The Effects of Early Life Stress on Impulsive and Risky Decision-Making Behaviors

A Dissertation
Submitted to
the Temple University Graduate Board

In Partial Fulfillment
of the Requirements for the Degree
DOCTOR OF PHILOSOPHY

by
Evelyn Ordoñes Sanchez
December 2021

Thesis Committee:

Dr. Debra Bangasser, Thesis Advisor, Temple University Psychology Department
Dr. Mathieu Wimmer, Temple University Psychology Department
Dr. Lisa Briand, Temple University Psychology Department
Dr. Vinay Parikh, Temple University Psychology Department
Dr. Thomas Olino, Temple University Psychology Department
Dr. Stan Floresco, The University of British Columbia, Psychology Department

ABSTRACT

Early life stress is a prevalent problem affecting many worldwide and can be experienced in a variety of ways, including limited access to resources as in many low socioeconomic status households. In humans, exposure to stress early in life is linked to various psychiatric conditions such as substance use disorder, attention-deficit/hyperactivity disorder, and gambling. One characteristic that these disorders share is elevated impulsivity. Impulsivity is a multifaceted construct and often behaviors are classified as either an impulsive choice (e.g., inability to delay gratification) or an impulsive action (e.g., inability to inhibit premature responses). In the first set of experiments presented here, we characterize the limited bedding and nesting model (LBN) of early life adversity in rodents, in which rat dams and their pups are housed in a limited resource environment from postnatal day (PND) 2 through 9. This model works by inducing stress in the dams, which alters their maternal care behaviors towards pups. As a result, this altered care can be stressful for the developing pups. By using this model, we hope to garner more insight into the long-term effects it may have on addiction-related phenotypes such as impulsivity and risky decision-making behaviors. We have found that LBN exposure promoted resilience to addiction-related phenotypes in adult male, but not female rats. Specifically, LBN reduced impulsive choice, morphine self-administration, and nucleus accumbens (NAc) glutamate transmission in males, effects not seen in females. Additionally, changes in NAc gene transcription unique to LBN males may contribute to resilience. We build on these findings in the second set of experiments, which explores whether LBN alters impulsive action, risky decision-making, and the gene transcriptome of the orbitofrontal cortex (OFC). We found that LBN increased impulsive action in males. Additionally, we found LBN exposure in rats across sex reduces risky choice. These changes in behavior were accompanied by highly specific changes in gene transcription in the OFC, which is a brain region that mediates both impulsive and risky decision-making behaviors. The identification of genes and signaling pathways that are altered by LBN in the male OFC lays the groundwork for future studies investigating the mechanisms by which early life stress alters addiction-related phenotypes.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Debra Bangasser for her invaluable advice, continuous support, and patience (especially with my overuse of puns) during my PhD. Her knowledge and experience have helped me greatly throughout my academic career. Another big thank you is for Dr. Lisa Briand, Dr. Mathieu Wimmer, and Dr. Stan Floresco for all the learning opportunities and collaborative projects we have worked on together. I would also like to thank Dr. Thomas Olino and Dr. Vinay Parikh for their feedback and support as my external readers. Lastly, I want to extend my thank you to Dr. Brian Trainor and Dr. Karen Aboody who first exposed me to research and helped propel me to where I am today.

I would also like to extend my gratitude to my family. My mom for always letting me practice my talks with her even if she didn't quite understand what I was talking about, Jacky for always being there when I wanted to grab a glass of wine to whine, and Alexandra for always sending me TikToks that made me laugh. They have been there for me all the step of the way. I would also like to thank present and past members of the Neuroendocrinology & Behavioral Laboratory, especially Cory Ardekani, Dr. Sam Eck, James Flowers II, and Dr. Alexia Williams. They made coming into lab everyday so much fun! Also, special shout out to Dr. Charlotte Bavley, Hannah Mayberry, Andre Touissant, Anna McGrath, Melissa Knouse, and Dr. Megan Wickens (these folks are awesome current and former 9th floor homies). Lastly, I would like to thank Rahwa Woldeyesus, Cindy Valladares, and Victor Vasquez for being such wonderful and supportive friends all these years.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1A: THE EFFECTS OF EARLY LIFE STRESS ON IMPULSIVITY

Impulsivity is acting without forethought. In some cases, it pays to act impulsively. For instance, when one is in a dangerous situation, acting impulsively (e.g., landing the first punch in a fight) may improve chances of survival. Additionally, acting on impulse can stimulate creative moments and allow one to seize opportunities that they may have otherwise missed. Most of the time, acting impulsively is not considered pathological. However, when impulsivity impacts everyday life, it can constitute a risk factor for a large number of life-threatening behaviors (Bari & Robbins, 2013; Everitt et al., 2008; Fineberg et al., 2014; Gut-Fayand et al., 2001). High impulsivity is associated with several psychiatric disorders including, attention-hyperactivity disorder (ADHD), bipolar disorder, and addiction (e.g., gambling disorder and substance use disorder, SUD) (Adler et al., 2017; Dawe & Loxton, 2004; Najt et al., 2007; Vest, Reynolds, & Tragesser, 2016).

Another factor associated with ADHD, bipolar disorder, and addiction is exposure to early life adversity (ELS) (Halevi, Djalovski, Vengrober, & Feldman, 2016; Herzberg et al., 2018; Syed & Nemeroff, 2017). Changes in cognition contribute to symptoms of these disorders, and ELS can produce long-lasting effects on cognition (Bath, 2020; Chen & Baram, 2016; Grassi-Oliveira, Honeycutt, Holland, Ganguly, & Brenhouse, 2016; Hedges & Woon, 2011; Krugers & Joëls, 2014; Pechtel & Pizzagalli, 2011). For instance, among adolescents receiving treatment for substance use disorder, more than 70% report a history of trauma (Deykin & Buka, 1997; Funk, McDermeit, Godley, & Adams, 2003). Additionally, childhood trauma has been shown to predict ADHD onset and is a risk factor for the persistence of ADHD symptoms into adulthood (Biederman et al., 1995; Sugaya et al., 2012; Vrijsen et al., 2018). Because impulsivity is a characteristic of these disorders, it is important to understand how stressful experiences in childhood may aggravate these symptoms. Much work has focused on how ELS impairs executive functioning, memory, and reward processing (Hostinar, Stellern, Schaefer, Carlson, & Gunnar, 2012; Pechtel & Pizzagalli, 2011). However, here we will detail how ELS can also affect impulsivity. We first will describe types of impulsive behaviors and the circuits that underly impulsive processes. Then, we will detail how ELS affects impulsivity and the purported mechanisms by which this can occur. Throughout we will compare preclinical to clinical findings and highlight, when known, sex differences in these effects. Importantly, a better understanding of how ELS affects impulsivity may reveal mechanisms that contribute to risk and resilience to certain psychiatric disorders.

Different Types of Impulsive Behaviors

Because impulsivity is a multifaceted construct, an attempt to categorize impulsivity has been to organize impulsive behaviors into three distinct types (Robbins & Dalley, 2017). The first type, waiting impulsivity, encompasses impulsive behaviors that require a subject to wait before getting a reward. Behaviors associated with this branch are measured using delay discounting, differential-reinforcement-of-low-rates-of-responding (DRL), and five-choice-serial-reaction-time tasks (5-CSRTT). For DRL and 5-CSRTT, impulsivity depends on the ability to prevent an inappropriate, premature response. Inability to withhold a premature response is termed an impulsive action. In delay discounting, impulsivity is associated with choosing a small, immediate reward over a large, delayed one. A subject's preference for instant gratification over delayed gratification is considered an impulsive choice. The second branch, risky impulsivity, refers to a subject's preference for uncertain but bigger outcomes. A task used to measure this type of behavior in rodents is the probability discounting task, which involves choosing between two levers: one dispenses a small reward every time it is pressed versus another which dispenses a larger reward sometimes (risky lever). Choosing the riskier option is indicative of higher levels of impulsivity and these responses can be considered a form of impulsive choice. However, there is some debate as to whether risky behavior is the same as impulsivity. For example, studies that use delay and probability discounting show that steeper discounting in one measure does not predict the results of the other, suggesting these processes are actually different (Herman, Critchley, & Duka, 2018; Holt, Green, & Myerson, 2003). Nevertheless, risk-taking is closely related to impulsivity and can predict the likelihood of one pursuing hazardous behavior (Donohew et al., 2000). Finally, the last type, stopping impulsivity, refers to the inability to stop a response after it has been initiated. Tasks commonly used to measure stopping behavior are go-no-go and stop-signal reaction time task. Given these tasks require stopping an initiated action, they also measure impulsive actions.

Differentiating between various forms of impulsivity not only has implications for experimental design but also for understanding risk factors for psychiatric disorders. There is evidence that different forms of impulsivity are associated with disorders to varying degrees. For example, some evidence suggests pathological gambling is associated with impulsive action but not impulsive choice (Brevers et al., 2012). In

ADHD, both impulsive choice and impulsive action are disrupted but these measures relate to different symptoms: increased impulsive choice is associated with a broad range of ADHD symptoms, including hyperactivity, while impulsive action is related more specifically to executive control (Solanto et al., 2001). Parsing impulsivity is also informative for understanding different aspects of SUD (Broos et al., 2012). In a rodent model of nicotine seeking, impulsive action was associated with an enhanced motivation for nicotine self-administration, while impulsive choice was associated with enhanced vulnerability to cue-induced relapse (Diergaarde et al., 2008). These distinctions are driven by the differences in circuitry, which will be detailed below. Collectively, these data underscore the value of studying distinct aspects of impulsivity.

Dopamine Modulates Impulsivity

Dopamine (DA) is linked to impulsivity in humans and rodents. Clinically, disorders with high impulsivity as a key feature are commonly associated with DA dysregulation (Ashok et al., 2017; Pettorruso et al., 2019; Rosa-Neto et al., 2005; Whitton, Treadway, & Pizzagalli, 2015). It is believed that hypodopaminergic function may contribute to drug abuse and underlie behavioral abnormalities observed in ADHD and bipolar disorder (Badgaiyan, Sinha, Sajjad, & Wack, 2015; Berk et al., 2007; Blum et al., 2008; D. A. Cousins, Butts, & Young, 2009; Fattore & Diana, 2016; Gold, Blum, Oscar-Berman, & Braverman, 2014; Sanna, Fattore, Badas, Corona, & Diana, 2021). Administration of psychostimulants, like amphetamine or methamphetamine, are often prescribed for ADHD or bipolar disorder to help reduce impulsivity symptoms (Perugi, Vannucchi, Bedani, & Favaretto, 2017; Wolraich et al., 2019). These types of drugs block DA transporters (DAT), which promotes increased DA levels in the brain (Kuczenski & Segal, 1997). Although these treatments can help alleviate impulsive symptoms in these disorders, nonmedical use of stimulants by people who do not have these disorders promotes impulsive behaviors (Grant, Redden, Lust, & Chamberlain, 2018; Messina et al., 2014).

By using rodent models, we can more specifically parse how changes in DA signaling impact different types of impulsive behavior. Acute administration of amphetamine in rodents often increases premature responses and impairs behavioral inhibition (Baarendse & Vanderschuren, 2012; Britton & Koob, 1989; Caballero-Puntiverio, Fitzpatrick, Woldbye, & Andreasen, 2017; Hayton, Maracle, & Olmstead, 2012; van Gaalen, Brueggeman, Bronius, Schoffelmeer, & Vanderschuren, 2006). However, some have found that acute doses of this drug can also reduce premature responding (Hayton et al., 2012). For instance, rats trained on a response inhibition task, which requires them to withhold pressing a lever until signaled to do so, amphetamine administration increases their impulsive action when the delay to respond is fixed, but reduces it when the delay is variable (Hayton et al., 2012). Therefore, acute doses of this psychostimulant alter impulsive action and its effect may depend on the subject's expectation of task demands. Chronic administration of other DA agonist drugs, like ropinirole hydrochloride, can also increase premature responding, especially when the cues to the reward are signaled (Tremblay, Barrus, Cocker, Baunez, & Winstanley, 2019). Cues predicting reward elicit activity in dopaminergic neurons, which has been shown to sensitize rats to the hyperlocomotor effects of stimulants (Zack, Featherstone, Mathewson, & Fletcher, 2014).

Interestingly, acute, low doses of stimulant administration can improve the ability to wait for large rewards (S. B. Floresco, M. T. Tse, & S. Ghods-Sharifi, 2008; Wade, de Wit, & Richards, 2000; Catharine A. Winstanley, Dalley, Theobald, & Robbins, 2003). For instance, low doses of amphetamine (0.01-0.25 mg/kg) can shift choice towards large, delayed rewards on delay discounting tasks, therefore reducing impulsive choice (S. B. Floresco et al., 2008; van Gaalen, van Koten, Schoffelmeer, & Vanderschuren, 2006). Similarly, DAT blockade, which also results in an increase in DA levels, reduces impulsive choice (van Gaalen, van Koten, et al., 2006). However, some studies have reported that elevations in DA do not always decrease or affect impulsive choice (Cardinal, Robbins, & Everitt, 2000; Evenden & Ryan, 1996; Helms, Reeves, & Mitchell, 2006; Zeeb, Soko, Ji, & Fletcher, 2016). In one study, high doses of amphetamine (1-3mg/kg) decreased lever pressing and chow consumption in a lever pressing/feeding choice procedure (M. S. Cousins, Wei, & Salamone, 1994). Therefore, lower doses, but not high levels of psychostimulants, increase choice for larger, delayed rewards.

Even though it is tempting to assume increases in DA levels decrease impulsive choice and increase impulsive action, it’s possible the effects of DA on impulsivity may be less linear and more like an inverted-U. In other words, depending on where one lands on the curve, shifts in DA levels, can improve or impair impulsivity symptoms (J. W. Dalley & Roiser, 2012). For instance, oral administration of methylphenidate reduces impulsivity only in high, but not low, impulsive rats as determined by the 5-CSRTT

(Caprioli et al., 2015). These results suggest that the efficacy of certain stimulant treatments is baseline-dependent and is comparable to the efficacy of psychostimulants in human populations. For instance, treatments that can benefit impulsive individuals (presumably with suboptimal baseline DA signaling) can also impair performance in people who have low levels of impulsivity (de Wit, Crean, & Richards, 2000; Petzold et al., 2019).

Genetic factors can also influence the effect of DA on impulsive behavior (J. W. Dalley & Roiser, 2012; Loos et al., 2010; Simon et al., 2013). For instance, lower dopamine 2 (D2) mRNA receptor expression in the nucleus accumbens (NAc) is correlated with higher impulsive action (Simon et al., 2013). These results are similar to positron emission tomography or autoradiography studies that show impulsive rats have reduced D2 receptor availability in the NAc (Dalley et al., 2007; Jupp et al., 2013). Similar reductions in ventral striatal D2 receptors are also found in highly impulsive methamphetamine-dependent individuals (Kohno et al., 2016; Lee et al., 2009; London, 2020). These findings suggest the expression of DA-related genes influence impulsive traits. Collectively, these studies highlight DA's involvement in impulsivity.

One caveat with these data is that they were collected only in male rodents. However, various reviews have highlighted the fact that there are sex-specific effects in dopamine circuitry that are influenced by gonadal, chromosomal, and even epigenetic factors (Becker & Chartoff, 2019; Eck & Bangasser, 2020; Kokane & Perrotti, 2020; Zachry et al., 2021). For instance, males, but not females, overproduce D1 and D2 receptors in the striatum early in development (Susan L. Andersen, Rutstein, Benzo, Hostetter, & Teicher, 1997; S. L. Andersen & Teicher, 2000). Additionally, even though baseline firing activity of DA neurons in the VTA of male and female rodents is similar (Locklear, Michaelos, Collins, & Kritzer, 2017; Rincón-Cortés & Grace, 2017), circulating levels of estradiol (E2) can affect neuronal activity. For instance, electrically stimulated phasic DA release in the VTA is higher in estrous females as compared to males or non-estrus females (Calipari et al., 2017). E2 also has been shown to regulate D1 receptor binding in the ventral striatum of female rats (Lévesque, Gagnon, & Di Paolo, 1989). Although these are only a handful of studies, these data illustrate that there are sex differences in the DA system and highlight the importance of including both sexes in experimental designs.

The NAc & Impulsivity

The NAc is a major component to the ventral striatum that receives DA and facilitates reward-seeking (Berridge & Robinson, 1998; Salamone, 1994; Trifilieff et al., 2013). The NAc is commonly subdivided into two parts: the core and shell (Zahm & Brog, 1992). Inputs from cortical and striatal brain areas display unique topographical organization throughout the NAc (Brog, Salyapongse, Deutch, & Zahm, 1993; H. J. Groenewegen, der Zee, te Kortschot, & Witter, 1987; Kelley & Domesick, 1982; Spooren, Veening, Groenewegen, & Cools, 1991). Dorsal structures like the anterior cingulate cortex (ACC), target the NAc core, while ventral structures like the basolateral amygdala (BLA) and infralimbic cortex target the NAc shell (Brog et al., 1993; H. J. Groenewegen, Wright, Beijer, & Voorn, 1999; Sesack, Deutch, Roth, & Bunney, 1989). Some structures, such as the orbitofrontal cortex (OFC), project to both the core and shell

(Brog et al., 1993). Efferent fibers of the NAc as a whole project to the midbrain, hypothalamus, and ventral pallidum as well as other motor systems (Brog et al., 1993; Henk J. Groenewegen & Russchen, 1984). As such, the NAc is a hub that receives and relays information to motor association sites in charge of carrying out appropriate behaviors.

NAc functions are regulated by DA. DA signaling in the NAc can occur via two classes of DA receptors. D1-like receptors (D1 and D5 receptors) stimulate postsynaptic adenylyl cyclase activity, while D2-like receptors (D2, D3, and D4 receptors) inhibit this signaling (Gingrich & Caron, 1993; Hopf, Cascini, Gordon, Diamond, & Bonci, 2003; Sibley & Monsma, 1992). In other words, when DA binds to a D1-expressing neuron this causes increased firing (Surmeier, Ding, Day, Wang, & Shen, 2007). In contrast, when DA binds to a D2-expressing neuron, this causes a reduction in firing.

Work from van Gaalen has highlighted the involvement of D1 and D2 receptors in regulating inhibitory control and impulsive decision-making. For instance, selectively antagonizing D1 receptors in the NAc core and shell reduces premature responding (T. Pattij, Janssen, Vanderschuren, Schoffelmeer, & van Gaalen, 2007). Likewise, systemic blockade of D1 receptors increases impulsive choice (van Gaalen, van Koten, et al., 2006). Antagonizing D2 receptors has no effect on behavior on its own, but when followed by systemic amphetamine administration, it can block premature responding and impulsive choice (T. Pattij et al., 2007; van Gaalen, van Koten, et al., 2006). These data illustrate that DA D1 and D2 receptors play important, but perhaps distinct roles, impulse control.

Impulsive action is modulated by DA signaling in the NAc. For example, low NAc D2 receptor expression, which reduces DA inhibitory drive, increases premature responding in rats (Jupp et al., 2013). Similarly, increasing DA signaling by reducing DAT function in the NAc increases impulsive action (Jupp et al., 2013). Interestingly, these effects are specific to the NAc shell and high DA release to the shell is associated with increased premature responding (Diergaarde et al., 2008). Moreover, lesions to the NAc shell block amphetamine-induced premature responding, which may disrupt DA related effects of this stimulant (E. R. Murphy, Robinson, Theobald, Dalley, & Robbins, 2008). These findings have led to the idea that premature responding is due to excess DA levels in the shell region of the NAc (J. W. Dalley & Robbins, 2017).

While increased dopaminergic drive in the NAc shell increases impulsive action, evidence suggests that decreased dopaminergic drive in the NAc core increases impulsive choice. Specifically, overexpression of DAT in the NAc core, which would lower levels of available DA, increases impulsive choice (Adriani et al., 2009). Additionally, D1 antagonism in the NAc core decreases sensitivity to reinforcer magnitudes (Yates & Bardo, 2017). In other words, blocking D1 signaling shifts preference away from large rewards even when its delivery is immediate. A potentially contrary finding is that reduced D2/3 receptor binding in the NAc core is associated with impulsive choice (Barlow et al., 2018). A reduction in postsynaptic D2/3 receptors would increase dopaminergic tone in the NAc. However, presynaptic D2 receptors on dopaminergic neurons act as autoreceptors, limiting DA synthesis and release (Beaulieu & Gainetdinov, 2011; Gingrich & Caron, 1993). Viral knockdown of D2 presynaptic receptors in the

VTA, the major source of dopamine for the NAc, increases choice impulsivity (Bernosky-Smith et al., 2018). Taken together, these studies support the idea that reducing dopaminergic drive in the NAc core increases impulsive choice.

Electrophysiology studies also support pharmacological data that DA signaling in the NAc regulates impulsivity (Pan, Schmidt, Wickens, & Hyland, 2005; M. R. Roesch, Calu, & Schoenbaum, 2007; Saddoris, Sugam, et al., 2015). Phasic DA release in the NAc tracks certain aspects of value-based decision-making (M. R. Roesch et al., 2007; Saddoris, Sugam, et al., 2015). This is interesting because phasic DA bursts are known to occur in response to reward-predictive cues (Day, Jones, Wightman, & Carelli, 2010; Schultz, Dayan, & Montague, 1997), whereas dips in DA firing are associated with omissions of expected reward (Saddoris, Sugam, et al., 2015). One study that only used female rats found that bursts and dips in phasic DA signaling could function as a teaching signal to facilitate reward-related learning (Steinberg et al., 2013). Indeed, studies that override or inhibit phasic DA signals have shown that this can change behavior in male rodents (Fitzpatrick et al., 2019; Colin M. Stopper, Tse, Montes, Wiedman, & Floresco, 2014). For instance, suppressing DA bursting that typically occurs when a rat chooses its preferred reward option will biases it to discontinue selecting that option in subsequent trials (Saddoris, Sugam, et al., 2015). Lastly, each subregion of the NAc encodes DA signaling differently: DA release to core is associated with tracking value of predicted outcomes, whereas DA release to shell tracks motivationally salient stimuli (Saddoris, Cacciapaglia, Wightman, & Carelli, 2015). These data indicate that neurons in the NAc core and shell can encode DA signaling differently and further supports the notion that each subregion has dissociable effects in regulating impulsivity. A schematic depicting the role of the NAc core and shell in impulsive action and impulsive choice is shown in Figure 1. Notably only one of the above studies used females instead of males and none compared the sexes, so more studies including both sexes are needed to identify any similarities/differences in DA signaling in the NAc.

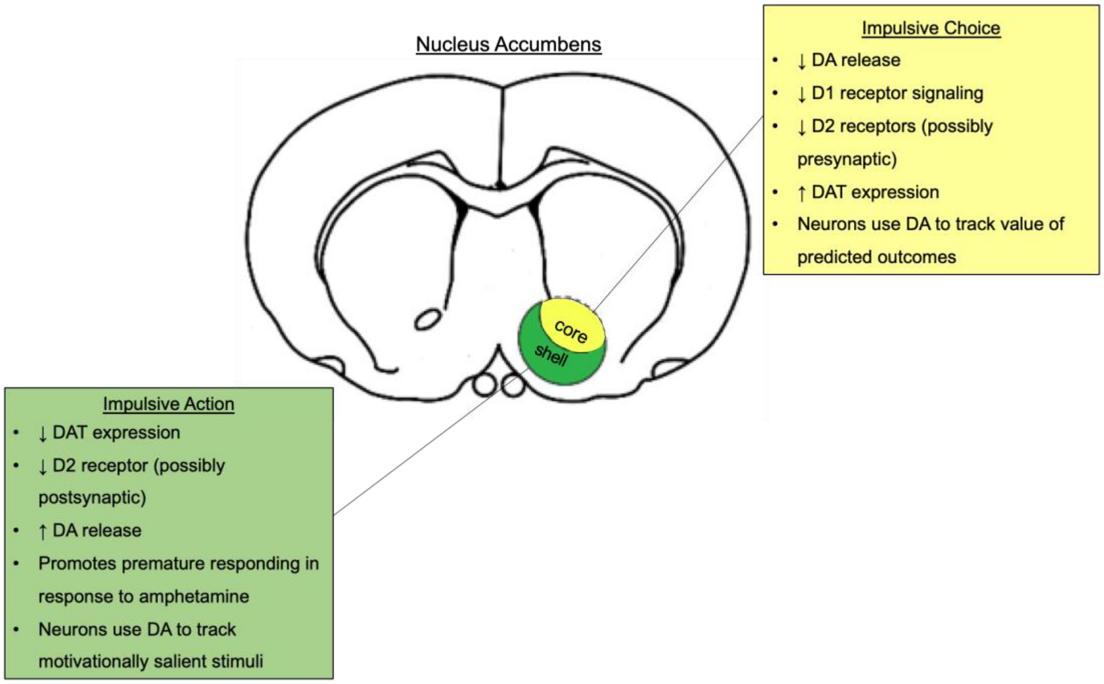


Fig. 1. A schematic showing how the NAc core and shell regulate aspects of impulsivity. Changes in the dopaminergic system that increase DA tone in the shell promote impulsive action. In contrast, changes in the dopaminergic system that decrease DA signaling in the core promote impulsive choice. DA, dopamine; D1 receptor, dopamine 1 receptor; D2 receptor, dopamine 2 receptor; DAT, dopamine transporter.

Cortical Involvement in Impulsivity

In addition to the NAc, different areas of the prefrontal cortex are associated with mediating different forms of impulsivity. In particular, the OFC and medial prefrontal cortex (mPFC) have received a lot of attention due to their involvement in different aspects of impulsivity (Dalley & Ersche, 2019; Dalley, Everitt, & Robbins, 2011). The type of impulsivity mediated by these areas differs and provides an anatomical basis for multiple impulsivity constructs.

The OFC is generally associated with impulsive choice in rodents and humans (Bechara, Tranel, & Damasio, 2000; Mar, Walker, Theobald, Eagle, & Robbins, 2011; Mobini et al., 2002; Tommy Pattij & Vanderschuren, 2008; Zeeb, Floresco, & Winstanley, 2010). Several studies have reported changes in impulsive behavior in OFC-lesioned rats. Some studies report OFC lesions make male and female rats more impulsive and less likely to wait for delayed rewards (Mobini et al., 2002; Rudebeck, Walton, Smyth, Bannerman, & Rushworth, 2006). These results suggest that the OFC is necessary for responding when rewards are delayed. Others report that OFC lesions make rats less impulsive, which may indicate it is necessary for devaluing the delayed reward (C. A. Winstanley, Theobald, Cardinal, & Robbins, 2004). Possible reasons for the discrepancies are attributed the heterogeneity of the OFC region as well as when the lesion took place (e.g., pre-training vs. post-training). For example, if the OFC is lesioned prior to a rat learning a rodent gambling task, it slows down its ability to reach task acquisition (Zeeb & Winstanley, 2011). OFC lesions that occur after training, however, have no effect on risky choices on this task. These findings point to the OFC being involved in learning and that lesions that occur early in training could impair its ability to keep track of outcome values to guide future responding. Single unit electrophysiological recordings also illustrate a role for the OFC in delay discounting behavior. Activity of different neurons in the OFC reflected a rat's preference for immediate, small rewards or large, delayed rewards (Roesch, Taylor, & Schoenbaum, 2006). These results suggest that different groups of neurons in the OFC track delayed rewards and outcome expectancies. Additionally, attenuating neural activity in the OFC, while simultaneously enhancing NAc neural activity, impairs impulse control (Meyer & Bucci, 2016). Collectively, these data provide evidence that the OFC modulates impulsive choice behavior.

Unlike the OFC, the mPFC is generally associated with impulsive action as it plays a role in restraining premature responses (Cho & Jeantet, 2010; B. Li, Nguyen, Ma, & Dan, 2020; Tommy Pattij & Vanderschuren, 2008). Specifically, projections from the dorsomedial PFC to the subthalamic nucleus (STN) can inhibit premature responding (B. Li et al., 2020). The STN is a structure implicated in motor control and STN lesions can increase impulsive action (Baunez, Nieoullon, & Amalric, 1995; Guillaumin, Serra, Georges, & Wallén-Mackenzie, 2021; Uslaner & Robinson, 2006). These results suggest that the mPFC can signal to downstream structures like the STN to suppress inappropriate motor driven responses. Functional disconnection of the mPFC-NAc pathway also increases impulsive actions, an effect attributed to disrupting this "top-down" control over behavior (Christakou, Robbins, & Everitt, 2004). One caveat with mPFC lesion studies is that sometimes they may include the ACC (Cho & Jeantet, 2010; Pezze, Dalley, & Robbins, 2009). The ACC itself is involved in many different processes related to impulsivity such as error detection (Bryden, Johnson, Tobia, Kashtelyan, & Roesch, 2011), cognitive control (Newman, Creer, & McGaughy, 2015), and response selection (Bussey, Everitt, & Robbins, 1997; Newman et al., 2015). It is thought that the ACC is involved in detecting situations where behavioral response is ineffective and processes this information to guide behavior. Lesions to the ACC increases premature responses and reduces accuracy on the 5-CSRTT (Chudasama et al., 2003; J. L. Muir, Everitt, & Robbins, 1996). Additionally, activation of layer-5 pyramidal cells of the ACC impairs behavioral disinhibition (van der Veen et al., 2021). Thus, both the mPFC and ACC modulate impulsive action.

