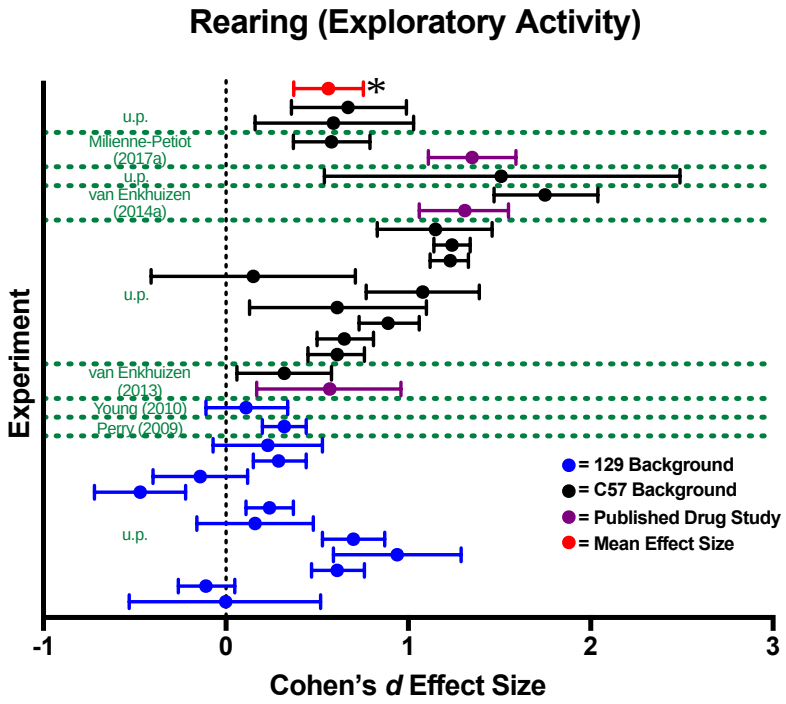

Figure 2.3. DAT KD mice exhibit a significant, reproducible increase in exploratory behavior, as measured by number of rearings in the BPM, compared with WT littermates across experiments. Each row represents a different experiment, and each point is the calculated effect size for that experiment. Blue points: data from DAT KD mice on a 129/SJ (129) background; black points: data from DAT KD mice on a C57/BL6J (C57) background. The dashed black vertical line denotes a Cohen's *d* effect size of zero. All black points are located to the right of the dashed line, whereas 3 blue points are to the left of the line. Homogeneity of effect sizes was tested using a Q statistic prior to mean effect size calculation. $Q(27)=62.0$ ($p<0.01$; $I^2=56.5$), indicating heterogeneity of effect sizes in the overall effect size pool. Using a random effects model, the calculated mean effect size was 0.56 (95% CI=0.37, 0.75, $Z=5.8$, *$p<0.0001$; red data point), reflecting a moderate, reproducible increase in rearing in DAT KD mice vs. WT littermates across experiments. When analyzed separately by background strain, the effect sizes from mice on a C57 background were homogeneous ($Q(14)=19.0$, $p>0.05$; $I^2=26.3$) and a mean effect size of 0.89 (95% CI=0.69, 1.10, $Z=8.5$, $p<0.0001$) was calculated. Effect sizes from mice on a 129 background were also homogenous ($Q(12)=14.2$, $p>0.05$; $I^2=15.5$), and a mean effect size of 0.24 was calculated (95% CI=0.05, 0.43, $Z=2.5$, $p<0.05$). Purple data points represent effect sizes calculated from published studies that included a drug treatment group (DAT KD on drug vs. WT littermates on drug). DAT KD mice treated with 15 mg/kg valproate chow for 28 days exhibited an increase in number of rearings vs. DAT KD mice treated with normal chow (black (control) and purple (drug) data points labeled "van Enkhuizen (2013)"). A reduction in number of rearings was observed in DAT KD mice treated with 30 mg/kg of a tyrosine hydroxylase inhibitor alpha-methyl-p-tyrosine (AMPT) for 4 days vs. DAT KD mice treated with vehicle (black (control) and purple (drug) data points labeled "van Enkhuizen (2014a)"). Treatment of DAT KD mice with 0.3 mg/kg of the $D_{2/3}$ antagonist brexpiprazole resulted in a substantial increase in number of rearings vs. DAT KD mice treated with vehicle (black (control) and purple (drug) data points labeled "Milienne-Petiot (2017)"). Data points are organized in ascending (bottom to top) chronological order based on date of data acquisition. Published data are denoted by: first author last name (year of publication); "u.p." = unpublished data.

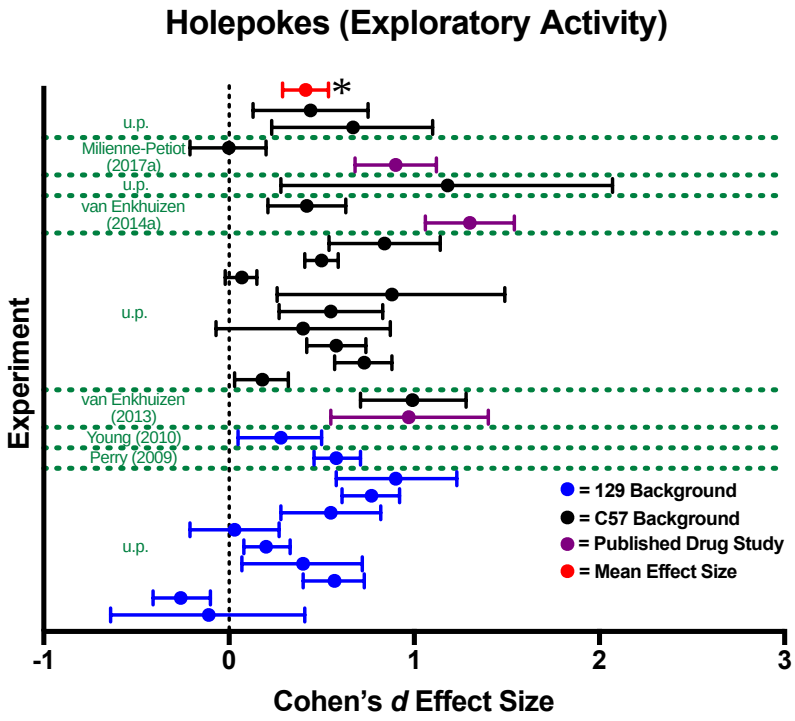

Figure 2.4. DAT KD mice exhibit a significant, reproducible increase in exploratory behavior, as measured by number of holepokes in the BPM, compared with WT littermates across experiments. Each row represents a different experiment, and each point is the calculated effect size for that experiment. Blue points: data from DAT KD mice on a 129/SJ (129) background; black points: data from DAT KD mice on a C57/BL6J (C57) background. The dashed black vertical line denotes a Cohen's *d* effect size of zero. Homogeneity of effect sizes was tested using a Q statistic prior to mean effect size calculation. $Q(25)=25.4$ ($p>0.05$; $I_2=1.6$), and the calculated mean effect size was 0.41 (95% CI=0.29, 0.54, Z=6.5, *$p<0.0001$; red data point), reflecting a moderate, reproducible increase in holepokes in DAT KD mice vs. WT littermates across experiments. Purple data points represent effect sizes calculated from published studies that included a drug treatment group (DAT KD on drug vs. WT littermates on drug). DAT KD mice treated with 15 mg/kg valproate chow for 28 days exhibited no change in number of holepokes vs. DAT KD mice treated with normal chow (black (control) and purple (drug) data points labeled "van Enkhuizen (2013)"). A substantial increase in holepokes was observed in DAT KD mice treated with 30 mg/kg of a tyrosine hydroxylase inhibitor alpha-methyl-p-tyrosine (AMPT) for 4 days vs. DAT KD mice treated with vehicle (black (control) and purple (drug) data points labeled "van Enkhuizen (2014a)"). Similarly, treatment of DAT KD mice with 0.3 mg/kg of the $D_{2/3}$ antagonist brexpiprazole resulted in a sizeable increase in holepokes vs. DAT KD mice treated with vehicle (black (control) and purple (drug) data points labeled "Milienne-Petiot (2017)"). Data points are organized in ascending (bottom to top) chronological order based on date of data acquisition. Published data are denoted by: first author last name (year of publication); "u.p." = unpublished data.

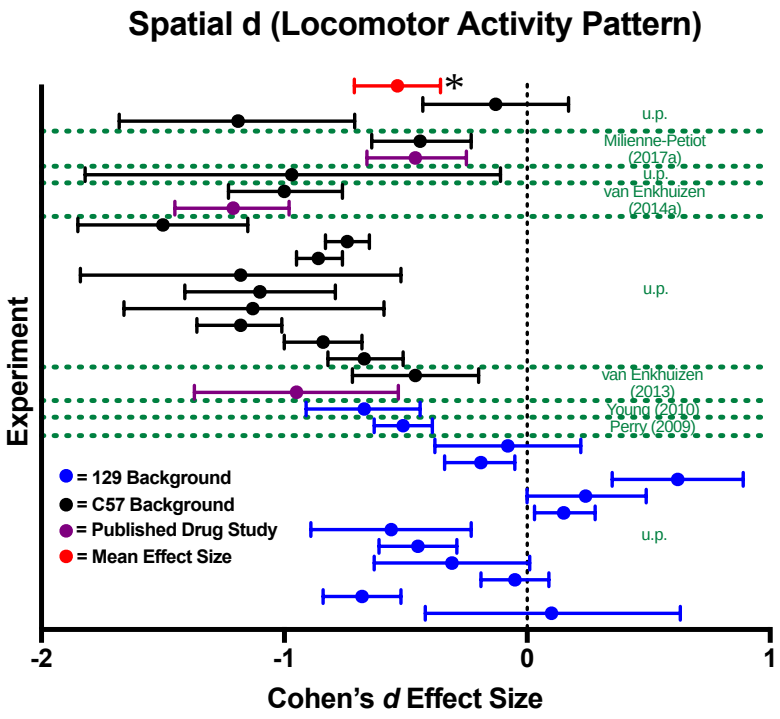

Figure 2.5. DAT KD mice exhibit a significant, reproducible reduction in spatial d in the BPM compared with WT littermates across experiments. Each row represents a different experiment, and each point is the calculated effect size for that experiment. Blue points: data from DAT KD mice on a 129/SJ (129) background; black points: data from DAT KD mice on a C57/BL6J (C57) background. The dashed black vertical line denotes a Cohen's *d* effect size of zero. All black points are located to the left of the dashed line, whereas 4 blue points are to the right of the line. Homogeneity of effect sizes was tested using a Q statistic prior to mean effect size calculation. $Q(27)=54.4$ ($p<0.01$; $I_2=50.4$), indicating heterogeneity of effect sizes in the overall effect size pool. Using a random effects model, the calculated mean effect size was -0.53 (95% CI= -0.71, -0.36, Z= -5.9, *$p<0.0001$; red data point), reflecting a moderate, reproducible decrease in spatial d in DAT KD mice vs. WT littermates across experiments. When analyzed separately by background strain, the effect sizes from mice on a C57 background were homogeneous ($Q(14)=12.1$, $p>0.05$, $I_2=0.0$), and resulted in a mean effect size of -0.83 (95% CI= -1.00, -0.66, Z= -9.7, $p<0.0001$). Effect sizes from mice on a 129 background were also homogenous ($Q(12)=16.9$, $p>0.05$, $I_2=29.0$), and a mean effect size of -0.21 was calculated (95% CI= -0.41, 0.00, Z= -1.9, $p>0.05$). Purple data points represent effect sizes calculated from published studies that included a drug treatment group (DAT KD on drug vs. WT littermates on drug). DAT KD mice treated with 15 mg/kg valproate chow for 28 days exhibited a reduction in spatial d vs. DAT KD mice treated with normal chow (black (control) and purple (drug) data points labeled "van Enkhuizen (2013)"). Similarly, a reduction in spatial d was also observed in DAT KD mice treated with 30 mg/kg of a tyrosine hydroxylase inhibitor alpha-methyl-p-tyrosine (AMPT) for 4 days vs. DAT KD mice treated with vehicle (black (control) and purple (drug) data points labeled "van Enkhuizen (2014a)"). Treatment of DAT KD mice with 0.3 mg/kg of the $D_{2/3}$ antagonist brexpiprazole resulted in no change in spatial d vs. DAT KD mice treated with vehicle (black (control) and purple (drug) data points labeled "Milienne-Petiot (2017)"). Data points are organized in ascending (bottom to top) chronological order based on date of data acquisition. Published data are denoted by: first author last name (year of publication); "u.p." = unpublished data.

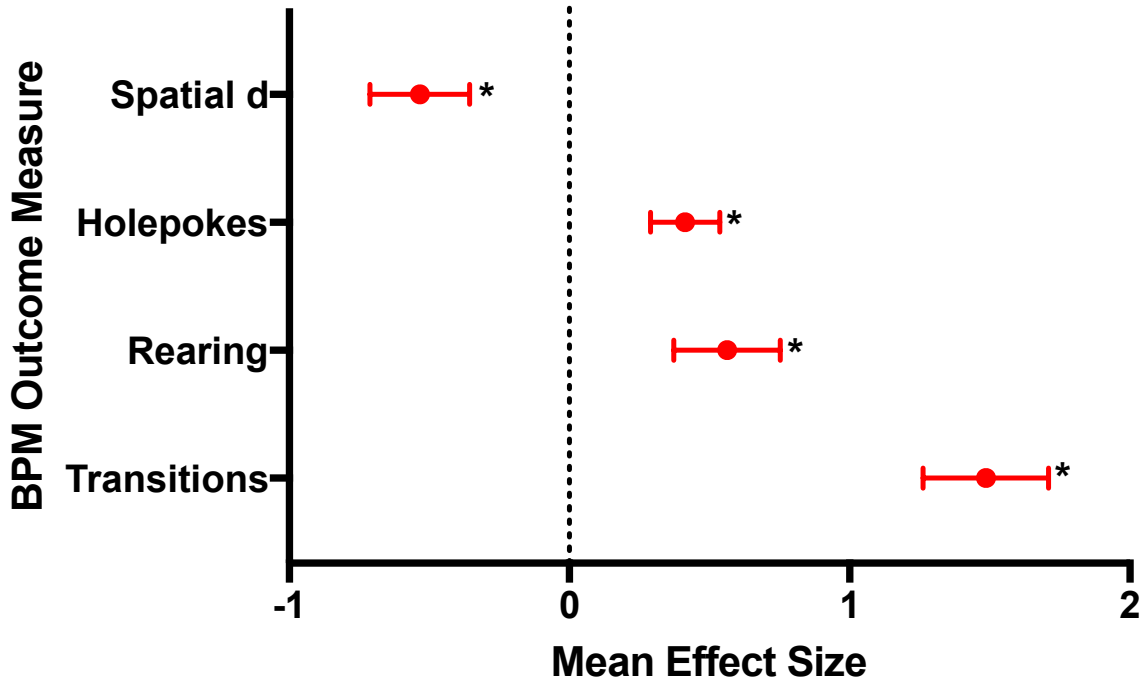

Figure 2.6. Summary of mean effect sizes generated for each BPM outcome measure. The dashed black vertical line denotes a mean effect size of zero. All 4 BPM outcomes were significantly altered in DAT KD mice vs. WT littermates, with increases in transitions, rearing, and holepokes, and a reduction in spatial d. Transitions: random effects model mean effect size=1.49 (95% CI=1.26, 1.71, Z=13.0). Rearing: random effects model mean effect size=0.56 (95% CI=0.37, 0.75, Z=5.8). Holepokes: random effects model mean effect size=0.41 (95% CI=0.29, 0.54, Z=6.5). Spatial d: random effects model mean effect size= -0.53 (95% CI= -0.71, -0.36, Z= -5.9). *p<0.0001

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Chapter 3: Using Dopamine Transporter Knockdown Mice in the Behavioral Pattern Monitor to Assess Putative Anti-Mania Drugs

Abstract

Bipolar disorder (BD) is a severe mental illness affecting 2% of the global population. Current pharmacotherapies provide incomplete symptom remediation, highlighting the need for novel therapeutics. BD is characterized by fluctuations between mania and depression, likely driven by shifts between hyperdopaminergia and hypercholinergia, respectively. Hyperdopaminergia may result from insufficient activity of the dopamine transporter (DAT), the primary mediator of synaptic dopamine clearance. The DAT knockdown (DAT KD) mouse recreates this mechanism and exhibits a highly reproducible hyperexploratory profile in the cross-species translatable Behavioral Pattern Monitor (BPM) that is: a) consistent with that observed in BD mania patients (i.e., hyperactivity, increased specific exploration, and straightened movements), and b) partially normalized across species by chronic valproate treatment. The DAT KD/BPM model of mania therefore exhibits high levels of face-, construct-, and predictive- validity for the pre-clinical assessment of putative anti-mania drugs. Three different drug regimens – chronic nicotine (nicotinic acetylcholine receptor (nAChR) agonist; 40mg/kg/d, 26d), subchronic suramin (anti-purinergic; 20mg/kg, 1×/wk, 4wks), and subchronic resveratrol (striatal DAT upregulator; 20mg/kg/d, 4d) – were administered to separate cohorts of male and female DAT KD- and wildtype (WT) littermate mice, and exploration was assessed in the BPM. Throughout, DAT KD mice exhibited robust hyperexploratory profiles relative to WTs. This hyperexploration (activity and specific exploration), was partially normalized only by nicotine. These results support the mania-like profile of DAT KD mice, which may be partially remediated by nAChR agonists via restoration of disrupted catecholaminergic/cholinergic equilibrium. Delineating the precise mechanism of action of nicotine could identify more selective therapeutic targets.

Introduction

Bipolar disorder (BD) is a life-long and often life-threatening mental illness that occurs in approximately 2% of the global population (Merikangas et al., 2011). BD is characterized by mood fluctuations between mania and depression (Grande et al., 2016) that may reflect extreme shifts between hyperdopaminergia and hypercholinergia, respectively (Ashok et al., 2017; van Enkhuizen et al., 2015). Homeostatic control of dopamine levels is primarily mediated by dopamine transporters (DAT), suggesting that this hyperdopaminergia may be driven by altered DAT expression/function in patients with BD.

Consistent with this potential for DAT alterations to give rise to BD-related behaviors, gene polymorphisms have been linked to BD pathogenesis (Greenwood et al., 2006, 2001; Pinsonneault et al., 2011), which may involve reduced DAT expression (Horschitz et al., 2005). Indeed, DAT binding and expression is reduced in the BD dorsal caudate (Anand et al., 2011) and frontal cortex (Rao et al., 2012), respectively. DAT knockdown (KD) mice, which express DAT at 10% of normal levels (Zhuang et al., 2001), model this hypoexpression phenotype and exhibit reproducible mania-like behaviors in the cross-species translatable Behavioral Pattern Monitor (BPM), including hyperactivity, increased exploration, and straightened path trajectories (Kwiatkowski et al., 2019; Perry et al., 2009; Young et al., 2007; Zhuang et al., 2001). These behaviors are consistent with those observed in acutely manic BD patients assessed in the human version of the BPM (Minassian et al., 2011; Perry et al., 2009). Critically, chronic valproate partially remediates this shared BPM profile in a manner that is consistent across species (Minassian et al., 2011; van Enkhuizen et al., 2013; Young and Dulcis, 2015), thus demonstrating the pharmacological predictive validity of the paradigm. This level of validation, in combination with the robust reproducibility of the hyperactivity profile of the DAT KD mouse (Kwiatkowski et al., 2019) and the construct- and face-validity indicated by the interspecies

similarities in DAT expression and locomotor profiles, enables the use of this paradigm to assess potential new therapies for aspects of BD mania.

Consistent with observations in the BPM (Milienne-Petiot et al., 2017; van Enkhuizen et al., 2013), current therapies provide an incomplete remediation of BD mania, with meta-analyses reporting only moderate effect sizes (Cipriani et al., 2011; Yildiz et al., 2011). Novel pharmacotherapies are therefore urgently needed for patients with BD. Efforts to address this need are hindered by a lack of knowledge of mania pathogenesis, regarding which several hypotheses have been formulated. The catecholaminergic-cholinergic balance hypothesis, for example, postulates that mania symptoms reflect low cholinergic versus catecholaminergic activity in the brain (van Enkhuizen et al., 2015). This hypothesis is supported by studies demonstrating that increasing cholinergic activation reverses symptoms of mania (Dulawa and Janowsky, 2019). Following this model, it may be possible for a nicotinic acetylcholine receptor agonist (e.g., nicotine, the active component of tobacco smoke) to exert a similar effect by directly increasing cholinergic activation. Interestingly, tobacco use is highly prevalent in BD populations – patients with histories of mania have a 3.9× greater likelihood of tobacco dependence than individuals without psychiatric illness (Grant et al., 2004). Nicotine may therefore play a role in the management and prevention of manic symptoms.

Alternative hypotheses of BD mechanisms include the purinergic hypothesis, which posits that disruption of purinergic signaling gives rise to mania symptoms (Machado-Vieira et al., 2002). Purines play an important role in the regulation of neurotransmission and metabolic processes (Zarate and Manji, 2008). Enhanced purinergic metabolism, as indicated by high levels of uric acid (UA), have been associated with greater impulsivity in humans and mice (Sutin et al., 2014), as well as with severe manic symptoms in patients with BD (Machado-Vieira et al., 2002). Indeed, two placebo-controlled clinical trials reported that reduction of UA formation via the addition of allopurinol, a xanthine oxidase inhibitor, to mood-stabilizing

pharmacotherapies significantly reduced manic symptoms in BD patients (Akhondzadeh et al., 2006; Machado-Vieira et al., 2008). Limiting the conversion of xanthine to UA in such a manner leads to elevations in upstream compounds in the purine degradation pathway, including the purine ribonucleoside adenosine, an agonist at the P1 G protein-coupled purinergic receptor (Cheffer et al., 2018). The A_{2A} subtype of P1 receptors is highly expressed in striatal neurons, and inhibitors of these receptors have been demonstrated to have neuroprotective effects (Boison, 2008). Suramin, which inhibits activation of heterotrimeric G-proteins and thereby blocks downstream signaling (including at the A_{2A} receptor), normalizes social behavior, novelty preference, and metabolism in a mouse model of autism (Naviaux et al., 2014), and may also prove beneficial to the treatment of BD mania.

Given the construct (Anand et al., 2011; Rao et al., 2012) and pharmacological predictive validity (van Enkhuizen et al., 2013) of the DAT KD mouse model of BD, directly addressing the most likely cause of the DAT KD mania-like behavioral profile, DAT hypoexpression, may itself yield important information regarding BD pathogenesis. Subchronic administration of resveratrol, a naturally occurring phytoestrogen with anti-apoptotic, anti-aging, and anti-oxidant properties, increases striatal DAT expression in female wildtype (WT) mice (Di Liberto et al., 2012). A similar treatment regimen may therefore increase DAT levels in DAT KD mice, and thereby normalize their mania-like profile in the BPM.

In order to assess the therapeutic potential of each of these drugs, separate cohorts of DAT KD mice and WT littermates were treated with: a) chronic nicotine, b) subchronic suramin, or c) subchronic resveratrol, and then assessed in the cross-species translatable BPM. Each of these treatments were hypothesized to partially remediate the mania-relevant behaviors observed in DAT KD mice in the BPM. The results of these investigations will serve to identify novel pathways and biological processes that may be targeted to ameliorate symptoms of mania.

Methods

Animals

DAT KD and WT littermates were bred in-house using heterozygous breeding pairs. Mice were housed in tetrads by genotype in transparent plastic boxes in a climate-controlled room maintained on a 12-hour light/dark schedule (7:00 AM-7:00 PM dark). Testing was conducted during the dark phase of the animals' light/dark schedules. Food and water were available *ad libitum*, except during testing. Mice were bred, raised, and maintained in a dedicated animal facility approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC). All procedures were approved by the University of California San Diego Animal Care and Use Committee.

Study 1: Chronic Nicotine

Animals

The effects of chronic nicotine on locomotor and exploratory behavior were assessed in male DAT KD- and wildtype (WT) C57BL6/J mice (DAT KD: N=30; 23-32 g; WT: N=27; 26-34 g). Mice were between 50 & 60 weeks of age at time of testing.

Procedure

DAT KD- and WT mice were chronically infused with either vehicle or (−)nicotine hydrogen tartrate at a rate of 40 mg/kg/day for 26 days, at which point they were assessed in the BPM for 60 min. Nicotine was dissolved in sterile 0.9% saline solution and was pH-adjusted to 7±0.5 using sodium hydroxide. Infusions were delivered by ALZET mini-osmotic pumps (Model 2004) at a pumping rate of 0.25 µL/h (±0.05 µL/h). Prior to surgical implantation, pumps were filled and primed in room temperature saline for 40-48 h. Mice were anesthetized with isoflurane (1-3% in oxygen) and were operated on using the following procedure. Ahead of the initial incision, the surgical site (area around the back of the neck) was shaved, and then

sterilized with betadine. Once sterilized, an incision was made and subsequently enlarged by blunt dissection to create a pouch large enough to accommodate the pump. The pump (pre-filled) was then inserted into this pouch, oriented such that the flow modulator was directed caudally. The incision was closed using 9 mm wound clips (MikRon Precision, Inc., Gardena, CA). Subcutaneous baytril (5 mg/kg) and flunixamine (2.5 mg/kg) were administered post-operatively in order to prevent infection and minimize pain. All drugs and reagents were acquired from Sigma Aldrich (St. Louis, MO). Drug doses were chosen based on previously reported nicotine effects in mice (Hall et al., 2015; Higa et al., 2017; Portugal and Gould, 2009).

Study 2: Subchronic Suramin

Animals

The effects of subchronic suramin on locomotor and exploratory behavior were assessed in male and female DAT KD- and WT littermate mice (DAT KD: N=30; females=15 (20-24 g), males=15 (23-28 g)) (WT: N=33; females=17 (19-24 g), males=16 (24-29 g)), all of which were 12-14 weeks of age at time of testing.

Procedure

DAT KD- and WT mice received weekly intraperitoneal injections of either suramin (20 mg/kg; DAT KD: females=6, males=10; WT: females=11, males=6) or vehicle (saline; DAT KD: females=5, males=9; WT: females=10, males=6) for 4 weeks. Mice were assessed in the BPM for 45 min on the day after the final injection. Drug doses were chosen based on previously reported suramin effects in mice (Naviaux et al., 2015, 2013).

Study 3: Subchronic Resveratrol

Animals

The effects of subchronic resveratrol on locomotor and exploratory behavior were assessed in two cohorts each of female DAT KD- and WT C57BL6/J mice, one cohort aged 50

weeks (N=24; DAT KD=11, 23-32 g; WT=13, 26-34 g) and the other aged 18-20 weeks (N=32; DAT KD=11, 20-22 g; WT=21, 19-23 g).

Procedure

DAT KD- and WT mice received daily intraperitoneal injections of either resveratrol (20 mg/kg; DAT KD=11, WT=18) or vehicle (25% DMSO, 25% ethanol, 50% saline; DAT KD=11, WT=16) for 4 days following a between-subjects design. Injections were administered at a volume of 5 ml/kg. All drugs/reagents were obtained from Sigma-Aldrich (St. Louis, MO). On the 7th day following the final injection, mice were assessed in the BPM for 60 min. The dose selected was the same dose reported to induce DAT upregulation in striatum of female mice (Di Liberto et al., 2012).