Like the NAc, these cortical regions receive dopaminergic input from the VTA (Oades & Halliday, 1987). DA in the cortex can also modulate impulsivity (Puumala & Sirviö, 1998). For instance, dopaminergic depletion in the OFC of rats decreases impulsive choice (Kheramin et al., 2004). This finding may indicate high levels of DA in the OFC may underlie discounting behavior. *In vivo* microdialysis data support this idea by finding increased levels of 3,4-dihydroxyphenylacetic acid (DOPAC) when rats make delay discounting judgements (C. A. Winstanley, Theobald, Dalley, Cardinal, & Robbins, 2006). Increases in DOPAC could reflect increased DA utilization occurring in the OFC during delay discounting. In regards to the mPFC, antagonism of D1 and D2 receptors reverses the effects of amphetamine-induced premature actions and improves timing of responses (Cheng & Liao, 2017). However, low D2 mRNA receptor expression in the prelimbic region of the mPFC is correlated with high impulsivity (Simon et al., 2013). These results highlight that DA signaling via these two receptors in the mPFC mediates impulsive action. Altogether, these data indicate that DA in the OFC and mPFC can also affect impulsivity.

Models of Early Life Stress

ELS in rodents can be studied using a variety of different models which include prenatal stress, as well as postnatal stress typically in the form of maternal separation or limited bedding/nesting (LBN). These models have helped researchers further understand stress effects on impulsive behavior. Models of prenatal stress aim to stress a pregnant dam during different time-points of gestation (e.g., early, middle, or late) by using various stressors (e.g., restraint, noises, and lights), and different lengths of time (e.g., 3 times a day vs. 3 weeks) (Mueller & Bale, 2008; Soares-Cunha et al., 2018; Van den Hove et al., 2006; Weston, Weston, Allen, & Cory-Slechta, 2014; Wilson, Schade, & Terry, 2012). Offspring of stressed pregnant dams typically exhibit elevated corticosterone release in response to an acute stress exposure (30 min of restraint), which indicates heightened hypothalamic-pituitary-adrenal (HPA) axis reactivity (Cory-Slechta, Virgolini, Thiruchelvam, Weston, & Bauter, 2004; Soares-Cunha et al., 2018). Maternal separation models are used as a postnatal stressor, and they disrupt dam-pup interactions by separating the pups from their mother for intermittent periods (e.g., 15 minutes to 24 hours per day) for one to three weeks postnatally (Millstein & Holmes, 2007; Molet, Maras, Avishai-Eliner, & Baram, 2014). Shorter variations of this model are said to result in increased levels of maternal care and increased stress resiliency in offspring, however longer variations induce impaired maternal care (Eck & Bangasser, 2020; Nishi, Horii-Hayashi, & Sasagawa, 2014). To control for variations in maternal care, some manipulations employ artificial rearing procedures, where pups are separated from dams and experimenters mimic various amounts of pup care (e.g., low or high) with "maternal licking-like stimulations" via a wet paintbrush (Burton et al., 2007; Lovic, Keen, Fletcher, & Fleming, 2011).

Another postnatal stressor is the limited bedding and nesting (LBN) manipulation, which is typically implemented during a pup's first week of life (postnatal day 2-9). This low resource environment aims to mimic aspects of poverty (Eck et al., 2019; Gilles, Schultz, & Baram, 1996; Molet et al., 2014). This manipulation induces stress in dams, altering their maternal care towards their developing pups (Ivy, Brunson, Sandman, & Baram, 2008; Rice, Sandman, Lenjavi, & Baram, 2008). One of the advantages of the LBN paradigm compared to maternal separation models is that it can induce changes in maternal care with limited external experimenter interventions. Rather than physically removing the dam from her pups to induce changes in maternal care, the LBN manipulation causes changes in care by altering the environment the animals are placed in. Additionally, data from human studies of chronic childhood stress, including war, famine, and neglect/abuse suggest that the mother is typically present, even though her behavior may be abnormal. Although few studies have begun to study or implement this model, it has strong translational potential (Walker et al., 2017).

Even though stress from these ELS models can dissipate quickly, exposure to these stressful manipulations early in life can cause long-lasting changes to reward-related networks (Dubé et al., 2015; Eck et al., 2019; Huang, 2014). Utilization of these models has also allowed researchers to better understand the neurobiological mechanisms underlying how stress persistently alters behavior. The type/severity of stress experienced, as well as the timing and duration of the stressor can influence its specific outcomes on the brain and behavior. Although different models of ELS may produce slightly different results, they have been useful for examining mechanisms that affect impulsive behavior.

Dissociable Effects of Early Life Stress on Impulsivity

Relatively few studies have examined how prenatal stressors affect impulsive action. One study did find that male and female rats exposed to prenatal stress made more premature responses on a challenging version of the 5-CSRTT (Wilson et al., 2012). Specifically, when the intertrial interval times increased, these rats were unable to withhold responding as compared to controls. In regards to postnatal stressors, work from Lovic and colleagues found that male and female rats that undergo a severe form of early life deprivation—artificial rearing with low simulated grooming—are unable to withhold premature responses as adults compared to control rats while performing DRL tasks (Lovic et al., 2011). Similarly, male rats which experienced maternal deprivation, make significantly more premature responses while performing the 5-CSRTT as compared to controls (Kentrop et al., 2016). Females however were not examined in this study. Together, these studies indicate that ELS can increase impulsive action. Table 1 summarizes the effects of ELS on impulsive action.

Stress Model	Species	Effect of ELS on Impulsive Action	Sex Affected	Citation
Motherless Rearing (i.e., artificial rearing) + Social Isolation	rats	increase	males	Lovic, Kleen, Fletcher, & Fleming, 2011
Motherless Rearing (i.e., artificial rearing) + Social Isolation	rats	increase	females	Lovic, Kleen, Fletcher, & Fleming, 2011
Maternal Separation	rats	increase	males	Kentrop et al., 2016
Variable Prenatal Stress	rats	increase	male & female data combined	Wilson et al., 2012

Table 1. Summary of ELS effects on impulsive action in male and female rodents.

In contrast to the enhancing effect of ELS on impulsive action, ELS tends to decrease impulsive choice. Specifically, maternal separation plus early social isolation reduces impulsive choice on delay discounting tasks in male but not female rats (Lovic et al., 2011). Reductions in impulsivity in delay discounting have been found in male, but not female rats that were reared in LBN (Ordoñes Sanchez et al., 2021). Prenatal stress and exposure to low levels of lead reduces impulsive choice in males but has no effects in females (Weston et al., 2014). In contrast, female, but not male rats, exposed to a surrogate mother while simultaneously being raised in a low resource environment early in life had reduced impulsivity in delayed discounting (Fuentes et al., 2014). These discrepancies between these studies in the sex impacted suggest that the social experience of having a surrogate mother may affect the sexes differently. Some studies find that ELS increases impulsive choice (Gondré-Lewis et al., 2016) but these findings are complicated by the fact that offspring were trained on operant binge drinking paradigms prior to learning delay discounting. These additional factors could affect the development of offspring as well as their behavior on these reward discounting measures. Taken together the findings suggest that postnatal ELS typically decreases impulsive choice, but studies utilizing prenatal stressors are needed. A summary of ELS effects on impulsive choice is found in Table 2.

Stress Model	Species	Effects of ELS on Impulsive Choice	Sex	Citation
Motherless Rearing (i.e., artificial rearing) + Social Isolation	rat	decrease	males	Lovic et al., 2011
Limited Bedding/Nesting	rat	decrease	males	Ordoñes Sanchez, Bavley et al., 2021
Limited Bedding/Nesting	rat	no effects	females	Ordoñes Sanchez, Bavley et al., 2021
Limited Bedding/Nesting	rat	decrease	females	Fuentes et al., 2014
Limited Bedding/Nesting	rat	no effects	males	Fuentes et al., 2014
Prenatal Restraint Stress + Lead Exposure	rats	decrease	males	Weston et al., 2014
Prenatal Restraint Stress + Lead Exposure	rats	no effects	females	Weston et al., 2014
Maternal Separation + Ethanol Exposure	rats	increase	male & female data combined	Gondré-Lewis et al., 2016

Table 2. Summary of ELS effects on impulsive choice in male and female rodents.

It is important to mention that some studies report that ELS can induce compulsive-like, rather than impulsive-like, behaviors (N. Boutros, Der-Avakian, Markou, & Semenova, 2017; Fuentes et al., 2014). Compulsive behaviors are repetitive, often purposeless, and lead to unfavorable outcomes. For instance, male offspring that experienced maternal separation showed increased perseverative responding (i.e., continued nose-poking after a correct response) on a 5-CSRTT task (Boutros et al., 2017). However, this experiment also included rats that were exposed to ethanol during adolescence (PND28-57), which could affect the interpretation of these results. Future studies should continue examining whether other prenatal and postnatal stressors also induce similar findings. Overall, the rodent literature demonstrates that ELS can lead to increases in impulsive action but decreases in impulsive choice.

Effects of ELS on the Mesolimbic System Can Mediate Impulsivity

As mentioned previously, there is a complex relationship between DA levels and impulsivity. Stimulants, which increase DA, increase impulsive action but decrease impulsive choice (Baarendse & Vanderschuren, 2012; Britton & Koob, 1989; Caballero-Puntiverio et al., 2017; S. B. Floresco, M. T. L. Tse, & S. Ghods-Sharifi, 2008; Hayton et al., 2012; van Gaalen, Brueggeman, et al., 2006; Wade et al., 2000). ELS can affect the mesolimbic DA system to increase dopaminergic drive (Baier, Katunar, Adrover, Pallarés, & Antonelli, 2012; Eck & Bangasser, 2020). For example, maternal separation increases the excitability of VTA DA neurons in female rats (males were not tested) (Spyrka et al., 2020). Maternal separation can also affect tyrosine hydroxylase (TH), the rate limiting enzyme of catecholamine synthesis. Specifically, maternal separation increases TH-immunoreactive cells in the VTA in adolescent male and adult female rats (Chocyk et al., 2011; Kapor et al., 2020). In the PFC and NAc, TH-immunoreactive fibers are denser following maternal separation in adolescent females (males were not tested) (Majcher-Maślanka, Solarz, Wędzony, & Chocyk, 2017). In studies that only tested male rats, prenatal stress increases DA transcription factors in the VTA and dopamine release in the NAc shell (the core was not analyzed) (Katunar, Saez, Brusco, & Antonelli, 2009; Silvagni et al., 2008). Although most of these studies do not directly compare males to females, the overall pattern is that ELS increases dopaminergic drive from VTA across sex.

In addition to increasing DA levels in the NAc, ELS can also alter DA receptor expression. Female, but not male, mice who experienced maternal separation plus social isolation show reductions in D1 receptor expression in the NAc (Sasagawa et al., 2017). A decrease in this receptor's expression in the NAc could reduce dopaminergic signaling in females and may help compensate the stress-induced increases in DA drive from the VTA. Changes in D1 expression are attributed to hypermethylation of the *Drd1a* promoter region, revealing an epigenetic modification that can explain this effect in females (Sasagawa et al., 2017). In contrast, exposure to a limited resource environment had no effect on D1 expression in the NAc shell in male and female rats (Fuentes et al., 2018). This discrepancy may be because only one subregion and not entire NAc was examined in this study. Alternatively, different types of early life adversity could have distinct effects on D1 receptors. ELS can also affect D2 receptor expression in the NAc but typically causes an increase in this receptor. In males (females were not tested), prenatal restraint stress as well as maternal separation alone or in combination with cocaine exposure increases D2 receptor expression in the NAc (Berger, Barros, Sarchi, Tarazi, & Antonelli, 2002; Gracia-Rubio et al., 2016; Romano-López et al., 2016), but see (Majcher-Maślanka et al., 2017). A stress-induced increase in D2 receptors, assuming postsynaptic, would inhibit the NAc, which may help control increased DA influx in the NAc. Higher expression of NAc D2 receptors following ELS may drive changes in impulsivity, because, as noted, reduced D2 receptor expression is positively correlated with poor inhibitory control (Cropley, Fujita, Innis, & Nathan, 2006; Hamidovic, Dlugos, Skol, Palmer, & de Wit, 2009).

Another regulator of dopaminergic function is DAT. In humans, low levels of DAT, which could increase DA signaling, are associate with poor inhibitory control (H. Kim, 2018; Sekiguchi, Pavey, & Dean, 2019; C. T. Smith et al., 2018). Maternal separation in spontaneously hypertensive rats, a rodent model of ADHD, increases the rate of DA clearance in the ventral striatum (Womersley, Hsieh, Kellaway, Gerhardt, & Russell, 2011). An increased rate of clearance is indicative of reductions in DAT function because less DA is being removed from the synapse. Consistent with this finding, male rats (females were not studied) that experience maternal separation show reduced DAT expression in the accumbens and display increased sensitization to amphetamine (Brake, Zhang, Diorio, Meaney, & Gratton, 2004; Meaney, Brake, & Gratton, 2002). Interestingly, female, but not male, rats exposed to maternal stress are less susceptible to methamphetamine induced decreases in striatal DAT content (Hensleigh & Pritchard, 2015). Thus, another mechanism by which ELS could impact impulsivity is via altering DAT function.

ELS also affects plasticity within the NAc (Monroy, Hernández-Torres, & Flores, 2010; Romano-López et al., 2016). Female rats that experienced prenatal stress in combination with motherless rearing have decreased expression of neuronal markers of plasticity such as synaptophysin and brain-derived neurotrophic factor in the accumbens (Burton et al., 2007). Prenatal stress with maternal separation increases dendritic MSN branching in both male and female rats (Muhammad, Carroll, & Kolb, 2012). Increased branching can provide more surface area for synaptic connections. These data illustrate that synaptic development is sensitive to different forms of ELS. What is unclear, however, is if ELS promotes inhibitory or excitatory synaptic connections. Electrophysiology data suggests male, but not female rats, exposed to LBN exhibit reductions in spontaneous excitatory postsynaptic currents (sEPSCs) in the NAc core (the shell was not assessed) (Ordoñes Sanchez et al., 2021). These findings could suggest that early life experiences can potentially reduce excitatory synapses and therefore, reduce glutamate transmission in the NAc. Reductions in glutamate transmission in the accumbens is associated with reduced impulsive choice in males (Ordoñes Sanchez et al., 2021; Yates & Bardo, 2017). These findings indicate that ELS can alter other aspects of accumbal plasticity in addition to regulating DA which could impact impulsivity.

It is important to mention that there is a disconnect between the detail in which the NAc has been studied in the ELS field vs. the impulsivity field. The impulsivity literature has revealed distinct functions of the NAc core and NAc shell, with a stronger role for NAc core in impulsive choice and the NAc shell in impulsive action (Dalley & Ersche, 2019; J. W. Dalley & Robbins, 2017; Ito, Robbins, & Everitt, 2004). Many ELS studies, however, often focus on the entire NAc or only examine one subregion. Future ELS studies need to better incorporate core/shell assessments to further our understanding of the mechanisms by which ELS alters impulsive behavior.

Effects of ELS on the Cortex Can Mediate Impulsivity

The cortex also plays a role in impulsive behavior, and ELS can affect the cortex. Recall that cortical DA signaling contributes to the expression of behavioral impulsivity (Loos et al., 2010; Puumala & Sirviö, 1998; C. A. Winstanley et al., 2006; C. A. Winstanley et al., 2010). ELS alters DA innervation of the cortex (Kunzler, Braun, & Bock, 2015). For example, maternal separation causes tyrosine hydroxylase (TH)-fiber density in the OFC to be higher in male, but not female, *Octodon degus* (Kunzler et al., 2015). Given that TH is a marker for DA projections, these data suggest ELS increases dopaminergic innervation in the OFC. Interestingly, in the same study density of TH fibers in the mPFC was lower in stressed males than control males with no effects found in females (Kunzler et al., 2015). These results indicate that ELS alters dopaminergic innervation in the cortex in a region-specific manner. ELS can also alter cortical DA receptors. Maternal separation in male rats (females were not tested) increased adolescent D1 expression but reduced adult D2 receptor expression on glutamatergic projection neurons from the prelimbic PFC (Brenhouse, Lukkes, & Andersen, 2013). However, in the prelimbic PFC projections specifically to the NAc, maternal separation caused a transient decrease in both D1 and D2 receptors in adolescence (Brenhouse et al., 2013).

Studies on the effects of ELS on DA in the PFC are limited, and much more research in this area is warranted.

In addition to changes in cortical dopamine, cortical glutamate dysfunction is associated with impulsivity in ADHD, SUD, bipolar disorder, and preclinical models (Jochen Bauer et al., 2018; J.-N. Li, Liu, & Li, 2020; Emily R. Murphy et al., 2012; Smaragdi, Chavez, Lobaugh, Meyer, & Kolla, 2019). As an example, people with ADHD have higher glutamate in the ACC than controls and these high glutamate levels are positivity correlated with impulsivity symptoms (J. Bauer et al., 2018). ELS can affect cortical glutamate. Primates reared in adverse conditions have higher ACC glutamatergic signaling than controls (Mathew et al., 2003). Glutamatergic tone in cortical regions is modulated by local parvalbumin (PV) GABAergic interneurons (Sohal, Zhang, Yizhar, & Deisseroth, 2009; S. M. Williams, Goldman-Rakic, & Leranth, 1992). Several lines of rodent research demonstrate that ELS reduces PV neurons in the mPFC and OFC (Goodwill et al., 2018; Grassi-Oliveira et al., 2016; Ohta et al., 2020). Interestingly the effect of ELS on cortical PV neurons differs by sex and developmental stage. Maternal separation reduces PV neurons of the mPFC transiently in juvenile female rats but causes a persistent decline of these neurons in male rats which starts in adolescents and continues into young adulthood (Grassi-Oliveira et al., 2016; Holland, Ganguly, Potter, Chartoff, & Brenhouse, 2014). In the OFC, LBN reduces PV neurons in female but not male mice (Goodwill et al., 2018). These ELS-induced changes in cortical PV have been linked to cognitive and social deficits, but they have not directly been linked to impulsivity, a gap that should be addressed in future studies. Collectively, these findings suggest that another mechanism by which ELS can alter impulsivity is by increasing cortical glutamate signaling.

Conclusion

How ELS affects impulsivity is just beginning to be elucidated and there remain many unanswered questions. However, the rodent literature demonstrates that ELS increases impulsive action and decreases impulsive choice. Yet, these findings are not totally aligned with human studies: while both rodent and human studies report that ELS increases impulsive action, in humans, adversity in childhood also typically increases impulsive choice (Duckworth, Kim, & Tsukayama, 2013; S. T. Kim et al., 2018; Lovallo et al., 2013). One possible explanation for why ELS effects on impulsive choice in the rodent versus human literature differ could be due to differences in the stressor timing and duration, and the types of stressors experienced. Most rodent models of ELS utilize one specific stress manipulation during a restricted developmental timeframe, in part, to identify sensitive windows (Molet et al., 2014; Nishi et al., 2014; Walker et al., 2017). However, stressful events experienced by children are not typically restricted to a limited developmental window (Lupien, McEwen, Gunnar, & Heim, 2009). Moreover, most children who experience ELS report multiple forms of adversity prior to adulthood (Arata, Langhinrichsen-Rohling, Bowers, & O'Brien, 2007; J. G. Green et al., 2010; Kessler et al., 2010; Katie A. McLaughlin et al., 2010; K. A. McLaughlin et al., 2012). Additionally, while most rodent models of ELS focus on some form of neglect (e.g., neglect of maternal care or physical resources), clinical studies also take into consideration stress stemming from other ELS subtypes (e.g., sexual, physical, and emotional abuse) (Katie A. McLaughlin et al., 2010; K. A. McLaughlin et al., 2012). Different types of early trauma can lead to different outcomes later in life. For instance, a meta-analysis found that childhood abuse was positively associated with the development of adult psychopathology, but not neglect stemming from caregivers unable to provide basic needs such as housing/shelter (Carr, Martins, Stingel, Lemgruber, & Juruena, 2013). Because most rodent models of ELS involve stress from neglect, they may not always capture the effects of childhood trauma in humans. One approach would be to try to develop rodent models that better capture multiple stressors for a more protracted period of development. Another approach is to study impulsivity in humans who have experienced briefer and milder forms of early adversity. Although there is some disconnect regarding impulsive choice between the rodent and human ELS studies, it does not mean the rodent research cannot be leveraged into better treatments for those suffering from conditions with impulsive choice as a feature. Using rodent models to discover mechanisms that reduce impulsivity may reveal novel treatments for disorders characterized by high impulsivity.

In conclusion, adverse experiences early in life can alter many cognitive processes, including impulsivity. Although the precise mechanisms by which this occurs are still not fully elucidated, regulation of the mesocortolimbic system by ELS clearly contributes to later alterations in impulsive behavior. Future studies which disentangle stressor type, duration, and developmental window as well as consistently compare males and females are needed to better understand conditions that promote vulnerability vs.

resilience to ELS. Leveraging these data can help improve therapeutics to treat several conditions including ADHD, bipolar disorder, and SUD.

CHAPTER 1B: RISKY DECISION-MAKING AND EARLY LIFE STRESS

Everyday decision-making can involve some level of uncertainty and risk. For example, a commonly encountered dilemma is determining whether or not to try a new menu option at a favorite restaurant. Even though the risks associated with this particular situation would not pose a threat to one's well-being (other than being potentially disappointed by the food choice), when risk taking is extreme it can be maladaptive and a characteristic of a number of neuropsychiatric disorders. High levels of risk taking can contribute to some of the adverse outcomes present in ADHD and gambling disorders (Bechara et al., 2001; Breyer et al., 2009; Thompson, Molina, Pelham, & Gnagy, 2007). Additionally, impaired risky decision-making is found in many substance-dependent populations, including people with cannabis (Casey & Cservenka, 2020), opiate (Brand, Roth-Bauer, Driessen, & Markowitsch, 2008), and alcohol (Brevers et al., 2014) addictions. A task used to measure risky decision-making in rodents is the probability discounting task. This task requires subjects to choose between a small/certain (safe) vs. a large/uncertain (risky) reward. Odds of earning the risky reward vary in the task (from really good to bad or vice-versa) and the selection of the risky reward is only advantageous when the likelihood of earning the reward is high (e.g., 50-100%). By using this task, we can better understand how different factors (e.g., genetics or environment) contribute to risky behavior and gain insight as to how these processes may go awry in pathological conditions.

Cortical areas of the brain regulate risky decision-making. Bilateral inactivation of the prelimbic region of the medial PFC (mPFC) increases risky choices in probability discounting tasks when odds of earning a reward go from good-to-bad (100→12.5%) but has an opposite effect when reward probabilities increase over a session (St. Onge & Floresco, 2010). When reward probabilities are fixed however, this area of the mPFC does not influence risky choice. Selectively disconnecting descending (but not ascending) PFC → basolateral amygdala (BLA) pathways also disrupts decision-making similar to bilateral inactivation of the mPFC (St. Onge, Stopper, Zahm, & Floresco, 2012). These findings suggest that the PFC plays a role in risky decision-making and tracks outcomes to promote optimal patterns of choice. Another area of the cortex, the medial OFC (mOFC) also plays a role in mitigating the impact that large, risky rewards exert on subsequent choice behavior. Inactivating the mOFC increases risky choices, irrespective of the order in which probabilities are presented over the course of a session (C. M. Stopper, Green, & Floresco, 2014). In this study, inactivating the mOFC did not affect impulsive choice, demonstrating selective involvement of this brain region with risky rather than impulsive choices. Additionally, the mOFC—BLA circuit facilitates the use of cue-dependent reward outcomes to influence decision-making (Lichtenberg et al., 2021). Disrupting mOFC → BLA projections prevents the ability to use environmental cues to make judgements on how advantageous a certain action might be, whereas disrupting BLA→ mOFC projections reduces a rodents ability to infer how advantageous it is to continue responding to a cue based on the current value of a reward (Lichtenberg et al., 2021). Altogether, the PFC and mOFC play a role mitigating risky choices and guiding decision-making to promote better choice patterns. One caveat with these studies is that they were all conducted in males and it is unclear if similar manipulations would induce the same effects in females.

In addition to the PFC and mOFC, the BLA is also involved in regulating risky decision-making. Bilateral inactivation of the BLA reduces risky choices and mitigates sensitivity to losses in male rats (females not examined) (Ghods-Sharifi, St. Onge, & Floresco, 2009; Orsini et al., 2017). In other words, if choice for a risky reward is unrewarded, on the next trial the rat will not continue selecting the risky reward and shift to smaller/certain reward option. Therefore, the BLA may encourage “go for it!”-like behaviors by minimizing the negative outcomes of risky choices. The effects of the BLA on risky decision-making appear to be mediated via interactions with the NAc, as inactivation of the NAc also reduces preference for larger, risky rewards (Colin M. Stopper & Floresco, 2011). Unlike the BLA however, inactivation of the NAc in males (females not examined) diminishes reward sensitivity and reduces the tendency to follow a rewarded risky choice with another risky choice (Colin M. Stopper & Floresco, 2011). Therefore, unlike the PFC which tries to guide appropriate and less risky behaviors, the BLA-NAc circuitry seems to promote the opposite.

Similar to impulsivity-related circuits, risky decision-making is influenced by dopamine (DA). DA signaling via PFC or NAc D1 receptors reduces the sensitivity to non-rewarded actions. For instance, blocking D1 receptors in the PFC or NAc reduces risky choice during probability discounting and these effects are driven by negative feedback sensitivity (St. Onge, Abhari, & Floresco, 2011; Colin M. Stopper, Khayambashi, & Floresco, 2013). In contrast, D2 receptor antagonism in the PFC prevents shifts in choice biases and increases risky choice when the odds of earning a reward descend (12.5%-100%) (St. Onge et al., 2011). Collectively, these results suggest that DA signaling via D1 receptors may reduce the negative outcomes associated with risky choices, whereas D2 receptors promote exploration of different options. Additionally, changes in phasic DA release can track decision outcomes during risky decision-making, wherein receipt of larger vs. smaller rewards is associated with increases in DA, and reward omissions cause phasic suppressions in DA levels (Sugam, Day, Wightman, & Carelli, 2012). Interestingly, overriding phasic bursts and dips in DA activity associated with decision outcomes can change subsequent choice behavior (Sugam et al., 2012), suggesting DA can convey information about outcomes that can affect future actions. Again, however, the use of females and comparison of both sexes data is lacking.

The involvement of the PFC, NAc, and DA in risky decision-making processes makes it tempting to believe this construct is similar to impulsivity. However, these processes are actually different. Human studies that test the connection between probability discounting and delay discounting find no correlation between the two (Andrade & Petry, 2012; Holt et al., 2003; Madden, Petry, & Johnson, 2009). These data suggest that these tasks measure different constructs and using one to predict the outcome of the another is inaccurate. Therefore, it is more appropriate to consider behaviors associated with risky decision-making separate from those associated with impulsivity.

Early life stress (ELS) also affects risky behavior. In humans, exposure to early adversity can predict future risky sexual behavior in young adults (Filipkowski, Heron, &

Smyth, 2016), shorten the onset of drug taking behaviors (Lacey, Zilanawala, Webb, Abell, & Bell, 2018), and is associated with poor decision-making in adulthood (Birn, Roeber, & Pollak, 2017). In rodents, studies on the effects of ELS on risky behavior are more limited. However, one study found that exposure to prenatal restraint stress in male rats (females not examined) reduces risk-taking behavior as measured using an elevated-plus maze (Gatta et al., 2018). In this study, reduced risk-taking was associated with more time spent time in the closed arms versus open arms of the maze. However, greater time spent in the closed arms of the maze could also be interpreted as greater anxiety-like behavior rather than risk-taking. In another study, exposure to limited bedding/nesting (LBN) early in life increases risk-taking in adolescent female, but not male, mice performing a predator-odor risk-taking task (PORT) (Viola et al., 2019). In the PORT task, risk taking was associated with the time animals took to cross an arena that smelled like coyote urine in order to reach a reward. The urine was supposed to trigger a potentially dangerous environment and shorter latencies indicated mice were more risk-taking. Although informative, this task was very short in nature (e.g., PORT test can last less than 60sec). The short duration of this task could cause floor effects and was even thought by the authors of the study to have influenced some of the results (Viola et al., 2019). To date, there are no rodent studies that have utilized probability discounting tasks to better understand how ELS changes risky choices and its underlying mechanisms. This is surprising given that most everyday decision-making involves weighing the risks and rewards associated with competing options. As such, investigating how stress early in life may shift bias towards or away from risky choices in male and female rats deserves more attention.

In conclusion, risky decision-making is a component of many disorders such as substance use disorder and gambling disorder. As such, it is necessary to understand how different factors, such as stress, contribute to risky behavior in order to gain insight as to how risky decision-making may go awry in pathological conditions. By doing so, we can improve interventions targeting risky behavior in stressed populations. Future studies that disentangle stressor type, duration, and developmental window as well as consistently compare males and females are also needed to understand conditions that promote vulnerability vs. resilience to ELS. Leveraging these data can help improve therapeutics to treat several conditions including ADHD, bipolar disorder, and improve abstinence from drugs or gambling.