Behavioral Pattern Monitor (BPM)

The unconditioned motor and exploratory behavior of DAT KD and WT mice was characterized using the Behavioral Pattern Monitor (BPM). The BPM comprised a 30.5 × 61 cm arena that was monitored by two sets of infrared photobeams, disruptions of which were recorded by microcomputer and used to ascertain animals' location and activity from moment to moment. Animals' X-Y coordinates were provided by a 12 × 24 grid of photobeams positioned 1 cm above the floor, while rearing behavior, either against the walls or into the air, was captured by a second array of 16 photobeams traversing the chamber at a height of 2.5 cm. The BPM also contained 11 photobeam-monitored apertures distributed across the walls and floor that mice could investigate via nose poke. The arena was enclosed by 38 cm-high Plexiglas walls that appeared opaque to the mice but permitted the passage of photobeams. 8 BPM chambers were used in the present studies, each of which were enclosed within ventilated sound-attenuating cabinets and illuminated by a single light source above the arena (producing 350 lux in the center, 92 lux in the corners). Photobeam arrays were sampled at 100-msec intervals.

The BPM provided a multivariate readout of general activity levels, specific locomotor behavior, exploration, and path patterns. General activity levels were reported by the *counts* variable – the total number of photobeam disruptions of any kind recorded during the session. Locomotor activity was ascertained from the *total distance traveled* within the session, and from the total number of times mice crossed from one region of the field to another (*transitions*). Exploratory behavior was quantified using the *holepokes* (number of investigatory nose pokes into the apertures) and *rears* measures. Animals' path trajectories were quantified by the *spatial d* metric, a measure of hierarchical/geometric organization of behavior in which values approaching 2 represent highly circumscribed movement patterns and values approaching 1 describe straight-line trajectories.

The effects of a) chronic nicotine and b) subchronic resveratrol on locomotor and exploratory behavior were characterized within 60 min BPM sessions, while the effects of subchronic suramin were assessed in 45 min sessions. Water was used to thoroughly clean the floors and walls of the chambers between runs and was subsequently wiped dry in random patterns in order to disrupt any residual scent trails left over from previous mice.

Statistics

Outcome measures of the BPM were analyzed via three- and four factor ANOVA for each of the three studies. Study 1 (chronic nicotine) data were analyzed using 20-min observation bin as a within-subjects factor and genotype and treatment as between-subjects factors. Study 2 (subchronic suramin) data were analyzed using 15-min bin as a within-subjects factor and genotype, sex, and treatment as between-subjects factors. Study 3 (subchronic resveratrol) data were analyzed via four factor ANOVA, with 20-min bin as a within-subjects factor, and genotype, treatment, and age as between-subjects factors. Statistically significant interactions between two or more factors ($p<0.05$), as well as near-significant interactions ($p<0.10$) predicted by *a priori* hypotheses, were investigated by follow-up ANOVAs. All data

were analyzed using SPSS 25.0 (Chicago, IL) and were represented graphically by mean and standard error of the mean.

Results

Study 1: Nicotine (Chronic)

Outcome variables of the BPM were analyzed via three factor ANOVA using 20-min observation bin as a within-subjects factor, and genotype and treatment as between-subjects factors. Main effects of genotype were observed on overall activity [counts; $F_{(1,48)}$=29.0, p<0.001; Fig. 3.1A] and on both measures of locomotion – transitions [$F_{(1,48)}$=21.2, p<0.001; Fig. 3.1B] and distance traveled [$F_{(1,48)}$=29.7, p<0.001; Fig. 1C]. No main effects of chronic nicotine treatment were observed on any of these measures [F's<1.3, n.s.]. Genotype × treatment interactions were observed on counts [$F_{(1,48)}$=4.6, p<0.05], transitions [$F_{(1,48)}$=4.5, p<0.05], and distance traveled [$F_{(1,48)}$=4.7, p<0.05], revealing that chronic nicotine reduced overall activity [$F_{(1,26)}$=6.3, p<0.05; Fig. 3.1A] and tendency for distance traveled [$F_{(1,26)}$=3.5, p=0.073; Fig. 3.1C] in DAT KD mice only.

Genotype did not affect holepoking [F<1.4, n.s.; Fig. 3.1D], though chronic nicotine tended to non-specifically reduce this behavior across genotypes [$F_{(1,48)}$=3.4, p=0.070]. Given that we had hypothesized *a priori* that nicotine would exert genotype-specific effects on holepoking, planned separate analyses of DAT KD and WT holepoking data were conducted. These analyses revealed a significant reduction of holepoking behavior in DAT KD mice only [$F_{(1,28)}$=6.0, p<0.05]. DAT KD mice reared significantly more frequently than WT mice [$F_{(1,48)}$=8.6, p<0.01; Fig. 3.1E], regardless of treatment [F<1, n.s.]. DAT KD mice demonstrated significantly lower spatial d than WT mice [$F_{(1,48)}$=11.4, p<0.01; Fig. 3.1F], indicating straighter

path trajectories. No main or interactive effects of treatment were observed on these measure [F's<1.2, n.s.].

Significant main effects of 20-min observation bin were observed on counts [$F_{(2,96)}$=76.5, p<0.001], transitions [$F_{(2,96)}$=58.4, p<0.001], distance traveled [$F_{(2,96)}$=83.9, p<0.001], holepoking [$F_{(2,96)}$=6.6, p<0.01], and rearing [$F_{(2,96)}$=18.8, p<0.001], indicating habituation-related decrements in each measure across the testing session. Significant bin × genotype [$F_{(2,96)}$=6.2, p<0.01] and bin × treatment interactions [$F_{(2,96)}$=3.2, p<0.05], but not a bin × genotype × treatment interaction, was observed on counts; the results of follow-up analyses were inconclusive. Similar interactions of bin and genotype [$F_{(2,96)}$=2.7, p=0.070] and of bin and treatment [$F_{(2,96)}$=2.6, p=0.080] were observed on transitions and distance traveled [bin × genotype: $F_{(2,96)}$=2.7, p=0.070; bin × treatment: $F_{(2,96)}$=2.6, p<0.080], though these effects failed to reach statistical significance.

Study 2: Suramin (Subchronic)

Primary outcome variables of the BPM were analyzed via four factor ANOVA, using 15-min observation bin as a within-subjects factor, and sex, genotype, and treatment as between-subjects factors. These analyses revealed the same effects of genotype on counts [$F_{(1,55)}$=52.4, p<0.001; Fig. 3.2A], transitions [$F_{(1,55)}$=17.7, p<0.001; Fig. 3.2B], and distance traveled [$F_{(1,55)}$=22.8, p<0.001; Fig. 3.2C] that were observed in the previous study. No main or interactive effects of sex or treatment were observed on these measures [F's<2.5, n.s.; Fig. 3.2A-C]. Significant main effects of sex [$F_{(1,55)}$=6.3, p<0.05] and genotype [$F_{(1,55)}$=7.5, p<0.01], as well as a sex × genotype interaction [$F_{(1,55)}$=5.6, p<0.05], were observed on holepoking, with male DAT KD mice demonstrating significantly less holepoking behavior than females [$F_{(1,28)}$=14.6, p<0.01; Fig. 3.2D]. Significantly more rears were performed by DAT KD mice than WT mice [$F_{(1,55)}$=15.7, p<0.001; Fig. 3.2E]. Neither sex nor treatment significantly affected rearing behavior, though a non-significant trend toward sex × genotype interaction was

observed on total rears [$F_{(1,55)}$=3.5, p=0.067]; follow-up analyses of this trend were not conducted. As in the previous studies, DAT KD mice demonstrated straighter path trajectories than WT controls, indicated by lower spatial d values [$F_{(1,55)}$=7.2, p<0.05; Fig. 3.2F]. No main or interactive effects of sex or treatment were observed on spatial d [F's<1, n.s.].

Main effects of bin were observed on counts [$F_{(2,110)}$=217.6, p<0.001], transitions [$F_{(2,110)}$=118.2, p<0.001] and distance traveled [$F_{(2,110)}$=203.3, p<0.001], indicating motor habituation across the session. Genotype significantly interacted with bin on counts [$F_{(2,110)}$=11.3, p<0.001], transitions [$F_{(2,110)}$=5.5 p<0.01], and distance traveled [$F_{(2,110)}$=5.6, p<0.01]. Follow-up analyses of these bin × genotype interactions were conducted, but the results did not reveal any markedly differential effect of genotype across the session – DAT KD mice exhibited significantly more activity than WT mice within each bin, as measured by each of these three variables [F's>14, p's<0.001]. Treatment significantly interacted with bin on transitions [$F_{(2,110)}$=4.1, p<0.05] and distance traveled [$F_{(2,110)}$=5.6, p<0.01], and non-significantly on counts [$F_{(2,110)}$=2.8, p=0.065]; follow-up analyses failed to identify any significant bin-specific effect of suramin [F's<1.3, n.s.]. Sex also interacted with bin on counts [$F_{(2,110)}$=17.5, p<0.001], transitions [$F_{(2,110)}$=12.8, p<0.001], and distance traveled [$F_{(2,110)}$=23.4, p<0.001]; given that these latter interactions were between sex and bin alone, and did not implicate either genotype or drug, follow-up analyses were not conducted. No three- or four-way interactions between bin and genotype, sex, or treatment were observed on any measures [F's<2.3].

Study 3: Resveratrol (Subchronic)

Primary outcome variables of the BPM were analyzed via four-factor ANOVA, using 20-min observation bin as a within-subjects factor and genotype, treatment, and age (52 weeks vs 18-20 weeks) as between-subjects factors. Age did not significantly interact with genotype or treatment on any measure (p's>0.10), so mice from both age groups were treated as a single cohort for purposes of interpretation. As in the nicotine and suramin studies, main effects of

genotype were observed on counts [$F_{(1,48)}$=24.3, p<0.001; Fig. 3.3A], transitions [$F_{(1,48)}$=18.0, p<0.001; Fig. 3.3B], and distance traveled [$F_{(1,48)}$=23.5, p<0.001; Fig. 3.3C]. No main or interactive effects of treatment were observed on any of these measures, however [F's<1.2, n.s.]. Main effects of genotype [$F_{(1,48)}$=10.5, p<0.01] and treatment [$F_{(1,48)}$=5.3, p<0.05] indicated more frequent holepoking behavior in DAT KD mice and in resveratrol-treated mice, with a weak, non-significant trend toward genotype × treatment interaction [$F_{(1,48)}$=2.8, p=0.098]; follow-up analysis of this interaction revealed that resveratrol increased holepoking in DAT KD mice only [$F_{(1,20)}$=4.8, p<0.05; Fig. 3.3D]. DAT KD mice also completed more rears than WT mice [$F_{(1,48)}$=19.9, p<0.001]; no main or interactive effects of treatment were observed on rearing behavior [F's<1.5, n.s.; Fig. 3.3E]. As in the nicotine and suramin studies, DAT KD mice displayed significantly lower spatial d than WT mice [$F_{(1,48)}$=5.4, p<0.05; Fig. 3.3F], indicating significantly straighter path trajectories, which were not significantly affected by resveratrol.

Main effects of 20-min observation bin were observed on counts [$F_{(2,96)}$=174.7, p<0.001], transitions [$F_{(2,96)}$=139.7, p<0.001], distance traveled [$F_{(2,96)}$=176.2, p<0.001], holepoking [$F_{(2,96)}$=11.8, p<0.001], and rearing [$F_{(2,96)}$=117.0, p<0.001], indicating a decrement in locomotor and exploratory activity across the testing session. Although bin did not significantly interact with genotype on counts [F<2.4, n.s.] or distance traveled [F<2.4, n.s.], a significant bin × genotype interaction was observed on transitions [$F_{(2,96)}$=3.6, p<0.05]; follow-up analyses were inconclusive. No two- or three-way interactions were observed between treatment and bin on these measures [F's<1, n.s.]. Bin did not significantly interact with genotype or treatment on holepoking or rearing behavior [F's<1.7, n.s.].

Discussion

When assessed in the cross-species translatable behavioral pattern monitor (BPM), dopamine transporter knock-down (DAT KD) mice reliably demonstrated a pattern of

hyperexploration consistent with the profile of patients with bipolar disorder (BD) mania. Specifically, in each of the three studies, DAT KD mice exhibited increased motor activity, increased specific exploration, and straightened path trajectories relative to wildtype (WT) littermate controls (Figs. 3.1-3.3), as do BD mania patients relative to healthy participants (Minassian et al., 2011, 2010; Perry et al., 2009). Chronic nicotine, but not subchronic suramin or resveratrol, partially normalized the hyperactivity (Fig. 3.1A-C) and reduced holepoking behavior in male DAT KD mice (Fig. 3.1D). The present findings illustrate the high fidelity of the mania-like profile of DAT KD mice (Kwiatkowski et al., 2019), as well as their utility in testing novel treatments. These data also support a role for long-term cholinergic agonism in the management of BD mania (Dulawa and Janowsky, 2019).

The BPM is a cross-species translational behavioral system that characterizes the unconditioned motor behavior of rodents and humans on three major axes: general activity/locomotion, exploration, and ambulatory path pattern. BD patients with acute mania exhibit a characteristic motor profile of hyperactivity, increased exploration, and straightened path trajectory in the BPM (Perry et al., 2009). This behavior is also seen in euthymic-state BD patients, albeit with smaller effect sizes (Minassian et al., 2011). The DAT KD mouse, a putative animal model of the DAT hypoexpression observed in post-mortem and PET imaging studies of BD brains (Anand et al., 2011; Rao et al., 2012), exhibits this same pattern of behavior with remarkable consistency (Kwiatkowski et al., 2019). Critically, chronic valproic acid attenuates the hyperactivity of DAT KD mice, consistent with valproate-medicated patients (Minassian et al., 2011; van Enkhuizen et al., 2013). This finding establishes the pharmacological predictive validity of the DAT KD model of mania which, together with the construct validity provided by the line's DAT hypoexpression and the face validity inherent to the cross-species BPM, facilitates prediction of the clinical efficacy of putative anti-mania drugs from their behavioral effects in the rodent BPM.

A recent meta-analysis of BPM data from >20 cohorts of DAT KD mice found that these mice reliably demonstrated significantly elevated levels of activity (transitions) and exploration (holepokes and rears) relative to WT littermates, as well as a significant shift toward straight-line path trajectories (lower spatial d) (Kwiatkowski et al., 2019). Similar inter-cohort consistency was observed across each of the three present studies, supporting this meta-analysis. Overall activity was uniformly elevated across male and female DAT KD mice relative to WT littermates (transitions, counts, and distance traveled), and consistently reduced spatial d indicated relative preference for linear movement. Overall exploratory behavior was also elevated across all cohorts of DAT KD mice, as measured by total rears in all three studies, and by holepoking in the suramin and resveratrol studies (albeit only in females in the former). Of the outcome variables examined in the recent meta-analysis, holepoking was found to be the least reliable (Kwiatkowski et al., 2019), so while male DAT KD mice did not demonstrate increased holepoking activity during the nicotine or suramin studies, the observed genotype effects on rearing, general activity, and spatial d nevertheless indicate robust mania-like profiles within those cohorts. Consistent with previous reports (Milienne-Petiot et al., 2017; Perry et al., 2009; van Enkhuizen et al., 2014, 2013; Young et al., 2010), no main or interactive effects of sex were observed on measures of general activity (counts, transitions, and distance traveled) during the suramin study (Fig. 3.2A-C), though as noted above, a significant sex $\times$ genotype interaction did reveal that only female DAT KD exhibited elevated holepoking.

The most striking findings support the premise that increasing cholinergic signaling via the non-specific nicotinic acetylcholine receptor (nAChR) agonist nicotine partially remediates the mania-like profile of DAT KD mice, as evidenced by genotype-specific reductions to counts (Fig. 3.1A), transitions (Fig. 3.1B), distance traveled (Fig. 3.1C), and holepoking (Fig. 3.1D). This general conclusion is in line with the catecholaminergic-cholinergic balance model of BD pathology, which posits that mania symptoms result from decreased cholinergic- vs.

catecholaminergic activity (van Enkhuizen et al., 2015). Chronic nicotine administration ultimately induces long-term upregulation of most classes of nAChRs (Gentry and Lukas, 2002), which may produce symptom remediation via restoration of catecholaminergic-cholinergic equilibrium. Indeed, this hypothesis is substantiated by findings that acetylcholinesterase inhibitors (e.g., physostigmine) also mitigate mania symptoms, ostensibly via a similar enhancement of cholinergic signaling (Dulawa and Janowsky, 2019) (such treatments are also likely to induce a depressive episode, however, an effect not observed following chronic nicotine). Importantly, nicotine does not affect all nAChR subtypes in the same manner, raising the possibility that its putative therapeutic effects are mediated by specific receptor subtypes rather than by gross enhancement of cholinergic signaling. For example, chronic nicotine induces *downregulation* of striatal α6-containing (α6*) nAChRs (Lai et al., 2005), a population of receptors involved in dopamine release (Salminen et al., 2004). Mice with gain-of-function mutations of the α6*nAChR exhibit similar characteristics to DAT KD mice, including hyperactivity and enhanced striatal dopamine transmission (Drenan et al., 2008). Though these similarities do not necessarily implicate specific α6*nAChR dysregulation in DAT KD mice, downregulation of this receptor class may nevertheless account for the nicotine-mediated modification of their behavioral profile, possibly via partial normalization of striatal dopamine signaling. Future work with specific nicotinic compounds applied directly to the striatum is needed to address this hypothesis.

Studies of the effects of nicotine on cognition in the DAT KD mouse are also warranted. In addition to the robust motor and exploratory profiles discussed above, BD mania is also characterized by inattention and risky decision making, as is readily apparent in the 5-choice continuous performance test (5C-CPT) and Iowa Gambling task, respectively. Both of these tasks are translatable across species, and indeed, DAT KD mice exhibit performance deficits that are consistent with those observed in BD mania patients (Young et al., 2019, 2011). Given

that nicotine improves CPT performance in healthy humans (Levin et al., 1998) and WT mice (Young et al., 2013), a logical next step would be to determine whether nicotine remediates cognitive deficits in DAT KD mice. Nicotine has already been noted to exert potentially pro-cognitive and protective effects in BD patients – cigarette-smoking BD inpatients scored higher on tasks of memory and language than their nonsmoking counterparts at time of admission and discharge (Caldirola et al., 2013) – further highlighting the pertinence of such an investigation. Nicotine withdrawal, meanwhile, worsens cognitive performance in both humans and animals (Hall et al., 2015; Higa et al., 2017) and places BD patients at an increased risk for symptom recurrence (Thomson et al., 2015), indicating a need for characterization of the effects of acute and chronic withdrawal in DAT KD mice. The results of such studies may serve to partially explain the lower smoking cessation rates observed amongst BD patients versus the general population (Jackson et al., 2015).

We did not observe any remediation of DAT KD mania-like behaviors following sub-chronic suramin administration. Although no studies have assessed the effects of suramin on BD patients, suramin has been shown to remediate symptoms of autism spectrum disorder (ASD) in both humans and mouse models. In a small study of male patients with ASD, suramin administration improved language and social interaction, and reduced repetitive behaviors (Naviaux et al., 2017); similarly, suramin restored normal social behaviors and novelty preference in mouse models of ASD (Naviaux et al., 2014, 2013). Although the present data do not suggest a role for suramin in the management of mania, reduction of purinergic signaling via other drugs, e.g., allopurinol, remediated mania symptoms in BD patients (Akhondzadeh et al., 2006; Machado-Vieira et al., 2008). Interpretation of the present data is limited by suramin's lack of receptor specificity – though popularly described as an inhibitor of the purinergic system, suramin antagonizes other receptor classes via destabilization of the ternary complex, including dopaminergic (Beindl et al., 1996), adrenergic (Huang et al., 1990), glutamatergic (Peoples and

Li, 1998), and opioid (Butler et al., 1988) receptors. The absence of effect on WT and DAT KD behavior may therefore be a cumulative result of suramin simultaneously interacting with opposing receptor classes, rather than of specific purinergic antagonism. Compounds that target specific purinergic receptors will be critical in determining the contribution of purinergic dysfunction to mania-related behaviors.

Subchronic resveratrol, previously demonstrated to upregulate striatal DAT expression in female WT mice (Di Liberto et al., 2012), did not significantly affect locomotion (Fig. 3.3A-C), rearing behavior (Fig. 3.3E), or path trajectory (Fig. 3.3F) in either WT or DAT KD mice, though it did increase holepoking behavior in DAT KD mice only (Fig. 3.3D). The absence of treatment $\times$ genotype interactions on locomotion variables – and especially the genotype-specific increase in holepoking – was surprising, as the DAT-enhancing effect of resveratrol was expected to directly address the genetic modification of DAT KD mice and at least partially remediate their behavioral abnormalities. This lack of normalization was not likely a result of sub-optimal study parameters – the present study implemented the same treatment regimen as that previously reported to increase striatal DAT expression (Di Liberto et al., 2012), and used DAT KD mice of the same sex and background (C57BL6/J). It therefore appears that upregulating striatal DAT is not sufficient to remediate mania-like symptoms in DAT KD mice. Given that the DAT gene is non-specifically knocked down in DAT KD mice, and the consequent reduction in DAT expression therefore not limited to a single structure (e.g., the striatum), the overall absence of effect of resveratrol may indicate that the mania-like profile of DAT KD mice is mediated by altered dopamine dynamics elsewhere in the brain. These conclusions are, however, entirely speculative, as direct measurement of striatal DAT levels would be necessary to confirm resveratrol-mediated DAT upregulation. Moreover, it remains unknown whether the effects of resveratrol on DAT expression are limited to the striatum, as studies investigating this interaction have so far only examined this one general area. Given the significantly decreased

DAT availability observed in the BD dorsal striatum (Anand et al., 2011) and frontal cortex (Rao et al., 2012), more targeted and comprehensive study is certainly justified.

The DAT KD mouse is a robust model of mania-relevant behaviors (Kwiatkowski et al., 2019) that, when assessed in the BPM, provides a pharmacologically valid means by which to screen potential anti-mania therapeutics. The present findings suggest a role for cholinergic agonism in the treatment of manic symptoms, specifically hyperactivity. Targeted studies of specific brain regions implicated in BD pathology are needed to elucidate the mechanisms underlying this attenuation. Future studies should also utilize nAChR subtype-specific compounds in order to identify receptor subtypes that may be targeted for the remediation of mania symptoms. The development and implementation of more targeted therapeutics for BD should improve treatment outcomes while concomitantly reducing side effects.

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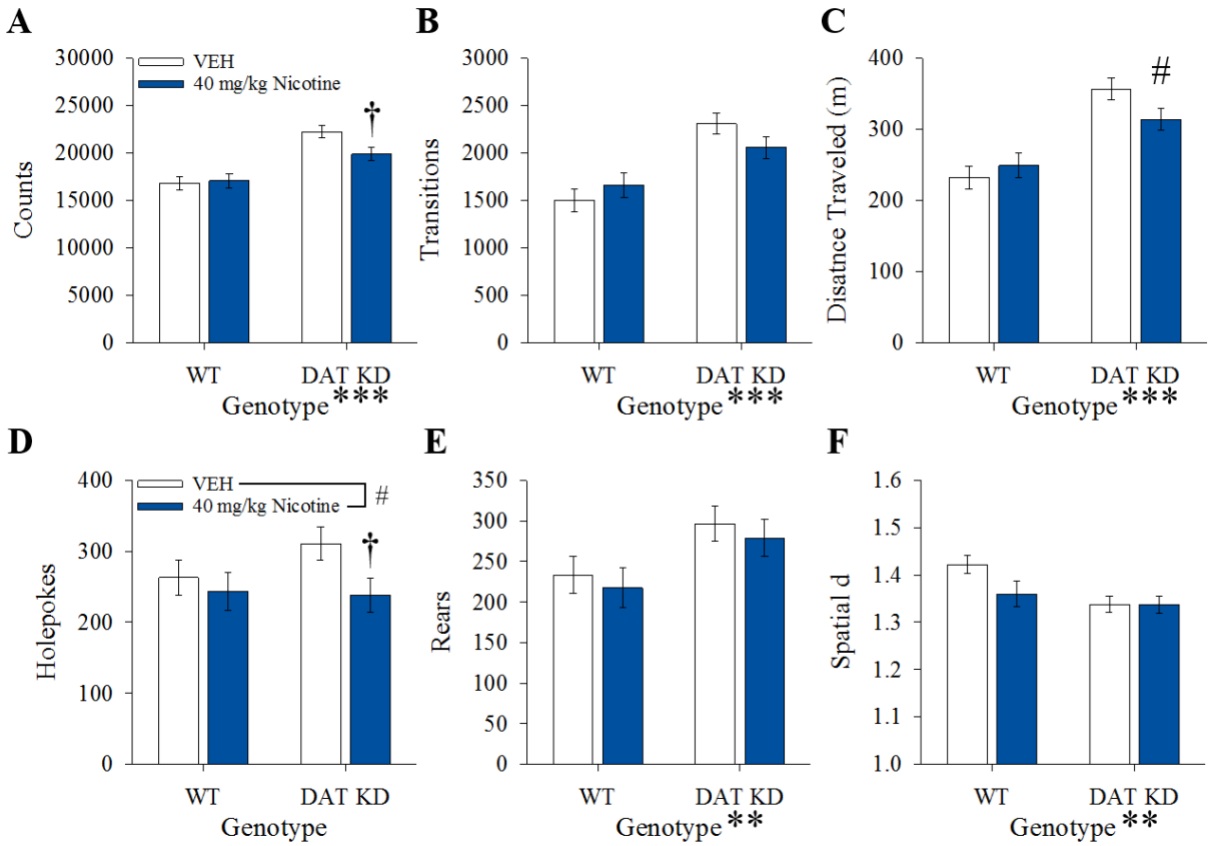

Figure 3.1. Chronic nicotine partially remediated the hyperactivity phenotype of DAT KD mice. DAT KD mice displayed increased levels of overall activity and locomotion relative to WT controls. Chronic nicotine decreased activity in DAT KD mice only, partially normalizing their characteristic hyperactivity (**A-C**). Genotype did not affect exploratory holepoking, though chronic nicotine tended to reduce this behavior across groups. Planned analyses revealed that nicotine significantly reduced holepoking in DAT KD mice only (**D**). DAT KD mice exhibited increased rearing behavior compared to WT mice, with no effect of nicotine (**E**). DAT KD mice demonstrated significantly straighter path trajectories than WT controls, as measured by spatial d, with no main or interactive effects of nicotine (**F**). Data presented as mean ±S.E.M. †=p<0.05 vs VEH; #=p<0.10 vs VEH; **=p<0.01; ***=p<0.001.