.

CHAPTER 2: EARLY LIFE STRESS PROMOTES RESILIENCE TO OPIOID ADDICTION-RELATED PHENOTYPES IN MALE RATS AND SEX-SPECIFIC TRANSCRIPTIONAL CHANGES

The opioid epidemic is a pervasive crisis, with negative impacts on individuals and society that are not abating, including a dramatic increase in opioid overdoses (Rudd, Aleshire, Zibbell, & Matthew Gladden, 2016). Given this crisis, many efforts have focused on identifying mechanisms that underlie vulnerability to opioid abuse. However, it is also important to consider factors that promote resilience to substance use disorder (SUD). If the molecular mechanisms underlying resilience are identified, they may guide the development of novel therapies to prevent opioid abuse.

Exposure to severe stress early in life is associated with the later development of SUD (Anda et al., 2006; Quinn et al., 2016). However, most people exposed to early life stress do not develop illnesses, and many are protected from later stressful experiences (Lyons, Parker, & Schatzberg, 2010; Masten, 2001). Some stress early in life can actually improve coping skills to deal with later stressors (Masten, 2001). These data are consistent with early studies in monkeys revealing that intermittent stress early in life has an “inoculating” effect that promotes the development of resilience in adulthood (Lyons et al., 2010). Since this early primate work, researchers have often turned to rodent models to identify mechanisms by which early life experience reprograms the brain. Some rodent studies support the stress inoculation hypothesis, while others find that early life stress increases vulnerability (Biggio et al., 2014; Hsiao et al., 2016; Santarelli et al., 2017). A recent synthesis of this literature found that such factors as the quality of maternal care, duration of the manipulation, developmental timing, and genetic background of the species contribute to these discrepancies (Murthy & Gould, 2018).

We used a model of early life stress, limited bedding/nesting (LBN) (Ivy et al., 2008), to test whether a low-resource environment early in development promotes resilience to addiction-related phenotypes in adulthood. In our version of LBN, we breed rats in-house rather than ship pregnant dams, which can cause prenatal stress (Sachs & Lumia, 1981). Then rat dams and postnatal day (PND) 2 to 9 pups are placed in an impoverished environment. We previously found that LBN alters maternal care by increasing pup-directed behaviors, such as nursing and pup grooming, while reducing self- care, such as self-grooming and resting outside the nest (Eck et al., 2019). This shift in maternal behavior is consistent with a hypervigilant phenotype (Gallo et al., 2019), and although it may be an attempt to compensate for the altered environment, offspring differ from controls. Specifically, progeny weigh less throughout development and exhibit endocrine changes that are particularly pronounced in male rats (Eck et al., 2019).

Here we extended our previous findings to determine how LBN affects addiction-related behaviors. We first assessed the effect of LBN on impulsive choice and morphine self-administration. Given the role of the nucleus accumbens (NAc) in these behaviors, we then tested the effect of LBN on NAc physiology. Finally, we evaluated LBN-induced changes in gene expression in the NAc. Given the shift in maternal care toward pups and the relatively mild nature of the model—altering only the postnatal environment for 7 d, versus prenatal and postnatal manipulations or postnatal manipulations that take several weeks (Banqueri, Méndez, & Arias, 2017)—we predicted that LBN would have an inoculating effect on addiction- related phenotypes.

Results

LBN Decreased Impulsive Choice in Male Rats

We first investigated whether LBN exposure altered impulsive choice, given that impulsivity is associated with SUD (Marino et al., 2013). Long–Evans dams and pups were placed in standard housing (ample bedding, two nestlets/ cage, one tunnel enrichment device/cage) or the LBN condition (no access to bedding materials, one paper towel for nesting, no enrichment) on PND2. Rats were transferred to normal housing on PND10 and tested as adults (PND 60 to 90) in all studies. Because we did not statistically compare the males and females, we are limited in making direct conclusions regarding sex differences.

For the delayed discounting task, rats must choose between a small/immediate reward and a larger/delayed reward. During training, there were no differences in the time it took LBN (n = 8) vs. control males (n = 10) to reach stable performance on delayed discounting (no effect of day [$F_{1.084,\ 17.343}$=2.858, P=.107]; no day × block interactions [$F_{6,\ 96}$= 1.012, p=.422]. Similarly, LBN females (n=10) were able to learn delayed discounting at the same rate as control females (n=9) no effect of day [$F_{1.213,\ 20.619}$= 1.839, p=.190]; no effect of day × block interactions [$F_{6,\ 102}$=1.555, p=.168]). For all statistics where sphericity was violated, we used Greenhouse Geisser corrections.

For delayed discounting, the percentage choice of the larger/delayed option relative to the smaller/no-delay option was calculated, such that a higher value indicated less impulsivity. There was a significant interaction for males ($F_{3,48} = 4.59$, $P = 0.007$). Sidak post hoc tests showed that LBN and control males performed similarly on the 0-s delay ($P = 0.812$), but that as delays increased, LBN males chose the larger/delayed reward more frequently (15 s, $P = 0.0005$; 30 s, $P = 0.0002$; 45 s, $P = 0.0012$), indicating reduced impulsive choice (Fig. 2A). Preference for larger/ delayed reward was similar in LBN and control females (delay: $F_{3,51} < 1$; LBN: $F_{1,17} = 1.54$, $P = 0.231$; interaction: $F_{3,51} = 0.496$, $P = 0.687$) (Fig. 2B).

Response latencies were not significantly different between LBN and control males (no effect of delay [$F_{1.802, 28.836} = 2.168$, $p=.137$], no effect of LBN [$F_{1, 16} = .016$, $p=.902$; interaction [$F_{1.802, 28.836} = .364$, $p=.676$] (Fig. 2C). Response latencies were also not significantly different between LBN and control females [no effect of delay [$F_{1.441, 24.502} = .823$, $p=.415$]; no effect of LBN [$F_{1, 17} = .217$, $p=.647$]; nor interaction [$F_{1.441, 24.502} = 1.294$, $p=.282$] (Fig. 2D). Lastly, there were no differences in the number of omissions made between LBN and control males (no effect of day [$F_{1.054, 16.857} = 2.184$, $p=.158$], no effect of LBN [F1, 16<1]; no interaction [F1.054, 16.857<1]. There were no differences in the number of omissions made between LBN and control females as well (no effect of day [F1.473, 25.033<1]; no effect of LBN [$F_{1, 17} = 2.825$, $p=.111$]; no interaction [$F_{1.473, 25.033} = .062$, $p=.890$].

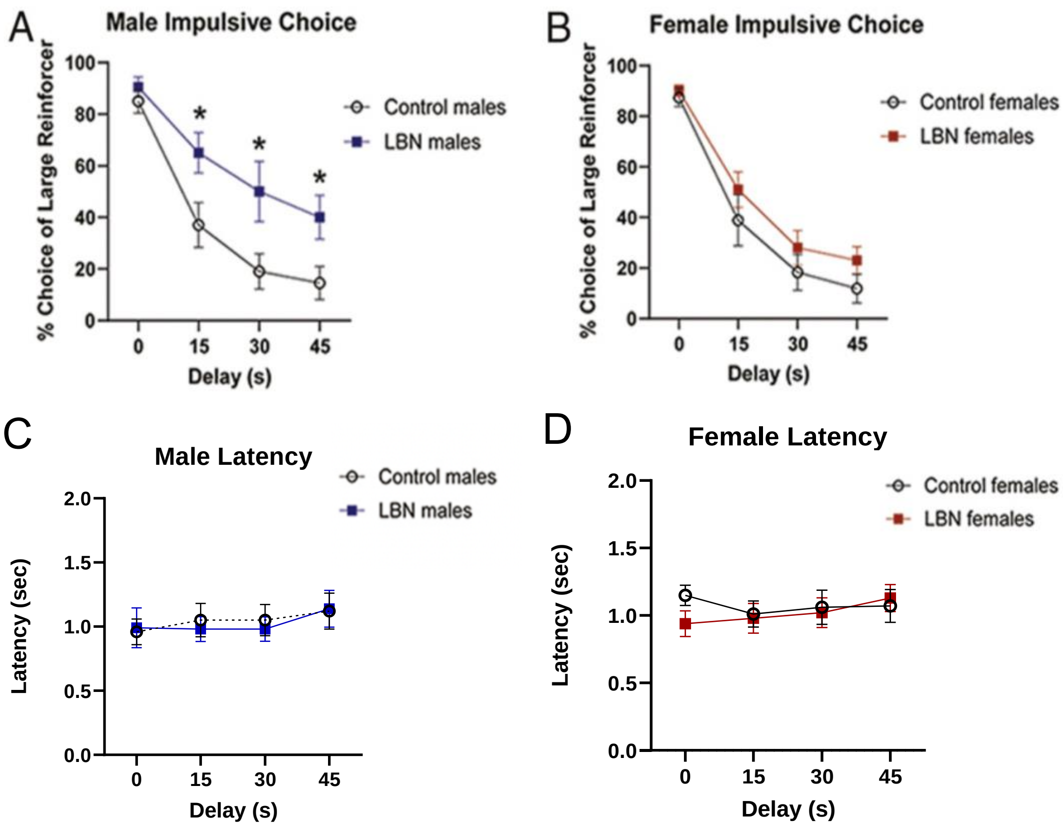


Fig. 2. LBN reduces impulsive choice in male rats. (A) LBN exposure reduced impulsive choice in male rats, as evidenced by the increase in preference of the large/delayed option at the 15s, 30 s, and 45s delays. *$p<0.05$. (B) Discounting did not differ significantly between LBN and control females. LBN exposure did not affect male (C) or female (D) latency to respond.

LBN Reduces Self-Administration of a Low Dose of Morphine in Male Rats

Lower impulsivity may reduce the risk for SUD, so we performed morphine self-administration studies using a low and high dose. For the low dose of morphine (0.25 mg/kg/infusion), LBN males (n = 5) earned a significantly fewer number of infusions within a 6-h period than their control counterparts (n = 7) on a fixed ratio (FR) 1 reinforcement schedule (LBN: $F_{1,10}$ = 5.974, P = 0.03; days: $F_{9,90}$ = 1.10, P = 0.39; interaction: $F_{9,90}$ <1) (Fig. 3A). On a progressive ratio (PR) schedule, LBN males earned a fewer number of infusions compared to control males (t_{10} = 2.44, P = 0.03) (Fig. 3B). In females (control, n = 5; LBN, n = 7), there were no significant differences in the number of morphine infusions at the 0.25 mg/kg/infusion dose between LBN and control rats (LBN: $F_{1,10}$ = 2.93, P = 0.12; days: $F_{9,90}$ = 4.26, P < 0.0001; interaction: $F_{9,90}$ = 1.00, P = 0.45) (Fig. 3C). LBN had no effect on PR for the low dose of morphine in females (t_{10} = 1.11, P = 0.29) (Fig. 3D).

LBN had no effect on drug-taking behavior using the high dose of morphine (0.75mg/kg/infusion) in males or females. Specifically, we found no differences in drug-taking between control (n=7) and LBN (n=11) males on an FR1 schedule (effect of LBN: [$F_{1,16}$=1.13, p=.304]; days of morphine IVSA: [F2.64,42.22=2.901, p=.0522]; interaction: [F9,144<1]) (Fig. 3E). There was also no effect of LBN on PR for the high dose (Fig.2F, effect of LBN: [t16=0.641, p=.53)] (Fig. 3F). Similarly, there were no differences in drug-taking for the high morphine dose between control (n=12) and LBN females (n=11) on an FR1 schedule (effect of LBN: [F1, 21<1]; days of morphine IVSA:

[$F_{2.51,52.77}$=4.321, p=.012]; interaction: [$F_{9,189}$=3.756, p=.0002]) (Fig. 3G). Breakpoint scores on PR for the high dose of morphine were not altered by LBN in females (effect of LBN: [t_{21}=1.8, p=.086] (Fig. 3H). A subset of the rats was tested for drug-seeking behavior in response to previously drug-paired cues at 7 d after the last self-administration session. During this one-hour test, morphine was not available and active lever presses were reinforced by presentation of the previously drug- paired cue light above the active lever. Females [t_{21}=0.3847, p=.7043] and males [Mann-Whitney test, p=.5494] from both groups showed similar responding during the cue tests, indicating that LBN does not impact drug-seeking behavior at this time point. Because the 0.75 mg/kg/ infusion morphine dose did not result in any group differences in morphine intake, this dose was used for subsequent physiology studies to prevent confounding the interpretation of these measures.

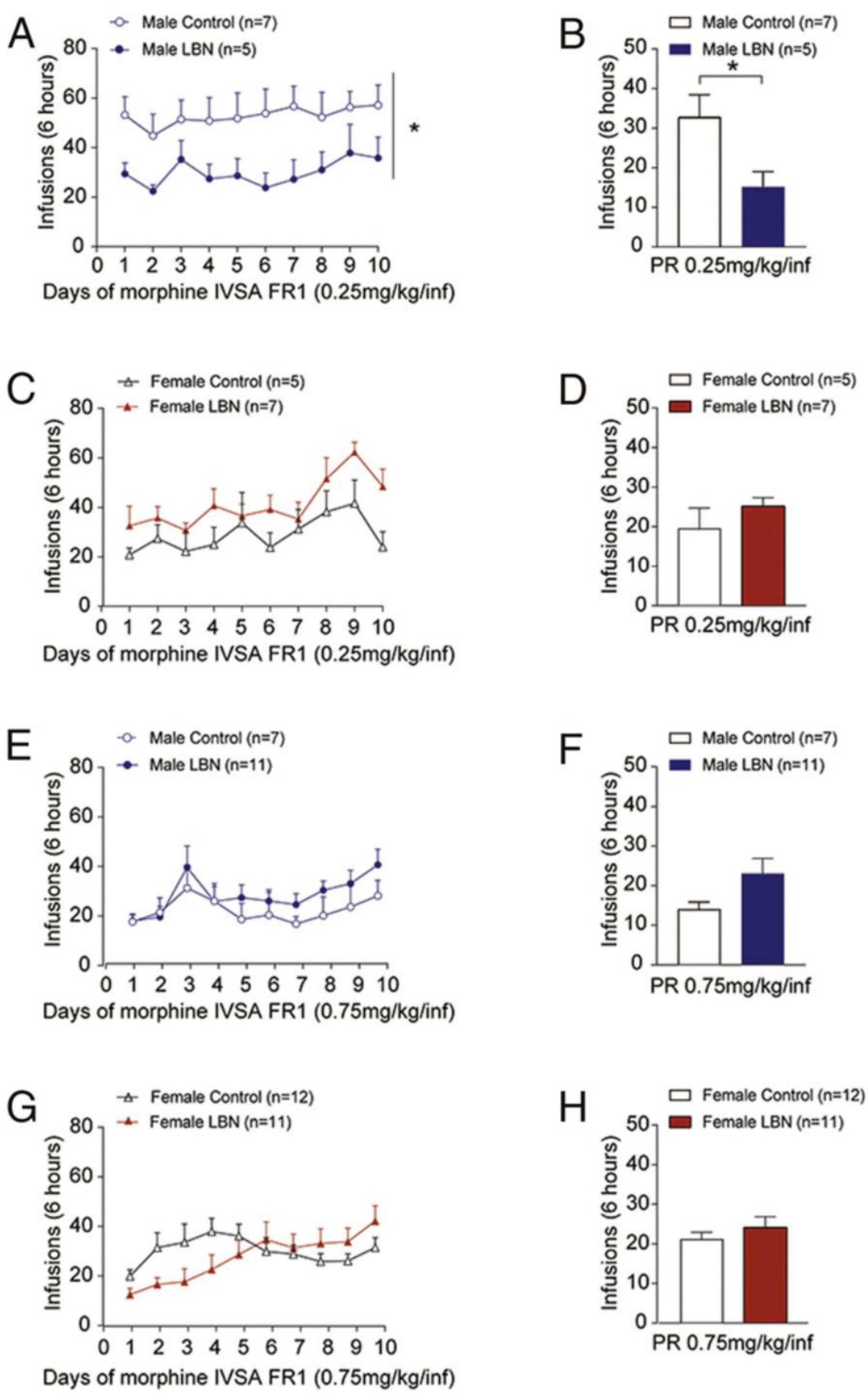

A
Male Control (n=7)
Male LBN (n=5)
Infusions (6 hours)
Days of morphine IVSA FR1 (0.25mg/kg/inf)
*
B
Male Control (n=7)
Male LBN (n=5)
Infusions (6 hours)
PR 0.25mg/kg/inf
*
C
Female Control (n=5)
Female LBN (n=7)
Infusions (6 hours)
Days of morphine IVSA FR1 (0.25mg/kg/inf)
D
Female Control (n=5)
Female LBN (n=7)
Infusions (6 hours)
PR 0.25mg/kg/inf
E
Male Control (n=7)
Male LBN (n=11)
Infusions (6 hours)
Days of morphine IVSA FR1 (0.75mg/kg/inf)
F
Male Control (n=7)
Male LBN (n=11)
Infusions (6 hours)
PR 0.75mg/kg/inf
G
Female Control (n=12)
Female LBN (n=11)
Infusions (6 hours)
Days of morphine IVSA FR1 (0.75mg/kg/inf)
H
Female Control (n=12)
Female LBN (n=11)
Infusions (6 hours)
PR 0.75mg/kg/inf

Fig. 3. LBN reduces self-administration of a low dose of morphine in male rats. LBN exposure reduced self-administration on FR1 (A) and PR (B) of a low dose of morphine (0.25 mg/kg/infusion) in male rats. *$P<0.05$. LBN did not significantly alter FR1 (C) or PR (D) for this low dose in females. FR1 and PR for the high dose of morphine (0.75 mg/kg/infusion) was not affected by LBN in male rats (E and F) or female rats (G and H).

LBN Leads to Alterations in Glutamate Transmission in the NAc Core of Male Rats

The NAc core (NAcC) mediates impulsive choice and self-administration (Banqueri et al., 2017), so we chose this region to explore physiological changes induced by LBN in males that could account for the behavioral findings. Spontaneous excitatory transmission in the NAcC of both naïve and morphine-experienced adult LBN rats was examined. LBN male rats had a decreased frequency of spontaneous excitatory postsynaptic currents (sEPSCs) compared to controls (8 to 12 cells/group from three rats/group) (LBN: $F_{1,32} = 4.20$, $P = 0.049$; morphine: $F_{1,32} = 3.42$, $P = 0.074$; interaction: $F_{1,32} = 1$) (Fig. 4A). While there were no effects of LBN on sEPSC amplitude in the males, there was a significant effect of morphine that may have been driven primarily by LBN males (LBN: $F_{1,35} = 0.32$, $P = 0.58$; morphine: $F_{1,35} = 4.26$, $P = 0.047$; interaction: $F_{1,35} = 3.98$, $P = 0.05$; Sidak's post hoc drug naïve LBN vs. morphine SA LBN, $P = 0.015$) (Fig. 4B). We examined the AMPA (α-amino-3-hydroxy-5-methyl-4- isoxazolepropionic acid)/NMDA (N-methyl-D-aspartate) ratio within the NAcC of drug naïve and morphine-experienced rats (eight to nine cells/group from three rats/group). The ratio between AMPA and NMDA receptors is a key factor governing plasticity of glutamatergic synapses and has been examined extensively in animal models of SUD (16–19). Morphine self-administration increased the AMPA/NMDA ratio in control males, but the LBN males did not show this drug-induced change (LBN: $F_{1,37}$ = 2.15, P = 0.15; morphine: $F_{1,37}$ = 9.45, P = 0.004; interaction: $F_{1,37}$ = 9.68, P = 0.004; Sidak's post hoc drug naïve control vs. morphine SA control, P < 0.001) (Fig. 4C). Females did not show any group differences in sEPSC frequency (7-13 cells/group from 3 rats/group) [effect of LBN, $F_{1,29}$=1.31, p=.26; effect of morphine, $F_{1,29}$=1.11, p=.30; interaction, $F_{1,29}$<1]. (Fig. 4E). No effect of early life adversity on sEPSC amplitude was detected in females as well [effect of LBN, $F_{1,29}$<1; effect of morphine, $F_{1,29}$=1.07, p=.31; interaction, $F_{1,29}$<1] (Fig. 4F). While there was no effect of LBN on the AMPA/NMDA ratio in females, we did detect a trend toward an increase following morphine (7 to 12 cells/group from three rats/group) (LBN: $F_{1,36}$ < 1; morphine: $F_{1,36}$ = 4.11, P = 0.050: interaction: $F_{1,36}$ = 1.36, P = 0.25) (Fig. 4G).

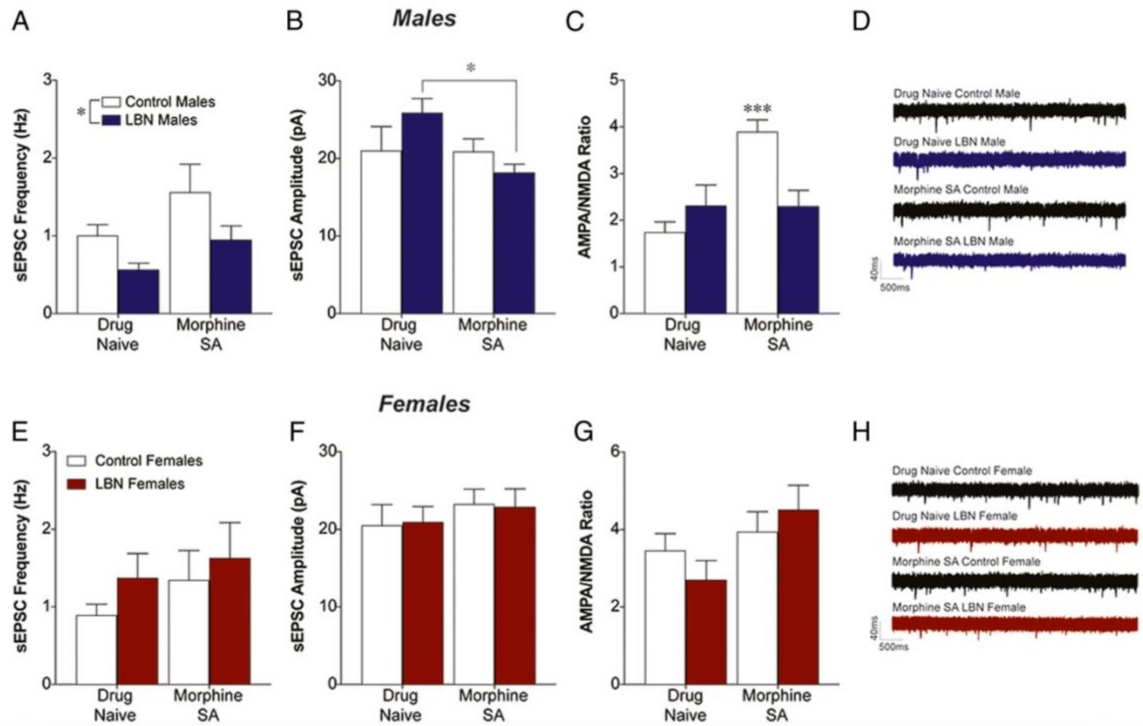


Fig. 4. LBN alters both baseline and morphine-induced glutamate transmission in male rats. (A) LBN exposure reduced sEPSC frequency in male rats regardless of morphine history. **P*<0.05. (B) sEPSC amplitude was lower following morphine exposure, and this effect was driven primarily by the LBN group. **P*<0.05, drug-naïve LBN males vs. morphine LBN males. (C) While no differences were seen in drug-naïve rats, control males exposed to morphine exhibited an increase in the AMPA/NMDA ratio that was not present in the LBN males. ****P*<0.001. (D) Example sEPSC traces from male rats. LBN exposure did not significantly affect sEPSC frequency (E), sEPSC amplitude (F), or AMPA/NMDA ratio (G) in females. (H) Example sEPSC traces from female rats.

LBN Causes Sex-Specific Changes in Gene Transcription in the NAc

To delineate molecular signatures in the NAc that may drive the LBN phenotypes in males, we conducted RNA-sequencing in punches from whole NAc from adult behaviorally naïve rats (male control, n = 4; female control, n = 5; male LBN, n = 5; female LBN, n = 6). We first used rank-rank hypergeometric overlap (RRHO) analysis to compare overall gene expression patterns in males and females induced by LBN. This analysis included all genes that were differentially expressed, including gene changes not reaching the threshold of statistical significance, and thus facilitated the agnostic comparison of gene expression patterns after LBN in males and females. Although there was significant overlap between both up- regulated and down-regulated genes between males and females (Fig. 5A; hot spots in bottom left and top right quadrants), we also found that LBN induces a unique signature in males and females. For example, many genes were up-regulated by LBN in males but down-regulated by LBN in females (Fig. 5A; bottom right quadrant).

We next narrowed our analysis to genes showing a significant difference between control and LBN and found 170 differentially expressed genes (DEGs) in males and 335 DEGs in females. These gene expression changes were largely sex-specific, with only 11 common genes altered by LBN in both sexes (Fig. 5B). Heatmaps sorted by fold change of LBN DEGs revealed different patterns of up-regulated and down- regulated genes between males and females: in males, 67.6% of identified DEGs were up-regulated following LBN, while in females, only 48.4% of DEGs were up-regulated (Fig. 5C and

D). Thus, LBN induces unique patterns of gene expression in males and females, rather than oppositely regulating a common set of genes.

To examine the biological processes altered by LBN, we used Kyoto Encyclopedia of Genes and Genomes (KEGG) and Panther Pathway analysis. The top Gene Ontology (GO) terms are displayed in Fig. 5E and F. Only three overlapping terms were identified between males and females (Fig. 5G). Within these GO terms identified in both sexes, largely different sets of DEGs were regulated within these networks, indicating unique transcriptional profiles following LBN in males and females. HOMER (Hypergeometric Optimization of Motif EnRichment) analysis of transcription factor binding sites was performed to identify potential master regulators affected by LBN that could account for the broad effects on transcription in the NAc. HOMER analysis found different transcription factor binding sites for the DEGs altered by LBN in males and females. Importantly, none of the transcription factors predicted to influence LBN effects in males or females were represented in the transcription factors predicted to influence baseline sex differences in the NAc (comparing control males and control females).

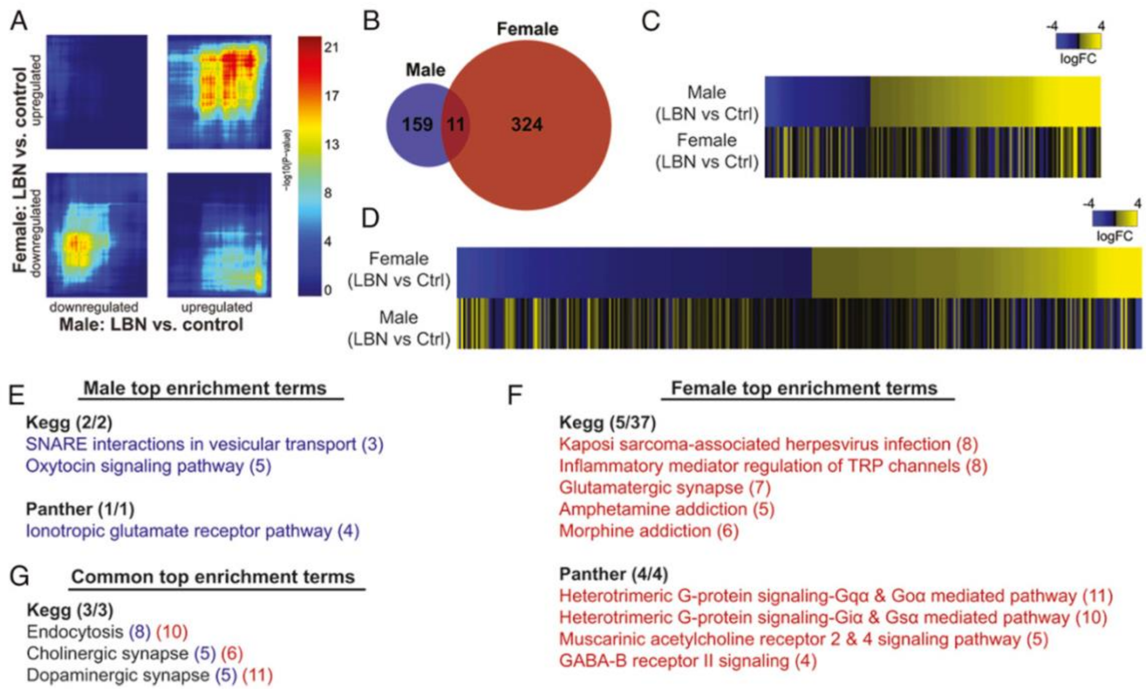


Fig. 5. LBN produces sex-specific changes in gene expression in the NAc. (A) Threshold-free comparison of DEGs by rank-rank hypergeometric overlap. Hotspots represent the overlap between the impact of LBN on gene expression in females and males. Hotter colors indicate more overlap. The upper right quadrant includes co–up-regulated genes, while the lower left quadrant represents co–down-regulated genes. The top left and bottom right quadrants represent genes that are changed by LBN in opposite directions in males compared to females. (B) LBN induces 170 DEGs in males and 335 DEGs in females, with 11 overlapping DEGs between the sexes. (C) Heatmap sorted by fold change of DEGs in males (Top) compared to the expression of those genes in females (Bottom). LBN reduced the expression of 55 genes and increased the expression of 115 genes in males. (D) Heatmap sorted by fold change of DEGs in females (Top) compared to the expression of those genes in males (Bottom). LBN increased the expression of 162 genes and decreased the expression of 173 genes. (E) KEGG and Panther enrichment terms identified in males following LBN. The number of DEGs within each term is in parentheses to the right of the term. (F) KEGG and Panther enrichment terms identified in females following LBN. The number of DEGs within each term is in parentheses to the right of the term. (G) KEGG enrichment terms identified in both males and females following LBN. The number of DEGs within each term is in parentheses to the right of the term, with males in blue and females in red.

Discussion

Early life stress can have an inoculating effect that promotes resilience in adulthood. Using the 7-d postnatal LBN manipulation, we revealed that LBN reduced impulsive choice and self- administration of a low dose of morphine in male rats. LBN also dampened NAcC glutamate transmission and prevented the morphine-induced increase in the AMPA/NMDA ratio, blocking this drug-induced plasticity in males. These physiological changes may underlie the resilience to addiction-related behaviors found in LBN males. Interestingly, LBN exposure in females did not significantly alter their behavior or NAcC physiology, so the effects of LBN on these phenotypes appear to be sex-specific. LBN also produced sex-specific changes in gene expression in the NAc. Overall, we show unique behavioral, electrophysiological, and molecular signatures associated with stress inoculation in LBN males.

LBN Reduces Impulsive Choice in Males

We examined the effects of LBN on impulsivity using a delay discounting task, a well- established assay for impulsive choice. LBN reduced impulsive choice in males. A similar effect was not found in females. This result complements another report that a different type of early life deprivation—artificial rearing with low simulated grooming—reduced impulsive choice in adult male rats but not in adult female rats (Lovic et al., 2011). Although in these two examples, early life manipulations had no effect on female impulsivity, a study that combined bedding restriction with exposure to a “substitute” mother found reduced impulsive choice in female rats (Fuentes et al., 2014). Males in that study were unaffected, suggesting that the social experience of having a substitute mother may have differential effects on impulsive choice in male vs. female animals later in adulthood. Collectively, these finding indicate that early life stress in rodents reduces impulsive choice, making their decision making more profitable. These changes would be beneficial in the long term, as such adaptations to an early stressful environment would prepare the organism to better cope with future stressors (Lyons et al., 2010).

LBN did not affect the acquisition of delayed discounting, indicating that LBN does not alter performance factors, such as activity or instrumental learning. Other studies have indicated that LBN exposure can impair learning (Brunson et al., 2005; Rice et al., 2008); however, those previous reports examined behaviors mediated by the hippocampus, whereas instrumental learning is not dependent on the integrity of this region (Corbit & Balleine, 2000). Thus, LBN may affect learning in a circuit-specific manner. Another measure unaltered by LBN in either sex was response latency. This finding contrasts with the effect of an immediate acute stressor on delay discounting, which increases response latencies in adult male rats (Shafiei, Gray, Viau, & Floresco, 2012) and implicates proximity to the stressful event as critical for this measure.

LBN Decreases Self-Administration of a Low Dose of Morphine in Males

High impulsivity is a risk factor for opioid misuse, whereas lower impulsivity may reduce the propensity to take drugs (Marino et al., 2013; Vest et al., 2016). Consistent with this idea, LBN not only reduced impulsive choice in male rats, but also reduced self-administration for the lower dose of morphine on FR1 and PR schedules.

The reduced responding under PR conditions indicates that LBN males may have diminished motivation and/or sensitivity to the reinforcing effects of this dose of morphine compared with controls. Another interpretation could be that LBN induces anhedonia in males; however, this interpretation is not supported by the delayed discounting data showing that LBN did not affect training for sucrose pellets and that LBN males were more likely to wait for the larger reward. In contrast to the low dose, self-administration of a higher dose of morphine was not affected by LBN exposure in males, suggesting that the changes in the reinforcing efficacy of morphine produced by LBN are dose-dependent. We chose two doses of morphine that are toward either the peak reinforcing efficacy (0.25 mg/kg/ infusion) or the nadir (0.75 mg/kg/infusion) of the dose–response curve for motivation to earn morphine infusions (Swain, Muelken, LeSage, Gewirtz, & Harris, 2018). This experimental design permitted the detection of either enhancement or suppression of morphine dosing following LBN. The lack of effect at the higher dose may reflect the fact that the motivation to earn infusions at the higher dose is lower in control animals.

Unlike the stress-inoculating effects observed in males, LBN had no significant effect on either dose of morphine self- administration in females. The lack of LBN effect on impulsive choice and morphine self-administration in females could suggest that the way in which we implement this manipulation does not impact them. Another possibility is that females are affected in other ways that we did not assess. Levis et al. (2019) examined the effects of LBN on heroin and remifentanil self-administration in female rats (males were not tested) and found no effect on the acquisition of self-administration but noted resistance to extinction and increased reinstatement (Levis et al., 2019). We found no difference between LBN and control males or females in cue-induced reinstatement after 1 wk of abstinence. None of the rats underwent extinction in our study, which could partly explain this discrepancy. However, in a behavioral economics task, LBN females had reduced demand elasticity for remifentanil, indicating they were willing to pay a higher price for the drug, an effect suggestive of higher motivation (Levis et al., 2019). We did not observe an effect of LBN on morphine PR in females for either dose, revealing no change in motivation for morphine. This result could be attributed to the different drugs used. However, another possibility is that differences in the implementation of the LBN model plays a role. As noted, in our manipulation, rats are bred in-house. In many other iterations of the model—including the one by Levis et al. (2019)—dams are shipped while pregnant, which is a stressor. Therefore, effects on addiction- related behavior in females may require both a prenatal and postnatal hit (i.e., two hits) of stress.

LBN Dampens Glutamate Transmission in Male Rats, an Effect Not Found in Females

LBN decreased presynaptic glutamate transmission in males, as indexed by a reduction in sEPSC frequency. This effect was observed in both drug-naïve and morphine-experienced males, reflecting an LBN-induced general dampening of glutamate transmission in males. Females did not exhibit any significant changes in sEPSC frequency following LBN. This result provides electrophysiological evidence for early life stress influencing glutamate transmission within the NAcC and complements previous work demonstrating that maternal separation stress leads to decreased GluA2 AMPA subunit expression in the NAc of males but not of females (Ganguly, Honeycutt, Rowe, Demaestri, & Brenhouse, 2019). Of note, many other studies have demonstrated stress-induced increases in glutamate transmission within the NAc, but those studies focused on recordings in the NAc shell and primarily examined the influence of stress exposure during adulthood (Khibnik et al., 2016; J. Muir et al., 2020; E. S. Williams et al., 2020).

LBN-induced decreases in glutamate transmission may be a key contributing factor to the behavioral effects of this manipulation. In support of this notion, reducing glutamate transmission with the NMDA receptor antagonist ifenprodil in male rats reduced impulsive choice during delay discounting (Yates & Bardo, 2017). Similarly, dampening of accumbal glutamate transmission leads to decreased opioid-seeking (Bossert, Gray, Lu, & Shaham, 2006) and decreased conditioned opioid reward (Baharlouei, Sarihi, Moradi, Zarrabian, & Haghparast, 2018). These results, juxtaposed with the behavioral and neurophysiological data reported here, suggest that reduced glutamate transmission in LBN males could contribute to the reduction in impulsive choice decrease in morphine self-administration.

Morphine Self-Administration Experience Enhances Glutamate Transmission in Male Rats, an Effect Blocked by LBN Exposure

In this study examining the electrophysiological consequences of morphine self-administration with the NAcC, control male rats exhibited an increase in the

AMPA/NMDA ratio within the NAcC following 13 d of morphine self-administration and then 7 d of forced abstinence. This change is consistent with an increased AMPA/NMDA ratio in the NAc shell of dopamine 1 (D1) receptor medium spiny neurons following experimenter-administered morphine in male mice (Hearing et al., 2016). Thus, it is possible that the D1 receptors are driving the effects observed here, although this remains to be explored in future studies.

Unlike in males, we did not find an effect of morphine self- administration on the AMPA/NMDA ratio in females. Given that morphine self-administration did not change the AMPA/NMDA ratio in females, other types of plasticity must underlie female self-administration behavior. These findings may also extend to other drugs of abuse, as there is no published evidence that cocaine increases the AMPA/NMDA ratio in female animals. Additionally, these studies demonstrate that LBN prevents the morphine-induced alteration in the AMPA/NMDA ratio in the NAcC of male rats. NAc AMPA receptors are necessary for the negative affective state induced by morphine withdrawal (Russell et al., 2016). As such, it is tempting to speculate that early life stress decreases morphine-taking in males by preventing the plasticity that leads to the negative affect that drives drug-seeking, but this idea needs to be tested.

LBN Induces Sex-Specific Epigenetic and Transcriptional Changes in the NAc

RNA-sequencing revealed sex-specific changes in gene expression induced by LBN in the NAc. Interestingly, these transcriptional changes were more pronounced in females, the sex that did not demonstrate any LBN-induced behavioral phenotypes. This finding indicates an active form of resilience in females and is consistent with previous animal models of stress. For example, while males are not susceptible to subchronic variable stress (SCVS), they demonstrate greater transcriptional changes within the NAc compared with females who are affected by SCVS (G. E. Hodes et al., 2015).

Gene set enrichment (GSE) analysis revealed that LBN had differential effects on the NAc transcriptome in males and females. Only three overlapping pathways were identified between males and females: endocytosis, cholinergic synapse, and dopaminergic synapse. Within these pathways, the genes responsible for the enrichment were distinct between males and females. Two pathways that were uniquely overrepresented in male DEGs, "SNARE interactions in vesicular transport" and "ionotropic glutamate receptor," are consistent with their LBN-induced changes in NAc glutamate transmission. Additionally, oxytocin signaling in the NAc is a well- known modulator of opiate addiction-like behaviors (Leong, Cox, King, Becker, & Reichel, 2018) and the "oxytocin signaling" pathway was represented only in male DEGs. In females, it was surprising that "glutamatergic synapse" and "morphine addiction" were in the KEGG enrichment terms, because LBN did not alter their glutamate transmission or morphine self-administration. These findings highlight the possibility that compensatory mechanisms reflected in the numerous female- specific pathways, such as "GABA-B receptor II signaling," may result in little observable phenotypes in females.

Consistent with our observation that LBN was accompanied by unique transcriptional signatures in the male and female NAc, potential master regulators engaged by LBN were unique to each sex. In LBN males, HOMER motif analysis identified KLF1, a transcription factor that interacts with glucocorticoid receptors to regulate its target genes (Voorhees et al., 2013). Chronic restraint stress similarly increased KLF1 in erythroid progenitor cells of mice (Voorhees et al., 2013). Transcription factors important for autophagy (E2F1) and lysosomal biogenesis (TFE3) (Gu et al., 2018; Martina et al., 2014) were likely regulators of DEGs in LBN males, raising the interesting possibility that baseline cellular clearance is altered (Raben & Puertollano, 2016). In females, thyroid hormone receptor beta (THRB) was a predicted master regulator of LBN-related DEGs in females. Thyroid hormone receptors can interact with repressive complexes, including histone deacetylases, in the absence of the hormone, while ligand binding results in conformational changes of the receptor and instead facilitates association with coactivators, including histone acetylases (Bernal, 2007). Thus, THRB may facilitate changes in gene expression following LBN through changes to the epigenetic landscape of its target genes. Importantly, the predicted transcription factors that may orchestrate the broad changes in gene expression elicited by LBN in males and females are distinct from those predicted to coordinate baseline sex difference in the NAc. The only potential exception is Rfx1 in females, since RFX family transcription factors were identified by HOMER when comparing control males to control females. Overall, these analyses highlight the potential master regulators in the NAc that warrant further investigation in the context of stress inoculation.

Conclusions

Here we have identified a stress inoculating effect of early life stress on addiction-related phenotypes in males. These finding lay the groundwork for further examination of the specific mechanisms underlying behavioral phenotypes of LBN in males and females at the behavioral, electrophysiological, and molecular levels. Importantly, understanding the mechanisms that promote resilience to SUD can guide the development of novel treatments to reduce opioid abuse.

Materials and Methods

Subjects & LBN

Long–Evans rats bred in-house from Charles River stock were assigned at random to be reared in LBN or control housing condition on PND2. Control animals were placed in standard housing conditions with access to bedding, 2 cotton nestlets, and 1 tunnel enrichment device. LBN animals were transferred to cages fitted with a custom stainless-steel metal platform to prevent access to bedding, only provided 1 paper towel for nesting material, and were denied enrichment as described (Rudd et al., 2016). On PND10 all rats were transferred into standard housing conditions and rats were weaned at PND21 into same-sex pairs. For all experiments, no more than four rats (two males, two females) were used from each litter. Rats were kept on a 12-hour reverse light-dark cycle (lights off at 1100h in breeding colony and 0830h after weaning) and given *ad libitum* food (LabDiet 5001) and water with one exception. For delayed discounting, food access was restricted to reduce body weight to 85% of free feeding weight, with target weights increasing incrementally weekly to allow for continued growth.

Delay Discounting

Delay discounting was adapted from previous work (C. M. Stopper et al., 2014). We used 8 operant conditioning chambers (30×24×30 cm, Med Associates Inc., USA), fitted with two retractable levers flanking a central food magazine that could deliver 45-

mg sucrose pellets (Lot: 1811251, TestDiet, USA). Animals were kept on a reverse light/dark cycle and testing always occurred in the dark. Before training, rats were pre-exposed to 40 sucrose pellets in their home cage. Training began with teaching rats to lever press under a fixed- ratio 1 (FR1) schedule until they reached a criterion of 50 presses in 30min, first for the left then right lever. Next rats were trained on one session of the reward magnitude discrimination where they learned to associate one lever with a large reward and the other with a small reward (but there were no delays for the large reward). Then rats were trained on the delayed discounting task. For both the reward magnitude discrimination and delay discounting phases, there were four blocks of 12 trials. Each block was comprised of two forced-choice trials of which only one lever was presented (one trial for each lever, in randomized order) followed by 10 free-choice trials (both levers were out). If the lever was not pressed in 10s, an omission was recorded. One lever was assigned as the small reward lever, which delivered 1 pellet after a single lever press. The other lever was assigned as the large reward lever, which delivered 4 pellets after a single lever press. The position of the large reward lever was counterbalanced between rats and both levers immediately retracted following a lever press selection. The delay to the large reward increased across the four blocks of trials (0s Block 1, 15s Block 2, 30s Block 3, and 45s Block 4) and began immediately after animals selected the large/delayed lever. The primary dependent measure of interest was the proportion of choices of the large/delayed lever. For each block, the proportion of choices was calculated by dividing the number of choices of the large/delayed reward lever by the total number of successful trials. Once stability was reached, rats were tested for an additional 3d and these sessions were analyzed. We defined stable performance on delay discounting when a mixed factors ANOVA revealed that there was no effect of day or day × block interaction for three consecutive training days as detailed in (2). Three male control rats did not choose the larger/delayed option 7 out of 10 times during the 0s delay after the rest of their cohort reached stable performance, so they were dropped from the experiment for failing to understanding the task contingencies, leaving the final total 37 rats.

Morphine Self-Administration

A different cohort of adult rats underwent intravenous catheter surgery. Anesthesia was performed using a combination of ketamine hydrochloride (90 mg/kg, i.p.) and xylazine (2.1 mg/kg, i.p.). Silastic catheters (SAI Infusion Technologies, Lake Villa, IL) were attached to a back implant and inserted into the right jugular vein. Post-surgery, animals received carprophen (5mg/kg i.p.) and were placed on heating pads until they awoke from anesthesia, then transferred to appropriate animal suites. After recovery (7 d), rats were trained to self-administer morphine for 6 h/d for 10 d on an FR1 reinforcement schedule; one active lever press resulted in one morphine infusion. An inactive lever with no programmed consequences was also present. After 10 d on FR1, rats were switched to a FR3 schedule, where three presses were required to earn a morphine infusion for two consecutive days. On day 13, animals were switched to a PR schedule, in which the number of active lever presses required to obtain an infusion increased steadily throughout the session. A subset of rats received a cue test, in which each lever press was reinforced by a previously drug-paired cue but morphine was not available, on day 7 of forced abstinence following 13 d of morphine self-administration (0.75 mg/kg/in- fusion).

Whole-Cell Recordings in the NAcC

Rats exposed to the cue test were euthanized 24 h later and compared with drug-naïve controls, which were taken from their home cage. The brain was removed and coronal slices (300 μm) of NAc tissue were prepared for recording. were cut with a Vibratome (VT1000S, Leica Microsystems) in an ice-cold artificial cerebrospinal fluid solution (ACSF), in which NaCl was replaced by an equiosmolar concentration of sucrose. ACSF consisted of 130 mM NaCl, 3 mM KCl, 1.25 mM NaH2PO4, 26 mM NaHCO3, 10 mM glucose, 1 mM MgCl2, and 2 mM CaCl2 (pH 7.2–7.4 when saturated with 95% O2/5% CO2). Slices were incubated in ACSF at 32–34 °C for 25 min and kept at 22–25 °C thereafter, until transfer to the recording chamber. The osmolarity of all extracellular solutions was 300–315 mOsm. Slices were viewed using infrared differential interference contrast optics under an upright microscope (Slice Scope Pro, Scientifica) with a 40 × water-immersion objective.

The recording chamber was continuously perfused (1–2 ml/min) with oxygenated ACSF heated to 32±1 °C using an automatic temperature controller (Warner Instruments). Picrotoxin (100 μM) was added to all solutions to block the GABAA receptor-mediated currents. Recording pipettes were pulled from borosilicate glass capillaries (World Precision Instruments) to a resistance of 4–7 MΩ when filled with the intracellular solution (whole-cell recordings) or to a resistance of 3–6 MΩ when filled with extracellular solution (field recordings). All recordings were conducted with a

MultiClamp700B amplifier (Molecular Devices). Intracellular solution contained (in mM): 100 $CsCH_3O_3S$, 50 CsCl, 3 KCl, 0.2 BAPTA, 10 HEPES, 1 $MgCl_2$, 2.5 phosphocreatine-2Na, 2 Mg-ATP, 0.25 GTP-Tris, 1 QX-314 (pH 7.2–7.3 with CsOH, osmolarity 280–290 mOsm). All sEPSC recordings were conducted in whole-cell voltage-clamp mode ($V_h = -70$ mV). Currents were low-pass filtered at 1 kHz and digitized at 20 kHz using a Digidata 1550A acquisition board and pClamp10 software (both from Molecular Devices). Access resistance (10– 30 MΩ) was monitored throughout the recordings by injection of 10 mV hyperpolarizing pulses and data were discarded if access resistance changed by >25% over the course of data acquisition. sEPSCs were detected using an automated sliding-template-based algorithm in pClamp 10. All detected events were verified by visual confirmation of a fast rise time and slower exponential decay to baseline. Mean sEPSC frequencies and amplitude were analyzed from 180s long trace segments. Evoked responses were triggered by 100 μs constant-current pulses generated by an A310 Accupulser (World Precision Instruments) and delivered at 0.06 Hz via a bipolar tungsten stimulation electrode positioned within 100 μm of the recorded cell. The amplitude of the current pulses was controlled by a stimulus isolator (WPI Linear Stimulus Isolator A395) and was adjusted to elicit monosynaptic responses in the range of 100–300 pA (the required stimulus intensity ranged from 15 to 80 μA). AMPA/NMDA current ratios were computed by dividing the mean peak eEPSC at -70mV (AMPA-mediated) by the mean amplitude at +40mV, 35 ms after the peak over a 2 ms window (NMDA-mediated). For all measures, cells from at least three animals within each group were used. Recordings were taken from cells within the NAcC.

Behavior and Physiology Data Analysis

Males and females were analyzed separately because we *a priori* predicted that LBN would have a bigger effect on males based on previous studies (Rudd et al., 2016; Santarelli et al., 2017) and we wanted the behavior and physiology comparisons to mirror the RNAseq data analysis that typically compares two groups (so here we separated males and females). As we do not compare the sexes for the behavior and physiology, we cannot conclude that there are sex differences and so limit our discussion of these findings to effects in males or females. Mixed factors ANOVAs were used for delay discounting with condition (control vs. LBN) as a between factor and delay (0s, 15s, 30s and 45s) as within factor. Mixed factors ANOVAs were also used for the FR1 self-administration data with (control vs. LBN) as a between factor and day as a within factor. Independent samples t-tests were used for PR. For electrophysiology studies, a 2×2 ANOVAs with condition (control vs. LBN) and drug (drug naïve vs. morphine SA) were conducted.

RNA Sequencing and Data Analysis

RNA was extracted from bilateral NAc punches and RNA quality was assessed via Nanodrop (260/280nm and 260/230nm). RNA quantity was measured using Quibit TM RNA HS Assay kit (Invitrogen, USA) following the manufacturer’s protocol. RNA quality was further assessed via RNA integrity number by Beijing Genomics Institute (BGI) Americas Corporation using an Agilent 2100 Bioanalyzer RNA 6000 nano kit (Agilent, USA). Samples with an RNA integrity number >8 were used for library preparation (performed by BGI Americas) and were sequenced on an Illumina HiSeq 4000.

Fastqc version 0.11.8 was used to evaluate the quality of reads (Andrews, 2015) with adaptors and nonpaired reads removed using Trimmomatic version 0.39 (Bolger, Lohse, & Usadel, 2014). RNA-seq analysis was performed as suggested by Sahraeian et al. (2017) (Sahraeian & *al.*, 2017). Briefly, to identify differences in gene expression, sequenced reads were aligned to the rn6 genome assembly using the Hisat2 software (Sirén, Välimäki, & Mäkinen, 2014) and were quantified using StringTie (Pertea & *al.*, 2015). DEGs were identified using an adjusted P value of <0.1 and a 50% change in the expression ($|\log2 \text{ fold change}|>0.58$) as cutoffs to determine significance. R statistical software (ver 4.0) was used for downstream analysis and visualization of the RNA-seq output including, but not limited, to hierarchical clustering analysis and drawing heatmaps.

The RRHO version 2 test (Cahill, Huo, Tseng, Logan, & Seney, 2018) evaluated the degree of overlap in gene signatures between sexes. The full table of comparisons between LBN samples and their control in each sex was extracted. The adjusted-p-values were multiplied by the sign of the effect to show up or down-regulated genes. These tables were used to make the RRHO heatmap. GSE analysis is a method to detect parts of genes that are over- represented in a large group of genes and may have an association with analyzed phenotypes (e.g. LBN treatment). GSE analysis was done using g:Profiler website service as previously described by (Reimand et al., 2019) and using DEGs from the male and female groups separately. KEGG pathways with an adjusted P value of <0.1

were selected. The WebGestalt online toolkit (Liao, Wang, Jaehnig, Shi, & Zhang, 2019) was used for Panther enrichment analysis.

CHAPTER 3: EFFECTS OF EARLY LIFE STRESS ON IMPULSIVE ACTION, RISKY DECISION-MAKING, AND THE ORBITOFRONTAL TRANSCRIPTOME IN MALE AND FEMALE RATS

Impulsivity is a multifaceted construct that encompasses various domains of behavioral inhibition. As such, researchers often classify impulsive behaviors as either an impulsive choice or an impulsive action. Impulsive choice is the inability to delay gratification, whereas impulsive action is the inability to withhold premature responses. By classifying behaviors in this way, researchers can measure various aspects of impulsivity associated with different psychiatric disorders. For instance, patients with ADHD often have problems in response inhibition which is closely related to impulsive action (Avisar & Shalev, 2011; Schachar et al., 2007; Schachar, Tannock, Marriott, & Logan, 1995). Additionally, having high impulsive action is negatively associated with willingness to quit smoking, while having high impulsive choice is associated with impairments in maintaining abstinence from smoking (McCarthy et al., 2016). Therefore, when examining impulsivity, it is important to measure both impulsive choice and impulsive action to better understand how it is affected by a range of factors (e.g., genetics, environment).

One type of decision-making process related to impulsivity is risky decision-making, which is the ability to weigh the risks needed to obtain a reward. Moderate amounts of risky behaviors can be advantageous and groundbreaking innovations are often made possible because someone took a risk. However, when people take too many risks, this type of behavior can lead to negative outcomes. Similar to impulsivity, excessive risk-taking frequently accompanies psychiatric conditions like ADHD, schizophrenia, addiction, major depression, & Parkinson's disease (Bechara et al., 2001; Ernst et al., 2003; Ludewig et al., 2003; Taylor Tavares et al., 2007; Kobayakawa et al., 2008). For instance, individuals with SUD often seek drug reinforcement despite elevated risk of adverse consequences on their well-being (Brand, Roth-Bauer, Driessen, & Markowitsch, 2008; Brevers, Bechara, Cleeremans, Kornreich, Verbanck, & Noël, 2014; Lane & Cherek, 2000).

Although there is a relationship between impulsivity and risky decision-making, these processes are different from one another. For example, studies that use delay and probability discounting show that steeper discounting in one measure does not predict the results of the other (Herman et al., 2018; Holt et al., 2003; Madden et al., 2009). These data suggest that these task measure different constructs. As such, it is more appropriate to measure behaviors associated with risky decision-making separate from those associated with impulsivity.

A brain region that can mediate aspects of both impulsivity and risk-based decision-making is the orbitofrontal cortex (OFC). Lesions to the OFC demonstrate it has a role in learning and keeping track of outcome values to guide future responding, which are important components for making proper decision-making (Zeeb & Winstanley, 2011). Even though the entire OFC plays a role in guiding behavior, separate sub-regions of the OFC can mediate distinct aspects of cost-benefit decision-making in humans and animals (Abela & Chudasama, 2013; Cardinal, Pennicott, Sugathapala, Robbins, & Everitt, 2001; Mar et al., 2011; Mobini et al., 2002). For instance, lesioning the medial

OFC versus the lateral OFC in adult male rats causes different effects in delay-discounting performance (Mar et al., 2011). Rats with lesions specific to the lateral OFC show increased impulsivity, whereas medial OFC-lesioned rats show decreased impulsivity. Different areas of the OFC therefore can mediate impulsive behaviors in diverse ways. Additionally, medial OFC inactivation in male rats does not change their impulsivity during delay-discounting but selectively increases risky choice during probability discounting (Stopper, Green, & Floresco, 2014). These data indicate that different areas of the OFC can mediate various forms of decision-making and that medial areas regulate risky decision-making behaviors whereas lateral areas are involved in impulsive choice.

An important risk factor that can affect impulsivity and risky behavior is exposure to early life stress. Early life experiences are predictive of changes in these behaviors in adulthood and one prevalent form of adversity is poverty. To model this form of stress in rodents, we use the limited bedding and nesting (LBN) paradigm, in which dams and their pups are exposed to a low resource environment from postnatal day (PND) 2-9. Compared to other rodent models of adversity, the LBN paradigm has high translational value and allows for better understanding of the neurobiology of stress-related disorders. We have previously found that LBN-exposure reduces impulsive choices in males but not females. Given these findings, here we tested whether LBN exposure would also alter impulsive action and risky decision-making in adult male and female rats. Because the orbitofrontal cortex (OFC) mediates these processes, we then examined whether LBN promotes gene expression changes in this brain region using RNA-sequencing (RNA-seq). We assessed a top gene target from our RNA-seq data using the fluorescent *in situ* hybridization technique, RNAScope.

Methods

Subjects

Long-Evans rats (Charles River Laboratories, Wilmington, MA) were bred in-house at Temple University. On PND2, litters were culled to 10 pups with an even male-female split where possible and were randomly assigned to either control housing or LBN housing conditions as described below. Animals were given *ad libitum* access to food and water while being kept on a 12h reverse light/dark cycle (lights off at 11:00AM in breeding colony and 8:30AM after weaning at PND21). At PND21, rats were weaned into same-sex and -stress condition pairs. Once rats reached adulthood (PND60+), food access was restricted to reduce body weight to 85% of free feeding weight, with target weights increasing incrementally weekly to allow for continued growth. All experiments occurred during adulthood and were approved by and in accordance with guidelines implemented by Temple University's Institutional Animal Care and Use Committee and the National Institutes of Health guidelines.

LBN

Dams with litters that were assigned to control housing conditions were reared in standard laboratory housing: access to bedding, 2 cotton nestlets, and 1 plastic tunnel enrichment device. LBN animals were transferred to cages fitted with a custom stainless-steel metal platform to prevent access to bedding, only provided 1 paper towel for nesting material, and were denied enrichment. Animals in both conditions were left undisturbed from PND2 through PND9. On PND10, all dams and litters were rehoused in clean control housing conditions. On PND 21, rats were weaned into same-sex pairs.