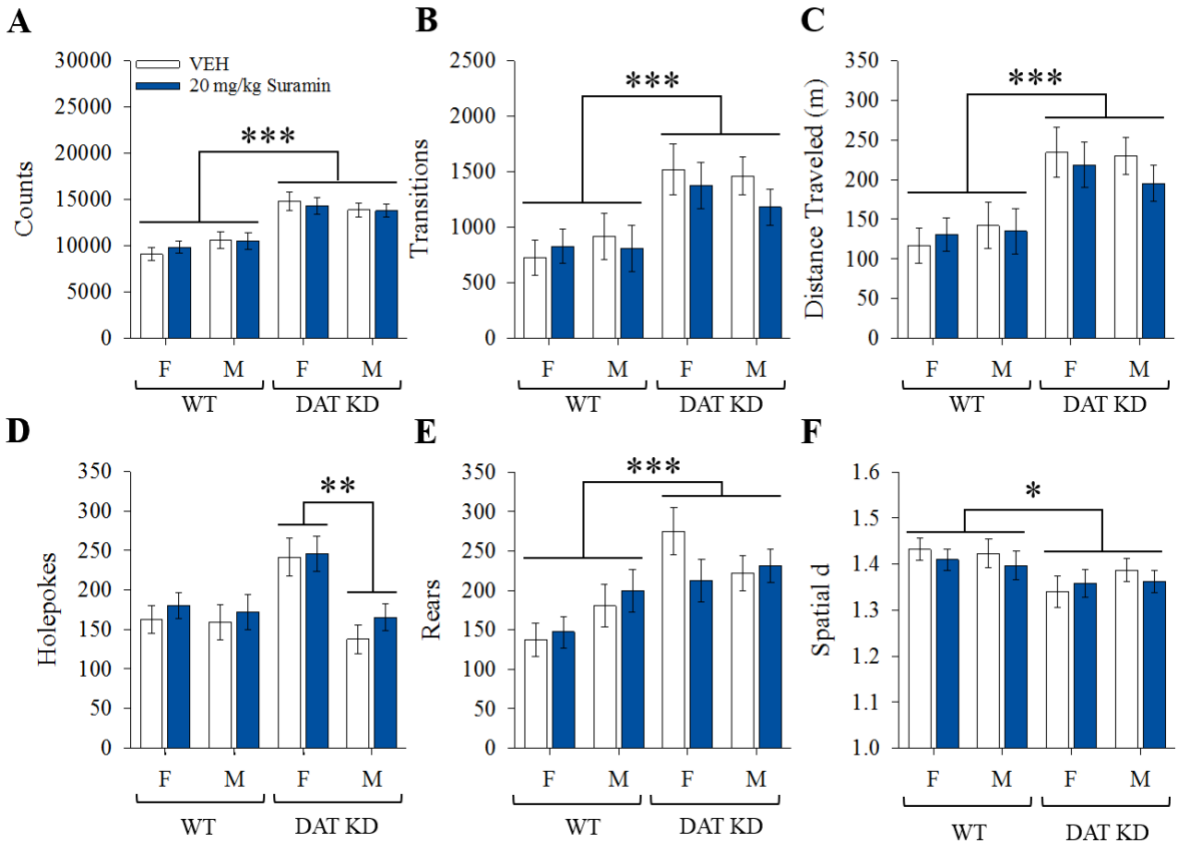

Figure 3.2. Subchronic suramin did not affect locomotor or exploratory behavior in DAT KD or WT mice. Regardless of sex or suramin treatment, DAT KD mice exhibited higher activity and locomotion than WT controls (**A-C**). Regardless of suramin treatment, male DAT KD mice exhibited lower levels of holepoking behavior relative to all other groups (**D**). DAT KD mice displayed more rearing behavior than WT littermates, but no effects of sex or suramin treatment were observed (**E**). DAT KD mice exhibited lower spatial d values than WT mice regardless of sex or suramin treatment. Data presented as mean ±S.E.M. *=p<0.05; **=p<0.01; ***=p<0.001.

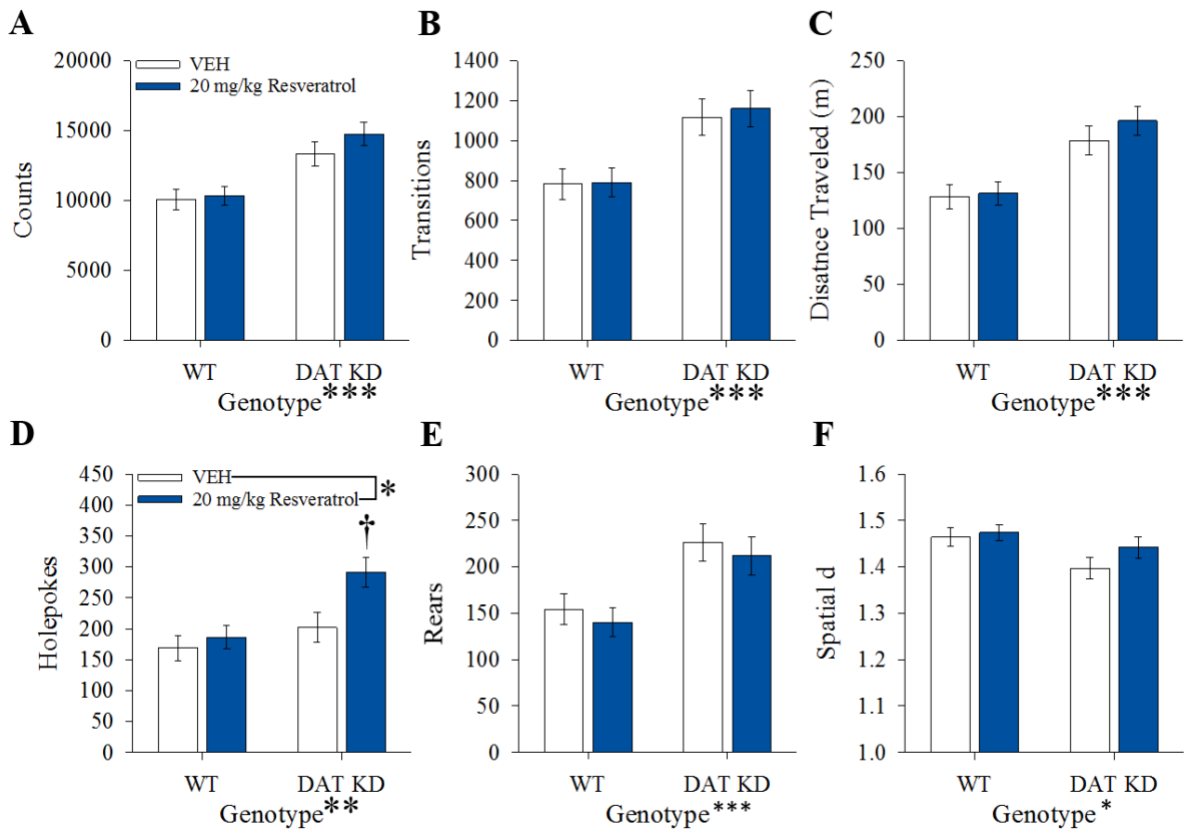

Figure 3.3. Subchronic resveratrol did not affect the hyperactive profile of DAT KD mice, but non-specifically increased exploration. DAT KD mice displayed a hyperactive profile that was resistant to the effects of subchronic resveratrol (**A-C**). Subchronic resveratrol increased exploratory holepoking behavior, in DAT KD mice only (**D**). DAT KD mice displayed increased rearing behaviors compared to WT mice, but no effect of resveratrol treatment was observed (**E**). DAT KD mice displayed lower spatial d values than WT mice, regardless of resveratrol treatment. Data presented as mean ±S.E.M. *=p<0.05; **=p<0.01; ***=p<0.001.

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Chapter 4: Examining Neurodevelopmental Gene x Environment Interactions Relevant to Psychiatric Disorders in Mouse Models

Abstract

A higher incidence of multiple psychiatric disorders occurs in people born in late winter/early spring. Reduced light exposure/activity level impacts adult rodent behavior and neural mechanisms, yet few studies have investigated such light exposure on gestating fetuses. A dysfunctional dopamine system is implicated in most psychiatric disorders, and genetic polymorphisms reducing expression of the dopamine transporter (DAT) are associated with some conditions. Furthermore, adult mice with reduced DAT expression (DAT-HT) were hypersensitive to short active (SA; 19:5 L:D) photoperiod exposure versus their wildtype (WT) littermates. Effects of SA photoperiod exposure during gestation in these mice have not been examined. We confirmed adult females exhibit a heightened corticosterone response when in SA photoperiod. We then tested DAT-HT mice and WT littermates in psychiatry-relevant behavioral tests after SA or normal active (NA; 12:12 L:D) photoperiod exposure during gestation and early life. SA-born WT mice exhibited sensorimotor gating deficits (males), increased reward preference, less immobility, open arm avoidance (females), less motivation to obtain a reward, and reversal learning deficits, vs. NA-born WT mice. DAT-HT mice were largely resilient to these effects, however. Future studies will determine the mechanism(s) by which SA photoperiod exposure influences brain development to predispose toward emergence of psychiatry-relevant behaviors.

Introduction

A higher incidence of multiple psychiatric disorders, including major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia (SZ), occurs in people born in late winter/early spring (Disanto et al., 2012; Torrey et al., 1996). Some factors arising from a winter gestation have been investigated to determine whether they drive outcomes that are relevant to psychiatric disorders, including maternal vitamin D deficiency and malnutrition (reviewed by

Cope et al., 2016). To-date, little investigation has been made of reduced light exposure/activity levels, despite evidence they can impact adult behavior and neural mechanisms in rats and mice (see below). Altered light exposure during the perinatal period also induces lasting changes in the circadian clock of rodent offspring (Ciarleglio et al., 2011). Relatively little investigation into the behavioral effects of altered perinatal photoperiod exposure has been conducted in such rodent offspring, although reduced immobility in the forced swim test (FST) has been reported (see Green et al., 2015). The question therefore remains as to what effect altered perinatal photoperiod exposure has on psychiatry-relevant behaviors in adults, that is behaviors observed in clinical psychiatric populations that can be observed in rodent models using cross-species translational tasks (Gilmour et al., 2013; Phillips et al., 2018; Robbins, 2017; Young et al., 2009, 2013, 2016; Young and Geyer, 2015; Young and Markou, 2015).

In adult rodents, exposure to short active (SA; winter-like; 19:5 light:dark (L:D)) photoperiod increased stress-related hormones as well as psychiatry-relevant behavioral changes (Dulcis et al., 2013). Specifically, after 7 days in a SA photoperiod, rats exhibited reduced time spent in the open arms of the elevated plus maze (EPM) and increased immobility in the FST, consistent with an anxiogenic- and depression-like profile. This profile coincided with neurotransmitter respecification occurring in the periventricular nucleus of the hypothalamus which in a standard condition expressed both tyrosine hydroxylase (TH), somatostatin (SST), and corticotropin-releasing factor (CRF), but in a SA condition expressed less TH, and more SST and CRF. A 14-day SA photoperiod exposure induced similar behavioral and neurotransmitter changes in mice, effects that were exaggerated in mice with reduced dopamine transporter (DAT) expression (Young et al., 2018). The effects of this stress-related response to winter-like-induced inactivity on gestation, however, have yet to be investigated.

While environmental factors such as winter gestation contribute to the development of psychiatric illnesses, genetic factors also play a role (Halldorsdottir and Binder, 2017). Without potential genetic susceptibility/resiliency, everyone born in the late winter/early spring would develop a psychiatric condition. As indicated above, reduced DAT expression induced a hypersensitivity to SA-photoperiod effects in adulthood (Young et al., 2018). Case-control, family cohort, and GWAS studies have repeatedly linked polymorphisms in SLC6A3, the gene encoding DAT, to multiple psychiatric conditions (Akutagava-Martins et al., 2016; Bidwell et al., 2011; Cartier et al., 2015; De Azeredo et al., 2014; Franke et al., 2010; Gadow et al., 2014; Gomez-Sanchez et al., 2016; Greenwood et al., 2016, 2013; Hawi et al., 2010; Huang et al., 2015, 2013, 2010; Kennedy et al., 2016; Pinsonneault et al., 2011; Zheng et al., 2012). Polymorphisms in DAT—the main homeostatic mechanism for synaptic DA clearance—are associated with BD (Greenwood et al., 2006, 2001; Huang et al., 2015; Pinsonneault et al., 2011), SZ (Greenwood et al., 2016; Huang et al., 2010; Kennedy et al., 2016; Zheng et al., 2012), and ADHD (Akutagava-Martins et al., 2016; Bidwell et al., 2011; De Azeredo et al., 2014; Franke et al., 2010; Gizer et al., 2009; Gomez-Sanchez et al., 2016; Hawi et al., 2010). Reduced DAT levels (~40%) were observed in euthymic BD patients, and reduced DAT expression was confirmed in postmortem frontal cortices of BD patients (Rao et al., 2012). Furthermore, DAT polymorphisms associated with psychiatric disease risk differentially modulate DAT gene promoter activity (Bamne et al., 2010; Talkowski et al., 2008). Hence, reduced DAT expression could confer a similar sensitivity to photoperiod-induced behaviors during gestation. A subset of studies have not found an association between DAT and these disorders (Bonvicini et al., 2016; Cosgrove et al., 2012; Huang et al., 2011; Yang et al., 2014); although a large number of studies support this association (above), and none determine potential interactive effects of season of birth.

To determine the effects of altered perinatal photoperiod on behavioral profiles in adulthood, we bred and raised mice in a SA vs. normal active (NA; 12:12 L:D) photoperiod. Additionally, we determined whether mice heterozygous for a DAT mutation that reduces DAT expression (DAT-HT) demonstrated an increased susceptibility to photoperiod-induced behavioral effects. We hypothesized that: 1) mice born/raised in SA photoperiod would exhibit psychiatry-relevant behavior vs. NA-born mice; and 2) DAT-HT mice would be hypersensitive to SA-induced behavioral changes compared to WT mice.

Methods

Dopamine Transporter Knockdown (DAT KD) Line

A 7.5-kb *Hin*dIII fragment with the first two DAT gene exons was excised from a phage DNA isolated from a mouse 129 Sv/J genomic library, and a *Not*I—*Asc*I cassette was inserted to generate the targeting construct. The insertion of an extra 4-kb DNA sequence (tTA-neo-tetO) reduced DAT expression levels. W9.5 embryonic stem cells were electroporated with the linearized targeting construct and injected into C57BL6/J blastocysts to generate chimeras. One chimera was mated with 129 Sv/J females to generate heterozygous mutants (DAT-HT) on a 129 Sv/J background (Zhuang et al., 2001). DAT-HT breeding pairs were sent to our laboratory from Columbia University and all subsequent mice resulted from a breeding colony in the vivarium at the University of California, San Diego (UCSD). Mice were bred until the DAT mutation was crossed over to a C57BL6/J background (>10 generations of backcrossing) and then maintained on this background. DAT-HT mice express ~50% of DAT compared to wildtype (WT) levels (Young et al., 2018).

Animals for Current Experiments

Male (20-30 g) and female (15-25 g) DAT-HT and WT littermates were generated from DAT-HT (sire)/C57BL/6J (dam) pairings after being placed in custom photoperiod chambers (see Photoperiod Exposure Paradigm section below). Behavioral testing began at approximately 10 weeks old (see Fig. 4.1b for timeline). When not in the photoperiod chambers, all mice were group housed (3-4/cage) and were maintained on a reversed day-night light cycle (lights on 7:00pm, off at 7:00am) in a temperature-controlled vivarium. All behavioral tests occurred during the active (dark) phase of the cycle. Food and water were provided *ad libitum*. All procedures and tests were approved by the UCSD Institutional Animal Care and Use Committee. The UCSD animal vivarium meets all federal and state requirements for animal care and was approved by the American Association for Accreditation of Laboratory Animal Care.

Plasma Corticosterone Assay

For confirmation of a SA-photoperiod stress-induced response at a hormonal level, adult female C57BL6/J mice were exposed to NA or SA photoperiod for 14 days, after which tail blood was collected for baseline plasma corticosterone (CORT) assessment. On day 15, tail blood was collected after a 2-hour acute restraint stress. Blood samples were analyzed for plasma CORT using a corticosterone rat/mouse ELISA kit (Rocky Mountain Diagnostics, Inc., Colorado Springs, CO, USA). A ratio of day 15 to day 14 plasma CORT levels was used for analyses.

Photoperiod Exposure Paradigm

Male DAT-HT and female C57BL/6J breeders were placed into custom photoperiod chambers programmed to a NA (12:12 L:D) cycle prior to pairing in order to acclimate to the chambers (6 cages per chamber). Ten photoperiod chambers are individually ventilated and illuminated to 130 lumens (measured in the center of the chamber) by three horizontal, programmable white LED strips equally spaced on each wall. After a 7-day acclimation period,

breeding triads (2 female: 1 male) were formed; half the breeding pairs remained in NA photoperiod, while half the breeding pairs were switched to SA photoperiod (19:5 L:D). Following a two-week pairing, dams were singly housed and maintained in their respective photoperiods (NA or SA) throughout pregnancy. The resulting 238 pups remained in either NA or SA photoperiod until weaning at P28. This photoperiod exposure was chosen to recreate the time period exposure of earlier research in this area (Green et al., 2015), as well as our work in adult photoperiod exposure inducing a stress-relevant response (Dulcis et al., 2013; Young et al., 2018; Young and Dulcis, 2015). This time period was further supported given that neurodevelopmental timelines between mice and humans indicate that milestones in humans (e.g., peak brain growth spurts, 36-40 weeks) occur in mice post birth (postnatal days 7-10) (Semple et al., 2013). Although not recreating the gradual change that occurs during human gestation, these conditions determine whether extreme photoperiod changes drive altered behavioral profiles in offspring. At P28, all pups were weaned by sex and genotype, moved into a standard vivarium room, and housed in NA photoperiod (12:12 L:D). For all subsequent behavioral testing, mice were maintained in NA photoperiod (see Fig. 4.1 for a schematic of experimental housing and testing timelines).

Behavioral Testing

To examine psychiatry-relevant behaviors induced by gestation/rearing in SA photoperiod, we assessed several domains commonly altered in patients with psychiatric disorders. Each test was performed as described below. The entire cohort of mice (N = 238) was tested in the behavioral pattern monitor (BPM), prepulse inhibition (PPI), and saccharin preference (SP) tests, whereas only half the cohort was used (n = 121) for the forced swim test (FST) and elevated plus maze (EPM). We used half the cohort for the latter two tests to minimize re-testing effects for other experiments (experiments not included here). An 8.5-week period elapsed between FST and EPM testing to minimize FST effects on EPM behavior (Fig.

1b). 16 weeks after EPM testing, a subset of these mice (n=74; 8-10 per group) were trained in the progressive ratio breakpoint task (PRBT) and Probabilistic Reversal Learning Task (PRLT). See “Operant Training Phases” section below for details, and Fig. 4.1b for timeline of operant training and testing.

Prepulse Inhibition (PPI): Sensorimotor Gating

Startle and PPI was tested using eight startle chambers (SR-LAB, San Diego Instruments, USA), each consisting of a Plexiglas cylinder (5 cm diameter) resting on a platform in a ventilated sound-attenuating box. Speakers located 33 cm above the cylinders produced all acoustic stimuli. Animal movements were transduced by piezoelectric accelerometers located underneath each cylinder, and data was stored and digitized by an interface and computer assembly. Mice were placed into startle chambers and underwent a 5-minute acclimation period prior to testing. Mice were exposed to a 65 dB background sound, as well as a light located on the chamber ceiling, continuously throughout the session.

Startle pulse duration was 40 ms, and prepulses were 20 ms in duration. The inter-trial interval (ITI) was on average 7 seconds (range: 3-12 s). The inter-stimulus interval (ISI) for prepulse trials was 100 ms (except for varying ISI trials below). Each session consisted of six blocks: 1) five 120 dB pulses, 2) prepulses (69, 73, 81 dB) preceding 100 dB pulses, 3) prepulses (69, 73, 81 dB) preceding 120 dB pulses, 4) varying ISI trials (73 dB prepulses preceding 120 dB pulses by 25, 50, 100, 200, and 500 ms, 5) startle trials (69, 73, 80, 90, 100, 110, 120 dB pulses), and 6) five 120 dB pulses. Startle responses to the 120 dB pulses from the first and sixth blocks were used to assess habituation across the session. PPI was calculated as a percentage for both the 100 dB and 120 dB pulses: %PPI = 100 – [(startle magnitude for prepulse+pulse/startle magnitude for pulse alone) x 100].

Behavioral Pattern Monitor (BPM): Locomotor Activity, Exploratory Behavior

Mice were tested in eight BPM chambers, each consisting of a 30.5 x 60 x 38 cm arena. Infrared beams designed to detect horizontal activity (e.g. number of transitions from one predefined region of the arena to another), vertical activity (e.g. rearing), and holepokes, tracked behavior across the 45-minute session. Behavioral data was acquired using Photobeam Activity System software (San Diego Instruments, USA). Custom programming was used to convert data into a format that could be analyzed using Biomedical Data Programs software (Statistical Solutions Inc., Saugus, MA). Detailed information regarding the chambers and testing methods have been previously published (Geyer et al., 1986; Perry et al., 2009; Risbrough et al., 2006; Young et al., 2010). Mice were placed in the upper left corner of the chamber at the beginning of the session and could freely explore the arena. Primary outcomes included number of transitions, rearing, holepokes, and spatial d (a value quantifying the geometrical pattern of locomotor activity across the session, whereby values near 1 reflect straight line paths, values closer to 2 reflect a circumscribed path, and values near 1.5 reflect a meandering path). These measures were chosen given the difference between people with BD mania, acute schizophrenia episode, and healthy participants, and the ability to recreate such altered profiles in mouse models (Young et al., 2016, 2007).

Saccharin Preference Test (SPT): Reward Preference, Hedonia-like Behavior

Mice had 72-hour unrestricted access to both a normal water bottle and a water bottle filled with 1% saccharin solution in their home cage (3-4 mice/cage). Both bottles were weighed three times (once/day) by experimenters, and percent saccharin preference/cage was calculated as the primary outcome. All cage values were averaged within each group and compared to the other group averages.

Forced Swim Test (FST): Despair-related Behavior

Animals were placed into a 4.0L beaker filled with 2.5L of room temperature water. Animals were unable to touch the bottom of the beaker. The beaker was enclosed on all sides

by a black box, and the experimenter observed behavior by video camera. The primary outcome variable, time spent immobile (seconds), was manually scored using ODLog software (Macropod Software) over the 5-minute session by people unblinded. Mice were judged "immobile" when not actively swimming. The first minute of data was subtracted to prevent obfuscation of perinatal photoperiod effects, as mice tend to be more active during the first portion of this test (Can et al., 2012).

Elevated Plus Maze (EPM): "Anxiety-like" Behavior, Risk Preference

Mice were placed in the center of the EPM at the beginning of the session. The EPM consisted of two open arms, two closed arms (Plexiglass walls covered with black paper), and an exposed center area. Mice could freely explore the maze for the entirety of the 5-minute session. Time spent in each open and closed arm, as well as in the center, was manually tracked using ODLog Software (Macropod Software). Primary outcomes included percent time spent in the open arms, percent time spent in the closed arms, and percent time spent in the center.

Operant Training Phases

Mice were initially trained in five-hole operant chambers (25 x 25 x 25 cm; Med Associates, St. Albans, USA). One week prior to initiation of Hab1 training, mice were food deprived to 85% of free-feeding body weight. During the first training phase (Hab1; 1 session/day), mice were conditioned to associate magazine illumination with the opportunity to collect 30 μl strawberry milkshake from the magazine. In the second training phase (Hab2; 1 session/day), mice were required to nose poke in any of the five illuminated holes to receive the reward (criterion: at least 70 responses for two consecutive days during 30-minute session). Once criterion was reached, mice moved into the testing phase.

Progressive Ratio Breakpoint Task (PRBT): Motivation to Obtain Reward

During a 60 min PRBT (tested on one day), mice had to make increasingly more holepokes into the central lit port in order to get a milkshake reward. The number of holepokes required to receive the reward increased in the following progression: 1, 2, 4, 7, 11, 16, 22, 29, 37, 46, 56, and 67 (see (Milienne-Petiot et al., 2017; Young and Geyer, 2010)). Mice had to respond three times at each ratio before moving on to the next ratio. If mice did not nose poke within 10 seconds, the chamber house light was illuminated for 4 seconds with all holes being inoperative until the next trial initiated. Primary outcome measure was breakpoint, defined as the last ratio completed before the end of the session. Secondary outcomes included total trials completed, mean reaction time, mean trial completion time, and mean response rate.

Probabilistic Reversal Learning Task (PRLT): Reversal Learning

During a 60 min PRLT (tested on one day), mice were presented with two lit ports associated with different contingencies: the target port was associated with a high probability of reward (80%) and low probability of punishment (20%). The non-target port was associated with a low probability of reward (20%) and high probability of punishment (80%). After 8 consecutive responses in the target port, criterion was met, and the target port became the non-target port and vice versa (reversal). If mice did not nose poke within 10 seconds, the chamber house light was illuminated for 4 seconds with all holes being inoperative until the next trial initiated. Primary outcome measure was number of reversals completed in the 60 min session. Secondary outcomes analyzed for this task have been previously published by our lab (Milienne-Petiot et al., 2017).

Operant Training Phase, PRBT, and PRLT Data Collection

Recording of responses in the operant training phases, as well as for PRBT and PRLT, was managed by a SmartCtrl Package 8-In/16-Out with additional interfacing by MED-PC for Windows (Med Associates, St. Albans, USA) using custom programming. Data was imported

into Microsoft Excel using MED-PC to Excel (Med Associates, St. Albans, USA), and subsequently used for analysis.

Statistical Analyses

Primary outcomes from behavioral experiments were analyzed using ANOVA, with between-subjects factors of sex, genotype, and perinatal photoperiod exposure. Analyses were collapsed across sex where no main or interactive effect of sex was observed. For SPT, a within-subjects factor of time was included to assess differences in saccharin preference across the 3-day test period. For PPI, intra-block PPI measures (varying pulse intensities, ISI, or habituation), were added to the analysis as within-subjects factors. BPM data was analyzed across the entire 45-minute test session, as well as in 15-minute time bins (as a within-subjects factor). For the plasma corticosterone study, an unpaired t-test was used to assess for photoperiod effects on CORT levels. Data was analyzed using SPSS 24.0 (IBM Corp., Armonk, NY), except for BPM data, which was analyzed using Biomedical Data Programs software (Statistical Solutions Inc., Saugus, MA).

Results

SA-Photoperiod Effects on Adult Female Plasma Corticosterone Levels

To determine whether the SA photoperiod drove a stress response in female mice, and hence had the potential to affect offspring, adult female mice were exposed to either NA or SA photoperiod for 14 days. Following this photoperiod exposure, SA-exposed adult female mice exhibited higher acute stress/baseline plasma corticosterone (CORT) ratios compared to NA-exposed females after a 2-hour acute restraint stress ($t_{(21)}= 2.6$, $p < 0.05$; Fig. 4.1c).

Sensorimotor Gating and Startle: Prepulse Inhibition (PPI)

Interactions with perinatal photoperiod were observed at both 100 dB and 120 dB. At 100 dB, a prepulse intensity x sex x genotype x perinatal photoperiod interaction was observed ($F_{(1.9,440)} = 4.9$, $p < 0.01$). Sex-specific analysis showed a prepulse intensity x genotype x perinatal photoperiod interaction in males, but not in females (males: $F_{(2,256)} = 4.7$, $p < 0.05$; females: $F_{(2,204)} = 1.4$, ns; Fig. 4.2a, 4.2b). Further restriction by genotype in males showed a prepulse intensity x perinatal photoperiod interaction in WT mice ($F_{(2,128)} = 4.0$, $p < 0.05$), with SA-born WT mice exhibiting reduced PPI compared to NA-born WT mice at the 73 dB prepulse level ($p < 0.01$). A main effect of photoperiod was also observed in male WT mice, with SA-born male WT mice exhibiting deficient PPI compared to NA-born male WT mice ($F_{(1,64)} = 5.5$, $p < 0.05$). There were no interactions with perinatal photoperiod observed in male DAT-HT mice; additionally, no main effect of perinatal photoperiod was observed ($F_{(1,64)} = 2.9$, ns). There was a main effect of genotype in female mice ($F_{(1,102)} = 9.4$, $p < 0.01$), with female DAT-HT mice exhibiting deficient PPI compared to female WT littermates. No significant interactions were seen in the pulse intensity or habituation curves at 100 dB.