Differential-Reinforcement-of-Low-Rates of Responding (DRL)

Testing took place in eight identical operant conditioning chambers (30×24×30cm, Med Associates Inc., USA), fitted with two retractable levers flanking a central food magazine that could deliver 45mg sucrose pellets (Lot: 1811251, TestDiet, USA). Animals were kept on a reverse light/dark cycle and testing always occurred in the dark. Before training, rats were pre-exposed to 40 sucrose pellets in their home cage. Training began with teaching rats to lever press under a fixed-ratio 1 (FR1) schedule until they reached a criterion of 50 presses in 30min, on the right lever. The left lever was inactive throughout the whole experiment. Lever press training lasted 2 days. Once rats learned to lever press, they transitioned to DRL schedules. During DRL, all animals had to wait a specific length of time before pressing the lever (5 sec for DRL-5, 10 sec for DRL-10, 15 sec for DRL-15). Premature responses were not rewarded and reset the DRL clock to zero. Each daily session lasted for a fixed duration of 45 minutes. For the first five days, the animals were tested on the DRL-5 schedule, followed by another block of 10 days of DRL-10, and then 10 days of DRL-15.

Probability Discounting

Pre-training

A separate group of control and LBN rats was trained on the probability discounting task. Behavioral testing occurred in the same eight operant chambers described for DRL. Instead of only one active lever however, two retractable levers were used for this task. One day prior to training, rats are introduced to 40 sucrose pellets in their home cage to habituate to the reinforcer. The following day, rats learned to press the left lever over 50 times. The next day, they learned to press the right lever. Lever press training was counter-balanced. Following lever press training, rats train to lever press retractable levers. This takes approximately three to five days. Each lever retracts if not pressed after 10 seconds of extension. Failure to press the lever when extended into the chamber results in the absence of a reward. Rats pass this phase in training until they make fewer than 10 omissions. Upon passing retractable lever training, the rats begin training on the actual discounting task.

Testing

Probability discounting measures the number of times a rat makes risky choices. Each session lasts 45 min and is broken down into five blocks. During each block, a rat chooses between two levers: one lever delivers one pellet always (certain lever), whereas the other delivers four pellets sometimes (risky lever). When the risky reward lever is chosen the probability of earning four pellets can vary throughout the session. Rats are exposed to two different versions of the task: ascending vs. descending. When rats train on the ascending version, the odds of earning the large reward start low but then increase (e.g., 6.25% in the first block, 12.5% in the second block, 25% in the third, 50% in the fourth, and 100% in the fifth). The reverse occurs in the descending program. Choice for the risky lever is only advantageous when the probability is 100% or 50%. At 25%, selecting the risky lever is just as advantageous as selecting the certain lever. As soon as the probabilities decrease to 12.5% or 6.25%, it is more advantageous for the animal to select the certain lever in order to get a higher payoff. Rats were trained until as a group they demonstrated stable baseline levels of discounting, which took approximately 25-30 days. Data from 3 consecutive days following stability were analyzed with a repeated-measures analysis of variance (ANOVA) and 2 within-subjects factors (day and trial block). If the effect of block was significant at the $p<.05$ level, but there was no main effect of day or day×block interaction ($p>.01$), animals were judged to have acquired stability.

Behavior Data Analysis

For DRL, we were interested in the following dependent measures during each of the three schedules: total number of rewards earned, total number of responses, and burst ratio. Burst ratio is the number of responses occurring within 3 sec of a previous response divided by the number of burst responses predicted by the corresponding negative exponential. An increase in burst ratio is an indicator of impulsive action. Data from DRL testing was analyzed using a mixed factors ANOVA with session as the within-subjects factor and condition (LBN vs. control) and sex (male vs. female) as a between-subjects factor. These computations were conducted for each DRL schedule. For probability discounting, the measure of interest was the proportion of choices directed toward the large/risky reward lever for each block of free-choice trials, factoring in trial omissions. The data from probability discounting was also analyzed using a mixed factors ANOVA with trial block as the within-subjects factor and condition and sex as between-subjects factor. These computations were conducted for all trials across the 5 blocks. We could not conduct a block-by-block analysis of these data because there were instances where the rats did not obtain the large reward at all during the low probability blocks. Response latencies and number of trial omissions were also analyzed using a mixed factors ANOVA with trial block as within-subject factor and condition and sex as between-subjects factor. We also calculated win-stay and lose-shift ratios from probability discounting data to examine if LBN affected reward feedback or negative feedback sensitivity. Win-stay was calculated by the number of times rat chose the large/risky lever after choosing the risky option on the preceding trial and dividing this by the total number of free-choice trials resulting in a win. For lose-shift, the number of times a rat shifted choice to the small/certain lever after choosing the risky option on the preceding trial and divided this by the total number of free-choice trials resulting in a loss. A multivariate ANOVAs was used for choice behavior analyses with win-stay and lose-shift ratios as the within-subjects factor and condition and sex as between-subjects factor. Because we *a priori* also predicted that LBN would have a bigger effect on males based on previous studies (Eck et al., 2019; Georgia E. Hodes & Epperson, 2019; Ordoñes Sanchez et al., 2021), we also split behavioral data by sex to investigate the effects of LBN in each the sex separately.

RNA Sequencing (RNA-seq) and Data Analysis

RNA was extracted from OFC tissue from adult rats (male control n=5; female control n=5; male LBN n=5; female LBN n=5). RNA quality was assessed via Nanodrop (260/280nm and 260/230nm). RNA quantity was measured using Quibit TM RNA HS Assay kit (Invitrogen, USA) following the manufacturer’s protocol. RNA quality was further assessed via RNA integrity number by Beijing Genomics Institute (BGI)

Americas Corporation using an Agilent 2100 Bioanalyzer RNA 6000 nano kit (Agilent, USA). Samples with an RNA integrity number >8 were used for library preparation (performed by BGI Americas) and were sequenced on an Illumina HiSeq 4000.

Fastqc version 0.11.8 was used to evaluate the quality of reads (Andrews, 2015) with adaptors and nonpaired reads removed using Trimmomatic version 0.39 (Bolger et al., 2014). RNA-seq analysis was performed as suggested by Sahraeian et al. (Sahraeian & *al.*, 2017). Briefly, to identify differences in gene expression, sequenced reads were aligned to the rn6 genome assembly using the Hisat2 software (Sirén et al., 2014) and were quantified using StringTie (Pertea & *al.*, 2015). DEGs were identified using an adjusted P value of <0.1 and a 50% change in the expression (|log2 fold change|>0.58) as cutoffs to determine significance. R statistical software (ver 4.0) was used for downstream analysis and visualization of the RNA-seq output including, but not limited, to hierarchical clustering analysis and drawing heatmaps.

The rank-rank hypergeometric overlap (RRHO) version 2 test (Cahill et al., 2018) evaluated the degree of overlap in gene signatures between sexes. The full table of comparisons between LBN samples and their control in each sex was extracted. The adjusted-p-values were multiplied by the sign of the effect to show up or down-regulated genes. These tables were used to make the RRHO heatmap. Gene set enrichment (GSE) analysis is a method to detect parts of genes that are overrepresented in a large group of genes and may have an association with analyzed phenotypes (e.g. LBN treatment). GSE analysis was done using g:Profiler website service as previously described (Reimand et al., 2019), using DEGs from the male and female groups separately. KEGG pathways with an adjusted P value of <0.1 were selected. The WebGestalt online toolkit (Liao et al., 2019) was used for Panther enrichment analysis. Males and females were analyzed separately because RNA-seq data analysis typically compares two groups.

Fluorescent ISH using RNA-Scope ® and Data Analysis

In order to validate RNA-Seq data, we used RNA-Scope. Brains from a separate group of behaviorally naïve control and LBN animals were collected and flash frozen. Brains were kept in a -80°C freezer until they were sectioned. Coronal brain slices (20 μm) containing the OFC were mounted onto Superfrost plus slides (Shandon, ThermoScientific) using a cryostat (Dolbey-Jamison) at -20°C. Note, before sectioning, brains were equilibrated in the cryostat for at least 45 minutes. Slides were stored at -80°C. RNAScope ® - registered trademark *in situ* hybridization (ISH) was conducted according to the Advanced Cell Diagnostics (ACD) user manual. Briefly, slides were fixed in 4% paraformaldehyde for 30 minutes at 4°C. Slides were then dehydrated with 50% ethanol (1× 5 min), 70% ethanol (1× 5 min), and 100% ethanol (2× 5 min). Slides were then left to air-dry at room temperature (RT) for 5 min. A hydrophobic barrier was drawn around the sections using a hydrophobic pen and allowed to dry for 15 min at RT. Sections were then incubated with Protease Pretreat-4 solution for 20 min at RT. Slides were washed with wash phosphate-buffered saline twice before being incubated with the appropriate probes for 2 hrs at 40°C in the HybEZ oven (Advanced Cell Diagnostics, ACD). The following probes were purchased from ACD: Rn-*Sox9*-C1 (SOX9 is the astrocyte cell marker, catalog #406851), Rn-*Agt*-O1-C2 (*AGT* marker, catalog #553841-C2), and Rn-*Rbfox3*-C3 (NeuN is the neuronal cell marker, catalog #436351-C3).

Following the incubation with the appropriate probes, slides were subjected to a series of amplification steps at 40°C in the HybEZ oven with 2× 2 min washes (w/agitation) in between each amplification step at RT. Amplification steps were as followed: Amp1 at 40°C for 30 min, Amp 2 at 40°C for 15 min, Amp3 at 40°C for 30 min, and Amp 4-Alt A at 40°C for 15 min. A DAPI-containing solution was applied to sections (one slide at a time) at RT for 30s. Finally, slides were cover slipped using ProLong Gold Antifade mounting media following application of DAPI and left to dry for 24hrs at room temperature. Slides were then stored at 4°C until imaging on an Olympus confocal microscope.

Quantification was done using Fiji/ImageJ software. Number of DAPI + *SOX9* or DAPI+*NeuN* cells were manually counted using the cell counter function. Cells were considered positive for the *SOX9* or *NeuN* probes if there was > 4 particles tightly clustered around the nucleus. Numbers of puncta for each experimental probe (*AGT*) was analyzed in a semi-automated fashion. First, regions of interest (ROI) were identified using cell counter freehand function only outlining the soma. ROI area was measured and used to generate a mask. The image containing puncta of experimental probe underwent thresholding and was made a binary. The ROI mask and binary were combined using the AND function in Image Calculator. Following combination, the area of puncta from the experimental probe within the mask and binary was calculated using Analyze Particle function. Independent samples t-tests were used for comparing lateral and medial values.

Results

Males lever press more than females on DRL

We first examined bursting responses, which are responses that occur faster than 3s. No differences in bursting responses were observed during DRL-5 [effect of session ($F_{1.535, 56.813}$=47.011, p<.001); no effect of session×sex ($F_{1.535, 56.813}$=.192, p=.767); no effect of session×LBN ($F_{1.535, 56.813}$=.167, p=.167); no effect of sex ($F_{1, 37}$=.202, p=.656); no effect of LBN ($F_{1, 37}$=.760, p=.389); no effects of sex×LBN ($F_{1, 37}$=.854, p=.361)]. No differences in bursting were found during DRL10 [effect of session (F3.467, 128.281=4.351); no effect of session×sex (F3.467, 128.281=.237, p=.895); no effect of session×LBN (F3.467, 128.281=1.730, p=.156); no effect of sex (F1, 37=3.450, p=.068); no effect of LBN (F1, 37=1.272, p=.267); no effects of sex×LBN ($F_{1, 37}$=.573, p=.454)]. No differences in bursting were found also during DRL15 [(no effect of session ($F_{2.933, 37.00}$=2.503, p=.064); no effect of session×sex ($F_{2.933, 37.00}$=.744, p=.525); no effect of session×LBN ($F_{2.933, 37.00}$=.692, p=.556); no effect of sex ($F_{1, 37}$=.001, p=.162); no effect of LBN ($F_{1, 37}$=.001, p=.980); no effect of sex×LBN (F1, 37=.005, p=.942)].

In terms of lever presses, we found that males lever pressed more than females during DRL-5 [effects of session (F2.684, 99.298=3.637, p=.019); no effects of session×sex (F2.684, 99.298=3.637, p=.541); no effects of session×LBN (F2.684, 99.298=.105, p=.945); effects of sex (F1, 37=5.806, p=.021); no effects of LBN (F1, 37=3.287, p=.078); no effects of sex×LBN (F1, 37=.091, p=.765)]. Males also lever pressed more than females when they were tested during DRL-10 [effects of session ($F_{4.486, 165.997}$=31.561, p<.001); no effects of session×sex ($F_{4.486, 165.997}$=.516, p=.745); no effects of session×LBN ($F_{4.486, 165.997}$=1.546, p=.185); effect of sex (F1, 37=4.449, p=.042); no effect of LBN ($F_{1, 37}$=3.146, p=.084); no effects of sex×LBN ($F_{1, 37}$=3.104, p=.086)]. However, no differences in lever pressing were found during DRL-15 [effects of session ($F_{3.825, 141.515}$=10.959, p<.001); no effects of session×sex ($F_{3.825, 141.515}$=.629, p=.635); no effects of session×LBN ($F_{3.825, 141.515}$=.820, p=.530); no effects of sex ($F_{1, 37}$=2.750, p=.106); no effects of LBN ($F_{1, 37}$=.131, p=.720); no effects of sex×LBN ($F_{1, 37}$=.130, p=.721)]. Summary of results is found in Table 3.

When we examined rewards earned, we found an interaction during DRL-5 [effect of session×LBN ($F_{3.370, 124.707}$=2.612, p=.048)]. Post-hoc LSD tests revealed, however, no significant effects due to LBN. No differences in rewards earned were found during DRL-10 [effects of session ($F_{3.999, 147.973}$=45.029, p=.023); no effects of session×sex ($F_{3.999, 147.973}$=.536, p=.709); no effects of session×LBN ($F_{3.999, 147.973}$=.536, p=.709); no effects of sex ($F_{1, 37}$=1.195, p=.281); no effects of LBN ($F_{1, 37}$=1.854, p=.182); no effects of sex×LBN ($F_{1, 37}$=1.602, p=.213)]. No differences in rewards earned was found during DRL-15 [effects of session ($F_{3.122, 115.511}$=23.562, p<.011); no effects of session×sex ($F_{3.122, 115.511}$=.312, p=.824); no effects of session×LBN ($F_{3.122, 115.511}$=.534, p=.667); no effects of sex ($F_{1, 37}$=.790, p=.380); no effects of LBN ($F_{1, 37}$=.002, p=.966); no effects of sex×LBN ($F_{1, 37}$=.081, p=.778)]. Summary of results is found in Table 3.

When assessing efficacy, we found no differences during DRL-5 [effects of session ($F_{4, 148}$=24.131, p<.001); no effects of session×sex ($F_{4, 148}$=.241, p=.915); no effects of session×LBN ($F_{4, 148}$=.571, p=.663); no effects of sex ($F_{1, 37}$=.748, p=.393); no effects of LBN ($F_{1, 37}$=1.656, p=.206); no effects of sex×LBN ($F_{1, 37}$=.568, p=.456)]. No differences in efficacy were found during DRL-10 [effects of session ($F_{3.385, 125.237}$=44.702, p<.001); no effects of session×sex ($F_{3.385, 125.237}$=.548, p=.672); no effects of session×LBN ($F_{3.385, 125.237}$=.999, p=.402); no effects of sex ($F_{1, 37}$=.748, p=.393); no effects of LBN ($F_{1, 37}$=1.656, p=.206); no effects of sex×LBN (F1, 37=.568, p=.456)]. No differences in efficacy were found during DRL-15 [effects of session ($F_{3.766, 139.325}$=21.092); no effects of session×sex ($F_{3.766, 139.325}$=.904, p=.459); no effects of session×LBN ($F_{3.766, 139.325}$=.596); no effects of sex ($F_{1, 37}$=.823, p=.370); no effects of LBN ($F_{1, 37}$=.029, p=.866); no effects of session×LBN ($F_{1, 37}$=.028, p=.867)]. For all statistics where sphericity was violated, Greenhouse Geisser corrections were used. A summary of the results is found in Table 3.

DRL Schedules	Measures			
	Bursting	Lever Pressing	Rewards	Efficacy
DRL-5	----	↑ M vs. F	----	----
DRL-10	----	↑ M vs. F	----	----
DRL-15	----	----	----	----

Table 3. Summary of sex differences found in DRL. Arrows indicate males lever press more than females during the DRL-5 and DRL-10 schedule. No effects found in any other schedule. M, males; F, females.

LBN Increases Indices of Impulsive Action in Males, An Effect Not Found in Females

When we examined bursting responses by separating data by sex, we found that LBN males (n=10) made more bursting responses than control males (n=12) during DRL-5 [interaction: ($F_{2.124,42.477}$=6.886, p=.002)] (Fig. 6A). Post-hoc LSD tests revealed differences in burst ratio were specific to the first 2 days of testing [day 1 (p=.011) and day 2 (p=.041)]. No differences in bursting responses were found between LBN and control males in DRL-10 [no effect of session ($F_{2.614, 52.286}$=1.802, p=.165); no interaction effect ($F_{2.614, 52.286}$=1.222, p=.309); no effect of LBN ($F_{1,20}$=1.225, p=.281)] or DRL-15 [no effect of session ($F_{2.838, 48.242}$=3.044, p=.04); no interaction effect ($F_{2.838, 48.242}$=1.234, p=.307); no effect of LBN ($F_{1,17}$=.199, p=.661)]. No differences in bursting responses were also found between control (n=8) and LBN females (n=11) during DRL-5 [effect of session ($F_{1.316, 22.365}$=15.526, p<.001); no interaction ($F_{1.316, 22.365}$=.258, p=.681); no effect of LBN ($F_{1,17}$=.001, p=.977)], DRL-10 [no effect of session ($F_{2.838, 48.242}$=3.044, p.040); no interaction ($F_{2.838, 48.242}$=1.234, p=.307); no effect of LBN (F1, 17=.199, p=.661)], or DRL-15 [no effect of session ($F_{4.161, 70.729}$=1.227, p=.307); no interaction ($F_{4.161, 70.729}$=1.723, p=.152); no effect of LBN (F1, 17=.006, p=.938)] (Fig. 6B).

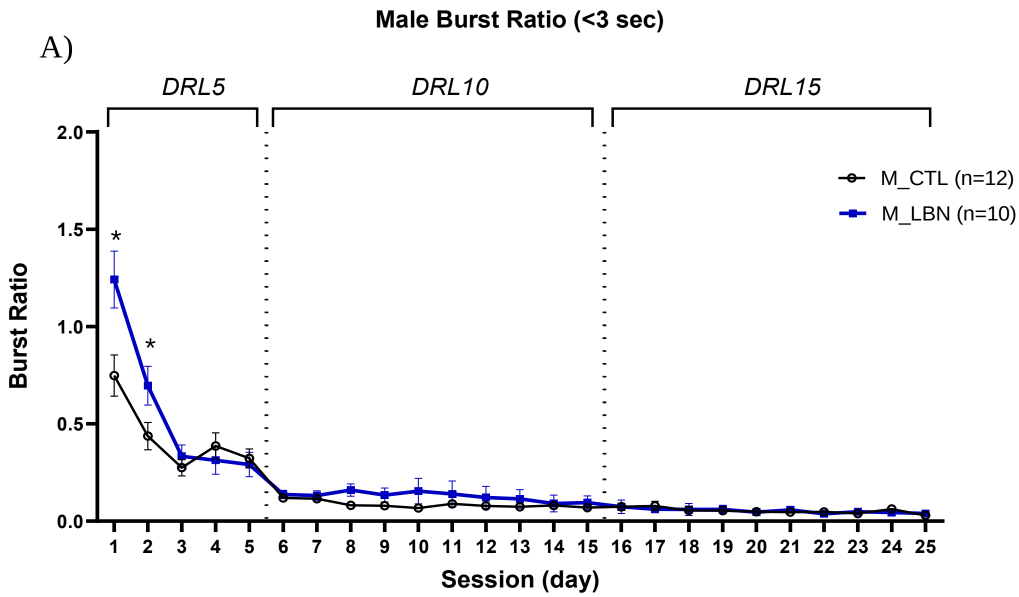
In regard to lever presses, LBN males made significantly more than controls during DRL-10 [effect of session ($F_{3.629,72.575}$=17.076, p<.001); no interaction effect ($F_{3.629,72.575}$=2.236, p=.079); effect of LBN ($F_{1,20}$=4.498, p=.047)] (Fig. 6C). No differences in lever pressing were found during DRL-5 [no effect of session: ($F_{4,80}$=

2.320, p=.064); no effect of LBN ($F_{1,20}$=2.783, p=.111); no interaction ($F_{4,80}$=.975, p=.426)] or during DRL-15 [effect of session ($F_{3.256, 65.123}$=5.215, p=.002; no interaction ($F_{3.256, 65.123}$=.721, p=.554); no LBN ($F_{1,20}$=.190, p=.667)]. LBN females lever pressing did not differ from controls during any DRL schedule [DRL-5: (no effect of session ($F_{2.048, 34.814}$=1.914, p=.162); no interaction (F2.048, 34.814=.617, p=.549); no effect of LBN ($F_{1, 17}$=.920, p=.351); DRL-10: (effect of session ($F_{3.505, 59.578}$=14.963, p<.001); no interaction ($F_{3.505, 59.578}$ =.491, p=.718); no effect of LBN ($F_{1, 17}$=.000, p=.990); DRL-15: [effect of session ($F_{3.672, 62.418}$=6.599, p<.001); no interaction ($F_{3.672, 62.418}$ =.835, p=.500); no effect of LBN ($F_{1, 17}$=.000, p=.998)] (Fig. 6D).

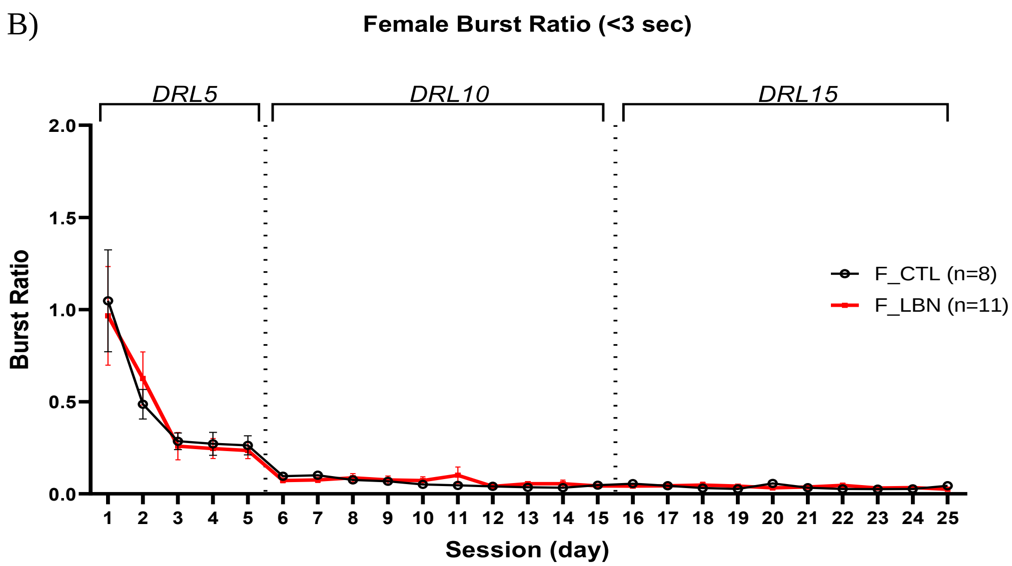
The number of rewards earned did not differ between LBN and control males during DRL-5 [effect of session ($F_{2.986, 59.723}$=18.035, p<.001); no interaction ($F_{2.986, 59.723}$=1.320, p=.276); no effect of LBN ($F_{1, 20}$=.533, p.474)], DRL-10 [effect of session ($F_{2.975, 59.502}$=20.917, p<.001); no interaction ($F_{2.975, 59.502}$=.928, p=.432); no effect of LBN ($F_{1, 20}$=2.799, p=.110)], or DRL-15 [effect of session ($F_{2.716, 54.312}$=12.831, p<.001); no interaction ($F_{2.716, 54.312}$ =.505, p=.662); no effect of LBN ($F_{1, 20}$=.048, p=.829) (Fig. 6E). No differences in total rewards earned between LBN vs. control females were found during DRL-5 [effect of session ($F_{2.688, 45.702}$=10.337, p<.001); no interaction ($F_{2.688, 45.702}$ =1.958, p=.139); no effect of LBN ($F_{1, 20}$=.271, p=.609)], DRL-10 [effect of session ($F_{4.167, 70.842}$=23.835, p<.001); no interaction ($F_{4.167, 70.842}$=.562, p=.698); no effect of LBN ($F_{1, 17}$=.007, p=.934)], or DRL-15 [effect of session ($F_{3.061, 52.035}$=11.169, p<.001); no interaction ($F_{3.061, 52.035}$=.370, p=.776); no effect of LBN ($F_{1, 17}$=.036, p=.851)] (Fig. 6F).

Despite LBN males pressing more than controls during DRL-10, their efficiency levels were not different during any schedule tested [DRL-5: (effect of session ($F_{4,80}$=12.129, p<.001); no interaction ($F_{4,80}$=.655, p=.625); no effect of LBN ($F_{1, 20}$=2.078, p=.165); DRL-10: (effect of session ($F_{2.677, 53.543}$=20.315, p<.011); no interaction ($F_{2.677, 53.543}$=20.315, p<.011); no effect of LBN ($F_{1, 20}$=1.626, p=.217); DRL-15: (effect of session ($F_{3.514, 70.281}$=11.385, p<.001); no interaction ($F_{3.514, 70.281}$ =.890, p=.464); no effect of LBN ($F_{1, 20}$=.015, p=.904)] (Fig. 6G). Female efficiency also did not differ by LBN at any schedule [DRL-5: (effect of session ($F_{4, 68}$=12.029, p<.011); no interaction ($F_{4, 68}$=.601, p=.663); no effect of LBN ($F_{1, 17}$=.147, p=.706); DRL-10: (effect of session ($F_{3.760, 63.918}$=24.292, p<.001); no interaction ($F_{3.760, 63.918}$=.549, p=.690); no effect of LBN ($F_{1, 17}$=.015, p=.015, p=.904); DRL-15: (effect of session ($F_{3.596, 61.133}$=10.593, p<.001); no interaction ($F_{3.596, 61.133}$=.483, p=.728), no effect of LBN ($F_{1, 17}$=.066, p=.800) (Fig. 6H). For all statistics where sphericity was violated, Greenhouse Geisser corrections were used.