At 120 dB, a sex x perinatal photoperiod interaction was observed ($F_{(1,230)} = 9.8$, $p <0.01$). Sex-specific analysis showed a main effect of perinatal photoperiod in males only ($F_{(1,128)} = 9.3$, $p < 0.01$; Fig. 4.2c), with SA-born males exhibiting reduced PPI compared to NA-born males. At each prepulse intensity, SA-born male WT mice exhibited deficient PPI compared to NA-born male WT littermates; this difference was only significant at the highest prepulse intensity in SA-born vs. NA-born male DAT-HT mice. No interactions or main effects were observed in female mice, except for a main effect of prepulse intensity (Fig. 4.2d). No sex x perinatal photoperiod interactions were observed in the pulse intensity or habituation curves at 120 dB.

Exploratory Behavior: Behavioral Pattern Monitor (BPM)

When data were analyzed in a single 45-minute time bin, no interactions with or main effects of perinatal photoperiod were observed in any BPM primary outcome measures (number of transitions, rearing, holepokes, or spatial d). A main effect of genotype was observed for number of transitions ($F_{(1,230)} = 13.9$, $p < 0.001$; Fig. 4.3a), rearing ($F_{(1,230)} = 28.0$, $p < 0.0001$; Fig. 4.3b), and spatial d ($F_{(1,230)} = 5.1$, $p < 0.05$; Fig. 4.3c). DAT-HT mice exhibited increases in number of transitions and rearings, as well as a reduction in spatial d, compared to WT littermates.

When data were analyzed in 15-minute time bins, a time x genotype x perinatal photoperiod interaction was observed for holepokes ($F_{(2,460)} = 3.1$, $p < 0.05$; Fig. 4.3d). All groups, regardless of genotype or perinatal photoperiod exposure, exhibited a significant increase in holepokes between the first and second 15-minute time bin (NA-born WT: $t_{(112)} = 2.4$, $p < 0.05$; NA-born DAT-HT: $t_{(116)} = 4.0$, $p < 0.01$; SA-born WT: $t_{(134)} = 2.7$, $p < 0.01$; SA-born DAT-HT: $t_{(104)} = 2.9$, $p < 0.01$). When examining for inter-group differences at each time point, no significant differences were observed during the first or second 15-minute time bin. During the final 15-minute time bin, NA-born DAT-HT mice completed significantly more holepokes compared to NA-born WT mice ($t_{(114)} = 2.3$, $p < 0.05$).

A time x perinatal photoperiod interaction for spatial d was observed when data were analyzed in 15-minute time bins ($F_{(2,240)} = 5.3$, $p < 0.01$; Fig. 4.3e). Both NA-born and SA-born mice exhibited an increased spatial d between the first and second 15-minute time bin (NA: $t_{(230)} = 3.9$, $p<0.01$; SA: $t_{(240)} = 4.2$, $p < 0.0001$). A further increase in spatial d was observed between the second and third 15-minute time bin in NA-born mice ($t_{(230)} = 2.3$, $p < 0.05$); this effect was not observed in SA-born mice ($t_{(240)} = 0.8$).

Reward Sensitivity: Saccharin Preference Test (SPT)

When tested as adults, mice born and raised in SA photoperiod tended to exhibit increased saccharin preference when compared to NA-born mice ($F_{(1,51)} = 3.2$, $p = 0.08$). No

interactions with sex or genotype were observed in the overall analysis. Given our a priori hypothesis of genotype-specific effects, we performed genotype-specific analyses of SP. SA-born WT mice exhibited increased saccharin preference compared to NA-born WT mice ($F_{(1,26)}$ = 7.8, $p < 0.05$; Fig. 4.4a), whereas there were no photoperiod effects in DAT-HT mice ($F_{(1,25)}$ = 0.0, ns; Fig. 4.4a).

Behavioral Despair: Forced Swim Test (FST)

When tested as adults, mice born and reared in SA photoperiod exhibited a trend toward decreased immobility in the FST, regardless of genotype, when compared to NA-born mice ($F_{(1,113)}$ = 3.8, $p = 0.06$). A main effect of sex was also observed, with males spending more time immobile compared to females ($F_{(1,113)}$ = 16.7, $p < 0.001$). Given our a priori hypothesis that these effects would be genotype-specific, we investigated photoperiod effects in each genotype. In WT mice, there was a main effect of photoperiod ($F_{(1,61)}$ = 5.2, $p < 0.05$; Fig. 4.4b), where SA-born WT mice exhibited decreased immobility compared to NA-born WT mice. In contrast, no photoperiod effect was observed in DAT-HT mice ($F_{(1,52)}$ = 0.4, ns; Fig. 4.4b).

Risk Preference/Anxiety Assessment: Elevated Plus Maze (EPM)

A sex x genotype x perinatal photoperiod interaction for percent time spent in open arms was observed in the EPM ($F_{(1,112)}$ = 6.0, $p < 0.05$). When males and females were analyzed separately, no interactions with or main effects of perinatal photoperiod were observed. In females only, a trend toward a genotype x perinatal photoperiod interaction was observed ($F_{(1,51)}$ = 3.7, $p = 0.06$). Female SA-born WT mice spent significantly less time in open arms compared to female NA-born WT mice ($t_{(29)}$ = 2.1, $p < 0.05$; Fig. 4.4c). SA-born female DAT-HT mice spent significantly more time in open arms compared to SA-born female WT mice ($t_{(26)}$ = 2.9, $p < 0.01$; Fig. 4.4c). A main effect of genotype was also seen in female mice, with DAT-HT mice spending more time in open arms compared to WT mice ($F_{(1,51)}$ = 6.5, $p < 0.05$). In male mice, SA-born

DAT-HT mice spent less time in open arms compared to SA-born WT mice ($F_{(1,36)} = 6.9$, $p < 0.05$; Fig. 4.4c).

Effort Valuation: Progressive Ratio Breakpoint Task (PRBT)

During Hab2 training, there was a sex x genotype x perinatal photoperiod interaction ($F_{(1,66)} = 5.9$, $p < 0.05$). SA-born male WT mice took significantly more sessions to meet criterion than all other groups ($p < 0.0001$ vs. female DAT-HT NA; $p < 0.001$ vs. female WT NA, female WT SA, male DAT-HT SA; $p < 0.01$ vs. female DAT-HT SA, male WT NA, male DAT-HT NA). In the PRBT, a genotype x perinatal photoperiod interaction was observed for breakpoint ($F_{(1,66)} = 6.1$, $p < 0.05$; Fig. 4.5a). SA-born WT mice exhibited a reduced breakpoint vs. NA-born WT mice ($F_{(1,36)} = 12.6$, $p < 0.01$) as well as vs. SA-born DAT-HT mice ($F_{(1,35)} = 10.5$, $p < 0.01$). Genotype x perinatal photoperiod interactions were also observed for most secondary outcome measures. SA-born WT mice completed fewer trials ($F_{(1,66)} = 5.3$, $p < 0.05$; Fig. 4.5b), had slower reaction times ($F_{(1,66)} = 5.6$, $p < 0.05$; Fig. 4.5c), took longer to complete each trial ($F_{(1,66)} = 5.5$, $p < 0.05$; Fig. 4.5d), and exhibited increased mean response rates ($F_{(1,66)} = 6.1$, $p < 0.05$; Fig. 4.5e) compared to all other groups. A main effect of perinatal photoperiod was also observed on mean reward latency, with SA-born mice taking longer to retrieve earned rewards than NA-born mice ($F_{(1,66)} = 13.8$, $p < 0.001$; Fig. 4.5f).

Reversal Learning: Probabilistic Reversal Learning Task (PRLT)

A genotype x perinatal photoperiod interaction was observed for number of switches completed during the PRLT session ($F_{(1,66)} = 6.9$, $p < 0.05$; Fig. 4.6a). SA-born WT mice completed fewer switches compared to NA-born WT mice ($F_{(1,36)} = 5.9$, $p < 0.05$; Fig. 4.6a). NA-born DAT-HT mice completed fewer switches compared to NA-born WT mice ($F_{(1,35)} = 5.0$, $p < 0.05$; Fig. 4.6a). For secondary outcome measures, a genotype x perinatal photoperiod interaction was observed for mean reward latency ($F_{(1,66)} = 7.6$, $p < 0.01$; Fig. 4.6b) and target win-stay ratio ($F_{(1,66)} = 7.3$, $p < 0.01$; Fig. 4.6c). Target win-stay ratio represents the probability of

choosing the target port after being rewarded from choosing the target port in the previous trial. SA-born WT mice exhibited an increased latency to collect rewards after being rewarded compared to both NA-born WT mice ($F_{(1,36)} = 7.8$, $p < 0.01$) and SA-born DAT-HT mice ($F_{(1,35)} = 7.4$, $p < 0.05$). SA-born WT mice exhibited a reduced target win-stay ratio compared to NA-born WT mice ($F_{(1,36)} = 5.6$, $p < 0.05$). NA-born DAT-HT mice also showed a reduced target win-stay ratio relative to NA-born WT mice ($F_{(1,35)} = 5.8$, $p < 0.05$).

Discussion

Exposure to short active (SA; 19:5 L:D) photoperiod increased corticosterone levels in adult female mice. Exposure to this SA photoperiod during pregnancy and early life induced psychiatry-relevant behavioral profiles in WT mice. Specifically, SA-born WT mice exhibited reduced PPI (males only), increased saccharin preference, reduced FST immobility, less time spent in open arms of EPM (females only), less motivation to obtain a reward (breakpoint; PRBT), and reversal learning deficits (switches; PRLT), compared to NA-born WT mice (see Table 4.1 for summary of behavioral results). No SA photoperiod effects were observed in the BPM, suggesting perinatal SA photoperiod exposure does not alter locomotor activity or exploratory behavior. Surprisingly, mice with reduced DAT expression (DAT-HT mice) that were hypersensitive to SA photoperiod effects in adulthood (Young et al., 2018), were largely resistant to the effects of gestational SA photoperiod exposure (with the exception of significant PPI deficits observed at the highest prepulse level, 81 dB; Table 4.1). Thus, recreating a winter-like SA photoperiod exposure in mice during gestation resulted in psychiatry-relevant behavioral abnormalities in adulthood, for which mice with reduced DAT expression were resilient.

The behavioral tests in the current study were selected to span a broad array of psychiatry-relevant behaviors consistent with those observed in patient populations. PPI deficits (reflecting sensorimotor gating deficits) have been observed in patients with SZ (Braff et al.,

1992; Swerdlow et al., 2014), obsessive-compulsive disorder (OCD) (Ahmari et al., 2012; Hoenig et al., 2005), and children/adolescents with ASD (Cheng et al., 2018). Cortico-striato-pallidopontine (CSPP) circuitry regulates PPI, and has been implicated in the pathology of multiple psychiatric disorders (Gunaydin and Kreitzer, 2016). SA perinatal photoperiod exposure may therefore influence CSPP circuitry (given the observed SA-induced PPI deficits), reflecting one way altered photoperiod exposure near birth might contribute to emergence of psychiatry-relevant behaviors in adulthood. Two of the tests included in this study (SPT, FST) are commonly used in animals to provide insight into depression-relevant behaviors. Given that anhedonia is a cardinal feature of MDD (Nelson and Charney, 1981), it is thought that the SPT measures such behavior in rodents (Der-Avakian et al., 2016), despite unaltered SPT in people with depression relative to healthy participants (Aguayo et al., 2012; Berlin et al., 1998; Dichter et al., 2010). Time spent immobile in the FST is a biological readout of altered behavior and has been associated with depression-relevant behaviors (e.g. behavioral despair) (Cryan and Holmes, 2005; Doron et al., 2014). In both tests, SA-born WT mice exhibited the opposite of expectations of 'anhedonic'-like profile, exhibiting increased saccharin preference and decreased FST immobility. While the relationship of these findings to aspects of depression remain unclear, the photoperiod gestation challenge affected behavior in these tests. The mechanism driving these effects remains unclear, but it is important to note that a similar photoperiod manipulation resulted in reduced FST immobility in a previous study (Green et al., 2015); this consistency across photoperiod studies (albeit with minor mouse background and 1 hour light change differences), from different laboratories supports the validity of these outcome measures. That study suggested the SA-born-induced reduction in immobility may have been as a result of a hyper-serotonergic dorsal raphe nuclei, but the dopaminergic system may also play a role in this effect given that mice with reduced DAT expression were resistant to this SA-born effect.

Although FST immobility has commonly been used as a metric for depression-relevant behaviors (e.g. despair), this interpretation may not accurately reflect what the behavior represents. Some suggest immobility is an adaptive learned response and reflects a coping strategy to the FST stressor (de Kloet and Molendijk, 2016). Others argue that reduced immobility may represent an anxiogenic behavioral profile, rather than an 'antidepressant' effect (Anyan and Amir, 2018). Therefore, the reduced immobility seen in SA-born mice could be an anxiogenic state or a maladaptive response to stress. An anxiogenic state is further supported by SA-born WT mice (females) spending less time in the open arms of the EPM. However, it is important to note that prior FST exposure has been shown to induce an anxiogenic behavioral profile in the EPM (in male mice) (Andreatini and Bacellar, 1999). As a result, we cannot rule out the possibility that FST testing influenced EPM results. Reduced breakpoint (PRBT) and impaired probabilistic learning (PRLT) are outcomes viewed as more proximate to amotivated behavior in SZ (Fervaha et al., 2013; James M Gold et al., 2013; Waltz and Gold, 2007) and depression (Hershenberg et al., 2016). Furthermore, these tests are more translationally relevant, as versions of these tasks have been implemented in both rodents and human populations. Reduced breakpoint from a PRBT predicts 24% of the variance of global cognitive functioning in patients with SZ, with lower breakpoints associated with greater severity of certain negative symptoms (anhedonia, avolition) (Gold et al., 2013). Impaired PRLT has also been observed in patients with SZ (Reddy et al., 2016). Given that FST immobility more than likely does not serve as an accurate measure of motivated behavior, the decreased FST immobility and reduced breakpoint observed in SA-born mice in the current study are not necessarily contradictory. That slowed latencies to collect rewards were observed in SA-born WT mice in both the PRBT and PRLT indicate a reduced motivation to exert effort to collect rewards. The lack of difference of overall activity levels in an exploratory chamber (BPM), suggests this amotivated behavior is specific to the collection of reward. Given the reduced win-stay behavior of SA-born WT mice, these deficits may also arise from impaired reward processing. Future

studies examining effort-related choice will likely be useful in determining the impact of this manipulation on motivated behaviors.

Some sex-specific results were observed in the current study. SA-born males (particularly WT males) exhibited reduced PPI, whereas SA-born female WT mice exhibited an anxiogenic profile in the EPM. These findings are consistent with other reports of sex-specific changes as a result of perinatal stress exposure, and how these differences might contribute to subsequent health outcomes of offspring (Sutherland and Brunwasser, 2018). One study showed greater prenatal maternal stressor sensitivity in male offspring for subsequent development of SZ spectrum disorders (Fineberg et al., 2016). Studies have suggested that female fetuses are more flexible in adapting to adverse intrauterine conditions compared to male fetuses, but that this adaptability comes with a tradeoff of increased susceptibility to anxiety/depression (Sandman et al., 2013). This female-specific vulnerability to developing depression-like behavior has also been shown in response to early life stress exposure using the limited bedding model to induce fragmented maternal care (Goodwill et al., 2018). Epidemiological studies have observed a winter/spring birth excess in people with depression (Brochard et al., 1994; Clarke et al., 1998; Disanto et al., 2012; Joiner et al., 2002), and one study reported a higher excess in women (peak excess of 31% in women born in March compared to women in general population comparison group) (Mino et al., 2000). Other studies report male vulnerability to this seasonality effect (Quesada and Nolasco, 2017), while some report no association between birth season and depression for either sex (Pérez-Rincón, 1991). Studies to date have been limited, and more work is required to understand seasonality of birth effects on risk for affective disorder development. The sexually dimorphic results we observed fit within the existing prenatal stress vulnerability literature. Given the observation of limited sex-specific effects in the DAT HT mice – which were largely resilient to SA photoperiod birth effects – strengthen the implication that the sex-specific effects of such birth on WT mice fit into the

literature. Future work should examine whether altered perinatal photoperiod exposure induces changes in maternal stress hormones (e.g. plasma corticosterone), which could be conferred to developing fetuses in a sex-specific manner.

Although we predicted that DAT-HT mice would be hypersensitive to SA-induced behavioral changes given their hypersensitivity in adulthood, they were resilient to photoperiod manipulation effects compared to their WT littermates. Investigations into the mechanisms underlying vulnerability (and, conversely, resiliency) to stress have recently become more numerous. Glucocorticoid administration during late gestation influences dopaminergic neural circuits, inducing an increased ratio of apoptotic to proliferative cells in the ventral tegmental area and subsequent reduction in dopaminergic input to the nucleus accumbens (Leão et al., 2007). While the effects of early life stress on dopaminergic circuitry are complex (reviewed here (Rodrigues et al., 2011)), the DAT mutation may protect against SA-induced behavioral changes by somehow counteracting photoperiod effects on developing dopaminergic pathways. The mechanism(s) underlying the observed resilience in DAT-HT mice remains unknown, however, and future work will determine how resilience is conferred.

In summary, SA perinatal photoperiod induces psychiatry-relevant behavioral profiles later in adulthood in mice, with vulnerability observed in WT mice. It is recognized that this photoperiod effect was consistent throughout but abrupt in its change, unlike changing light exposure in humans. Future studies will determine the mechanism(s) by which SA photoperiod exposure influences brain development to predispose toward emergence of psychiatry-relevant behaviors, as well as attempt to recreate gradual change effects on adult offspring. First, identifying the window of SA photoperiod exposure necessary to induce these behavioral effects will be critical to narrowing in on potential mechanisms (gestation vs. early life vs. a combination of the two time periods). If SA photoperiod exposure during gestation only is capable of inducing behavioral changes in offspring, stress-related changes in placental structure/gene

expression might be contributing mechanisms. The placenta plays an active role in fetal brain development (Zeltser and Leibel, 2011), and maternal environment perturbations (e.g. hormone levels, nutritional state) are transmitted to the fetus via changes in placental function (Jansson and Powell, 2007). In adult rodents, SA photoperiod exposure results in elevated plasma corticosterone (Dulcis et al., 2013), and exposure to elevated levels of maternal corticosterone affects placental growth and gene expression (Cuffe et al., 2012). These gene expression changes may alter the transplacental signals received by the developing fetus, and by extension, the developing brain. Furthermore, maternal signals are capable of affecting long-term gene expression changes in offspring via induction of epigenetic mechanisms (Seckl, 2004). If behavioral changes occur after SA exposure during early life (post-gestational), it may point toward other mechanisms. Retinal projections reach the suprachiasmatic nucleus (central clock of the circadian system) shortly after birth (Astiz and Oster, 2018). Perinatal photoperiod-induced changes in retinal function (Jackson et al., 2014) and circadian rhythms (Ciarleglio et al., 2011) have been previously reported, and may contribute to observed behavioral changes in offspring by influencing physiological crosstalk with the stress system (Astiz and Oster, 2018), thereby altering stress responses in adulthood. Many other potential mechanisms exist, and likely it is a combination of pre- and postnatal influences converging to produce SA-induced behavioral profiles. The interaction between environmental factors and developing neural circuitry is a critical part of the etiology of many psychiatric disorders; a better understanding of these complex processes will allow for the development of more effective therapeutics.

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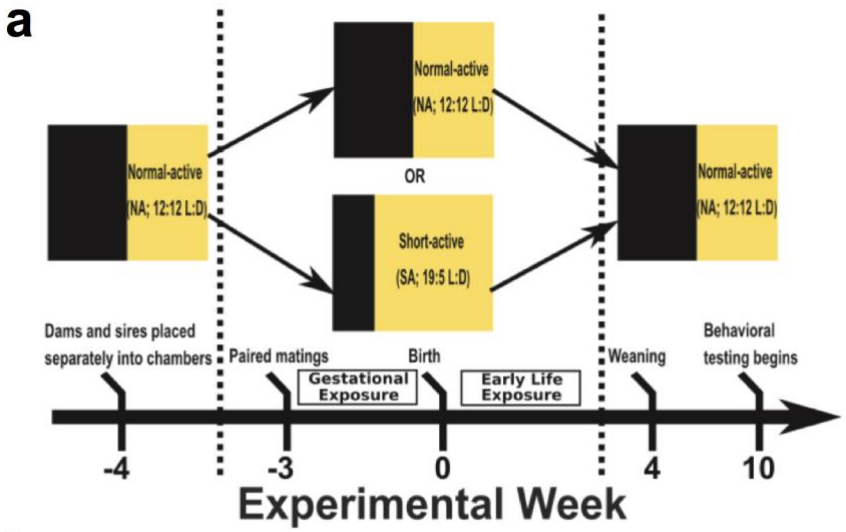
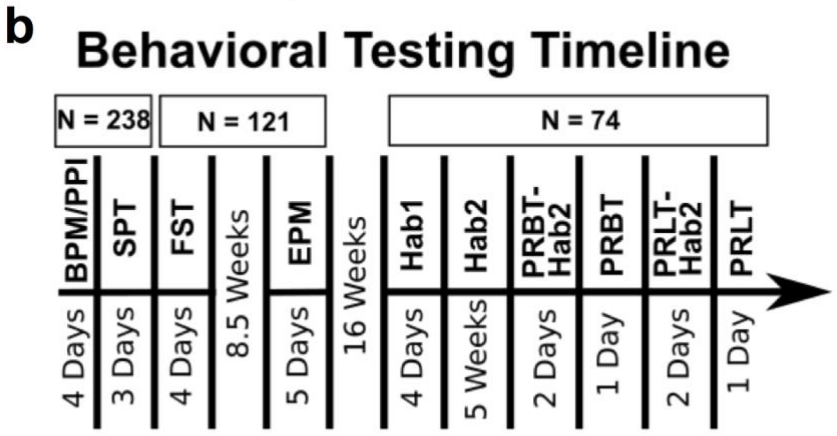
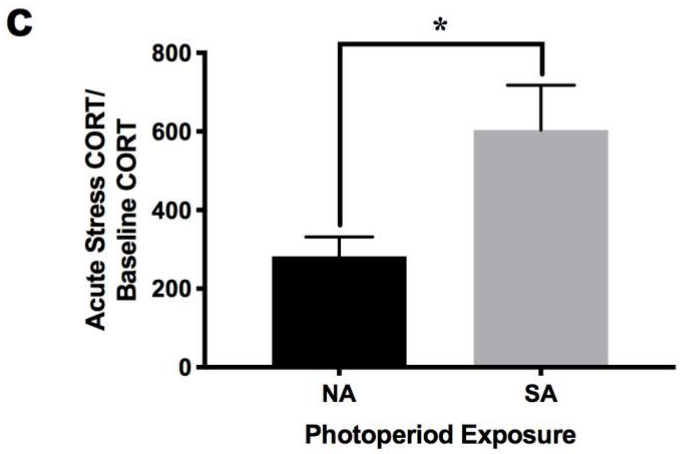
Chapter 4 contains material that has been submitted for publication to *Scientific Reports*. The dissertation author was the primary investigator and author of this material. Co-authors are

as follows: Molly A. Kwiatkowski*, Zackary A. Cope*, Maria L. Lavadia, Chuck J. A. van de Cappelle, Davide Dulcis, and Jared W. Young. *indicates co-first authorship

Figure 4.1. Schematic of experimental design and confirmation of short active photoperiod-induced stress response in adult females. (**a**) Schematic of the perinatal photoperiod exposure experimental timeline. Males heterozygous for a mutation in the dopamine transporter (DAT-HT) and female C57BL/6J (C57) breeders were placed separately into photoperiod chambers programmed to a normal active (NA; 12:12 light:dark (L:D)) cycle for 1 week. Following acclimation, breeding triads (2 female: 1 male) were formed at which point half the breeding pairs were moved to a short active (SA; 19:5 L:D) photoperiod. After 2 weeks, dams were singly housed and maintained in their respective photoperiods (NA or SA) throughout pregnancy into early life exposure (until weaning at P28). After weaning, offspring were housed in NA photoperiod (reversed L:D cycle) until behavioral testing began at 10-11 weeks old. (**b)** The behavioral testing timeline is described. All pups generated (N=238) were tested in the behavioral pattern monitor (BPM), prepulse inhibition (PPI), and saccharin preference test (SPT). Subsequently, half (N=121) were tested in the forced swim test (FST). After an 8.5-week washout period, they were tested in the elevated plus maze (EPM). After a 16-week period to minimize FST effects, a subset of the cohort (N=74) were food deprived to 85% of free-feeding body weight, then trained in operant chambers and tested in the progressive ratio breakpoint task (PRBT) and probabilistic reversal learning task (PRLT). For Hab1, mice learned to associate magazine illumination with milkshake reward. For Hab2, mice were required to nose poke in any of the five illuminated holes to receive the milkshake reward. All mice were run on Hab2 for 2 days prior (PRBT-Hab2) to the single PRBT session, and 2 days prior (PRLT-Hab2) to the single PRLT session. (**c**) Confirmation of a SA photoperiod-induced stress response in females, wherein adult females exposed to SA photoperiod exhibited elevated plasma corticosterone (CORT) levels in response to acute restraint stress compared to NA-exposed female mice. Data presented as mean +S.E.M., *$p<0.05$ where indicated.


a
Normal-active
(NA; 12:12 L:D)
OR
Normal-active
(NA; 12:12 L:D)
Short-active
(SA; 19:5 L:D)
Normal-active
(NA; 12:12 L:D)
Dams and sires placed
separately into chambers
Paired matings
Gestational
Exposure
Birth
Early Life
Exposure
Weaning
Behavioral
testing begins
-4
-3
0
4
10
Experimental Week


b
Behavioral Testing Timeline
N = 238
N = 121
N = 74
BPM/PPI
SPT
FST
EPM
Hab1
Hab2
PRBT-Hab2
PRBT
PRLT-Hab2
PRLT
4 Days
3 Days
4 Days
8.5 Weeks
5 Days
16 Weeks
4 Days
5 Weeks
2 Days
1 Day
2 Days
1 Day


c
*
800
600
400
200
0
Acute Stress CORT/
Baseline CORT
NA
SA
Photoperiod Exposure

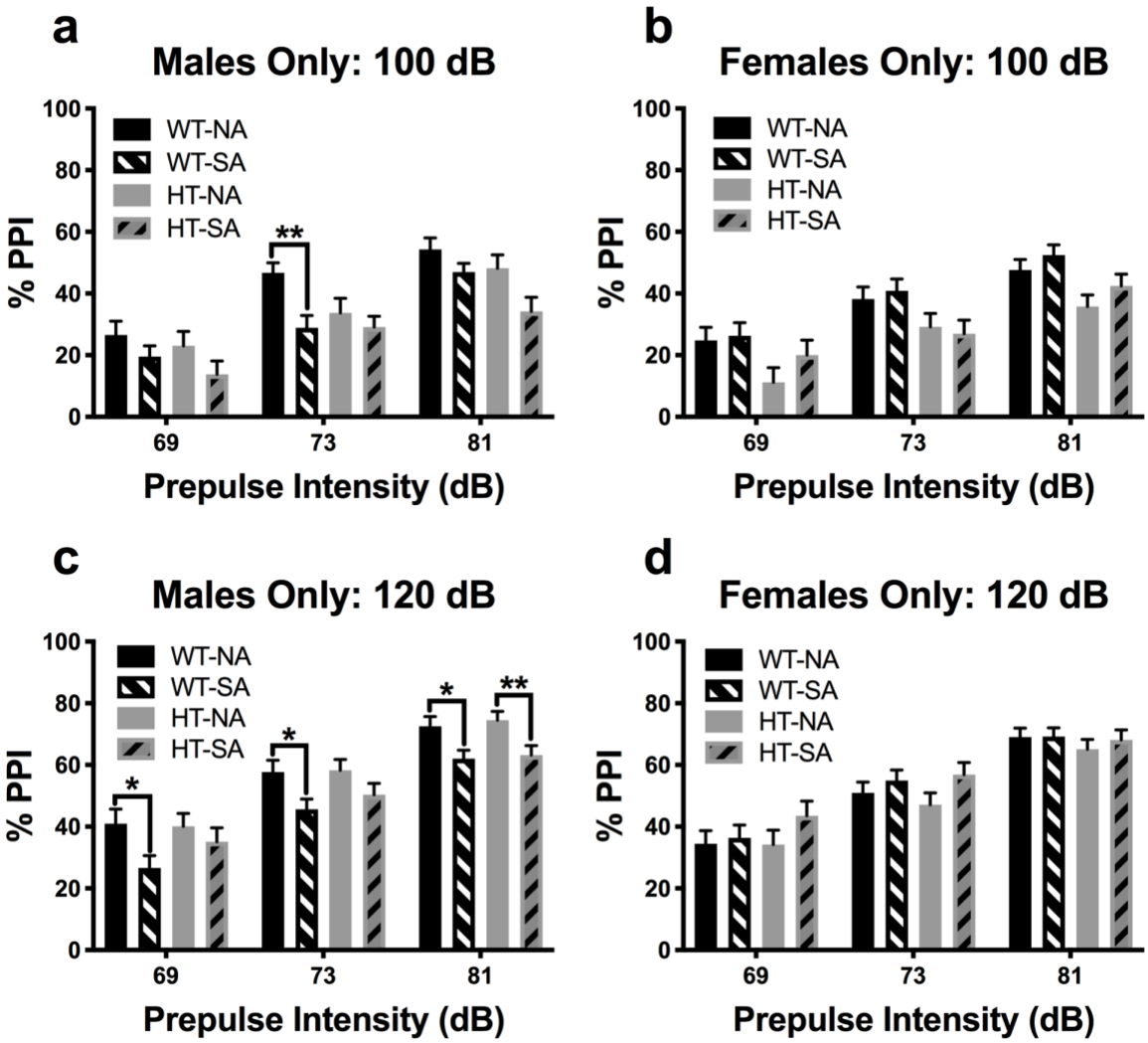


Figure 4.2. Short active (SA; 19:5 L:D) photoborn wildtype (WT) mice exhibit male-specific deficits in PPI. (**a**) Male SA photoperiod-born mice exhibited reduced prepulse inhibition (PPI) compared to normal active (NA; 12:12 L:D)-born male mice at a 100 dB startle pulse. This effect was specific to WT mice, not their dopamine transporter heterozygous (HT) littermates (**b**) This SA-photoborn-induced deficient PPI of WT mice was not seen in females, nor was there an effect in HT mice. Interestingly, female HT mice exhibited deficient PPI compared to female WT mice. **(c)** This pattern was recapitulated at 120 dB startle pulses wherein SA photoborn male WT mice exhibited deficient PPI, with little to no effect in HT mice (except at the 81 dB prepulse level). (**d**) Similarly, no interactions with or main effect of photoperiod were observed in female mice, either in WT or HT mice. Data presented as mean + S.E.M., *$p<0.05$, **$p<0.01$ where indicated.

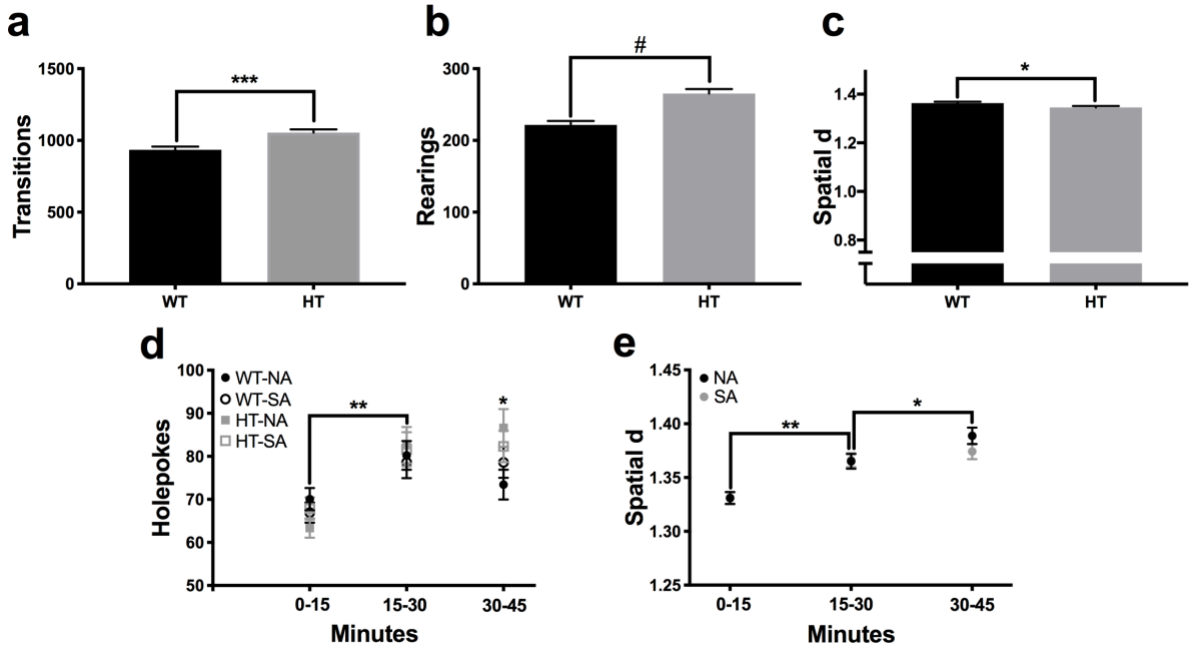


Figure 4.3. Short active (SA; 19:5 L:D) photoperiod did not induce changes in locomotor activity or exploratory behavior, as measured in the behavioral pattern monitor (BPM). (**a**) Photoperiod birth neither affected, nor interacted with genotype to affect transitions, (**b**) rearing, or (**c**) spatial d. Dopamine transporter heterozygous (HT) mice exhibited more transitions, rearing, and lower spatial d (more straight-line movement) than their wildtype (WT) littermates, however. (**d**) Across 15-min time bins, all groups exhibited increased holepoking from the first to the second time bin, while normal active (NA; 12:12 L:D) photoborn HT mice holepoked more than their NA-born WT littermates in the final time bin. (**e**) Both NA- and SA-born mice exhibited an increased spatial d between the first and second time bin, while NA-born mice exhibited further increased spatial d in the final time bin. Data presented as mean ±S.E.M., *p<0.05, **p<0.01, ***p<0.001, #p<0.0001 where indicated.

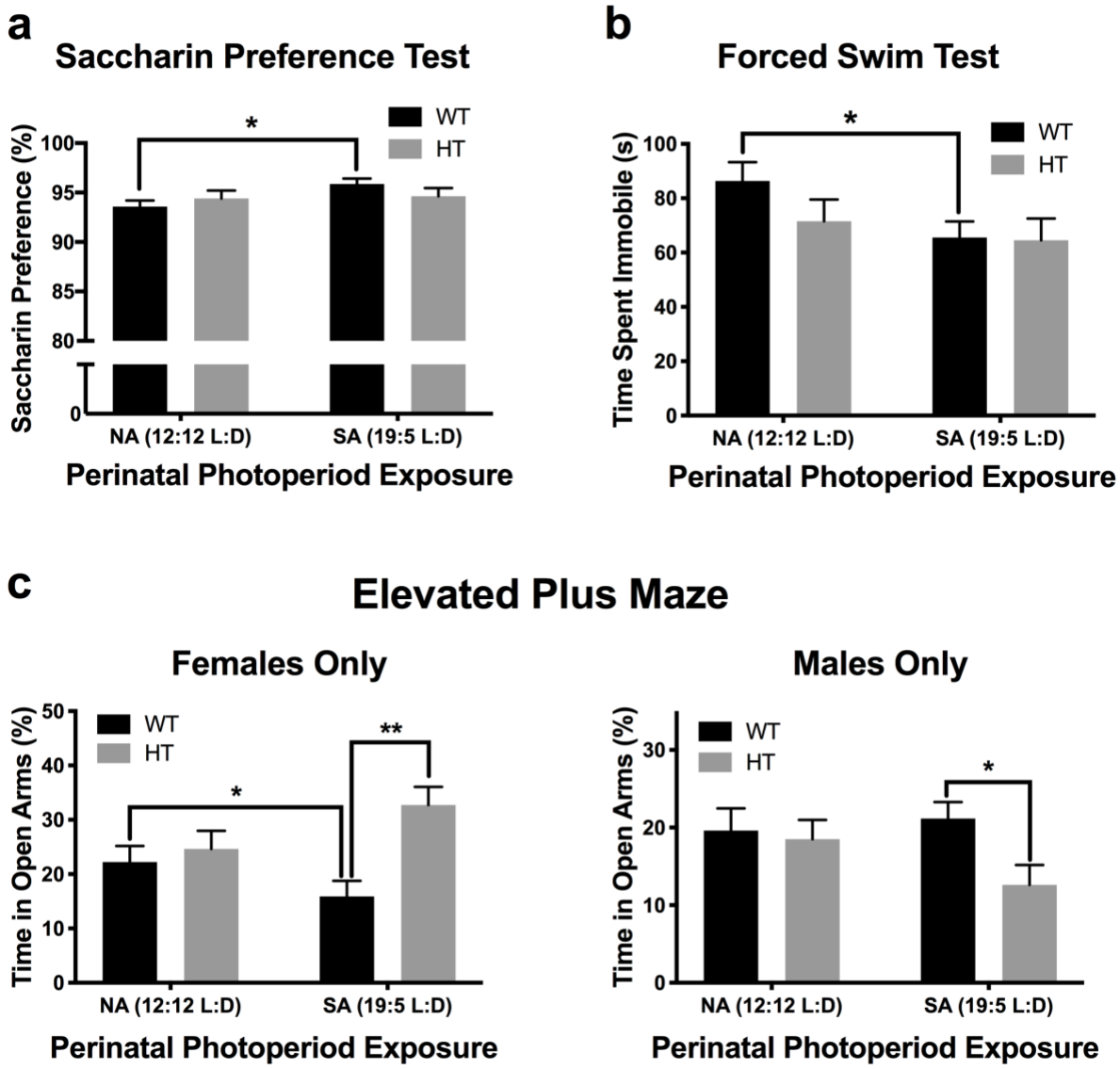
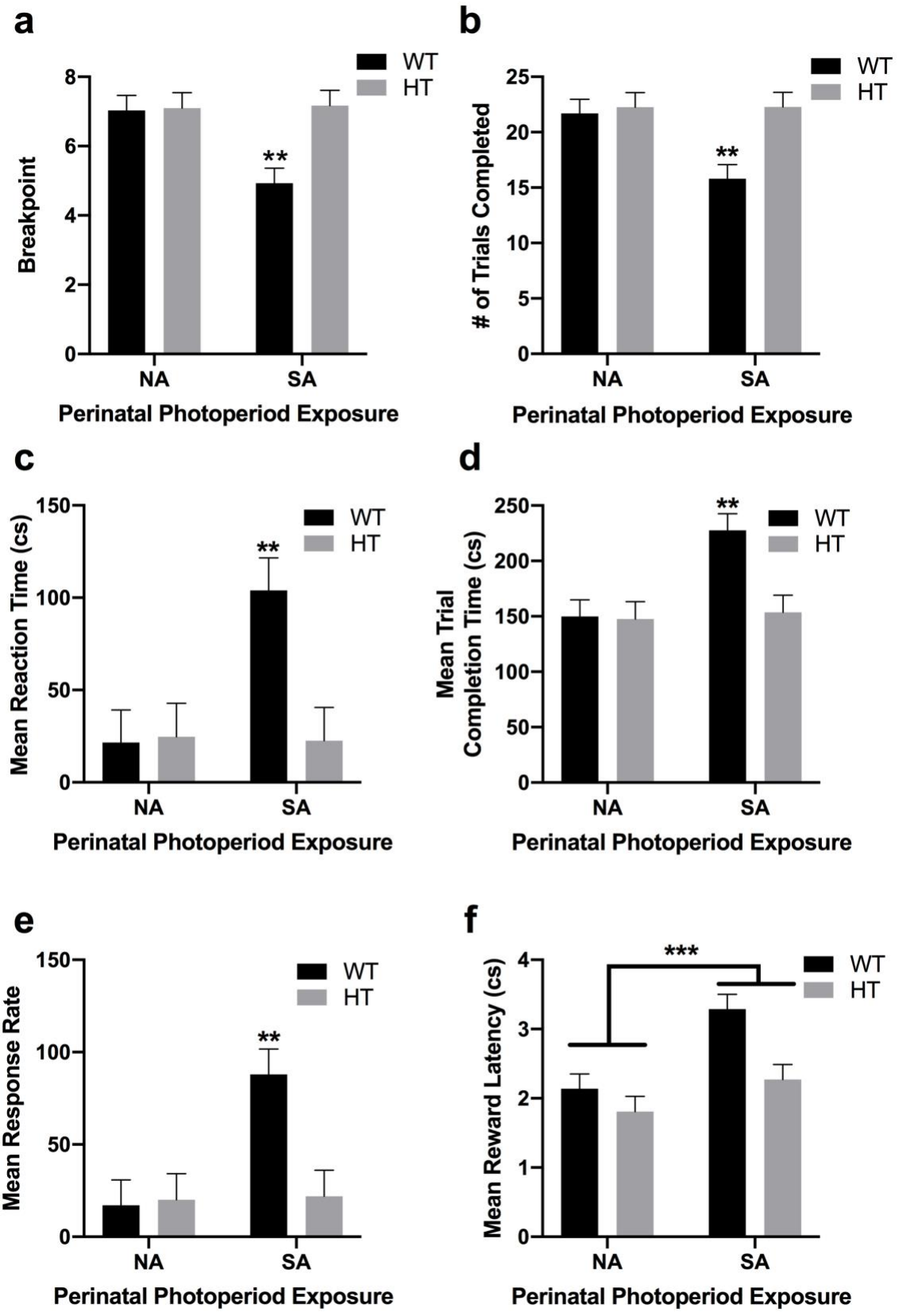


Figure 4.4. Short active (SA; 19:5 L:D) photoperiod born-induced changes on ethologically-relevant behaviors. (a) SA-born wildtype (WT) mice exhibited increased saccharin preference compared to normal active (NA; 12:12 L:D)-born WT mice, while dopamine transporter heterozygous (HT) mice were resilient to this effect. **(b)** SA-born WT mice exhibited decreased immobility compared to NA-born WT mice, with HT mice again unaffected. **(c)** In females (left), SA-born WT mice spent significantly less time in open arms compared to female NA-born WT mice, while SA-born female HT mice spent significantly more time in open arms compared to SA-born female WT mice. In males (right), male SA-born HT mice spent less time in open arms compared to male SA-born WT mice. Data presented as mean +S.E.M., *$p<0.05$, **$p<0.01$ where indicated.

Figure 4.5. Short active (SA; 19:5 L:D) photoperiod induced a reduction in willingness to work for a reward in wildtype (WT) mice. (**a**) SA-born WT mice exhibited a reduced breakpoint, (**b**) thus completed fewer trials, (**c**) exhibited longer reaction times, and (**d**) took longer to complete trials compared to all other groups. (**e**) SA-born WT mice had increased response rates, compared to all other groups. (**f**) Importantly, SA-born mice took longer to collect earned rewards than NA-born mice, largely driven by SA-born WT mice. No main effect of dopamine transporter heterozygous (HT) genotype, or effect of photoperiod on HT mice, was observed for any measure. Data presented as mean +S.E.M., **p<0.01, ***p<0.001 where indicated.


a
WT
HT
Breakpoint
8
6
4
2
0
**
NA
SA
Perinatal Photoperiod Exposure
b
WT
HT
# of Trials Completed
25
20
15
10
5
0
**
NA
SA
Perinatal Photoperiod Exposure
c
WT
HT
Mean Reaction Time (cs)
150
100
50
0
**
NA
SA
Perinatal Photoperiod Exposure
d
WT
HT
Mean Trial Completion Time (cs)
250
200
150
100
50
0
**
NA
SA
Perinatal Photoperiod Exposure
e
WT
HT
Mean Response Rate
150
100
50
0
**
NA
SA
Perinatal Photoperiod Exposure
f
WT
HT
Mean Reward Latency (cs)
4
3
2
1
0
***
NA
SA
Perinatal Photoperiod Exposure

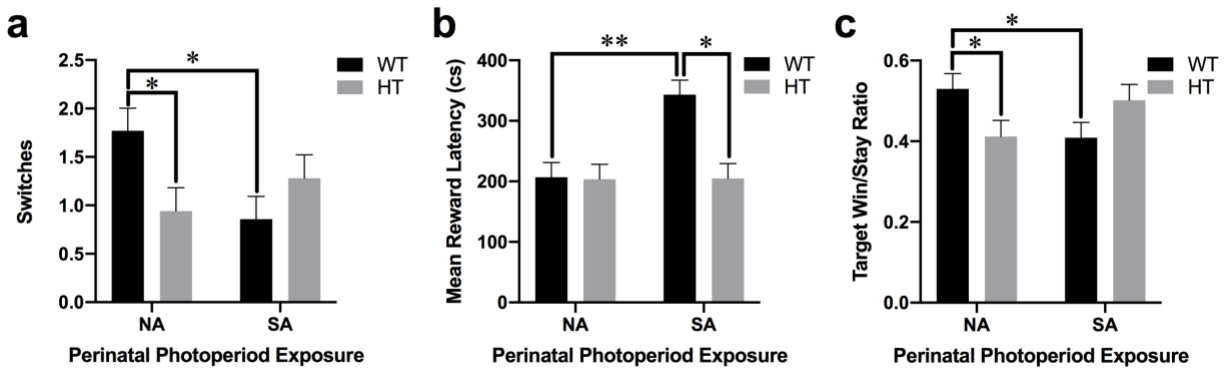


Figure 4.6. Short active (SA; 19:5 L:D) photoperiod induced impaired probabilistic learning in wildtype (WT) mice. (**a**) SA-born WT mice completed fewer switches compared to normal active (NA; 12:12 L:D)-born WT mice. NA-born dopamine transporter heterozygous (HT) mice completed fewer switches compared to NA-born WT mice. (**b**) SA-born WT mice took longer to collect earned rewards than NA-born WT mice and SA-born DAT-HT mice. (**c**) SA-born WT mice were less likely to choose the target after being rewarded from choosing the target in the previous trial (target win/stay ratio) compared to NA-born WT mice. NA-born DAT-HT mice also exhibited a reduced target win/stay ratio compared to NA-born WT mice. Data presented as mean +S.E.M., *p<0.05, **p<0.01 where indicated.

Table 4.1. Behavioral effects of short active (SA; 19:5 L:D) photoperiod exposure during gestation and early life in mice heterozygous for a mutation in the dopamine transporter (DAT-HT) and wildtype (WT) littermates. PPI = prepulse inhibition; BPM = behavioral pattern monitor; SPT = saccharin preference test; FST = forced swim test; EPM = elevated plus maze; PRBT = progressive ratio breakpoint task; PRLT = probabilistic reversal learning task.

Behavior (Test)	Potential Relevance to Human Condition	Effect in WT (vs. NA-born WT)	Effect in DAT-HT (vs. NA-born DAT-HT)
Sensorimotor gating (PPI)	Deficits seen in patients with schizophrenia (SZ) (Braff et al., 1992; Swerdlow et al., 2014), obsessive-compulsive disorder (OCD) (Ahmari et al., 2012; Hoenig et al., 2005), autism spectrium disorder (ASD) in children/ adolescents (Cheng et al., 2018)	100 & 120 dB: ↓ PPI (males only)	100 dB: no effect 120 dB: ↓ PPI (males only; 81 dB prepulse level only)
Locomotor activity/ Exploration (BPM)	Hyperlocomotion, hyperexploration, and altered motor activity patterns seen in patients with mania (Henry et al., 2013; Minassian et al., 2011; Perry et al., 2009)	**None**	**None**
Reward preference, Hedonia (SPT)	Used in rodent models as a measure of anhedonia (Der-Avakian et al., 2016); anhedonia cardinal feature of major depressive disorder (MDD) (Nelson and Charney, 1981)	↑ SP	**None**
Despair-related behavior (FST)	Commonly used in rodent models as metric for depression-like behaviors seen in humans (e.g. despair) (Cryan and Holmes, 2005; Doron et al., 2014)	↓ immobility	**None**
"Anxiety-like" behavior, risk preference (EPM)	Used in animal models to measure anxiety behavior seen in humans (Bourin, 2015; Lister, 1987)	↓ time spent in open arms (females only)	**None**
Motivation to obtain reward (PRBT)	Decreased breakpoint in patients with SZ (Gold et al., 2013; Wolf et al., 2014), patients with MDD less willing to expend effort to obtain reward (Treadway et al., 2012)	↓ breakpoint (less motivated)	**None**
Reversal learning (PRLT)	Impaired reversal learning in SZ (Reddy et al., 2016; Waltz and Gold, 2007), OCD (Tezcan et al., 2017)	↓ reversal learning	**None**

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Chapter 5: Prenatal Short Active Photoperiod Alone Induces Abnormal Behaviors: Potential Placental Mechanisms

Abstract

Given that both prenatal and postnatal short active (SA; 19:5 L:D) photoperiod exposure was used in Chapter 4 to induce behavioral deficits, the forced swim test (FST) was utilized as a quick functional readout to determine whether prenatal vs. postnatal SA exposure could induce similar behavioral changes. Prenatal SA exposure induced a reduction in FST immobility like that observed following prenatal + postnatal photoperiod exposure, whereas postnatal SA exposure did not affect immobility. SA-born mice exhibited deficits in a cross-species translatable effort-based decision-making task (EBDMT), as they preferred to work less for a smaller reward instead of exerting more effort for a larger reward. This deficit is similar to deficits observed in patients with schizophrenia using a human version of the EBDMT. Low-dose d-amphetamine (0.1 mg/kg) partially remediated this deficit in SA-born mice. A trend toward a main effect of photoperiod was observed on the average amount of chow required to maintain 85% baseline weight during the EBDMT, which was driven by SA-born males requiring more food restriction to maintain 85% baseline weight compared to NA-born males. These preliminary data indicate a potential long-term metabolic disturbance in SA-born males. At embryonic day (E) 12.5, SA-exposed male embryos were heavier than NA-exposed male embryos. At E18.5, both male and female SA-exposed embryos were heavier than NA-exposed embryos. Placental function is critical to the developing fetus and can transmit information regarding changes in maternal state by altering 1) stress response, 2) nutrient/oxygen transport, 3) inflammatory response, and/or 4) epigenetics. Previous work in rodent models has shown that maternal stress alters expression of stress-responsive genes in the placenta. Therefore, SA-induced changes in placental gene expression will be assessed in the future to identify early mechanisms by which prenatal SA exposure impacts fetal neurodevelopment, including assessment of placental genes involved in nutrient transport and metabolic processes.

Introduction

Short active (SA; 19:5 light:dark (L:D)) photoperiod exposure during gestation and early life (embryonic day (E) 0 through postnatal day (P) 28) induces behavioral abnormalities in wildtype (WT) mice on a C57BL6/J background, but mice heterozygous for a mutation in the dopamine transporter (DAT-HT) gene were resilient to these effects (Chapter 4). The length of exposure used in that study spanned a wide range of neurodevelopmental milestones that could contribute to these behaviors (Semple et al., 2013). Identifying the time window(s) required to drive the observed behavioral abnormalities remains critical to delineate the underlying neural mechanisms. The majority of neurogenesis in the mouse brain occurs prior to birth; neurons that comprise layers II-VI of the cerebrocortex (E11-16), initiation of neuron formation in areas CA1, CA3, and dentate gyrus of the hippocampus (E11), and peak neurogenesis levels in the nucleus accumbens (E16) all occur prenatally (Chen et al., 2017; Finlay and Darlington, 1995). The pituitary gland, which participates in the stress response by stimulating the corticosterone secretion from adrenal glands, forms and develops from E8.5-E13.5 (Chen et al., 2017). By E17, corticothalamic connections are established (Chen et al., 2017). Hence, depending on the gestational timing of environmental manipulations, different neural processes would be affected, potentially leading to varied behavioral outcomes.

While neurogenesis in the mouse brain occurs primarily during the prenatal period, most granule cells in the dentate gyrus (85%) form postnatally (Altman and Bayer, 1990). In addition to neurogenesis, a period of rapid synaptogenesis and subsequent elimination via apoptosis occurs from P0-P14 in the mouse, and is critical for neural circuitry to function normally (Verney et al., 2000; Waites et al., 2005). Myelination initiates around birth and proceeds postnatally with a region-specific time course: myelination begins by P6 in the medulla oblongata, pons, and thalamus, and by P12 all white matter tracts have started myelination processes (including cortex, basal ganglia) (Chen et al., 2017). Finally, the suprachiasmatic nucleus of the

hypothalamus receives retinal projections shortly after birth (Astiz and Oster, 2018). Changes in retinal function (Jackson et al., 2014) and circadian rhythms (Ciarleglio et al., 2011) have been reported following altered perinatal photoperiod exposure, and therefore may contribute to observed behavioral changes. Whether prenatal, postnatal, or a combination of both exposures, is required for the observed SA photoperiod-induced behaviors, will help to identify what aspects of neurodevelopment are affected by this manipulation. In this chapter, I utilized the ethologically relevant forced swim test (FST) to determine whether prenatal (E0-P0) or postnatal (P0-P28) SA photoperiod exposure alone could induce behavioral deficits in offspring. The FST was chosen as a first test for two reasons: 1) offspring exposed to SA photoperiod during gestation and early life (E0-P28) exhibited reduced immobility (Chapter 4), and 2) it is a quick, functional readout of behavioral changes arising from prenatal vs. postnatal SA exposure. These results will indicate whether prenatal SA exposure, and the neurodevelopmental processes occurring embryonically, are more relevant in driving SA-induced behaviors observed later in adulthood compared to postnatal mechanisms.