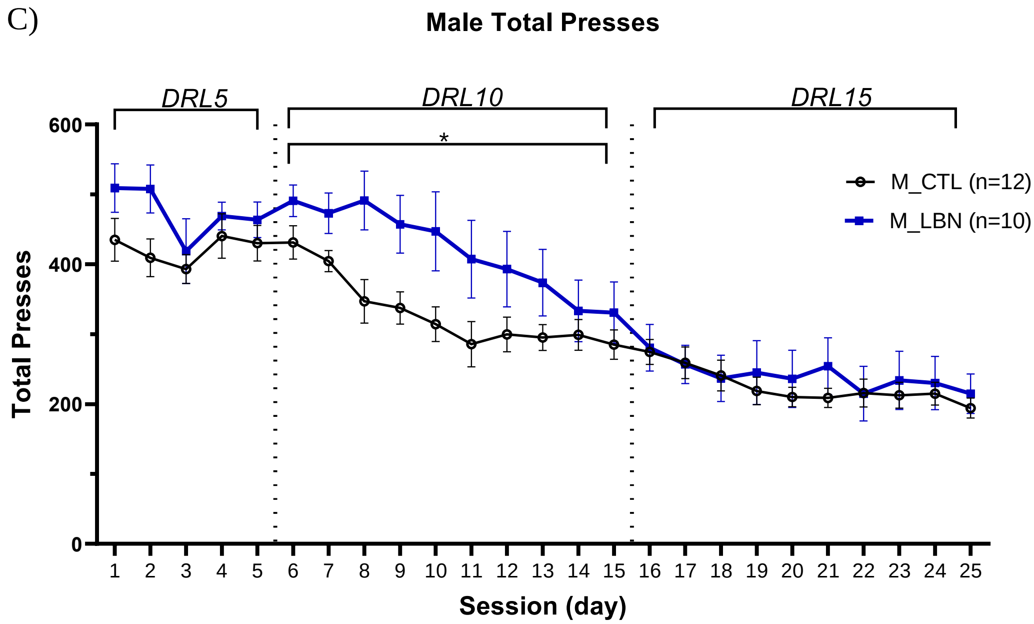
A)


Male Burst Ratio (<3 sec)
DRL5
DRL10
DRL15
2.0
1.5
1.0
0.5
0.0
Burst Ratio
*
*
M_CTL (n=12)
M_LBN (n=10)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Session (day)

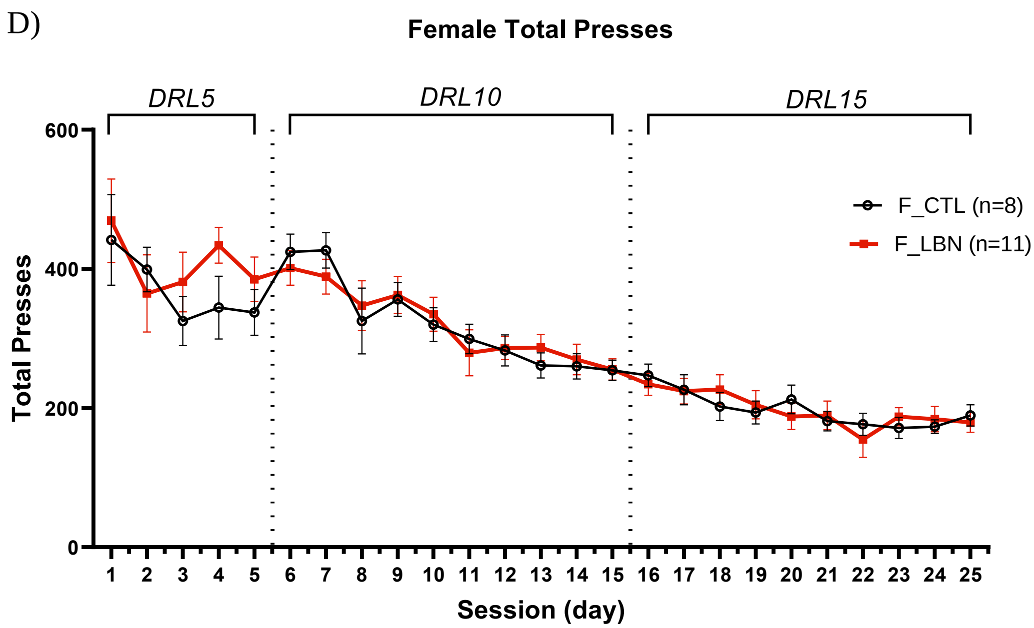
B)


Female Burst Ratio (<3 sec)
DRL5
DRL10
DRL15
2.0
1.5
1.0
0.5
0.0
Burst Ratio
F_CTL (n=8)
F_LBN (n=11)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Session (day)

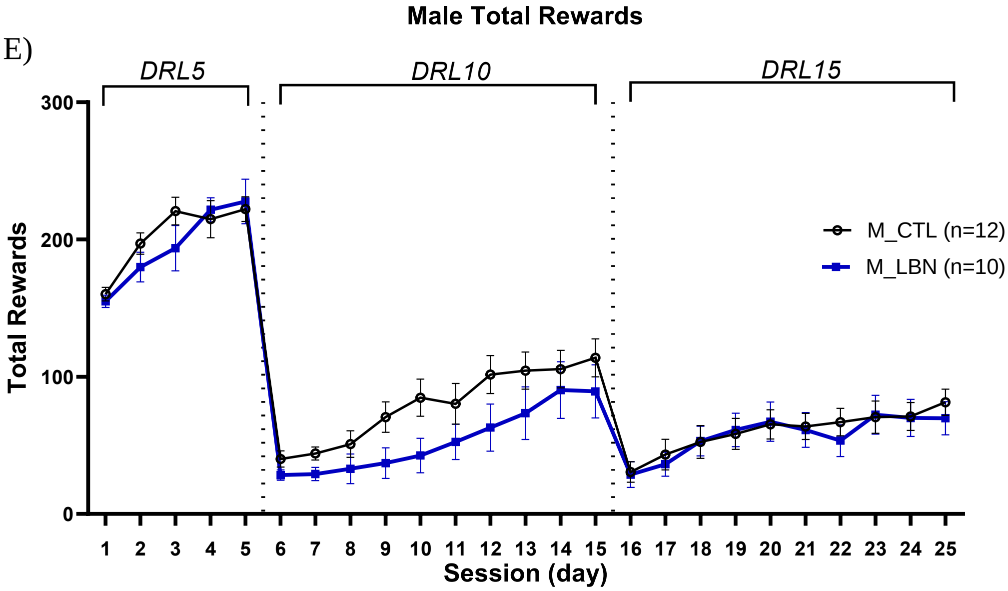
C)


Male Total Presses
DRL5
DRL10
DRL15
*
M_CTL (n=12)
M_LBN (n=10)
Total Presses
Session (day)

D)

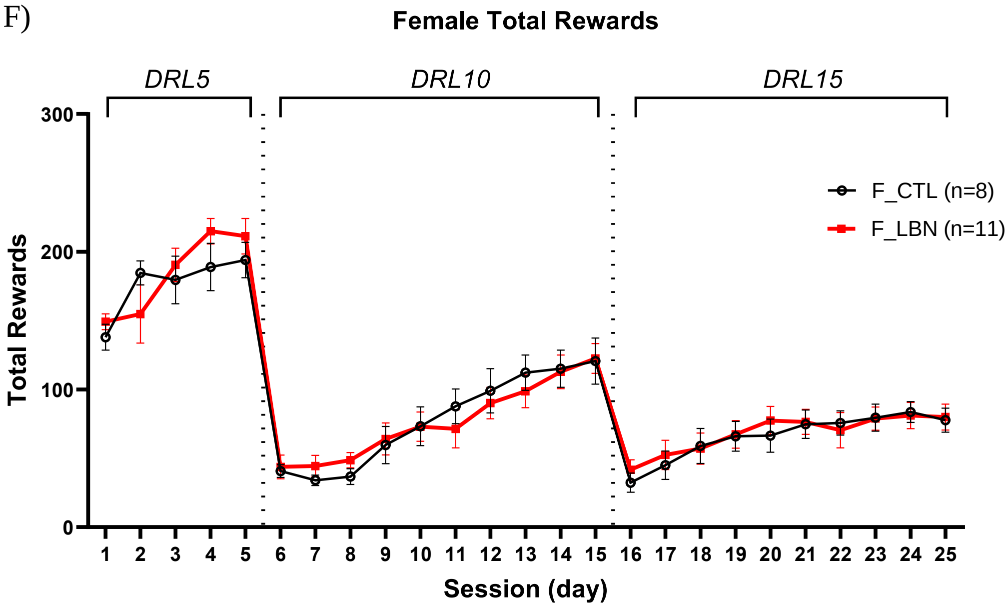

Female Total Presses
DRL5
DRL10
DRL15
F_CTL (n=8)
F_LBN (n=11)
Total Presses
Session (day)

E)



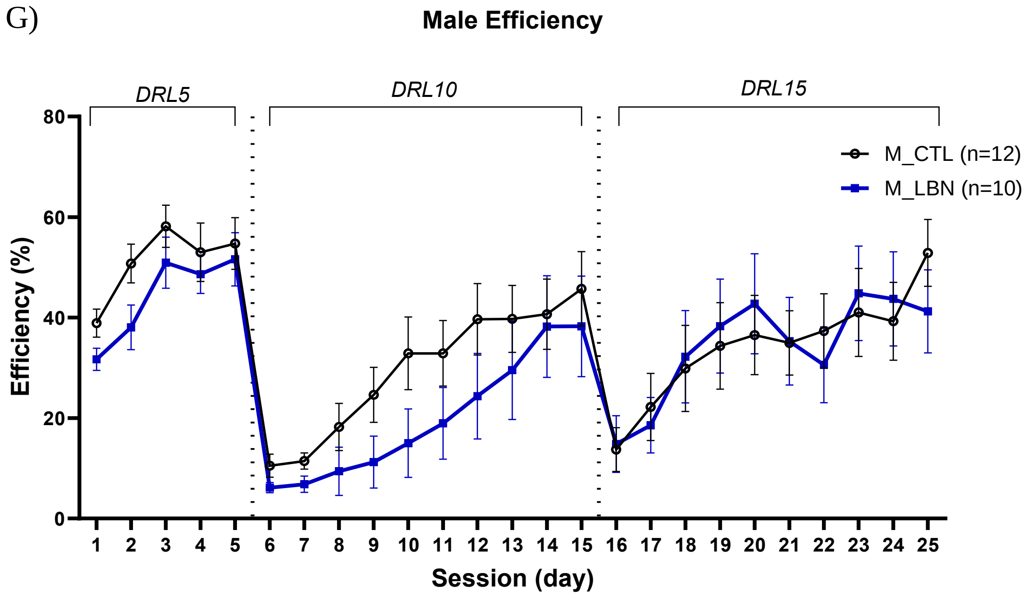
Male Total Rewards
DRL5
DRL10
DRL15
Total Rewards
300
200
100
0
M_CTL (n=12)
M_LBN (n=10)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Session (day)

F)



Female Total Rewards
DRL5
DRL10
DRL15
Total Rewards
300
200
100
0
F_CTL (n=8)
F_LBN (n=11)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
Session (day)

G)


Male Efficiency
DRL5
DRL10
DRL15
M_CTL (n=12)
M_LBN (n=10)
Efficiency (%)
Session (day)
0
20
40
60
80
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

H)

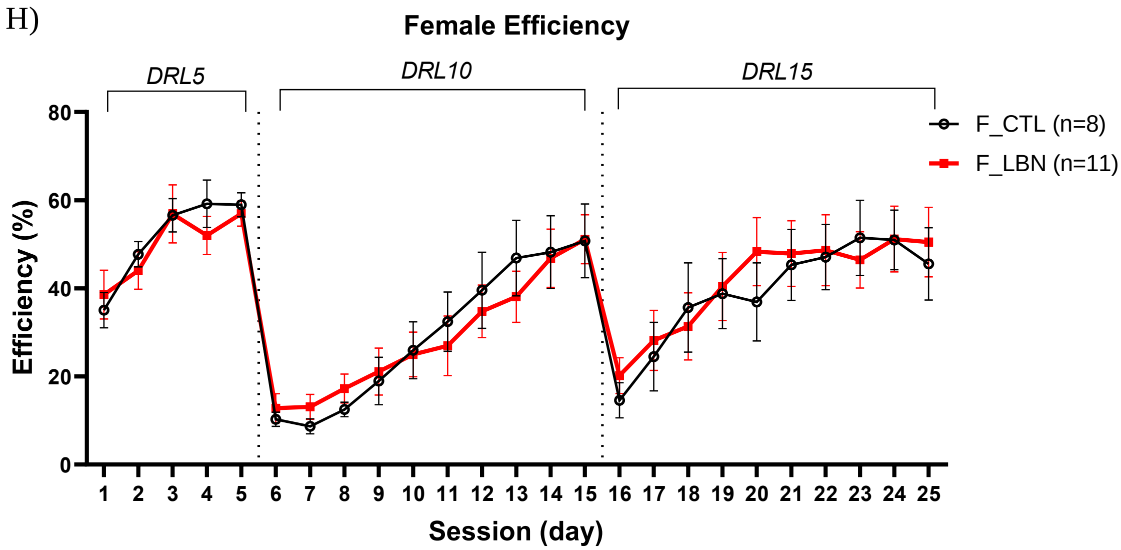

Female Efficiency
DRL5
DRL10
DRL15
F_CTL (n=8)
F_LBN (n=11)
Efficiency (%)
Session (day)
0
20
40
60
80
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

Fig. 6. LBN increases indices of impulsive action in males but not females. (A) LBN exposure increased burst ratio responding in males during DRL-5 [interaction: F (1,20)=6.886, p<.001)]. Post-hoc LSD tests revealed differences in burst ratio were specific to the first 2 days of testing [day 1(p=.011) and day 2 (p=.041)]. (B) LBN did not affect female bursting responses during any DRL schedule. (C) LBN males made significantly more lever presses than controls during DRL-10 [condition: F (1,20)=4.498, p=.047], but no differences in lever pressing were found during DRL-5 or DRL-15. (D) LBN females lever presses just as much as control females. (E) LBN males earned just as many rewards as control males. (F) LBN females earned just as many rewards as control females. (G) No differences between LBN and control male efficiency levels at any schedule. (H) No differences between LBN and control female efficiency levels at any schedule.

All Animals Exposed to LBN Make Fewer Risky Choices

In total, 98 rats were used for probability discounting experiments. One rat was excluded from data because they did not learn to select the risky lever at the highest probability (100%) over 7 times. Therefore, 97 rats were used for the analysis. Prior to splitting data by sex, we analyzed data together and did not separate data by task variant. We found that all animals exposed to LBN displayed reduced risky decision-making on the probability discounting task [effect of block×LBN ($F_{2.844, 261.607}$=2.963, p=.035)] (Fig. 7A). Post-hoc LSD tests revealed this difference was specific to when the odds of earning the reward were 25% (p=.043) and 12.5% (p=.038). Response latencies did not differ by LBN [effect of block ($F_{4, 376}$=3.138, p=.015); no effect of block×sex ($F_{4, 376}$=1.363, p=.246); no effect of LBN ($F_{1, 94}$=1.619, p=.206)] (Fig. 8A). Omissions also did not differ due to LBN exposure [no effect of block ($F_{1.928,181.275}$=2.701, p=.072); block×sex ($F_{1.928,181.275}$=1.701, p=.146); no effect of LBN ($F_{1, 94}$= 1.133, p=.290)] (Fig. 9A).

Additionally, LBN and control animals win-stay [$F_{1, 88}$=.190, p=.664] and lose-shift [$F_{1, 88}$= .236, p=.693] tendencies did not differ (Fig. 10A). Risky choice behavior did not differ by sex [effect of block (F2.845, 267.410=461.246, p<.001); no effect of block×sex ($F_{2.844, 261.607}$=1.171, p=.321); no effect of sex ($F_{1, 94}$=1.684, p=.198)]. Sex also did not affect win-stay [$F_{1, 88}$= .157, p=.693] and lose-shift [$F_{1, 88}$= 1.808, p=.182] tendencies.

LBN Does Not Alter Male or Female Risky Behavior (Data Split by Sex)

When we split data by sex, we found no LBN effects on risky choices made by males during ascending [effect of block ($F_{2.108, 52.708}$=129.350, p<.001); no block×LBN ($F_{2.108, 52.708}$=1.744, p=.183); no effect of LBN ($F_{1, 25}$= 1.788, p=.193)] or descending [effect of block ($F_{2.649, 66.222}$=150.875, p<.01); no block×LBN ($F_{2.649, 66.222}$=.630, p=.579); no effect of LBN ($F_{1, 25}$=.127, p=.725)] task variants (Fig. 7B & C). LBN also did not affect the risky choices made by females in either ascending [effect of block ($F_{2.504, 45.068}$=96.661, p<.011); no block×LBN ($F_{2.504, 45.068}$=.738, p=.512); no effect of LBN ($F_{1, 18}$=1.003, p=.330)] or descending [effect of block ($F_{2.992, 59.834}$=89.854, p<.001); no block×LBN ($F_{2.992, 59.834}$=1.182, p=.324); no effect of LBN ($F1_{, 20}$=.798, p=.382)] versions of the task (Fig. 7D & E).

LBN also had no effects on response latencies made by males during ascending [no effect of block ($F_{1.68, 41.88=}$.734, p=.463); no block×LBN ($F_{1.68, 41.88=}$.670, p=.492); no effect of LBN ($F_{1, 25=}$.002, p=.968)] or descending [no effect of block ($F_{1.88, 46.88=}$3.50, p=.010); no effect of block×LBN ($F_{1.88, 46.88=}$1.011, p=.367); no effect of LBN ($F_{1, 25}$=.037, p=.850)] (Fig. 8B &C). LBN also had no effects on response latencies made by females during ascending [no effect of block ($F_{1.32, 23.671}$=1.401, p=.242); no block×LBN ($F_{1.32, 23.671}$=1.604, p=.222); no effect of LBN ($F_{1, 18}$=8.70, p<.05)] or descending [no effect of block ($F_{1.60, 31.929}$=3.246, p=.016]no effect of block×LBN ($F_{1.60, 31.92}$=1.787, p=.139); no effect of LBN ($F_{1, 20}$=.410, p=.529)] (Fig. 8D &E).

LBN had no effect on the number of omissions made by males on either ascending [no effect of blocks ($F_{4, 100}$= .794, p=.532); no effect of block×LBN ($F_{4, 100}$= .794, p=.532); no effect of LBN ($F_{1, 25}$= .794, p=.381)] or descending [no effects of block ($F_{4, 100}$= .753, p=.558); no effect of block×LBN ($F_{4, 100}$= 1.253, p=.294); no effect of LBN ($F_{1, 25}$= .003, p=.959)] (Fig. 9B & C). LBN also did not affect the number of omissions made by females in either ascending [no effect of block ($F_{4, 72}$= 1.942, p=.113); no effect of block×LBN ($F_{4, 72}$= 1.942, p=.113); no effect of LBN ($F_{1, 18}$= 1.942, p=.180)] or descending [no effect of block ($F_{4, 80}$= 1.714, p=.155); no effect of block×LBN ($F_{4, 80}$= 1.120, p=.353); no effect of LBN ($F_{1, 20}$=.66, p=.424)] versions of the task (Fig. 9D & E).

When we examined patterns of choice behavior, LBN did not affect win-stay [$F_{1, 25}$=.029, p=.866] or lose-shift [$F_{1,25}$= 1.458, p=.239] ratios in males when performing the descending version of the task (Fig. 10B). During the ascending version of the task, LBN also did no alter male win-stay [$F_{1,25}$=.118, p=.734] or lose-shift [$F_{1,25}$=.767, p=.390] ratios (Fig. 10C). In females, LBN did not affect win-stay [$F_{1,20}$=.652, p=.429] or lose-shift [$F_{1,20}$=.013, p=.909] ratios during the descending task variant (Fig. 10D). In the ascending version of the task, LBN did not affect female lose-shift ratios [$F_{1,18}$=.263, p=.615], but seemed to affect win-stay as we detected a trend towards reduced win-stay ratios [$F_{1,18}$=4.180, p=.056] (Fig. 10E).

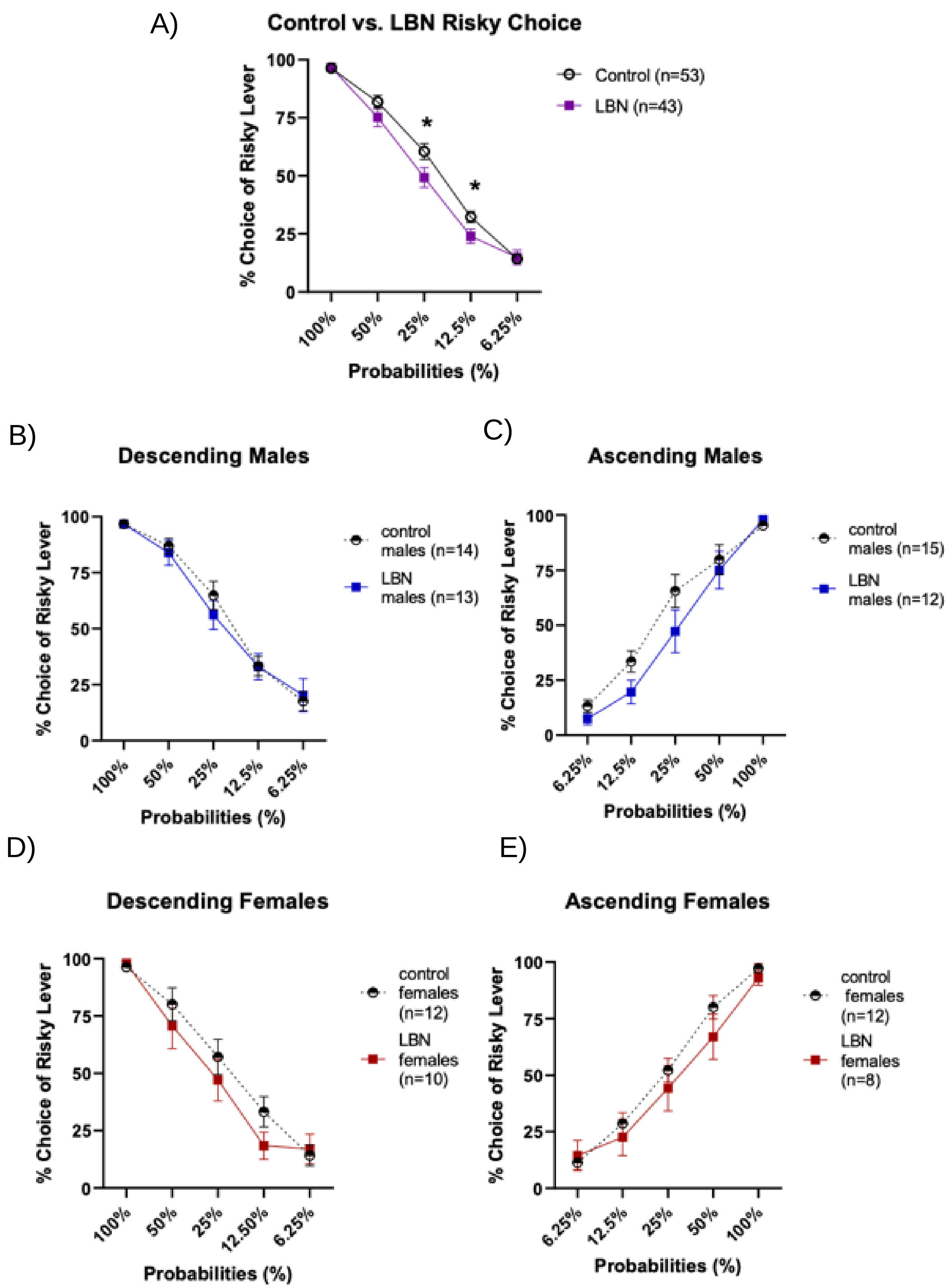

A)
Control vs. LBN Risky Choice
% Choice of Risky Lever
Probabilities (%)
100%
50%
25%
12.5%
6.25%
Control (n=53)
LBN (n=43)
B)
Descending Males
% Choice of Risky Lever
Probabilities (%)
control males (n=14)
LBN males (n=13)
C)
Ascending Males
% Choice of Risky Lever
Probabilities (%)
control males (n=15)
LBN males (n=12)
D)
Descending Females
% Choice of Risky Lever
Probabilities (%)
12.50%
control females (n=12)
LBN females (n=10)
E)
Ascending Females
% Choice of Risky Lever
Probabilities (%)
control females (n=12)
LBN females (n=8)

Fig. 7: LBN effects on risky decision-making in male and female rats. (A) All LBN rats choose the risky lever less than all controls when the probability of earning large rewards were low [$F(4,376)=2.845$, $p=.024$]. Post-hoc LSD tests revealed this difference was specific to when 25% ($p=.043$) and 12.5% ($p=.038$) probabilities were presented. When analyzed by task version and sex, LBN had no effect on male (B & C) or female (D & E) choice of the risky lever in either ascending or descending versions of PD.

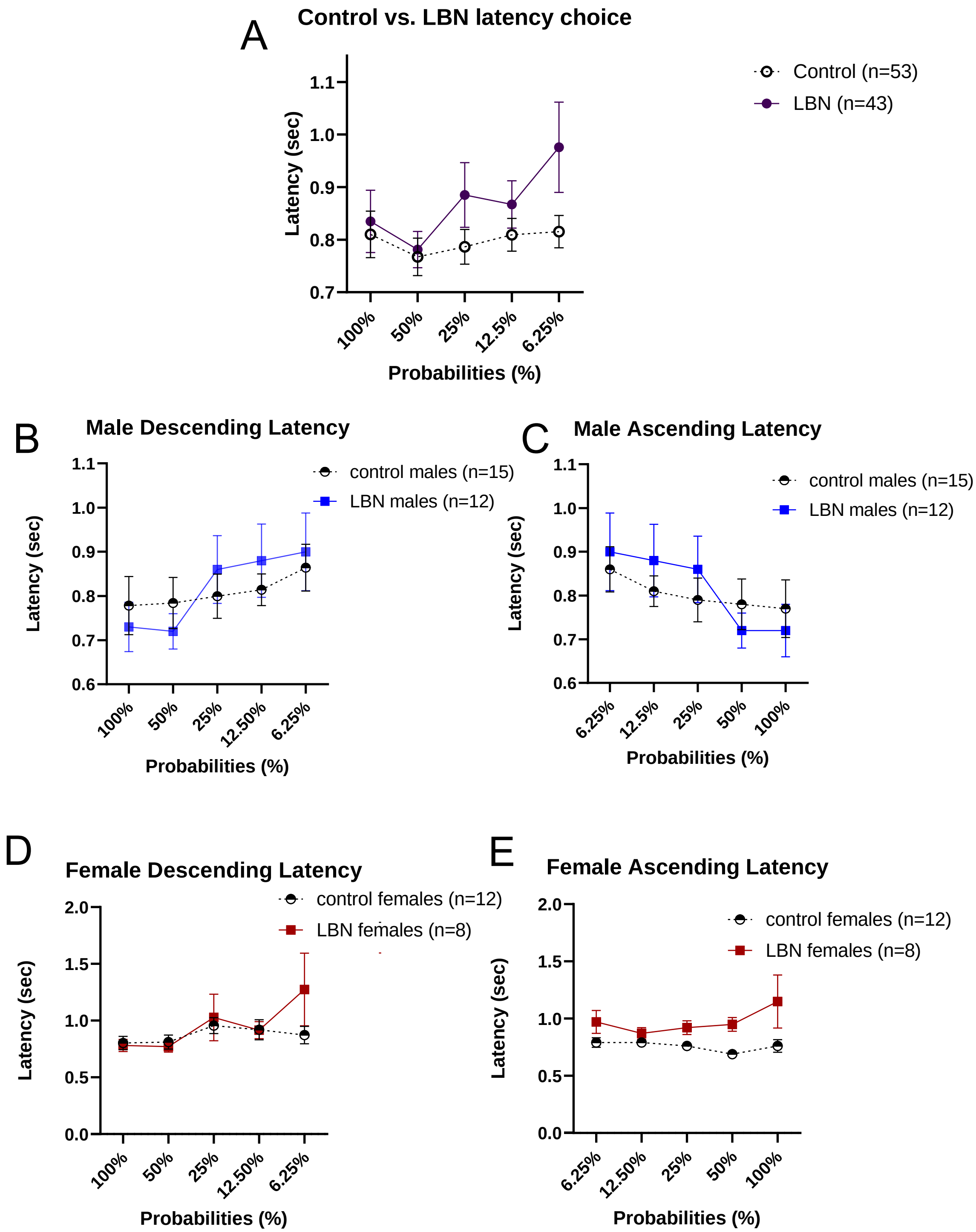


Fig. 8.: LBN effects on response latency. Response latencies did not differ due to LBN exposure (A). Similarly, LBN exposure did not affect response latencies when data was split by sex (B-E).

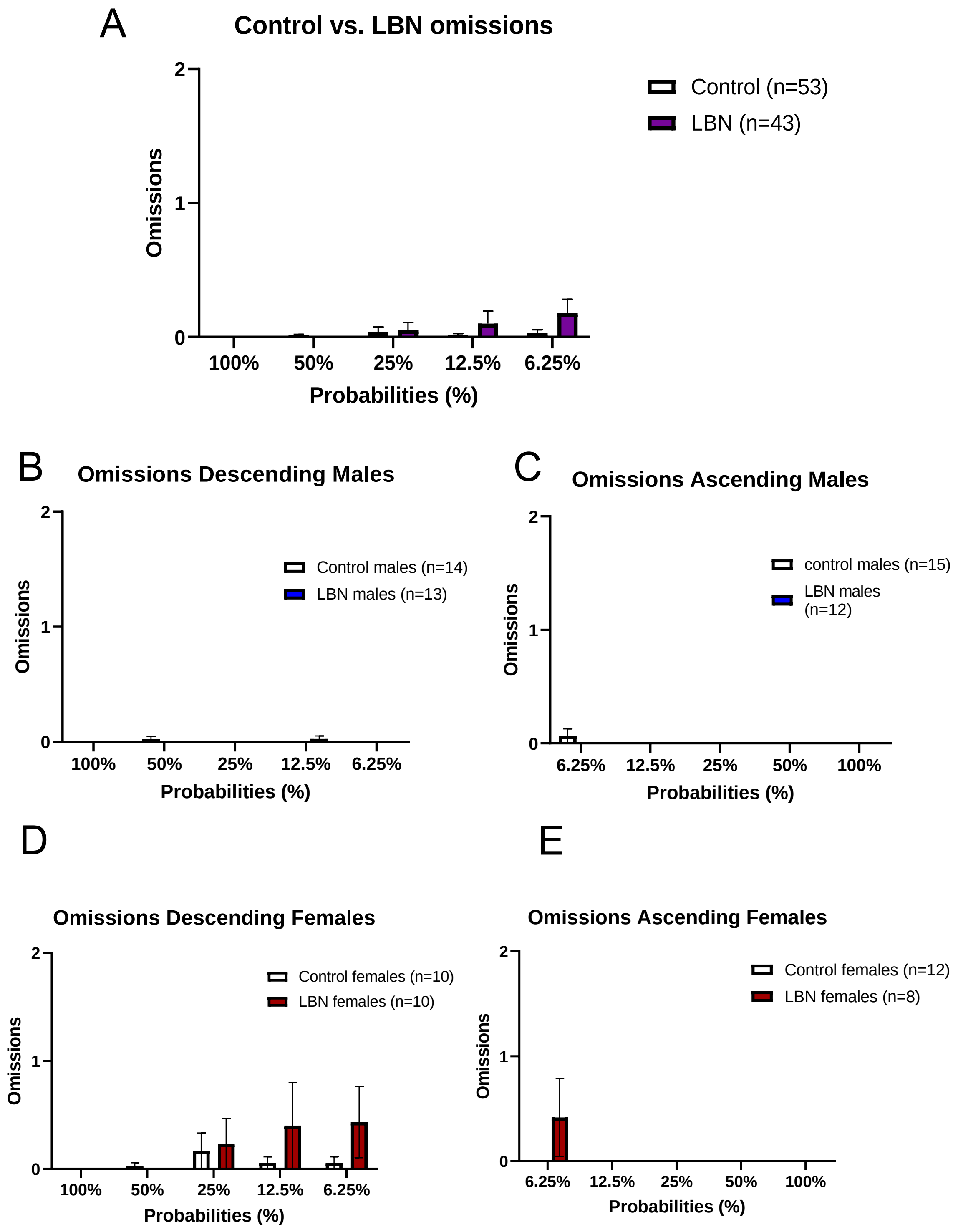


Fig. 9.: LBN effects on number of omissions. Response latencies did not differ due to LBN exposure (A). Similarly, LBN exposure did not affect response latencies when data was split by sex (B-E).