Given this information, I wanted to better understand the SA photoperiod-induced reduction in motivation to obtain a reward observed in the progressive ratio breakpoint task (PRBT; Chapter 4) in the context of prenatal SA exposure only. I utilized an effort-based decision-making task (EBDMT) that has been used in both human and animal models to better understand reward-based decision-making. In one example of the human version of the task, participants are asked to choose between making a small amount of money (e.g. $1) by completing 20 button presses or making more money by completing 100 button presses (Gold et al., 2013). Patients with schizophrenia exhibit relatively constant choice behavior across reward levels and probabilities compared to healthy participants, reflecting an inability to use information related to reward magnitude and probability of receiving a reward to guide reward-based decision-making (Gold et al., 2013; Treadway et al., 2015). In a rodent version of the

EBDMT, rats make a choice between a lever associated with a small reward after minimal physical effort (low-reward (LR) lever) or a lever associated with a larger reward obtained after higher physical effort (high-reward (HR) lever) (Floresco et al., 2008). I assessed mice with prenatal SA vs. NA photoperiod exposure in an EBDMT and found SA-induced deficits in reward-based decision-making. I then determined whether systemic administration of amphetamine would remediate these deficits, given evidence in the rat literature showing dopaminergic influence on the task (Floresco et al., 2008; Hosking et al., 2015; St. Onge and Floresco, 2009). I hypothesized that mice with prenatal SA exposure would exhibit reduced preference for the HR lever as effort requirements increased, relative to NA-exposed controls, and that amphetamine treatment would rescue this deficit.

Methods

Animals and Photoperiod Exposure Paradigm

Two groups of male and female C57BL6/J mouse breeders (8-10 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). One group was used to generate photoborn mice for the forced swim test, and the other group to generate photoborn mice for the effort-based decision-making task. The first group of breeders was placed into photoperiod chambers programmed to a NA (12:12 L:D) cycle for a 1-week acclimation period. Photoperiod chambers are ventilated and illuminated to 130 lumens (as measured from the center of each chamber) by three horizontal white LED strips on each wall. After the acclimation period, breeding triads (2 female: 1 male) were formed and split into three groups of photoperiod exposures. The control group was maintained in NA photoperiod during both the prenatal (E0-P0) and postnatal (P0-P28) period. The prenatal group was exposed to SA (19:5 L:D) photoperiod from gestation and switched into NA photoperiod on P0. The postnatal group was exposed prenatally to NA photoperiod and switched into SA photoperiod on P0. All

breeding triads across groups remained together for 2 weeks to mate, after which dams were singly housed. Dams and offspring were maintained in their postnatal photoperiod exposures (NA for control and prenatal group, SA for postnatal group) until P28, when litters were weaned by sex and prenatal photoperiod exposure and maintained in a standard vivarium room. All mice were maintained in NA photoperiod for subsequent behavioral testing (see Fig. 5.1 for experimental housing schematic). After obtaining behavioral results from the first breeding cohort, only the control and prenatal groups were used for the subsequent round of breeding (to generate photoborn mice for the effort task).

Forced Swim Test (FST)

Animals from the first breeding cohort (N = 23) were placed into a 4.0L beaker filled with 2.5L of room temperature water. Animals were unable to touch the bottom of the beaker. The beaker was enclosed on all sides by a black box, and the experimenter observed behavior by video camera. The primary outcome variable, time spent immobile (seconds), was manually scored using ODLog software (Macropod Software) over the 5-minute session by people unblinded. Mice were judged "immobile" when not actively swimming.

Statistical Analysis

The first minute of data was subtracted to prevent obfuscation of photoperiod effects, as mice tend to be more active during the first portion of this test (Can et al., 2012). Data were analyzed via one-way ANOVA using SPSS 24.0 (IBM Corp., Armonk, NY) with sex and photoperiod exposure as between-subjects factors. One mouse was excluded from the NA control group as an outlier, defined as being greater or less than two standard deviations away from the mean.

Operant Training Phases

Animals from the second breeding cohort (N = 28) were initially trained in operant boxes with two retractable levers (21.6 x 18.1 x 12.7 cm; Med Associates, St. Albans, USA). One week prior to initiation of training, mice were food deprived to 85% of free-feeding body weight. During the first training phase (Hab1; 1 session/day), mice were conditioned to associate magazine illumination with the opportunity to collect a strawberry milkshake reward from the magazine. In the second training phase (Hab2; 1 session/day), mice were required to press either lever to receive the reward (criterion: at least 70 responses for two consecutive days). Once criterion was reached, mice moved into the testing phase.

Effort-Based Decision-Making Task (EBDMT)

In the effort-based decision-making task (EBDMT), mice chose between pressing a low effort (LE) lever once for 40 μl of strawberry milkshake (low reward; LR) or a high effort (HE) lever more times (number of presses increased across blocks) for 120 μl of milkshake (high reward; HR; Figure 5.2A). A session consisted of 160 trials or one hour, whichever was achieved first. Trials were arranged in 5 blocks containing 32 trials each. Mice had 45 seconds to complete each trial. The first 6 trials were forced choice trials, followed by 10 free choice trials, 6 forced trials, and 10 free choice trials. In forced choice trials, only one lever extended, and mice had to respond on that lever to obtain the associated reward, gaining information on changing contingencies. In free choice trials, both levers extended, and mice had to make an active choice between the LE and HE levers. In Block 1, the HE lever press requirement was 2, and this requirement increased across the five blocks (# of presses required = 4, 8, 12, and 16 for Blocks 2-5, respectively). The LE lever press requirement was 1 across blocks. If mice chose the LE lever, both levers retracted and the 40 μl of strawberry milkshake reward was delivered. If mice chose the HE lever, the LE lever retracted and mice were able to complete the additional lever press requirement associated with the HE lever. If mice met the requirement before the 45-second trial period elapsed, the 120 μl of milkshake was delivered. If the additional lever

press requirement was not met, the trial was counted as a failure, and resulted in an 8 second timeout where the chamber lights came on until the next trial initiated. If mice did not make a lever choice within 45 seconds, the trial was recorded as an omission, and mice underwent an 8 second timeout until the next trial initiated. See Figure 5.2A-C for EBDMT experimental design.

Drug Study

Once baseline performance was assessed, d-amphetamine sulfate (Sigma, St. Louis, MO, USA) was dissolved in 0.9% saline and administered (i.p.) in a volume of 5 ml/kg to mice using a within-subjects design. Two low doses of amphetamine (AMPH) were used: 0.1 mg/kg and 0.3 mg/kg, as well as saline vehicle. These doses were chosen based on previous work in the lab (MacQueen et al., 2018). On drug test days, pre-injection time was 10 minutes. Two baseline sessions were conducted before the first drug test day, and two baseline sessions were conducted between each drug test day. No injections were administered during baseline sessions. Order of dose administration was counterbalanced across mice to avoid effects related to the order of drug doses.

Statistical Analyses

The primary outcome was % HE lever choices made in free choice trials, excluding failure and omission trials. Secondary outcomes included average latency to choose a lever, average latency to retrieve earned rewards, omissions, and failures. To assess baseline performance stability, two analyses were conducted. First, a repeated measures ANOVA on % HE lever choices in Block 1 was performed across a 3-day period using day as a within-subjects factor to determine that there were no significant differences in Block 1 performance across that period. Second, a repeated measures ANOVA on % HE lever choices in Block 1 vs. Block 4 was conducted with block and day as within-subjects factors to determine if there was a block effect, indicating task acquisition (Block 5 was not used for analyses as not all mice completed this block during the 60-minute session). Using a criterion of a certain value for % HE lever

choice in Block 1 was not used, as some mice had stable, lower % HE lever choices. Using such a criterion would incorrectly exclude mice that learned the task but were electing to choose the LE lever more.

Once performance stability was determined, baseline performance was analyzed using data from the third day of the stable 3-day period. Data were analyzed by repeated measures ANOVA, with block as a within-subjects factor, and sex and prenatal photoperiod exposure as between-subjects factors. For the amphetamine drug study, data were analyzed using a repeated measures ANOVA, with drug dose and block as within-subjects factors, and prenatal photoperiod exposure as between-subjects factor. All data were analyzed using SPSS 26.0 (IBM Corp., Armonk, NY, USA).

Adult Weight Analyses

To determine whether mice with perinatal SA exposure exhibited body weight differences later in adulthood, data from two cohorts of mice were used. Body weights from Cohort A (behavioral data presented in Chapter 4, exposed to SA photoperiod prenatally and postnatally) was used to assess SA effects at 10 weeks old and again at 5 months old. Body weights from Cohort B (behavioral data presented in current chapter, prenatal SA exposure only) was used to assess SA effects at 4 months old. Additionally, the average amount of chow required to maintain 85% of baseline body weight during the EBDMT was assessed using Cohort B. The amount of chow per cage was divided by the number of mice in each cage to obtain an estimate of amount of chow consumed per mouse. These values were then averaged across a 3-day period during baseline EBDMT performance. Univariate ANOVAs were conducted with sex and photoperiod exposure as between-subjects factors. In cases where Levene's test of homogeneity of variances was significant, Welch's ANOVA was used to assess sex and photoperiod effects.

Results

Prenatal SA exposure reduced immobility in the forced swim test (FST)

A main effect of photoperiod exposure was observed for time spent immobile in the forced swim test (FST; $F_{(2,21)} = 6.6$, $p < 0.01$; Fig. 5.3). Mice in the prenatal SA exposure group exhibited reduced immobility compared to both NA-born mice ($p < 0.05$) and mice with postnatal SA exposure only ($p < 0.01$). NA-born mice were not significantly different from the postnatal SA group.

Prenatal SA exposure reduced preference for high effort option in effort-based decision-making task (EBDMT)

There were no main effects of or interactions between sex or prenatal photoperiod exposure on number of sessions to reach Hab2 criterion (sex: $F_{(1,24)} = 0.2$, photo: $F_{(1,24)} = 1.6$, interaction: $F_{(1,24)} = 0.1$; Fig. 5.4A). A main effect of block was observed for % high effect (HE) lever choices during free trials ($F_{(3,72)} = 24.0$, $p < 0.001$; Fig. 5.4B). % HE lever choices significantly decreased across the first four blocks, with the exception of between Blocks 1 and 2 (Block 1 vs. 2, $p = 0.08$; Block 1 vs. 3, $p < 0.001$; Block 1 vs. 4, $p < 0.001$). Regardless of block, prenatal SA exposure reduced preference for the HE lever compared to NA-born mice ($F_{(1,24)} = 4.5$, $p < 0.05$). A main effect of block was observed for average choice latency ($F_{(2,50)} = 8.8$, $p < 0.001$; Fig. 5.4C). Mice took longer to choose which lever to respond on in Blocks 3 and 4 compared to Block 1 (Block 1 vs. 3, $p < 0.01$; Block 1 vs. 4, $p < 0.001$). This difference was also significant between Blocks 2 and 4 ($p < 0.05$). A main effect of block was also observed on average reward latency ($F_{(1.6,39.4)} = 4.5$, $p < 0.05$; Fig. 5.4D), with mice taking longer to retrieve earned rewards in Block 4 compared to Block 1 ($p < 0.01$) and Block 2 ($p < 0.05$). No main effects of or interactions between block, sex, or prenatal photoperiod exposure were observed on number of omissions (Fig. 5.4E). There was a main effect of block on number of failures ($F_{(2,49)} = 3.8$, $p < 0.05$; Fig. 5.4F), with significantly more failures in Block 4 compared to Block 1

($p < 0.05$) and Block 2 ($p < 0.05$). While this was a main effect, the overall number of failures was low across all blocks and did not differ by group.

Low-dose amphetamine (AMPH) partially remediated the reduced effort preference in mice with prenatal SA exposure

A drug x photoperiod x block interaction was observed following systemic AMPH administration ($F_{(6,150)} = 2.5$, $p < 0.05$; Fig. 5.5). A main effect of drug was observed in mice with prenatal NA photoperiod exposure ($F_{(2,28)} = 5.1$, $p < 0.05$). Both 0.1 mg/kg and 0.3 mg/kg reduced % HE lever choices in free choice trials compared to vehicle, and this reduction was significant in Block 2 for vehicle vs. 0.3 mg/kg ($p < 0.05$) and in Block 3 for both doses of AMPH (vehicle vs. 0.1 mg/kg: $p < 0.05$; vehicle vs. 0.3 mg/kg, $p < 0.01$). A main effect of drug was also observed in mice with prenatal SA photoperiod exposure ($F_{(1.4,15)} = 7.9$, $p < 0.01$). 0.3 mg/kg AMPH reduced % HE lever choices relative to vehicle in Blocks 1-3 ($p < 0.05$). In Block 3, however, 0.1 mg/kg increased % HE lever choices relative to both vehicle ($p < 0.05$) and the higher 0.3 mg/kg dose ($p < 0.01$), indicating an amphetamine-induced increase in HE preference in SA-born mice with low-dose AMPH.

Perinatal SA exposure does not induce changes in body weight later in adulthood

Perinatal SA exposure did not induce changes in body weight in Cohort A at 10 weeks old (Fig. 5.6A) or 5 months old (Fig. 5.6B). A main effect of sex was observed at both time points, with males being heavier than females (10 weeks: $F_{(1,122)} = 247.4$, $p < 0.001$; 5 months: $F_{(1,60)} = 55.6$, $p < 0.001$). The baseline body weight data at 4 months old from Cohort B violated Levene's test of homogeneity of variances, so Welch's ANOVA was used to assess photoperiod effects in this dataset. No effect of photoperiod was observed (Fig. 5.6C). Males were heavier than females, however ($F_{(1, 31)} = 38.5$, $p < 0.001$). There was a trend toward a photoperiod effect on the average amount of chow required to maintain mice at 85% baseline weight during the EBDMT ($F_{(1,9)} = 3.6$, $p = 0.09$; Fig. 5.6D). No effect of sex was observed ($F_{(1,9)} = 2.6$). However,

there was a clear qualitative difference between males and females, with SA-born males requiring more food restriction to maintain 85% baseline weight compared to NA-born males, and NA- and SA-born females requiring the same level of food restriction (Fig. 5.6D).

Discussion

Mice that were born during a prenatal-only SA photoperiod exposure continued to exhibit behavioral abnormalities even in adulthood, consistent with patients with psychiatric conditions. Here, I demonstrated that mice with prenatal SA exposure exhibited reduced immobility in the forced swim test (FST), as well as reduced willingness to exert more physical effort for a larger reward in an effort-based decision-making task (EBDMT). EBDMT deficits were partially reversed by low-dose amphetamine. Thus, abnormal behaviors resulting from prenatal SA exposure indicate that prenatal mechanisms play an important role in inducing the behavioral effects observed in Chapter 4, and that postnatal mechanisms may contribute less to these effects. SA-born mice exhibited reduced immobility in the FST, consistent with observations from Chapter 4 as well as previously published work using similar photoperiod manipulations (Green et al., 2015). The reproducibility of this finding validates our model and indicates that further assessment of behaviors resulting from prenatal SA exposure alone is necessary.

Effortful motivation to obtain a reward was assessed in NA- vs. SA-born mice using a cross-species translatable effort-based decision-making task (EBDMT). SA-born mice chose the low effort (LE) lever more than NA-born mice regardless of the effort differential between the two levers (no effect of block). This observation is similar to EBDMT performance in patients with schizophrenia who exhibit relatively constant choice behavior, choosing to exert less effort to obtain a smaller reward rather than engaging in more effort to obtain a large reward (Gold et al., 2013; Treadway et al., 2015). Systemic administration of low-dose d-amphetamine (AMPH; 0.1 mg/kg) partially remediated these deficits in SA-, but not NA-born mice. Conversely, 0.3

mg/kg worsened performance relative to vehicle in both NA- and SA-born mice. This bimodal AMPH effect is consistent with previous work in rats showing that low-dose AMPH (0.125 mg/kg or 0.25 mg/kg) increased willingness to work harder for a reward (increased proportion of high effort lever choices made), whereas higher doses (0.50 mg/kg) had the opposite effect (Floresco et al., 2008). Recent work has shown that overexpressing striatal dopamine D2 receptors in mice (D2R-OE) increased sensitivity to effort costs (Filla et al., 2018). Specifically, D2R-OE mice preferred to maintain a sustained action (lever holding) over initiating a lever press repeatedly. Filla et al. also showed that during goal-directed effort-based tasks, less dopamine is released in the ventral striatum of D2R-OE mice. Therefore, the impaired effort-based decision-making in these mice is consistent with the rat literature showing increased sensitivity to effort costs following D2R antagonism (Floresco et al., 2008; Hosking et al., 2015). These studies point toward hypodopaminergia in the ventral striatum during goal-directed behavior as a potential mechanism underlying SA-induced deficits in effort-based decision making. In vivo microdialysis in SA-born mice performing the EBDMT would test this hypothesis. An increase in the percentage of high effort lever choices made in the EBDMT following low-dose AMPH infusion into the ventral striatum would also support this idea. The systemic route of AMPH administration used in the current study, however, limits the ability to determine specific brain regions and neural circuits affected by the prenatal SA photoperiod manipulation.

The reduced preference for the HE lever observed in SA-born mice could indicate an impaired ability to learn the EBDMT relative to NA-born mice. There were no photoperiod effects on number of sessions to reach Hab2 criterion, however (Fig. 5.4A), indicating that SA photoperiod did not impair the ability to associate lever pressing with obtaining a reward. When comparing baseline performance values to vehicle-treated groups in the AMPH study, NA-born mice exhibited similar % HE choice values, except Block 3 (Block 1 baseline vs. AMPH-vehicle: 83% vs. 80%; Block 2: 77% vs. 81%; Block 3: 68% vs. 77%; Block 4: 54% vs. 51%). SA-born

vehicle-treated mice in the AMPH study exhibited higher % HE choice in Blocks 1, 2, and 4 compared to baseline (Block 1 baseline vs. AMPH-vehicle: 68% vs. 82%; Block 2: 64% vs. 73%; Block 3: 50% vs. 52%; Block 4: 34% vs. 45%), which may indicate that SA-born mice were slower to learn the contingencies of the HE and low effort (LE) levers. Future work on photoborn mice in the EBDMT may require more training days prior to the assessment of baseline performance. The reduced preference observed for the HE lever could have also resulted from SA-induced effects on satiety. If SA-born mice reached satiety faster than NA-born mice, then that might manifest as reduced preference for the larger reward. It would be expected that if satiety played a role in this effect that reduced HE preference would be more extreme in later blocks. However, there was not a photoperiod x block interaction on % HE choices, as the reduced preference in SA-born mice was not block-dependent. Furthermore, previous work in the lab using other operant tasks has shown that the strawberry milkshake reward does not show a satiety curve, making satiety less of a concern. Another limitation of the current study is that AMPH acts on both the dopamine transporter (DAT) and the norepinephrine transporter (NET), with a sixfold selectivity for NET over DAT (Han and Gu, 2006). Therefore, partial remediation of EBDMT deficits might be related to the effects of norepinephrine rather than dopamine. Future pharmacological studies will use more selective compounds to distinguish between these two neurotransmitter systems.

While SA exposure did not affect baseline body weight later in adulthood in two cohorts of mice, there was a trend toward a photoperiod effect on the average amount of chow required to maintain mice at 85% of baseline weight during the EBDMT, which appeared to be driven by SA-born males requiring more extreme food restriction compared to NA-born males. This may indicate a long-lasting metabolic dysfunction induced by prenatal SA exposure. Previous work showed that male mice exposed postnatally to SA photoperiod (18:6 L:D; 0-4 weeks old) exhibited altered plasma metabolomic profiles later in adulthood (Uchiwa et al., 2016). While

these effects were a result of postnatal exposure, prenatal SA exposure may also induce such effects. Future work will increase sample sizes to see whether this trend becomes significant and will assess mechanisms by which prenatal SA exposure might induce such effects (e.g. plasma metabolomics assessment in pregnant dams, assessing placental gene expression changes related to nutrient transport).

Overall, these results suggest that prenatal SA exposure induces changes in underlying neural circuitry that persist into adulthood and promote the emergence of psychiatry-relevant behaviors. More work needs to be done to fully characterize the prenatal vs. postnatal influences on SA-induced behaviors, but currently prenatal mechanisms seem to predominately contribute to abnormal behaviors in this model. Given the stress-inducing properties of the SA photoperiod manipulation in adult rodent models (Dulcis et al., 2013; Chapter 4), looking toward the prenatal stress literature will also help guide future work on this mouse model.

Future Directions: Toward Mechanistic Origins

In this chapter, I showed that prenatal SA exposure can induce behavioral abnormalities in adulthood, raising the question of how altered maternal photoperiod during gestation is transmitted to the fetus to influence neurodevelopment. Accumulating research shows that the placenta plays an active role in transmitting information regarding maternal environmental conditions to the developing fetus (e.g. changes in hormonal and stress levels, nutritional state) (Jansson and Powell, 2007; Nugent and Bale, 2015). The placenta expresses glucocorticoid (GC) receptors (GRs) throughout pregnancy and serves as a protective barrier to the fetus from excessive GC signaling from the maternal side (e.g. corticosterone). A major component of this barrier is placental expression of the enzyme 11β-hydroxysteroid dehydrogenase type 2 (HSD2) which catalyzes the conversion of corticosterone into its inactive form, 11-dehydrocorticosterone. 11β-HSD2 is abundantly expressed in the fetal rodent brain around mid-

gestation, acting to protect immature neurons from the maturation effects of GC exposure; 11β-HSD2 expression sharply declines toward the end of gestation, when neurogenesis is complete in the majority of brain regions (Brown et al., 1996; Diaz et al., 1998). Therefore, perturbations in the regulation of this placental GC barrier could negatively impinge on early fetal neurodevelopmental processes. In fact, prenatal exposure to chronic restraint stress reduced placental HSD2 expression and activity in rats (Mairesse et al., 2007), leading to potential excessive fetal GC exposure. HSD2 null offspring exhibit an anxiogenic/risk averse profile in the EPM (Holmes et al., 2006), confirming that fetoplacental HSD2 plays a role in programming affective behaviors in offspring. Given that the stress-inducing effects of SA photoperiod exposure in adult rodents has been determined (Dulcis et al., 2013; Chapter 4), it is possible that gestational SA exposure induces aberrant maternal stress responses, thereby altering the placental GC barrier function and enabling fetal behavioral programming.

Placental function is critical to the developing fetus and can transmit information regarding changes in maternal state by altering 1) stress response, 2) nutrient/oxygen transport, 3) inflammatory response, and/or 4) epigenetics. Previous work in rodent models has shown that maternal stress alters expression of stress-responsive genes in the placenta. For example, in one study, exposure to physiological stressors during pregnancy altered expression of 5 out of 6 stress-responsive genes assessed, including downregulation of *Nr3c1* (GR), *Nr3c2* (mineralocorticoid receptor), *Hsd11b2* (codes for HSD2), *Crhr1* (corticotropin releasing hormone receptor 1), and *Ogt* (O-linked *N*-acetylglucosamine (GlcNAc) transferase) (Briffa et al., 2017). Furthermore, the downregulation of placental *Hsd11b2* following prenatal stress exposure has been replicated (Jensen Peña et al., 2012; Mairesse et al., 2007). Ogt has also been confirmed as a biomarker of prenatal stress exposure in more recent studies (Howerton et al., 2013; Howerton and Bale, 2014), and is known to affect a variety of cellular functions, including direct modification of core histone proteins (potentially inducing epigenetic alterations in the fetal

genome). Expression of genes coding for growth factors and nutrient transporters is altered by prenatal stress exposure in rodent models (Briffa et al., 2017; Mueller and Bale, 2008), providing a link between maternal stress and placental regulation of nutrient transport (and, as a result, fetal development). Prenatal stress also induces placental inflammation in a sex-specific manner (males > females) through upregulation of pro-inflammatory cytokines and chemokines (Bronson and Bale, 2014). While studies indicate male predominance with this effect, elevations in placental proinflammatory cytokines (e.g. interleukin (IL)-1β) have been reported in females as well (Gur et al., 2017). Genes known to play a role in epigenetic regulation have also been shown to be upregulated by maternal stress (e.g. *Dnmt3a* (Jensen Peña et al., 2012)) and may play a critical part in transmitting long-lasting behavioral effects of maternal environmental perturbations to offspring. Maternal environmental perturbations can alter a wide range of placental gene expression pathways that may be sex-specific and have relevance to fetal neurodevelopment, potentially explaining the sex-specific effects on behavior (Chapter 4). The question remains as to whether prenatal SA photoperiod exposure can induce changes in placental gene expression, and whether placental mechanisms drive SA-induced behaviors in adulthood.

To address whether prenatal SA photoperiod exposure can drive relevant changes in placental gene expression in a sex-specific manner, I designed an experiment using the same prenatal photoperiod exposure paradigm used for the EBDMT in this chapter to assess placental gene expression changes using RNA-Sequencing (RNA-Seq). Briefly, C57BL6/J mice were time-mated in NA or SA photoperiod and placentas from pregnant dams were evaluated at E12.5 and E18.5 (presence of vaginal plug indicated E0.5 of gestation). These time points were selected based on previously published work on prenatal stress effects on placental gene expression (Bronson and Bale, 2014; Howerton et al., 2013; Mueller and Bale, 2008; Nugent et al., 2018), for RNA-Seq dataset comparison with published datasets. Following dam euthanasia,

embryonic and placenta weights were obtained. Placentas were bisected, and half was placed into RNAlater (Ambion Inc., Austin, TX, USA) and stored at -80°C for RNA isolation and RNA-Seq. Total RNA was purified using the MirVana Total RNA kit (Ambion). The Qubit RNA Broad Range Assay kit and Qubit Fluorometer (Thermo Fisher Scientific) was used to determine RNA concentration, and RNA integrity was analyzed using a Bioanalyzer (Agilent). Samples with RIN>8.0 were selected for RNA-Seq. RNA was reverse transcribed into cDNA, and used to sex placentas using previously published primers (McFarlane et al., 2013). In total, 10 placentas/photoperiod/time point (n = 5 male placentas, n = 5 female placentas) were selected from n = 2-3 litters for RNA-Seq. RNA samples were sent to the UCSD Genomics Core for library prep and RNA-Seq. See Fig. 5.7 for schematic of RNA sample preparation.

RNA will be depleted of rRNA and then used to construct random-primed strand-specific RNA-Seq libraries, which will then be subjected to paired-end 100 bp sequencing at a depth of 60 million mapped reads/sample, multiplexing 96 samples per lane of a NovaSeq 6000 flowcell. Reads will be mapped to the reference transcriptome (Refseq) using STAR (Dobin et al., 2013). Normalization and differential analysis will be performed using DESeq (Anders and Huber, 2010). Principle component analysis (PCA) will be performed using the Qlucore Omics Explorer 3.1 (Lund, Sweden); four two-group comparisons will be performed: 1) female NA- vs. SA-exposed (E12.5), 2) female NA- vs. SA-exposed (E18.5), 3) male NA- vs. SA-exposed (E12.5), and 4) male NA- vs. SA-exposed (E18.5). Genes that exhibit significant differential expression between these groups will be identified. Gene Ontology enrichment analysis for Biological Process will be performed using Metascape. Affinity Propagation (AP) algorithm in R will be used to cluster differentially expressed genes using Pearson correlations as the similarity measure to identify other transcriptional signatures.

Sex-specific analyses of photoperiod effects on embryo and placenta weights were conducted using Mann-Whitney-U tests. For E12.5 males, a significant effect of photoperiod

was observed for embryo weight ($U = 24$, $p < 0.05$; Fig. 5.8A), with SA-exposed embryos weighing more than NA-exposed embryos. No photoperiod effects were observed for E12.5 male placenta weight or in E12.5 females (Fig. 5.8A, 5.8B). At E18.5, SA-exposed embryos weighed more than NA-exposed embryos in both males ($U = 24$, $p < 0.01$; Fig. 5.8C) and females ($U = 24$, $p < 0.05$; Fig. 5.8C). No photoperiod effects were observed on E18.5 placenta weights in either sex (Fig. 5.8D). One study found that birth weights of people born in late winter/spring are higher compared to people born during other times of the year (Selvin and Janerich, 1971), lending validity to this finding. Table 5.1 lists genes of interest that have shown to be regulated in the placenta by maternal stress, as well as the predicted direction of SA-induced placental effects. These genes include stress-related genes, immune-related genes, growth factors and nutrient transporters, as well as genes related to epigenetic control. In addition to these genes, this RNA-Seq dataset will provide a wealth of information regarding other potentially unknown genes influenced by prenatal SA photoperiod exposure and will help guide the design of future mechanistic studies targeted at understanding SA-induced changes in neural circuitry and function. Furthermore, understanding how prenatal maternal environmental perturbations impact placental gene expression and subsequent programming of offspring behavior in adulthood is of utmost importance when considering early intervention strategies to prevent the development of psychiatric disorders.

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teaching me how to collect and process placenta samples for RNA-Seq. Thank you to Tony Bui for helping me with my first rounds of Qubit and BioA.

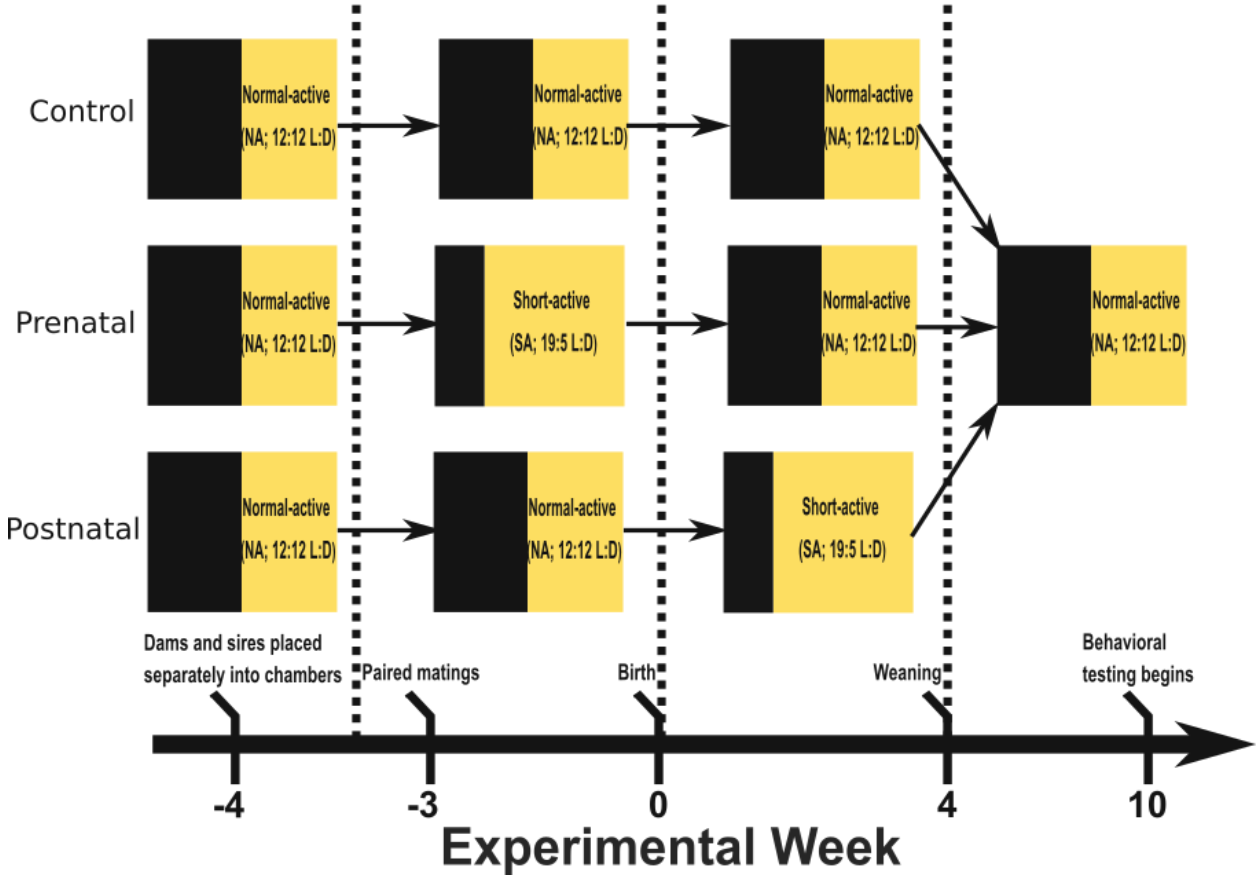
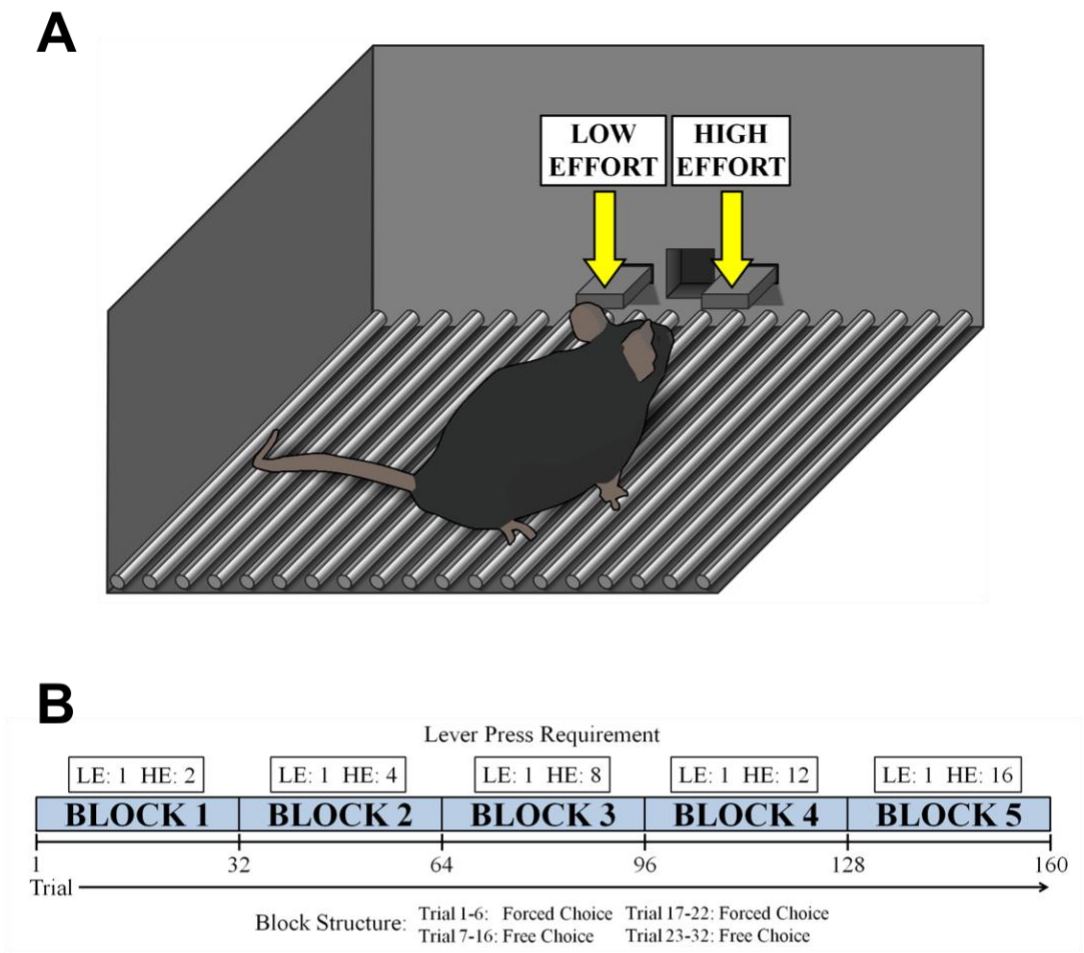


Figure 5.1. Schematic of photoperiod exposure experimental timeline used for forced swim test (FST). C57BL6/J breeders were placed separately into photoperiod chambers programmed to a normal active (NA; 12:12 light:dark (L:D)) cycle for 1 week. Breeding triads (2 females: 1 male) were then formed, and three photoperiod exposure groups were created. The control group was exposed prenatally (embryonic day (E) 0-postnatal day (P) 0) and postnatally (P0-P28) to NA photoperiod (top row). The prenatal group was exposed prenatally to short active photoperiod (SA; 19:5 L:D) and postnatally to NA photoperiod (middle row). The postnatal group was exposed prenatally to NA photoperiod and postnatally to SA photoperiod (bottom row). All groups were weaned at P28 and maintained in NA photoperiod until behavioral testing began in adulthood. Generating the effort-based decision-making (EBDMT) cohort followed a similar paradigm, except the postnatal group was excluded based on FST results.

Figure 5.2. Experimental design of effort-based decision-making task (EBDMT). (A) In the EBDMT, mice chose between a low effort (LE; fixed ratio (FR) = 1) lever and a high effort (HE; FR = 2, 4, 8, 12, 16) lever. Responding on the LE lever resulted in a smaller reward (40 μl strawberry milkshake), whereas choosing and completing the HE lever press requirement resulted in a larger reward (120 μl milkshake). (B) Each EBDMT session was one hour or 160 trials, whichever was achieved first. Sessions were organized into five blocks, each containing 32 trials. Mice had 45 seconds to complete each trial. The first six trials were forced choice trials, followed by 10 free choice trials, 6 forced choice, and 10 free choice trials. In forced trials, only one lever extended and mice were forced to respond on that lever to receive a reward. In free choice trials, both levers extended and mice made an active choice between in the LE and HE levers. Across blocks, the LE lever always required one lever press to receive the smaller reward. The HE lever press requirement increased across blocks such that it took 2, 4, 8, 12, and 16 lever presses to obtain the larger reward. (C) In free choice trials, if mice chose the LE lever, both levers retracted and the small reward was delivered. If mice pressed the HE lever, the LE lever retracted, and mice had the remainder of the trial time to meet the additional lever press requirement. If the requirement was met within the trial time limit (45 seconds), the larger reward was delivered. If the requirement was not met, the trial counted as a failure, and the chamber lights illuminated for an 8-second timeout. If neither lever was pressed within 45 seconds, the trial counted as an omission, and an 8-second timeout was initiated before the next trial began.


A
LOW EFFORT
HIGH EFFORT
B
Lever Press Requirement
LE: 1 HE: 2
LE: 1 HE: 4
LE: 1 HE: 8
LE: 1 HE: 12
LE: 1 HE: 16
BLOCK 1
BLOCK 2
BLOCK 3
BLOCK 4
BLOCK 5
1
32
64
96
128
160
Trial
Block Structure:
Trial 1-6: Forced Choice
Trial 7-16: Free Choice
Trial 17-22: Forced Choice
Trial 23-32: Free Choice
C
Both Levers Extend
Low Effort Lever Pressed
No Lever Pressed Within 45 s
High Effort Lever Pressed
Both Levers Retract
Low Effort Lever Retracts
Additional Lever Press Requirement Not Met
Additional Lever Press Requirement Met
Omission
Failure
Reward ×1
Timeout – 8 s
Timeout – 8 s
Reward ×3

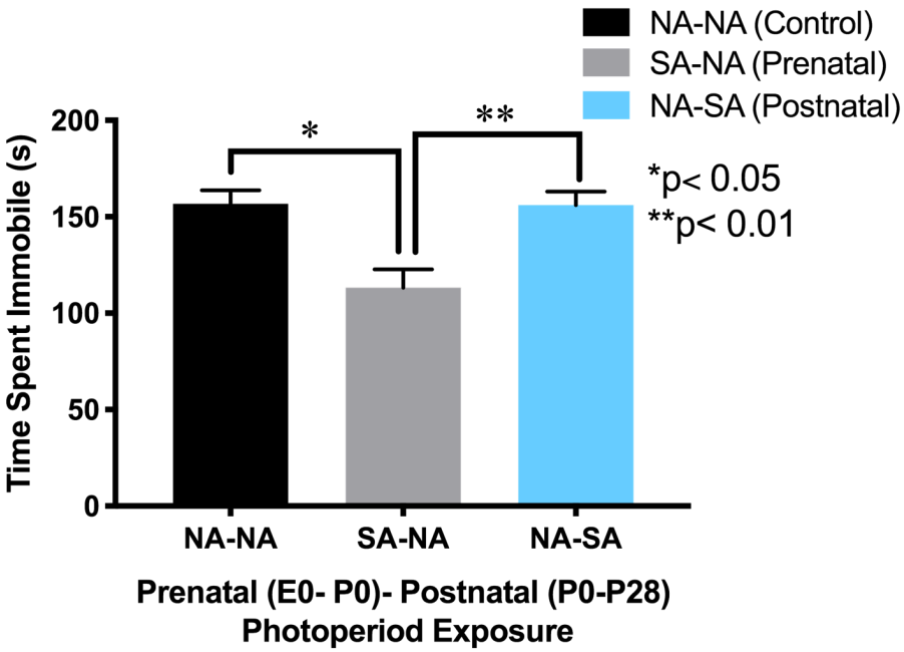
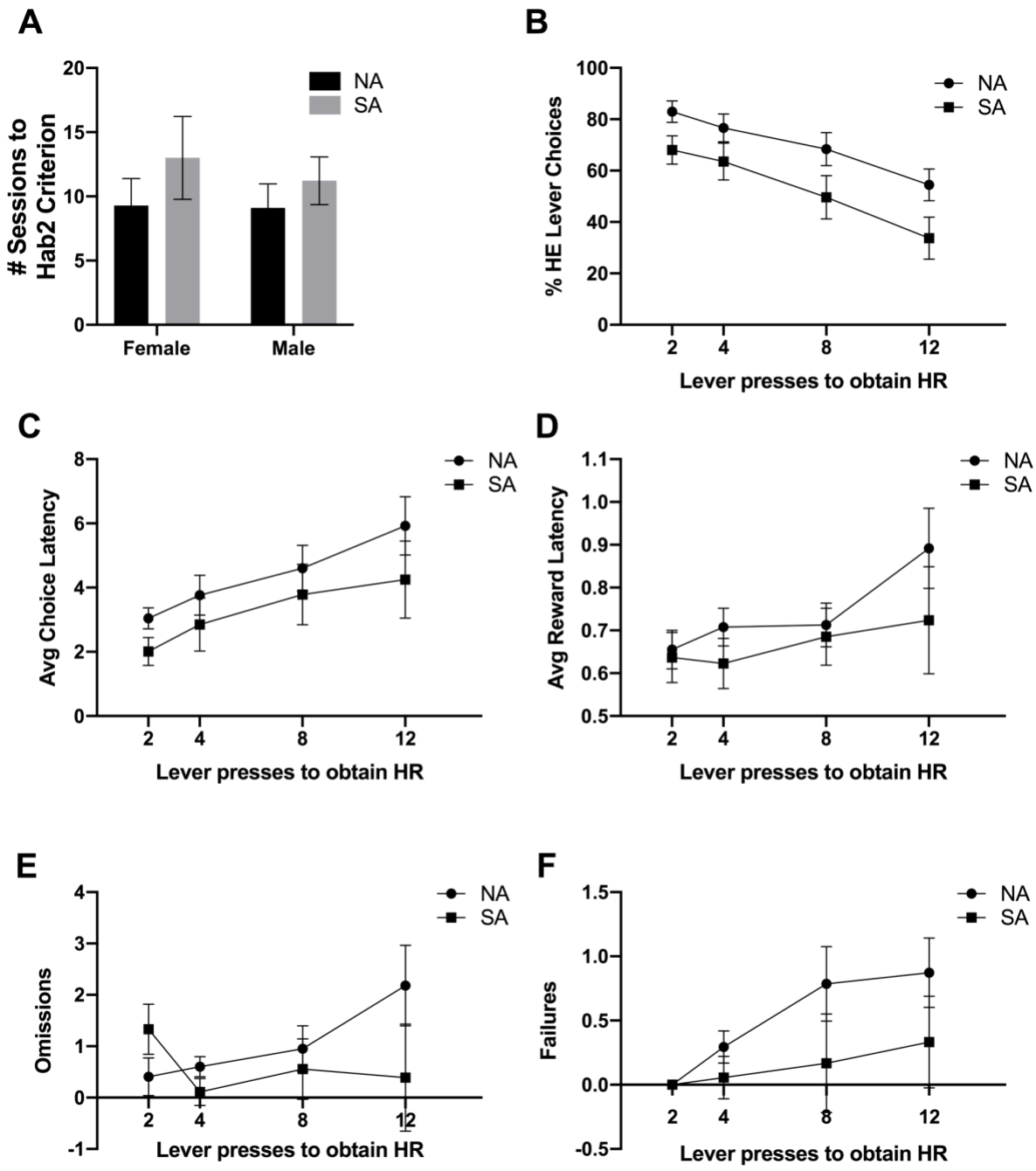


Figure 5.3. Mice with prenatal short active (SA; 19:5 light:dark (L:D)) photoperiod exposure exhibited reduced immobility in the forced swim test (FST) compared to normal active (NA; 12:12 L:D)-born mice and mice with postnatal SA exposure. Mice were exposed to one of three photoperiod conditions during the perinatal period. Control mice (black bar) were exposed prenatally (embryonic day (E) 0 – postnatal day (P) 0) and postnatally (P0-P28) to NA photoperiod. Mice in the prenatal group (gray bar) were exposed prenatally to SA photoperiod and postnatally to NA photoperiod. Mice in the postnatal group (blue bar) were exposed prenatally to NA photoperiod and postnatally to SA photoperiod. Mice in the prenatal group exhibited reduced immobility compared to mice in the control and postnatal groups. Data presented as mean +S.E.M., *$p < 0.05$, **$p < 0.01$ where indicated.

Figure 5.4. Prenatal short active (SA; 19:5 light:dark (L:D)) photoperiod exposure induced deficits in effort-based decision-making compared to normal active (NA; 12:12 L:D) photoperiod-exposed mice. (A) No main effects of sex or prenatal photoperiod exposure were observed on number of sessions to reach Hab2 criterion. (B) Mice with prenatal SA exposure exhibited reduced willingness to exert more effort for a higher value reward (HR), as reflected by a reduced preference for the high effort (HE) compared to NA-born mice, regardless of block ($F_{(1,24)} = 4.5$, $p < 0.05$). (C) A main effect of block was observed for average choice latency, with mice taking longer to choose which lever to respond on at higher lever press requirements associated with the HE lever (increased choice latency when lever presses required to obtain HR = 8 and 12 vs. 2). (D) A main effect of block was also seen with average reward latency. Mice took longer to retrieve earned rewards when lever presses to obtain HR = 12 vs 2 or 4. (E) No main effects of or interactions with prenatal photoperiod exposure, block, or sex were seen on number of omissions. (F) There was a main effect of block on number of failures, with more failures when the HE lever press requirement = 12 vs. 2 or 4. The number of failures across blocks, however, was low. Data presented as mean +S.E.M.


A
Sessions to Hab2 Criterion
20
15
10
5
0
NA
SA
Female
Male
B
% HE Lever Choices
100
80
60
40
20
0
NA
SA
2
4
8
12
Lever presses to obtain HR
C
Avg Choice Latency
8
6
4
2
0
NA
SA
2
4
8
12
Lever presses to obtain HR
D
Avg Reward Latency
1.1
1.0
0.9
0.8
0.7
0.6
0.5
NA
SA
2
4
8
12
Lever presses to obtain HR
E
Omissions
4
3
2
1
0
-1
NA
SA
2
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Lever presses to obtain HR
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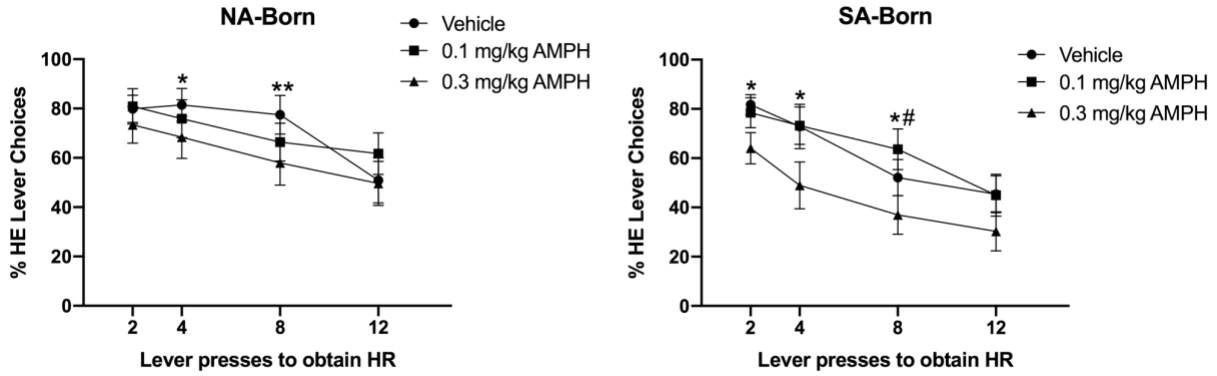

Figure 5.5. Low-dose d-amphetamine (AMPH) partially remediated effort-based decision-making deficits in SA-born mice. A drug x photoperiod x block interaction was observed following systemic AMPH administration ($F_{(6,150)} = 2.5$, $p < 0.05$). In NA-born mice (left), both 0.1 and 0.3 mg/kg reduced the percentage of high effort (HE) lever choices in free choice trials compared to vehicle when the HE lever press requirement was 4 or 8. In SA-born mice (right), 0.3 mg/kg AMPH reduced the percentage of HE lever choices made compared to vehicle when the HE lever press requirement was 2, 4, and 8. When the HE lever press requirement was 8, 0.1 mg/kg AMPH increased the percentage of HE lever choices made compared to both vehicle and 0.3 mg/kg AMPH, indicating improved EBDMT performance in SA-born mice. Data presented as mean +S.E.M., *$p < 0.05$ vehicle vs. 0.3 mg/kg; **$p < 0.01$ vehicle vs. 0.3 mg/kg; #$p < 0.05$ vehicle vs 0.1 mg/kg.

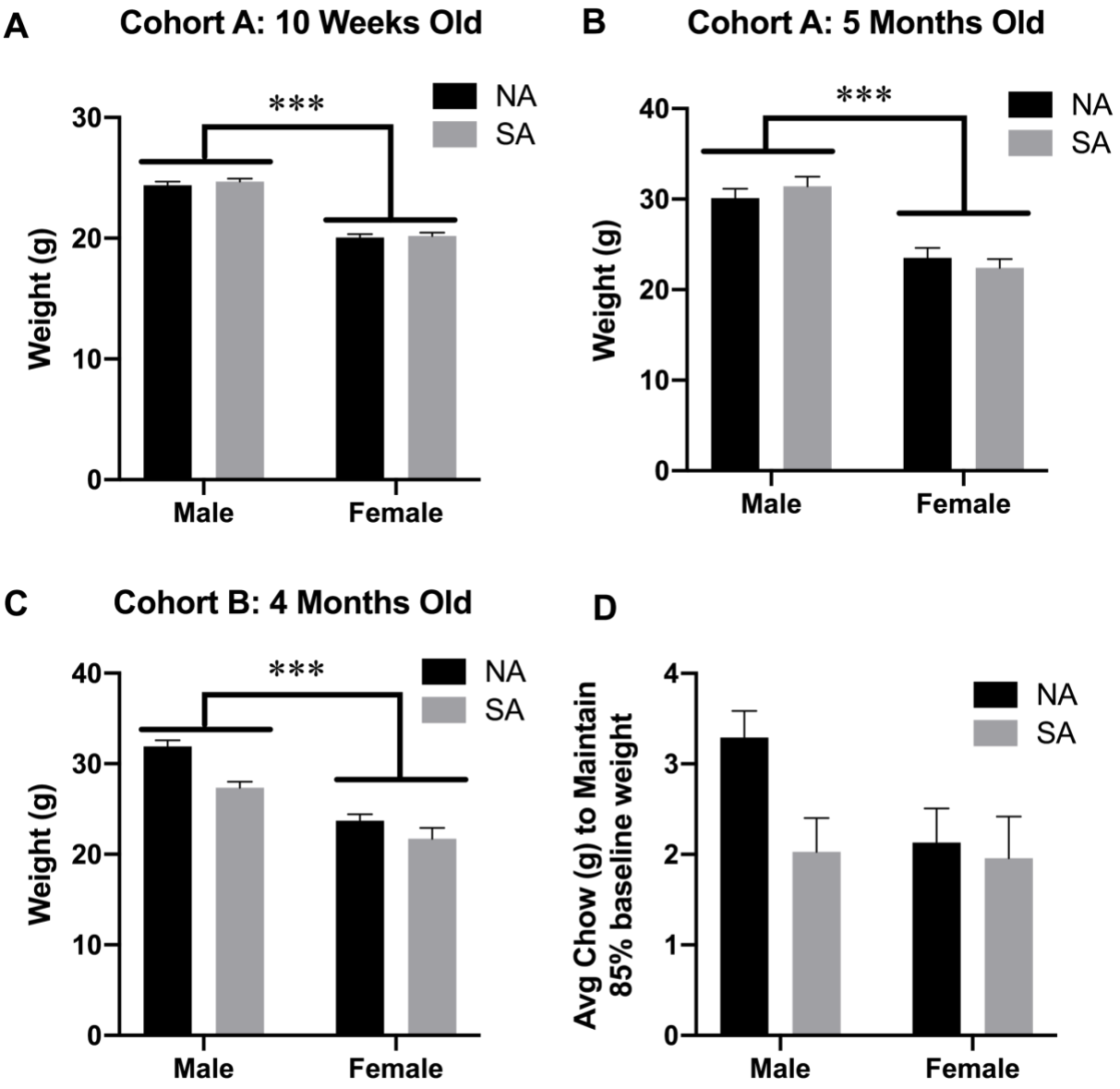

Figure 5.6. Perinatal SA exposure does not affect baseline body weight later in adulthood. Perinatal SA exposure does not induce alterations in baseline body weight at 10 weeks old (A) or 5 months old (B) in mice with prenatal and postnatal SA exposure (Cohort A). In mice with SA exposure only during the prenatal period (Cohort B), baseline body weight was unaffected by photoperiod exposure when assessed at 4 months old (C). During the effort-based decision-making task (EBDMT), mice were maintained at 85% of baseline weight. A trend toward a photoperiod effect was observed on the average amount of chow required to maintain 85% baseline weight (D), driven by SA-born males requiring more extreme food restriction compared to NA-born males.