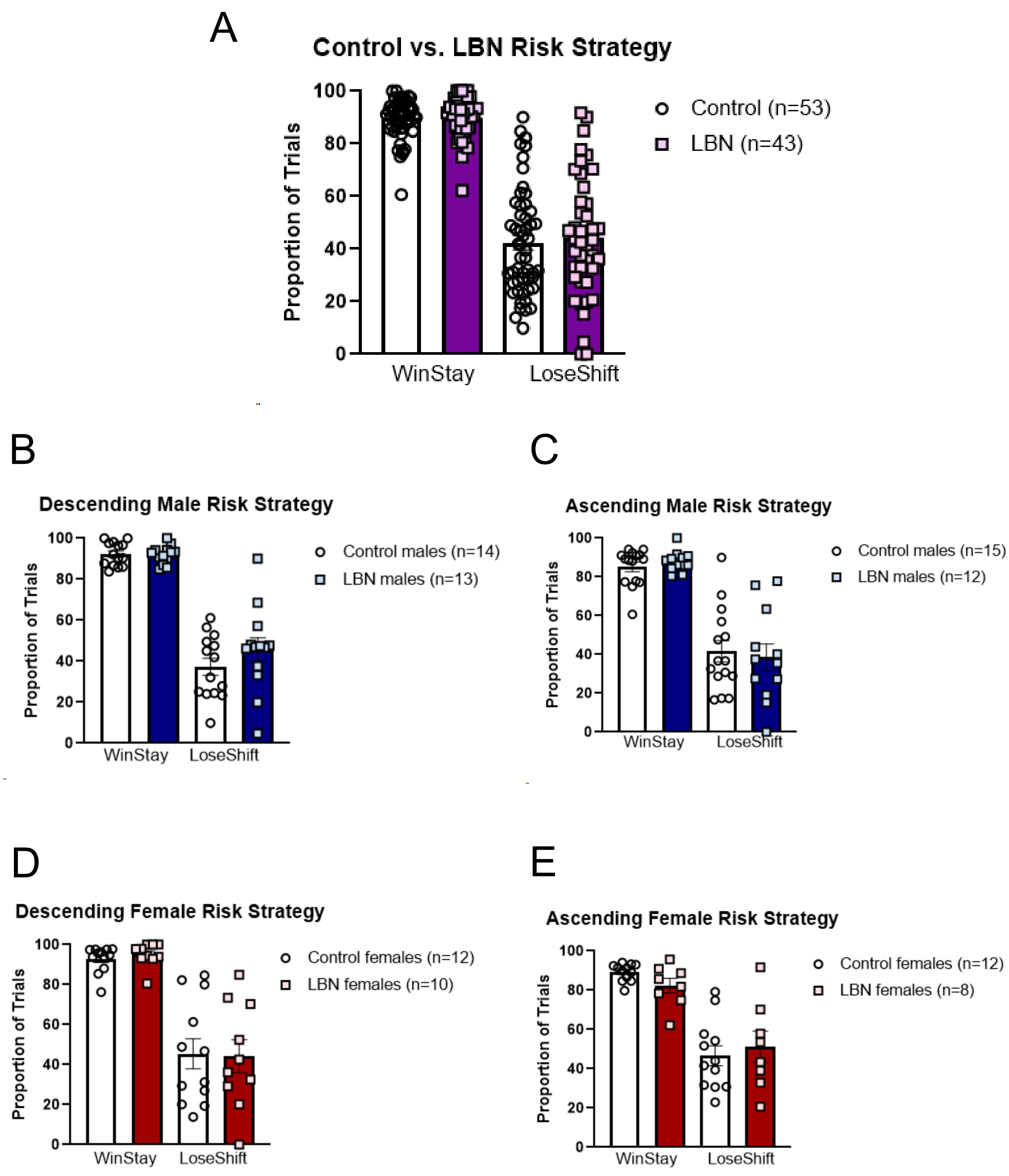


Fig. 10.: LBN effects on choice strategies. Patterns of choice did not differ due to LBN exposure (A). Similarly, LBN exposure did not affect response latencies when data was split by sex (B-E).

LBN Causes Sex-Specific Changes in Gene Transcription in the OFC

To delineate molecular signatures in the OFC that may drive the LBN phenotypes observed, we conducted RNA-seq in punches from the whole OFC from adult behaviorally naïve rats (male control n=5; female control n=5; male LBN n=5; female LBN n=5). We first used RRHO analysis to compare overall gene expression patterns in males and females induced by LBN. This analysis included all genes that were differentially expressed, including gene changes not reaching the threshold of statistical significance, and thus facilitated the agnostic comparison of gene expression patterns after LBN in males and females. We found little overlap between both up- and down-regulated genes between males and females (Fig. 4A; blue bottom left and top right quadrants), but we also found that LBN induces a unique signature in males and females. For example, many genes were upregulated by LBN in males as compared to females (Fig. 4A; bottom right quadrant).

We next narrowed down our analysis to genes showing a significant difference between control and LBN and found 161 differentially expressed genes (DEGs) in males and 138 DEGs in females. These gene changes were largely sex-specific, with only 18 common genes altered by LBN in both sexes (Fig. 4B). Heatmaps sorted by fold change of LBN DEGs revealed different patterns of up-and down-regulated genes between males and females: in males, LBN increased the expression of 98 genes while in females only 59 genes were upregulated (Fig. 4C, D). Lastly, KEGG pathway analysis revealed unique biological processes altered by LBN in both males and females (Fig. 4E, F). Thus, LBN exposure appeared to induce unique patterns of gene expression in males and females, rather than oppositely regulating a common set of genes.

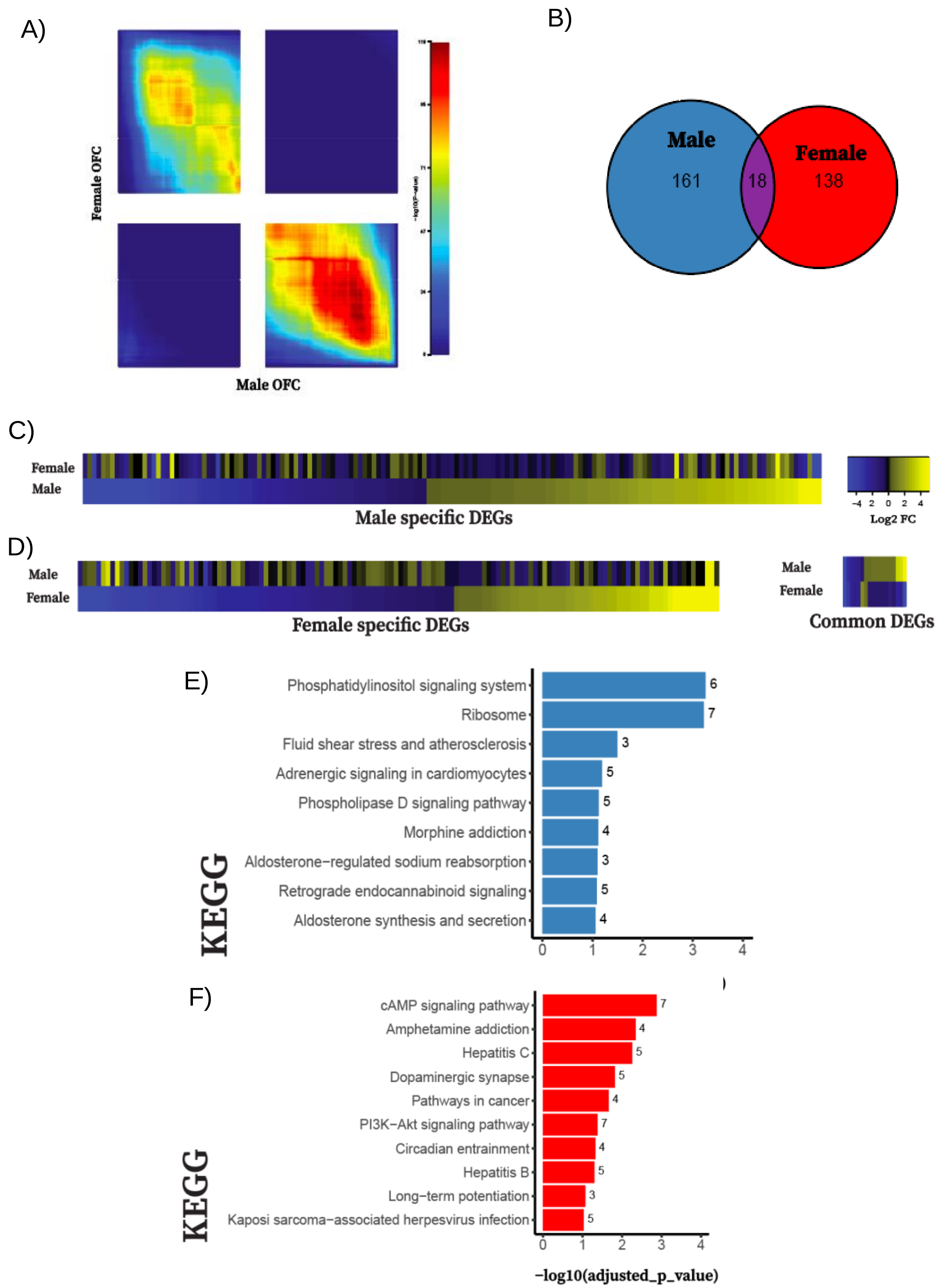

A)
Female OFC
Male OFC
B)
Male
161
18
Female
138
C)
Female
Male
Male specific DEGs
-4 2 0 2 4
Log2 FC
D)
Male
Female
Female specific DEGs
Male
Female
Common DEGs
E)
Phosphatidylinositol signaling system
Ribosome
Fluid shear stress and atherosclerosis
Adrenergic signaling in cardiomyocytes
Phospholipase D signaling pathway
Morphine addiction
Aldosterone−regulated sodium reabsorption
Retrograde endocannabinoid signaling
Aldosterone synthesis and secretion
6
7
3
5
5
4
3
5
4
0 1 2 3 4
KEGG
F)
cAMP signaling pathway
Amphetamine addiction
Hepatitis C
Dopaminergic synapse
Pathways in cancer
PI3K−Akt signaling pathway
Circadian entrainment
Hepatitis B
Long−term potentiation
Kaposi sarcoma−associated herpesvirus infection
7
4
5
5
4
7
4
5
3
5
0 1 2 3 4
KEGG
−log10(adjusted_p_value)

Fig. 11. LBN produces sex-specific changes in gene expression in the OFC. (A) Threshold-free comparison of DEGs by rank-rank hypergeometric overlap. Hotspots represent the overlap between the impact of LBN on gene expression in females and males. Hotter colors indicate more overlap. The upper right quadrant includes co-upregulated genes while the lower left quadrant represents co-downregulated genes. The top left and bottom right quadrants represent genes that are changed by LBN in opposite direction in males compared to females. (B) LBN induces 161 DEGs in males and 138 DEGs in females, with 18 overlapping DEGs between the sexes. (C) Heatmap sorted by fold change of DEGs in males (top) compared to the expression of those genes in females (bottom). LBN increased the expression of 98 genes and decreased the expression of 63 genes. (D) Heatmap sorted by fold change of DEGs in females (top) compared to the expression of those genes in males (bottom). LBN increased the expression of 59 genes and decreased the expression of 79 genes. (E) Top KEGG enrichment terms identified in males following LBN (blue). (F) Top KEGG enrichment terms identified in females following LBN (red).

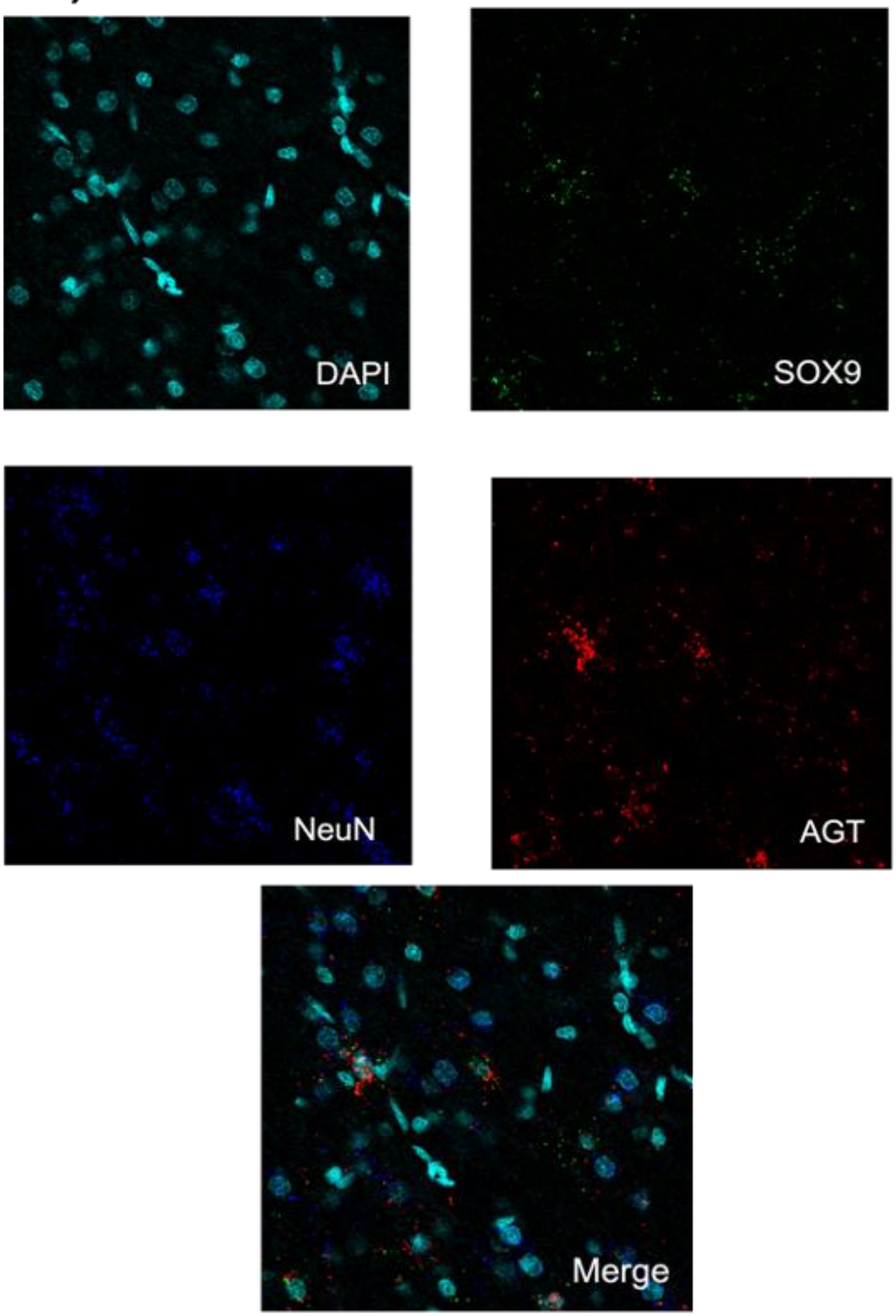
AGT is Primarily Located in Astrocytes and its Expression Does Not Differ Between OFC Subregions

Previous studies have shown that LBN exposure affects addiction-like behaviors in males but not females (Ordoñes Sanchez et al., 2021). As such, for our RNA-seq validation studies we were interested in selecting a gene that could potentially explain the changes in male behavior we observe induced by LBN. One gene target that caught our attention from the OFC RNA-seq data was angiotensinogen (*AGT*). *AGT* was significantly upregulated by LBN in males, but not females. Additionally, *AGT* contributed to the enrichment of “aldosterone synthesis and secretion”, a KEGG enrichment term identified in males but not females. Therefore, if this gene is significantly altered by LBN in males, and LBN exposure tends to affect male behaviors, we hypothesized this gene may play a role in the LBN effects typically found in adult males. To carry out our validation studies we used RNAScope fluorescent *in situ* hybridization, which permitted us to identify the cell types that express this gene in the OFC as well as measure how much of it is expressed in different OFC subregions. Given that the medial and lateral subregions of the OFC play a role in risky decision-making or impulsivity, we hypothesized that increased expression of *AGT* in either area could potentially correlate to LBN-induced changes in either impulsive choice or risky choice behavior.

Prior work in rodents report that *AGT* is predominantly expressed in astrocytes (O'Callaghan et al., 2011; Sherrod, Liu, Zhang, & Sigmund, 2005; Stornetta, Hawelu-Johnson, Guyenet, & Lynch, 1988), however, these studies have primarily been done using male rodents and lack any comparison or inclusion of female data. Here we found that over 93% of SOX9 cells co-expressed AGT in control (n=5) and LBN males (n=7) (Fig. 9B, C, & F). Fewer cells (less than 7%) co-expressed NeuN and *AGT* regardless of control vs. LBN exposure in males (Fig. 9D, E, & G). Lastly, control (n=5) and LBN females (n=5) also had more *AGT* present in astrocyte cells (over 89%) (Fig. 9H, I, & L) than in neuronal cell types (less than 16%) (Fig. 9J, K, & M). As such, *AGT* in the OFC of males and females is predominantly expressed in astrocyte cell types rather than neuronal cell types.

In addition to identifying which cell types in the OFC express *AGT*, we were also interested in seeing if there are any differences in how much *AGT* is expressed in medial or lateral OFC subregions. Recall, different areas of the OFC play a role in impulsivity or risky decision-making. Therefore, if *AGT* is expressed in one area more than another, this could further our understanding as to why LBN has certain effects on impulsive or risky behavior. We found that LBN and control male *AGT* expression in the medial [$t(10)=.043$, $p=.967$] and lateral OFC [$t(10)=.081$, $p=.937$] did not differ (Fig. 10A). LBN and control female *AGT* expression also did not differ if measured in the medial [$t(8)=.401$, $p=.699$] or lateral OFC [$t(8)=1.228$, $p=.254$] (Fig. 10B).

A)

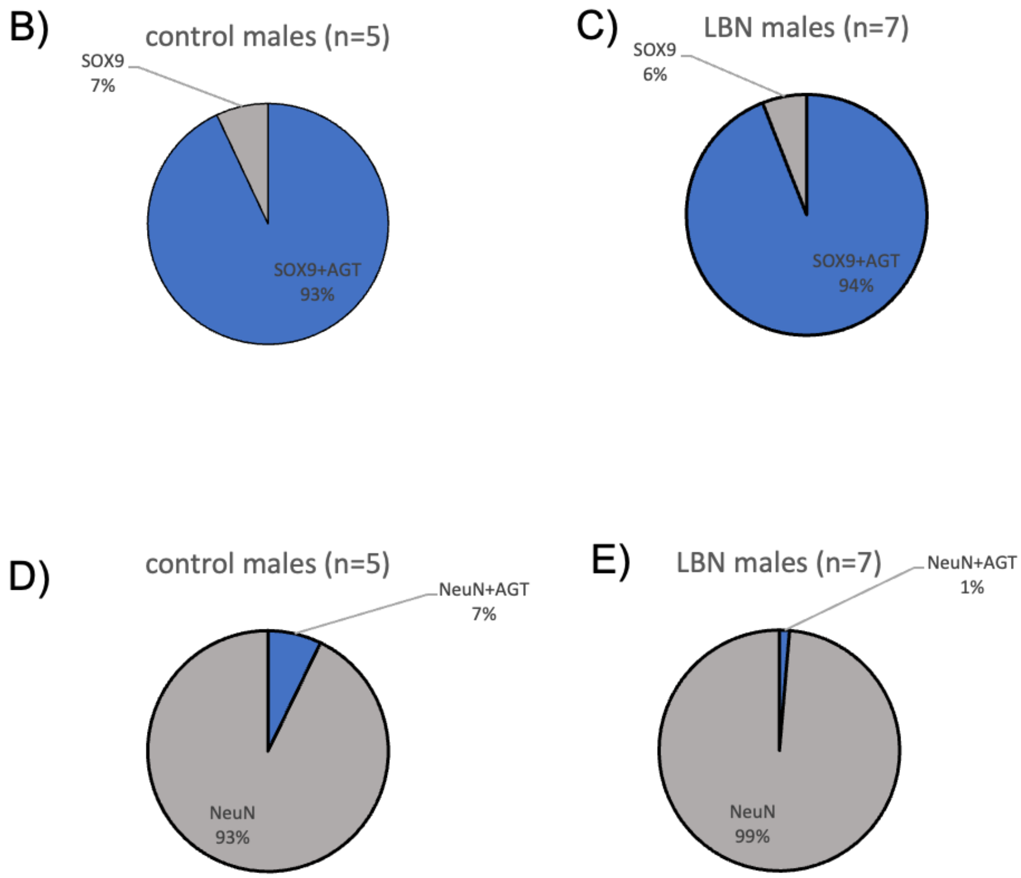


B)

C)

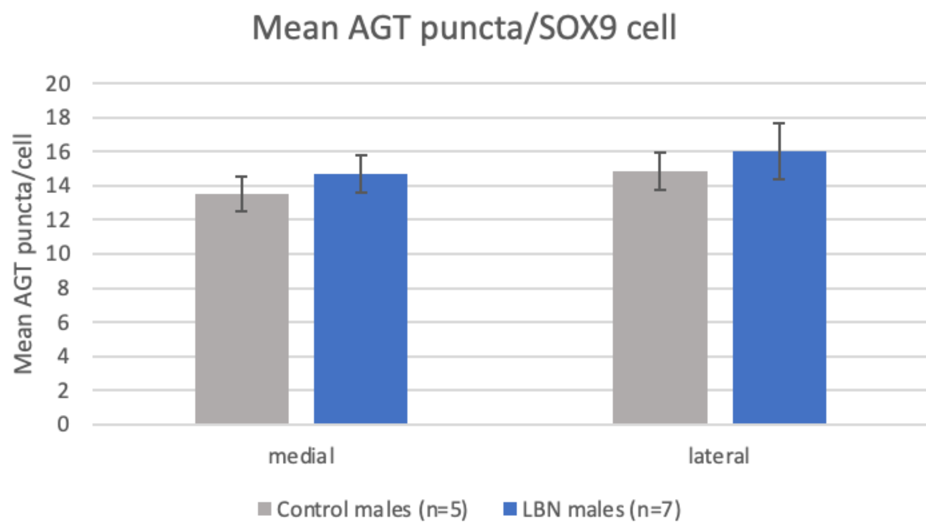
D)

E)

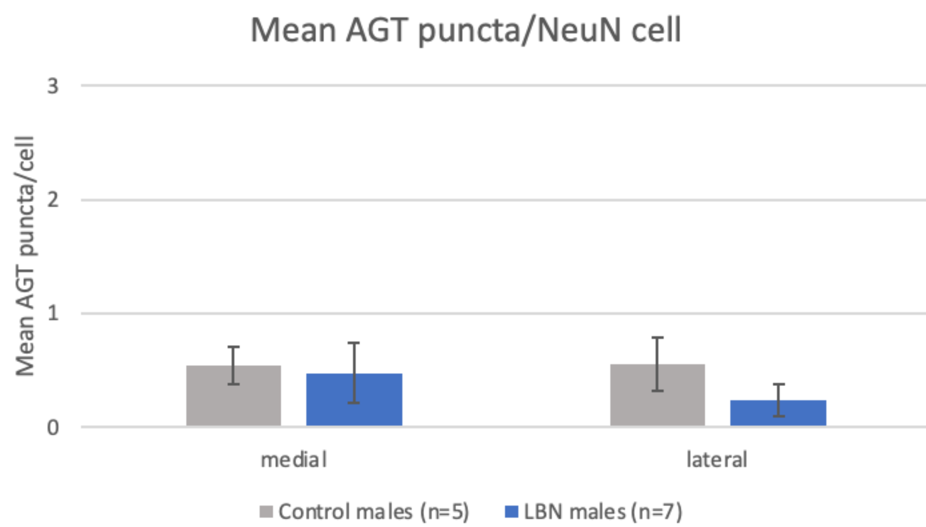


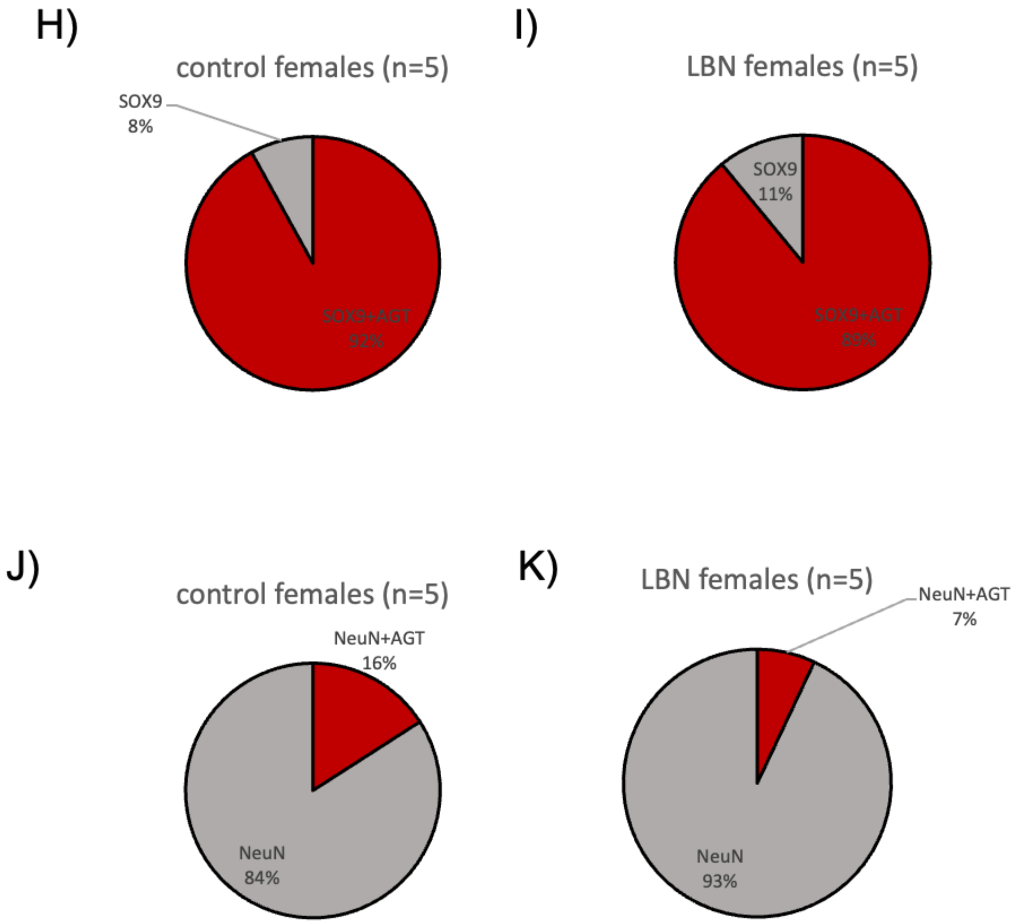
control males (n=5)
SOX9
7%
SOX9+AGT
93%
LBN males (n=7)
SOX9
6%
SOX9+AGT
94%
control males (n=5)
NeuN+AGT
7%
NeuN
93%
LBN males (n=7)
NeuN+AGT
1%
NeuN
99%

F)


Mean AGT puncta/SOX9 cell
Mean AGT puncta/cell
20
18
16
14
12
10
8
6
4
2
0
medial
lateral
Control males (n=5)
LBN males (n=7)

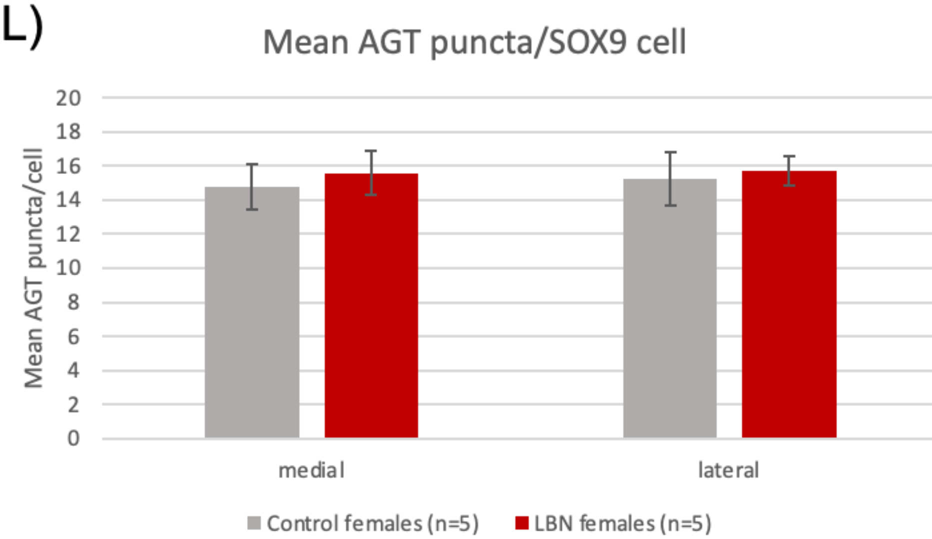
G)