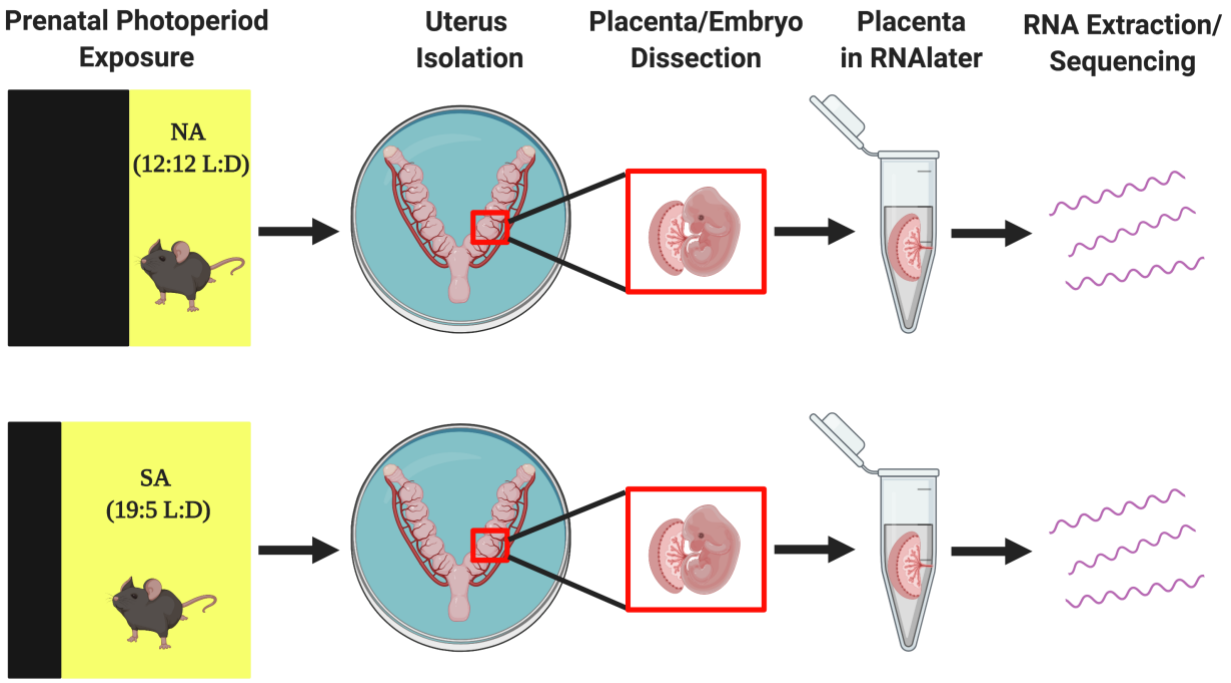

Figure 5.7. Experimental design for assessment of short active (SA; 19:5 light:dark (L:D)) photoperiod-induced placental gene expression changes. Timed-matings were set-up in either normal active (NA; 12:12 L:D) or SA photoperiod. Dams were euthanized at embryonic day (E) 12.5 or E18.5, and uterus isolation performed. The uterine wall was dissected away to isolate the embryo and placenta. Embryos and placentas were separated, and embryo and placenta weights were obtained. The placenta was stored in RNAlater at -80°C for subsequent RNA extraction and sequencing.

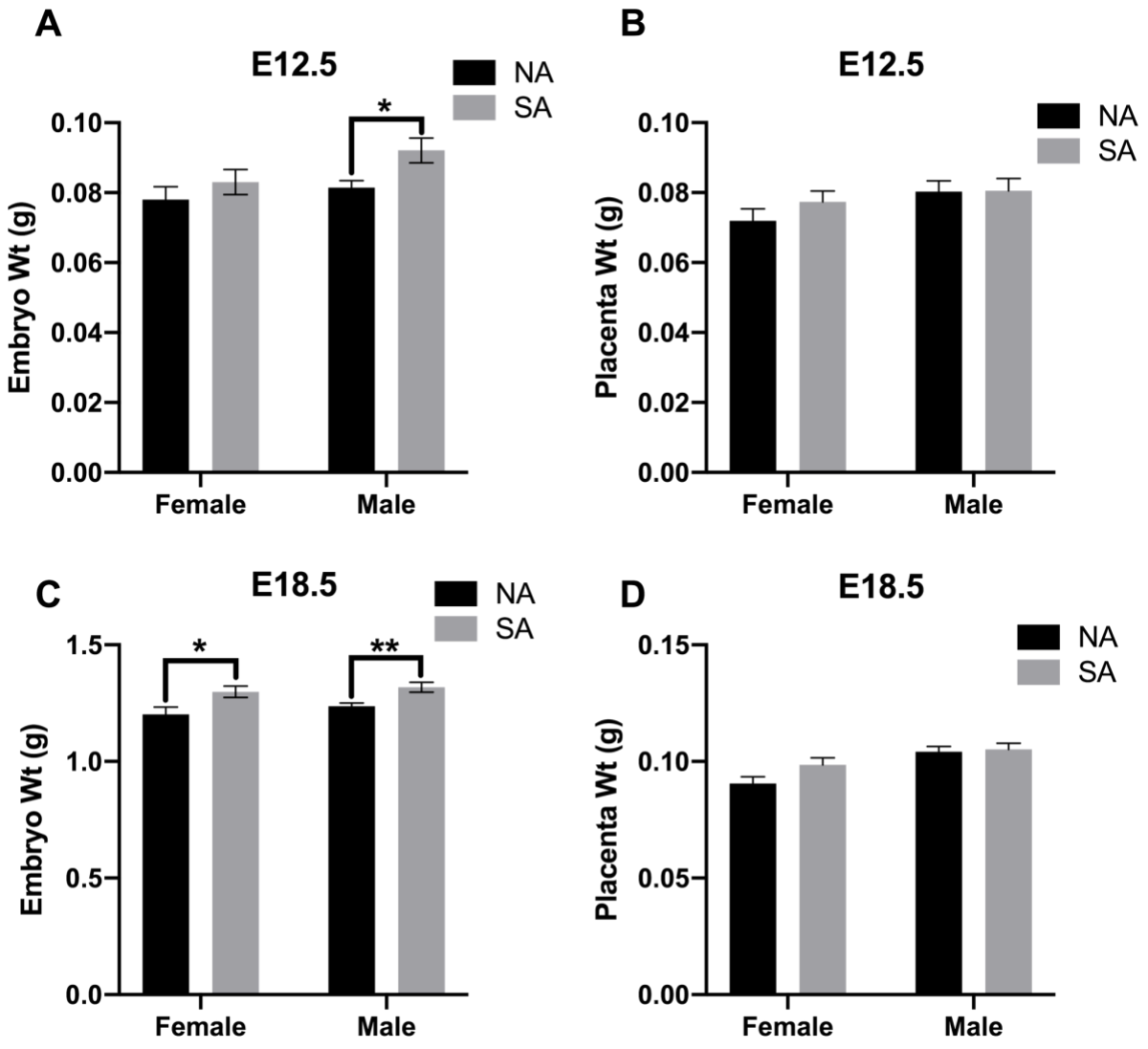

Figure 5.8. Prenatal SA photoperiod exposure increases embryo weight compared to NA-exposed embryos at both embryonic day (E) 12.5 and E18.5. (A) At E12.5, SA-exposed male embryos weighed more than NA-exposed male embryos. No photoperiod effect was observed on female embryo weight. (B) At E12.5, no photoperiod effects were observed on placenta weight in either sex. (C) At E18.5, SA-exposed embryos weighed more than NA-exposed embryos in both males and females. (D) At E18.5, no photoperiod effect on placenta weight was observed in either sex. Data presented as mean + S.E.M., *$p < 0.05$, **$p < 0.01$.

Table 5.1. Genes of interest that are regulated in the placenta by maternal stress, and predicted direction of short active (SA; 19:5 light:dark (L:D) photoperiod-induced effects. Placental genes shown to be regulated by maternal stress will be analyzed using RNA-Sequencing (RNA-Seq) data generated from normal active (12:12 L:D) and SA photoperiod-exposed placenta samples collected at embryonic day (E) 12.5 and E18.5. Predicted direction of SA-induced effects is included, as well as potential sex-specific effects in parentheses.

Gene Category	Gene	Predicted SA Effect	Gene Category	Gene	Predicted SA Effect
Stress-Related	*Nr3c1*	↓	Growth Factors/ Nutrient Transporters	*Slc2a1*	↓
	Nr3c2	↓		*Slc2a3*	↓
	Hsd11b2	↓		*Slc2a4*	↓
	Crhr1	↓		*Igf2*	↓
	Ogt	↓ (M > F?)		*Ppara*	↑ (M), ↓ (F)
Immune-Related	*Il1b*	↑		*Bdnf*	↓ (F > M?)
	Il6	↑		*Igfbp1*	↑ (M), ↓ (F)
	Il2ra	↑ (M > F?)	Epigenetic Control	*Dnmt1*	↑ (F > M?)
	Ccl5	↑ (M > F?)		*Dnmt3a*	↑
	Ccr7	↑ (M > F?)		*Dnmt3b*	↑
	Cxcl10	↑ (M > F?)		*Mbd1*	↑

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Chapter 6: Final Discussion

Final Discussion

Translating novel therapeutics for psychiatric disorders from animal models to patient populations has been particularly problematic, with efficacious treatments in animal models failing in clinical trials (reviewed by Kaffman and Krystal, 2012). This failure in translation is likely due to multiple factors. Traditionally, psychiatric disorders have been classified into diagnostic categories (e.g. “schizophrenia”) based on clinical rating scales rather than objective endpoints, resulting in heterogenous patient populations that might not all respond to the same treatment. Furthermore, our understanding of the pathophysiology of most psychiatric disorders remains limited. The wide range of symptoms observed in patients within a single diagnostic category, combined with the lack of objective biomarkers of disease, has made the generation of animal models particularly difficult and led to poor translational value (Kaffman and Krystal, 2012; Nestler and Hyman, 2010). As a result, the field of psychiatric research has undergone a shift from adhering to diagnosis-based research (e.g. “schizophrenia”) to conducting research based on observable behaviors that span diagnostic categories (Insel et al., 2010). For example, instead of attempts at generating an “animal model of schizophrenia”, studying amotivation through the lens of epidemiological factors that increase risk for disease might aid in identifying neural substrates and novel therapeutic targets that help patients who exhibit this symptom regardless of diagnostic category. In this way, we can begin to create model animals with better construct and predictive validity. Reproducibility of behavioral outcomes from such models, particularly in psychiatry where diagnoses are currently behavior-based, is essential to developing more effective treatments for patients.

In this dissertation, the dopamine transporter knockdown (DAT KD) mouse line was demonstrated to be a robust model of mania-relevant behaviors when utilized in the cross-species translatable behavioral pattern monitor (BPM; Chapter 2). Dopamine system dysfunction has been implicated in most major psychiatric disorders (Dichter et al., 2012), with

multiple genome-wide association studies (GWAS), case-control studies, and family cohort studies linking polymorphisms in *SLC6A3*, the gene encoding DAT, to bipolar disorder (BD) (Greenwood et al., 2006, 2001; Huang et al., 2015; Pinsonneault et al., 2011), schizophrenia (Greenwood et al., 2016; Huang et al., 2010; Kennedy et al., 2016; Zheng et al., 2012), and attention deficit hyperactivity disorder (ADHD) (Akutagava-Martins et al., 2016; Bidwell et al., 2011; De Azeredo et al., 2014; Franke et al., 2010; Gizer et al., 2009; Gomez-Sanchez et al., 2016; Hawi et al., 2010). Furthermore, DAT hypoexpression has been observed in post-mortem and PET imaging studies of BD brains (Anand et al., 2011; Rao et al., 2012), lending construct validity to DAT KD mice as a tool for modeling BD-relevant behaviors. Pharmacological predictive validity has also been demonstrated with the DAT KD mouse line in the BPM, as chronic valproic acid remediated the hyperactivity of DAT KD mice, consistent with valproate-medicated patients in the human BPM (Minassian et al., 2011; van Enkhuizen et al., 2013). Therefore, the DAT KD mouse line in the BPM is a robust, reproducible model for mania-relevant behaviors (Kwiatkowski et al., 2019; Chapter 2), exhibiting both construct and predictive validity, making it a suitable model for testing novel anti-mania therapeutics.

In light of the robustness in this model, in Chapter 3, the DAT KD/BPM model was used to test novel treatments aimed at reducing the hyperactivity and hyperexploration exhibited by DAT KD mice in the BPM. Results from these experiments suggest a role for long-term cholinergic agonism in the treatment of mania, as DAT KD mice exhibited reduced hyperactivity following chronic systemic nicotine administration (Chapter 3). The catecholaminergic-cholinergic balance model of BD hypothesizes that mania symptoms result from decreased cholinergic vs. catecholaminergic activity (van Enkhuizen et al., 2015), and remediation of DAT KD hyperactivity via nicotine administration suggests that restoring that balance could benefit patients with BD mania. Reversal of mania symptoms through cholinergic activation via acetylcholinesterase inhibitor administration has been reported in the literature (Carroll et al.,

1973; Dulawa and Janowsky, 2019; Janowsky et al., 1972), though risk for experiencing a depressive episode was increased (an effect not observed after chronic nicotine treatment). In rodents, presynaptic nicotinic acetylcholine receptors (nAChRs) regulate dopaminergic activity in the striatum (Brimblecombe et al., 2018; Cachope et al., 2012; Exley and Cragg, 2008). Nicotine can initially enhance burst firing of dopamine neurons in the striatum through nAChR desensitization (Rice and Cragg, 2004; Zhou et al., 2001). With chronic nicotine administration, long-term upregulation of most classes of nAChRs is observed (Gentry and Lukas, 2002). This nAChR upregulation might partially remediate the striatal hyperdopaminergia observed in DAT KD mice through suppression of dopamine neuron burst firing. It is difficult to generalize the effects of chronic nicotine administration, however, as effects may be receptor subtype-specific (Lai et al., 2005; Marks et al., 2014). Future work should determine how nicotinic regulation of dopamine neuron activity is altered when dopaminergic tone in the striatum is chronically elevated, as observed in DAT KD mice. Furthermore, testing whether chronic nicotine administration directly to the striatum remediates DAT KD hyperactivity needs to be assessed. A better understanding of brain region, neural circuit, and receptor subtype differences is required to identify therapeutic cholinergic targets that will result in the greatest benefit for patients with BD while minimizing side effects. Using this reproducible model of mania-relevant behaviors, identified targets may be more successful when translated to the clinic.

While examining adult rodent models of psychiatry-relevant behaviors provides useful information about disease states, many psychiatric disorders have neurodevelopmental origins (Gardener et al., 2009; Kloiber et al., 2020; Owen et al., 2011) that result from gene x environment interactions occurring as early as the perinatal period (reviewed by Esposito et al., 2018). Short active (SA; 19:5 L:D) photoperiod exposure induces depression-relevant behaviors and elevated baseline stress hormones in adult male rodents (Dulcis et al., 2013; Young et al., 2018). An elevated stress response to acute restraint stress was also observed in adult female

mice (Chapter 4), raising the possibility that SA photoperiod exposure during gestation might contribute to early gene x environment interactions that drive the development of psychiatry-relevant behaviors in offspring. Given work characterizing behavior in adult DAT KD mice using cross-species translatable tasks (Kwiatkowski et al., 2019; Milienne-Petiot et al., 2017; Perry et al., 2009; Young et al., 2011), and work showing elevated stress responses induced by SA photoperiod exposure (Dulcis et al., 2013; Chapter 4), Chapter 4 examined whether the DAT mutation interacted with altered perinatal photoperiod exposure to induce a hypersensitivity to development of psychiatry-relevant behaviors later in adulthood in offspring. Gestational and early life exposure to SA photoperiod induced sensorimotor gating deficits, reduced forced swim test (FST) immobility, less time spent in the open arms of the elevated plus maze (EPM; females only), less motivation to obtain a reward, and reversal learning deficits (Chapter 4) in WT mice, but DAT-HT mice were largely resilient to these effects. Genetic factors that impart resiliency to stressful environmental factors have been reported in both humans (Brummett et al., 2012; Donner et al., 2012; Ptáček et al., 2011; Segman et al., 2002; Skelton et al., 2012) and rodents (Ognibene et al., 2007). Polymorphisms in the gene encoding DAT have been associated with risk/resiliency for developing post-traumatic stress disorder (PTSD) following trauma exposure (Segman et al., 2002), supporting the idea that DAT alterations might imbue resiliency to effects of stress. Future work will determine the neural circuitry underlying resiliency to the behavioral effects of SA photoperiod exposure observed in DAT-HT offspring, and other genes linked to psychiatric disorders will be assessed to determine whether they contribute to risk or resilience to stressful environmental factors.

The susceptibility to SA-induced behavioral effects observed in WT mice was expected given the effects observed in adult rodents. Reduced immobility in the FST was previously reported using a similar gestational photoperiod manipulation (Green et al., 2015), supporting the inter-laboratory reproducibility, and thus validity, of this finding. Deficits in cross-species

translatable tasks such as the progressive ratio breakpoint task (PRBT; reduced breakpoint) and the probabilistic reversal learning task (PRLT; less switches completed), tasks that have been used to measure amotivated behavior in patients with schizophrenia (Fervaha et al., 2013; Gold et al., 2013; Reddy et al., 2016; Waltz and Gold, 2007), enable further investigation into seasonality of birth effects on behavior in psychiatric populations. It is important to note that SA-born mice did not exhibit the same behavioral profile as DAT KD mice in certain tasks. For example, DAT KD mice had an elevated breakpoint in the PRBT (Cagniard et al., 2006), whereas SA- born mice had a reduced breakpoint (Chapter 4). This discrepancy indicates that the dopaminergic system may be influenced by perinatal SA photoperiod in a distinct manner from the developmental effects of carrying the DAT mutation. It also suggests that other neurotransmitter systems (e.g. serotonin, norepinephrine) may be influenced by perinatal SA exposure. However, given that d-amphetamine partially remediated prenatal SA-induced effort-based decision-making task (EBDMT) deficits (Chapter 5), dopaminergic alterations may still play a role in the pathogenesis of SA-induced behaviors. Future work should utilize specific pharmacological agents to determine the role of dopamine in these behaviors, as well as the role of other neurotransmitter systems.

Given that prenatal and postnatal SA exposure was used in Chapter 4 to induce behavioral effects, potential mechanisms underlying SA-induced behaviors were broad. Sex-specific results observed in Chapter 4, including reduced prepulse inhibition (PPI) in SA-born males and an anxiogenic EPM profile observed in SA-born females (Chapter 4), pointed toward prenatal stress mechanisms as these findings are consistent with sex-specific changes resulting from prenatal stress exposure (reviewed by Sutherland and Brunwasser, 2018). PPI deficits have been observed in patients with schizophrenia (Braff et al., 1992; Swerdlow et al., 2014), and male offspring are more susceptible to developing schizophrenia spectrum disorders after prenatal stress exposure (Fineberg et al., 2016). Female offspring, on the other hand, are more

flexible in adapting to prenatal stress at the expense of subsequent increased susceptibility to developing anxiety/depression (Sandman et al., 2013). Maternal stress has been associated with increased risk for development of schizophrenia spectrum disorders in offspring (reviewed by Lipner et al., 2019). Furthermore, the behavioral effects of environmental factors associated with increased risk for psychiatric disorder development, such as maternal inflammation, have been shown to be timing and sex-dependent in humans (Mac Giollabhui et al., 2019). Therefore, the sex-specific differences observed fit into the existing prenatal stress literature and suggest similar mechanisms might be contributing to SA-induced behavior.

Following prenatal SA exposure, reduced FST immobility was replicated and reduced willingness to work for a larger reward was observed in an EBDMT (Chapter 5). Reproducibility of behavioral findings in psychiatric research is important for improving translational value of preclinical work and ensuring inter- and intra-laboratory consistency, as discussed in Chapter 2. Importantly, postnatal SA exposure did not reduce FST immobility (Chapter 5), indicating that prenatal mechanisms are more relevant to SA-induced effects. Given the relative resiliency of DAT-HT mice to perinatal SA exposure, dopaminergic mechanisms might contribute to the susceptibility of WT mice to photoperiod manipulation. This idea is supported by the results that low-dose d-amphetamine partially improved EBDMT performance in SA-born mice (Chapter 5). Assessing whether other pharmacological agents known to increase release of dopamine, such as nicotine (Benowitz, 2010), will remediate SA-induced behaviors will add to evidence that dopaminergic dysfunction contributes to photoperiod effects, and therefore should be included in future studies. In rats, exposure to glucocorticoids (GCs) during late gestation influences dopaminergic circuits, leading to reduced dopaminergic input to the nucleus accumbens (Leão et al., 2007). The DAT mutation might protect against SA-induced behavioral changes, leaving WT mice vulnerable to such effects. However, whether SA-induced behavioral effects are mainly driven by prenatal stress mechanisms remains a question that future work will address.

Recognition that the placenta has an active role in fetal brain development (Zeltser and Leibel, 2011), and in transmitting information regarding changes in maternal environment to the fetus (Jansson and Powell, 2007), has generated renewed interest in the role of the placenta. In particular, much work has assessed placental gene expression changes related to prenatal stress and its contribution to psychiatry-relevant behaviors (Bronson and Bale, 2014; Howerton and Bale, 2014; Mueller and Bale, 2008; Nugent et al., 2018). The placenta is responsive to maternal stress hormones, as it expresses GC receptors (GRs) during gestation and serves to protect the fetus from excessive maternal GC signaling. 11β-hydroxysteroid dehydrogenase type 2 (HSD2), a large component of this protective barrier, catalyzes the conversation of corticosterone into its inactive form, 11-dehydrocorticosterone. Neurogenesis in the fetal mouse brain occurs primarily during gestation (Chen et al., 2017; Finlay and Darlington, 1995), and 11β-HSD2 expression peaks around mid-gestation, acting to shield new neurons from the maturation effects of GCs (Brown et al., 1996; Diaz et al., 1998). Exposure to elevated levels of maternal stress hormones affects placental growth and gene expression (Cuffe et al., 2012), and maternal stress has been shown to alter expression of stress-responsive genes, including downregulation of *Nr3c1* (GR), *Nr3c2* (mineralocorticoid receptor), *Hsd11b2* (codes for HSD2), *Crhr1* (corticotropin releasing hormone receptor 1), and *Ogt* (O-linked *N*-acetylglucosamine (GlcNAc) transferase) (Briffa et al., 2017). Therefore, stress-induced disruptions in regulation of the placental GC barrier could negatively affect fetal neurodevelopment.

In addition to affecting placental expression of stress-responsive genes, prenatal stress affects other gene networks in rodents, including those involved in nutrient/oxygen transport (Briffa et al., 2017; Mueller and Bale, 2008), inflammatory response (Bronson and Bale, 2014; Gur et al., 2017), and epigenetics (Jensen Peña et al., 2012). Table 5.1 lists genes of interest that are regulated by maternal stress in the placenta, and the predicted direction of effect in SA-exposed placentas. The pending placental RNA-Sequencing (RNA-Seq) data from SA-exposed

placental samples will help guide future mechanistic studies aimed at identifying neural circuitry mediating SA-induced behaviors. Initial data showing increased weight in SA- vs. NA-exposed mouse embryos at both embryonic day (E)12.5 and E18.5 (Chapter 5) indicate that prenatal stress-induced metabolic dysfunction might be contributing to subsequent development of psychiatry-relevant behaviors. Seasonality of birth weight has been demonstrated in humans, with higher birth weights in late winter/spring compared to the rest of the year (Selvin and Janerich, 1971), lending validity to this finding. Additionally, the trend toward a photoperiod effect on the average amount of chow required to maintain 85% baseline weight during the EBDMT, with SA-born males requiring more extreme chow restriction compared to NA-born males (Chapter 5), also points to a potential long-term metabolic dysfunction induced by prenatal SA photoperiod exposure. In adult male mice, a more extreme short active photoperiod (21:3 L:D) induced a significant increase in corticosterone-induced weight gain (Kawai et al., 2018), indicating that similar photoperiods might be able to influence energy metabolism, perhaps via altered stress responses. In C57BL6/J male offspring exposed to a short active photoperiod (18:6 L:D) during early life (0-4 weeks old), body weight was significantly higher compared to mice exposed to a long active (6:18 L:D) photoperiod (Uchiwa et al., 2016). At 10 weeks old, SA-exposed mice exhibited altered plasma metabolomic profiles compared to LA-exposed mice, including higher levels of metabolites in the glycolytic pathway (e.g. pyruvic acid). Furthermore, expression of peroxisome proliferator-activated receptor δ (PPARδ), a gene involved in fatty acid metabolism, was reduced in skeletal muscle in SA-exposed mice (Uchiwa et al., 2016). Insulin acts in skeletal muscle to activate glycolytic pathways and decrease fatty acid oxidation (regulated by PPARδ) (Dimitriadis et al., 2011), indicating that insulin-related processes might be altered in SA-exposed mice. While these findings were a result of postnatal SA photoperiod exposure, some of these mechanisms might contribute to behavioral effects observed following prenatal SA exposure. Maternal metabolic dysfunction (e.g. diabetes) can

induce dysregulation of placental insulin receptors (InsR) (Colomiere et al., 2009; Desoye et al., 1992; Petropoulos et al., 2015). In mice, placental InsR deficiency induced increased stress response to acute restraint stress, as well as PPI deficits, in male offspring only (Bronson et al., 2017). In the same study, placental InsR deficiency induced repression of genes related to steroid hormone synthesis, amino acid metabolism/transport, and serotonin synthesis/clearance in the placenta. If SA photoperiod exposure induces maternal metabolic dysfunction (perhaps due to chronodisruption or stress-related processes), placental gene expression could be altered thereby causing changes in fetal neurodevelopmental programming. The pending placental RNA-Seq dataset enables answering preliminary questions regarding this possibility (e.g. assessing placental expression of InsR in NA- vs. SA-exposed placentas). Future work will determine maternal metabolic function in SA- vs. NA-exposed dams via plasma metabolomic analyses, stress response to acute restraint in NA- vs. SA-exposed offspring, and gene expression changes in the fetal brain that might contribute to programming of neurodevelopment.

This dissertation demonstrated the DAT KD mouse line in the BPM to be a robust, reproducible model of mania-relevant behaviors (Chapter 2) that can be used to test novel therapeutics (Chapter 3). Chronic nicotinic receptor agonism was identified as a potential anti-mania treatment option, though more research must be done to identify specific receptor subtypes and neural circuits mediating these benefits to minimize risks. DAT-HT mice demonstrated resiliency to behavioral effects of perinatal SA photoperiod exposure, whereas WT mice exhibited SA-induced behaviors across a wide range of ethologically-relevant and cross-species translatable tasks (Chapter 4). The prenatal period was identified as more relevant to inducing these behaviors, and prenatal SA exposure was shown to increase fetal weight in mice at E12.5 and E18.5 (Chapter 5). Pending placental RNA-Seq data will allow for identification of putative mechanisms underlying prenatal SA-induced behaviors. Taken

together, this translational work spans genetic, neurodevelopmental, and pharmacological approaches to better understand genetic and environmental influences on emergence of psychiatry-relevant behaviors. Using cross-species tasks with reproducible behavioral outcomes allows for findings to be more easily translated to human populations, increasing the efficacy of this work toward developing psychiatric therapeutics. More work is necessary to identify the neural circuitry mediating these effects, as well as to determine the mechanisms that allow for prenatal SA photoperiod programming of subsequent behavior in adulthood.

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