Mean AGT puncta/NeuN cell
Mean AGT puncta/cell
3
2
1
0
medial
lateral
Control males (n=5)
LBN males (n=7)



H)
control females (n=5)
SOX9
8%
SOX9+AGT
92%
I)
LBN females (n=5)
SOX9
11%
SOX9+AGT
89%
J)
control females (n=5)
NeuN+AGT
16%
NeuN
84%
K)
LBN females (n=5)
NeuN+AGT
7%
NeuN
93%

L)



M)

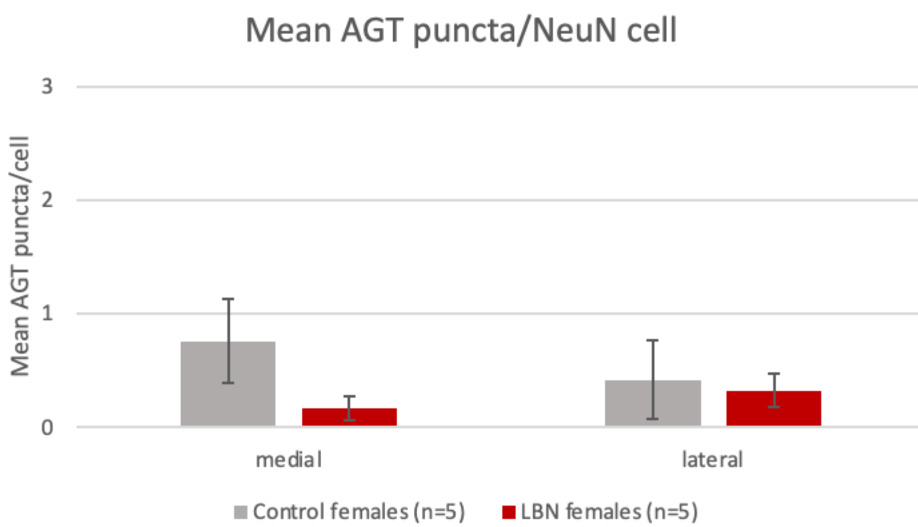
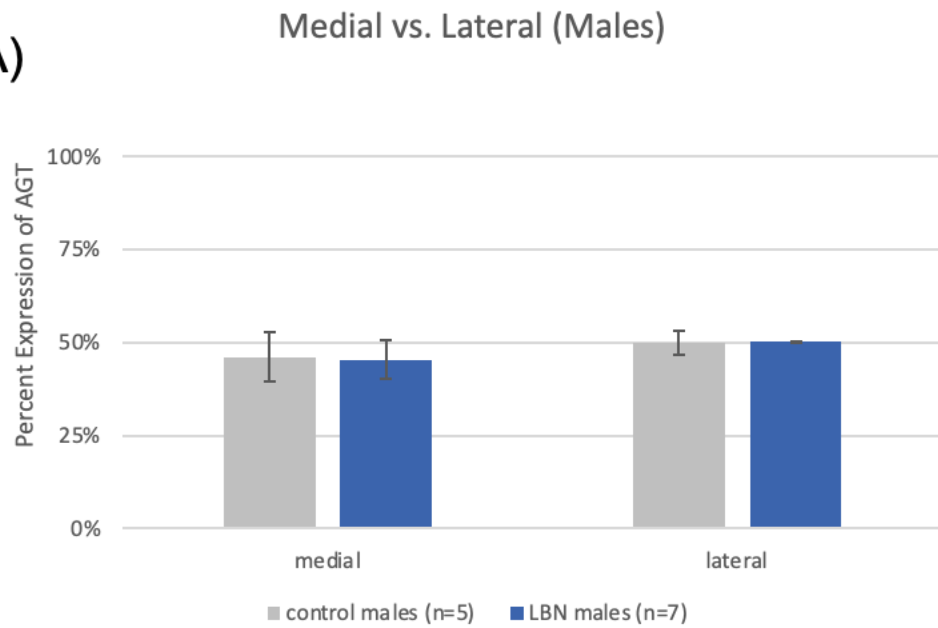


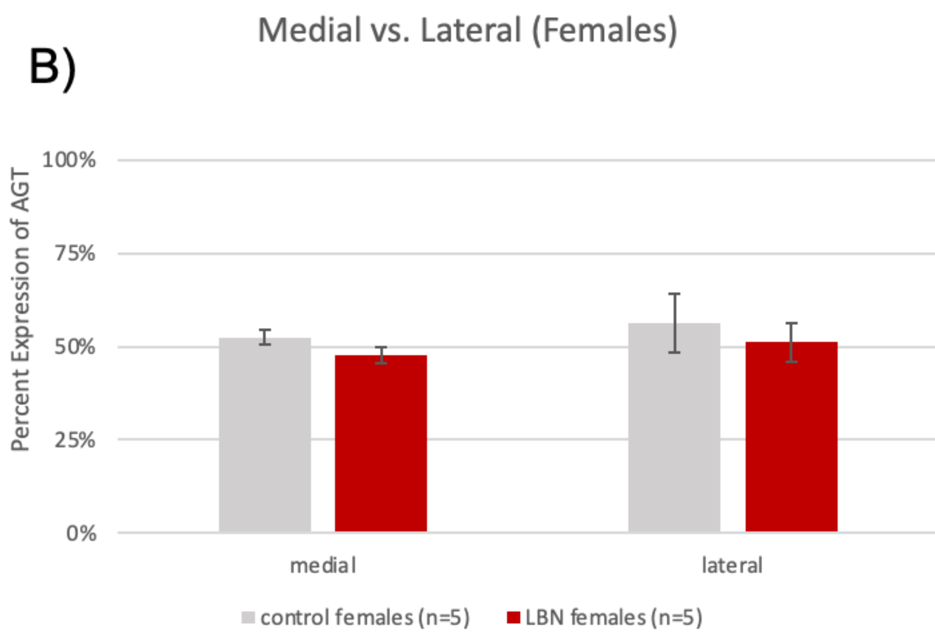
Fig. 12. *AGT* in the OFC is primarily found in astrocytes. (A) Representative images of coronal sections showing where *AGT* (red) is located. SOX9 (green) was our glial marker, NeuN (blue) was our neural marker, DAPI (cyan) was used as a counterstain to show nuclei. *AGT* expression in males, regardless of condition, is predominantly found astrocytes (B, C, & F) than neuronal cell types (D, E, & G). In females, regardless of condition, *AGT* is predominantly expressed in astrocytes (H, I, & L) than neuronal cell types (J, K, & M).

A)



Medial vs. Lateral (Males)
Percent Expression of AGT
100%
75%
50%
25%
0%
medial
lateral
control males (n=5)
LBN males (n=7)

B)



Medial vs. Lateral (Females)
Percent Expression of AGT
100%
75%
50%
25%
0%
medial
lateral
control females (n=5)
LBN females (n=5)

Fig. 13. LBN does not alter *AGT* expression in either the medial vs. lateral OFC. (A) Percent *AGT* expression between control and LBN males in medial and lateral subregions. (B) Percent *AGT* expression between control and LBN females in medial and lateral subregions.

Discussion

Early life adversity can alter the risk/resilience for disorders linked to changes in impulsive or risky behavior. In our LBN manipulation, rats are exposed to a low resource environment for a brief period during postnatal development and tested in adulthood. Previously, we found that the LBN model reduces impulsive choice in male but not female rats, suggestive of an "inoculation" effect against addiction-related behaviors in males (Ordoñes Sanchez et al., 2021). In this study, we show that LBN also alters behaviors associated with impulsive action and risky decision-making. LBN males displayed higher impulsive action than controls initially, but this effect disappeared with continued testing. No effects were found in females. In contrast, all animals exposed to LBN displayed reduced risky choice during probability discounting. Additionally, LBN induced changes in gene expression within the OFC in a sex-specific manner. Of the many genes we could select for our validation studies, we chose *AGT*, which is a gene significantly upregulated by LBN in males but not females. Although we found *AGT* was expressed more in astrocytes than in neuronal cells, we found no differences in *AGT* expression between medial vs. lateral subregions of the OFC due to LBN in males or females. Collectively, these data show unique behavioral and molecular signatures associated with LBN in male and female rats.

LBN Increases Impulsive Action in Males, An Effect Not Found in Females

We first examined the effects of LBN on impulsivity using a DRL task, a well-established assay for impulsive action. When data was combined, we found no effects of LBN, but we did see that males lever pressed more often than females. This sex difference was apparent early in testing but disappeared during the last DRL schedule. Interestingly, no differences were observed in any other measure (bursting, rewards earned, or efficiency). These results suggest males actively tried to earn more rewards, but they did not necessarily outperform females. Other studies also find that males on DRL tasks tend to respond more than females (Beatty, 1973; van Hest, van Haaren, & van de Poll, 1987). However, unlike our findings, males in these studies are also less efficient than females, suggesting females typically outperform males on DRL. One possibility for the discrepancies between these studies with ours could be attributed to differences in task design. In other studies, rats primarily start off DRL with longer schedules (e.g., 20 secs), whereas our rats started off testing with a shorter wait time (e.g., 5 secs). It could be that if we started the task immediately with a longer schedule, we would have also been able to find differences in efficiency between the sexes.

When we split DRL data by sex, we found that LBN caused males to be more impulsive than control males (albeit initially). LBN males only produced more bursting responses during the first two days of testing that later returned to control levels. Higher bursting is indicative of higher impulsive action. LBN males also lever pressed more than controls when rats were switched from a shorter (5 sec) to a longer (10 sec) wait time schedule. Although LBN males displayed some elevations in measures of impulsivity, through continued training, they were just as efficient as controls and earned similar number of rewards. Thus, LBN males did not necessarily perform more poorly than control males.

It is important to remember that not all forms of impulsivity are maladaptive, and in some cases, it does pay off to act impulsively. Acting on impulse may allow one to seize opportunities that may have otherwise passed by. These results may reflect an adaptive response in males to experiencing stress early in life. Animals that are raised in environments with limited access to resources are likely to live out the rest of their lives in those same conditions. This means that these animals may experience fewer opportunities (e.g., finding food or a mate) and therefore acting quickly may improve an animal's chances of survival. This possibility is supported by work from our lab that found LBN males initially exhibit enhanced acquisition of sex behavior as compared to controls (Eck et al., unpublished observations). Despite an initial and subtle difference, LBN males did not differ in overall performance from controls. Thus, LBN exposure may promote adaptive behaviors in males.

It was surprising to find LBN had no effects in females. One possibility could be that females are resilient. However, studies that incorporate more severe early life stressors, such as maternal separation with social isolation, find that female impulsivity in DRL increases (Lovic et al., 2011). It could be that by using a more severe stress manipulation like that of the Lovic study, we would have been able to see similar results.

Therefore, a stressor such as LBN may be insufficient to alter female behavior, but more aversive stressors that deprive the pup from their mother and other littermates could.

LBN Exposure Can Reduce Risky Behavior

Some forms of early life stress can reduce impulsivity, which can be beneficial to the survival of offspring and improve decision-making (Fuentes et al., 2014; Lovic et al., 2011; Ordoñes Sanchez et al., 2021). Here we examined if the same effect would be observed in tasks measuring risky behavior. We found that all animals that were exposed to LBN made fewer risky choices than their control counterparts. This effect was specific to when the odds of obtaining a reward were less than 50%, which indicates that LBN animals made more conservative choices than their control counterparts.

It was interesting to find, however, no sex differences in risky behavior in our data. Yet, others have found that male rats make more riskier choices than females during probability discounting tasks (Islas-Preciado et al., 2020). One possibility for these differences in outcomes could be due to slight differences in task contingencies and shipping stress. Rats used in our experiment were bred in house and trained on more trials/5 blocks, whereas those in Islas-Preciado et al. (2020) were shipped for their study and trained on fewer trials and only 4 blocks (Islas-Preciado et al., 2020). Although these are few differences, they could have contributed to the discrepancies observed between the two studies.

LBN had no effect on response latencies in males or females, and regardless of the task variant they were trained on. These results suggest that LBN did not impair how quickly the animals made a choice on the task despite the order of probabilities changing throughout a session. Number of omissions made also did not differ between groups, suggesting all animals regardless of condition or sex, were engaged in the task.

Lastly, we measured win-stay and lose-shift behaviors. Higher win-stay behaviors are interpreted as increased sensitivity to positive feedback, whereas higher lose-shift scores are interpreted as increased sensitivity to negative feedback (Iyer, Kairiss, Liu, Otto, & Bagot, 2020; St. Onge et al., 2011). By identifying how animals choose in this task, we can potentially assess differences in how animals learn from their environment. All animals tested in our study displayed more win-stay than lose-shift behaviors tendencies. Even when data was split, no differences were observed between LBN males and control males. No differences were also observed between LBN females and control females. In another study, however, males exposed to chronic unpredictable stress in adulthood display more win-stay behaviors than unstressed males on a competitive choice task (Matisz, Badenhorst, & Gruber, 2021). Even though the LBN model we use is also considered a chronic stressor (Walker et al., 2017), it could be that proximity to the stressful event is critical for this measure. Whether similar effects in choice behavior are observed in females after stress are unclear as no studies to date include or compare both sexes.

LBN Causes Sex-Specific Changes in Gene Transcription in the OFC

RNA-seq revealed sex-specific changes in gene expression induced by LBN in the OFC. Interestingly, these transcriptional changes were more pronounced in males, the sex that often demonstrated an LBN-induced behavioral phenotype. Additionally, a majority of DEGs in males were upregulated in response to LBN, whereas in females the majority was downregulated in response to LBN. Others report, however, that gene expression changes in the prefrontal cortex (PFC) in response to LBN is associated with transcriptional down-regulation in male rats (females not examined) (N. F. O. Green, Maniam, Riese, Morris, & Voineagu, 2021). Multiple reasons such as rat strain (e.g., Long Evans vs. Sprague Dawley), method of tissue collection (e.g., brain tissue collected without or following terminal anesthesia) and tissue sample (e.g., OFC vs. PFC) could account for these differences. Nevertheless, these data illustrate that gene expression changes in the cortex are sensitive to early life stress.

GSE analysis revealed that LBN had differential effects on the OFC transcriptome in males and females. KEGG pathway analysis revealed various pathways uniquely overrepresented in male, but not female, DEGs. Two pathways of interest are "aldosterone synthesis and secretion" and "aldosterone-regulated sodium reabsorption," which were significantly enriched only in males and could be responsible for driving behavioral changes induced by LBN in this sex. Aldosterone is part of the renin-angiotensin-aldosterone system (RAAS) and it is synthesized in response to increases in sympathetic activity (Gordon, Küchel, Liddle, & Island, 1967). Additionally, studies that reduce aldosterone secretion by blocking angiotensin converting enzyme (ACE) activity, report reductions in impulsive action in male mice that display behavioral abnormalities similar to ADHD (Porter, Pillidge, Grabowska, & Stanford, 2015). These findings suggest that different components of the RAAS can affect impulsive-related processes. It is still unclear, however, if RAAS also affects risky decision-making processes. In females, it was surprising to find "dopaminergic synapse" as a KEGG enrichment term because dopamine signaling is typically involved in the expression of impulsive and risky behaviors. However, LBN did not affect female behavior in DRL and only mildly affected it in probability discounting. Therefore, these findings highlight the possibility that compensatory mechanisms reflected in the numerous female-specific pathways, such as “cAMP signaling pathway,” may result in the little observable phenotypes in females. Thus, these data highlight that LBN exposure appears to induce unique patterns of gene expression in males and females, rather than oppositely regulating a common set of genes.

AGT Is Present in Astrocytes and its Expression Does Not Differ Between Medial vs. Lateral Areas of the OFC

One gene we selected for our validation studies was *AGT* due to it being significantly upregulated by LBN in males but not females. Previous studies have shown that LBN exposure increases male, but not female, addiction-like behaviors (Ordoñes Sanchez et al., 2021). Therefore, if this gene is significantly upregulated by LBN, and LBN most often affects male behaviors, this gene may underlie a mechanism contributing to LBN-induced phenotypes.

Another reason we choose *AGT* as a validation target was because it was contributing to the significance of the “aldosterone synthesis and secretion” pathway, a KEGG enrichment term found in males. *AGT* serves as a precursor of angiotensin II in the RAAS, which can then exert effects on the adrenal glands to stimulate the release of aldosterone (Raghavendra, Chopra, & Kulkarni, 1999). Aldosterone is responsive to sympathetic nervous system activity and regulates blood pressure (Gordon et al., 1967).

Under mild periods of stress, release of aldosterone increases and helps control blood pressure. This is believed to help an organism survive under periods of stress and prepare it for future threats (Ayada, Toru, & Korkut, 2015). However, chronic stress can lead to increased blood pressure, which can increase the risk of future cardiovascular problems. Lastly, manipulating components of the RAAS system can also reduce impulsive behavior, in particular impulsive action (Porter et al., 2015). Because *AGT* plays a role in stress reactivity, impulsive behavior, and was significantly upregulated by LBN in males but not females, we were very excited to select *AGT* as a validation target.

Prior work in rodents report that *AGT* is predominantly expressed in astrocytes (O'Callaghan et al., 2011; Sherrod et al., 2005; Stornetta et al., 1988), however, these studies have primarily been conducted only using male rodents and lack any comparison or inclusion of female data. Our findings support what is reported in the literature by showing that *AGT* is primarily found in astrocytes more so than neurons but also that this applies to both sexes. Because astrocytes are considered essential for the maintenance of the blood-brain barrier (Heithoff et al., 2020) and *AGT* plays a role in blood pressure control, it also makes sense for this gene to be located in cells that are responsible for blood vessel maintenance.

We next examined whether the expression of *AGT* differed across OFC subregions. If more *AGT* is present in one subregion vs. another, this could possibly explain some of the behavioral phenotypes we have observed induced by LBN in males. However, we found no differences in *AGT* expression in lateral vs. medial subregions in the OFC. This did not validate findings from RNA-Seq that indicated that LBN upregulated the expression of *AGT* in males. In addition to astrocytes, *AGT* in the frontal cortex can also be found in perivascular fibroblast-like cells (Saunders et al., 2018; Vanlandewijck et al., 2018). Although their main function is poorly understood, some work suggests fibroblast-like cells work alongside astrocytes to help support the blood-brain barrier (Sweeney, Ayyadurai, & Zlokovic, 2016). Therefore, it could be that if we probed for this cell-type we would have been able to replicate the effects seen from RNA-seq.

Conclusions

Here we have examined how early life stress affects impulsivity and risky decision-making in adult rats. Our results suggest that LBN may promote adaptive behaviors and improve decision-making. Although initially unexpected, these findings add to a growing body of literature supporting the stress inoculation hypothesis: that some early adversity promotes later resilience. Lastly, our molecular findings lay the groundwork for further examination of the specific mechanisms underlying behavioral phenotypes induced by the LBN model in males and females. Being able to identify new targets and mechanisms that promote resilience to addiction-related phenotypes can guide the development of novel treatments for stressed populations.

CONCLUSIONS

Mild amounts of impulsivity or risky behavior often pose no threat to one's well-being and can even allow one to seize opportunities that they may have otherwise missed. However, when these behaviors become maladaptive, it can have detrimental effects on one's health (Bari & Robbins, 2013; Everitt et al., 2008; Fineberg et al., 2014). Elevated impulsivity and risky decision-making are associated with several psychiatric disorders such as attention-deficit/hyperactivity disorder (ADHD), bipolar disorder, and addiction (e.g., gambling disorder and substance use disorder, SUD) (Adler et al., 2017; Dawe & Loxton, 2004; Najt et al., 2007; Vest et al., 2016). As such it is important to understand the mechanisms contributing to these effects in order to develop effective treatment and prevention therapies.

Recall, that impulsivity and risky decision-making are regulated by the mesocorticolimbic dopamine (DA) system, consisting of projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and areas of the cortex (e.g.., medial prefrontal cortex, mPFC, and orbitofrontal cortex, OFC). As detailed in Chapter 1A and B, this system can regulate the expression of impulsive and risky behaviors. Interestingly, early life stress can alter behaviors associated with both of these processes. For instance, in rodents, early life stress generally increases impulsive action and reduces impulsive choice behaviors. Additionally, stress exposure alters risky behavior in rodents. Therefore, it could be that early life stress impacts the mesocorticolimbic DA system, which can then impact the expression of impulsive and risky behaviors.

Early life stress, however, can come in many different forms and levels of severity in humans. Additionally, the timing in which stress is experienced during development can span from prenatal to early postnatal. This variety in experience is reflected in the different types of early life stress models that have been developed in rodents. The experiments reported in Chapter 2 characterize one model of early life stress, the limited bedding and nesting model (LBN), in rats. Notably, we found that LBN exposure promoted resilience to addiction-related phenotypes in adult male, but not female, rats. Specifically, LBN reduced impulsive choice as well as morphine self-administration. Given the role of the NAc in these behaviors, we then examined whether LBN exposure altered NAc core electrophysiology. We found that LBN in males decreased the frequency of spontaneous excitatory postsynaptic currents in comparison to controls, which is indicative of a decrease in presynaptic glutamate transmission. Additionally, chronic morphine self-administration led to an increase in the AMPA/NMDA ratio in control males. However, LBN males did not show this same drug-induced change in the NAc. LBN females did not show any of these physiology differences.

In order to delineate potential molecular mechanisms that contribute to stress-inoculation in males, we then performed NAc RNA-sequencing. We found sex-specific changes in differentially expressed genes (DEGs) following LBN in the NAc core. Pathway analysis revealed many distinct pathways in males vs. females. Of note, “SNARE interactions in vesicular transport” and “ionotropic glutamate receptor” pathways were uniquely over-represented in male DEGs and could account for the altered glutamate transmission in the NAc of LBN males. These findings reveal novel mechanisms underlying stress inoculation of addiction-related behavior in males.

In the set of experiments presented in Chapter 3, we further examine the effect of LBN on impulsive action, risky decision-making, and the gene transcriptome of the orbitofrontal cortex (OFC). Our first behavioral findings are in line with what was discussed in Chapter 1, such that stress exposure early in life generally increases impulsive action behaviors in adulthood. However, in this case an increase in impulsive action may be an adaptive response to exposure to early life stress. Other studies report that monkeys exposed to brief intermittent stress early in life perform similar and eventually better than controls on a response inhibition task (Parker, Buckmaster, Justus, Schatzberg, & Lyons, 2005). Although we did not see improvements in our study, we did observe stressed males were able to adjust their behavior as controls. These results support the idea that not all forms of stress lead to impairments in cognitive control. While females appeared resilient to LBN effects, it could be that utilizing more severe stress manipulations, we would have been able to see increased impulsive behavior. Future studies should investigate what mechanisms could contribute to this resiliency in females as this could have important implications for understanding sex-differences in stress responses.

When we examined risky decision-making, we found that all LBN exposed animals, regardless of sex, were less risky than controls. Specifically, LBN exposed animals tended to select the small reward, certain lever more often than the large reward, risky lever when the odds of obtaining reward were less advantageous. These findings are in line with our previous observations from Chapter 2 that LBN can promote resiliency to addiction-related phenotypes. However, when data was split by sex, the effect of LBN disappeared. In studies that only include male rats, combined exposure to ethanol vapor during adolescence and chronic social defeat stress in adulthood also do not alter risky choice (Nathalie Boutros, Der-Avakian, Semenova, Lee, & Markou, 2016). It could be that stress exposure may only have a mild effect in biasing risky choice on probability discounting tasks.

In addition to measuring how LBN affects risky choices, we also examined win-stay and lose-shift behaviors. All animals tested in our study displayed more win-stay than lose-shift tendencies. These results suggest that LBN rats engaged in similar patterns of choice behavior as controls. Some work suggests, however, that chronic unpredictable stress experienced for 35 days can exaggerate win-stay tendencies in stressed males as compared to controls (Matisz et al., 2021). It could be that if we had used a more severe stressor and for a longer time, then we would have also seen elevations in win-stay tendencies in stressed animals. Sometimes win-stay tendencies pay off when this strategy leads to rewards. However, when this strategy is no longer optimal, it is more appropriate to shift to another strategy (i.e., lose-shift). Choice strategies are often impaired in individuals with substance use disorder or bipolar disorder (R. Smith et al., 2020; Urošević, Halverson, Youngstrom, & Luciana, 2018). As such, future studies should continue to investigate how diverse forms of stress alter choice behavior strategies in males and females as this could inform and improve interventions more specifically targeting active learning strategies.

RNA-sequencing data also revealed sex-specific changes in DEGs following LBN in the OFC. These findings are similar with our previous observations from Chapter 2 that LBN can promote sex-specific changes in the transcriptome of the NAc core. These findings are interesting given that the NAc and OFC both are involved in impulsive and risky behavior. Because early life stress can affect gene expression within these regions in different ways across sex, this could explain why LBN induces such different behavioral effects in males and females. In humans, it is not uncommon to find opposing transcriptional signatures between the sexes. For instance, opposing transcriptional signatures are identified in samples of brain tissue from women and men diagnosed with major depressive disorder (Labonté et al., 2017; Seney et al., 2018). These results suggest responses to stress can differ by sex and highlight the need for incorporating sex as a biological variable in study designs. The assumption that what occurs in males equates to what is occurring in females should not be made (A. V. Williams & Trainor, 2018), whether the research be in humans or in rodent models.

One of the genes we selected for our validation study was *angiotensinogen (AGT)*, which was significantly upregulated by LBN in males but not females, and also contributed to the KEGG enrichment of the "aldosterone synthesis and secretion" pathway. *AGT* is sensitive to stress and manipulation of the renin-angiotensin-aldosterone system can affect impulsive behavior (Porter et al., 2015; Raghavendra et al., 1999). By using RNAScope, we confirm that males and females, regardless of stress condition, predominantly express *AGT* in astrocyte, but not neuronal, cell types in the OFC. These results were exciting because we are the first to include females in order to address whether *AGT* in the brain is also expressed in astrocytes like male data suggests. However, when we examined if *AGT* is expressed differently across OFC subregion (i.e., medial vs. lateral) we found no differences. It could be, however, that if we had probed for another cell-type, such as fibroblast-like cells, that also expresses *AGT,* we would have been able to replicate the LBN effects found by RNA-Seq. Additionally, it is possible that other genes significantly upregulated by LBN in males may be driving the changes in behaviors induced by this manipulation. Identification of genes that are altered by early life stress and impact OFC-mediated behaviors represents an additional direction for future investigation.

In summary, the present studies characterize the impact of LBN on addiction-related phenotypes such as impulsivity, risky decision-making, and morphine self-administration. These studies also examine how LBN alters the NAc electrophysiology, NAc transcriptome, and OFC transcriptome. Much more research is needed to understand the mechanisms by which LBN impacts impulsive and risky decision-making behaviors in males and females. Here we identify multiple possible mechanisms for LBN-induced changes via RNA-sequencing analysis of the NAc and OFC, which are critical for regulation of impulsive and risky behaviors. These findings lay the groundwork for future studies to investigate the mechanisms by which LBN alters behavior and brain function. They also add to the body of literature characterizing the ways in which various experiences of early life stress alter impulsivity and risky decision-making in males and females. Understanding these mechanisms will lead to a better understanding of how stress can affect these processes in various psychiatric disorders and contribute to the development of better treatment and prevention interventions.

REFERENCES

Abela, A. R., & Chudasama, Y. (2013). Dissociable contributions of the ventral hippocampus and orbitofrontal cortex to decision-making with a delayed or uncertain outcome. *Eur J Neurosci, 37*(4), 640-647. doi:10.1111/ejn.12071

Adler, L. A., Faraone, S. V., Spencer, T. J., Berglund, P., Alperin, S., & Kessler, R. C. (2017). The structure of adult ADHD. *Int J Methods Psychiatr Res, 26*(1). doi:10.1002/mpr.1555

Adriani, W., Boyer, F., Gioiosa, L., Macrì, S., Dreyer, J. L., & Laviola, G. (2009). Increased impulsive behavior and risk proneness following lentivirus-mediated dopamine transporter over-expression in rats' nucleus accumbens. *Neuroscience, 159*(1), 47-58. doi:10.1016/j.neuroscience.2008.11.042

Anda, R. F., Felitti, V. J., Bremner, J. D., Walker, J. D., Whitfield, C., Perry, B. D., . . . Giles, W. H. (2006). The enduring effects of abuse and related adverse experiences in childhood. A convergence of evidence from neurobiology and epidemiology. *European archives of psychiatry and clinical neuroscience, 256*(3), 174-186. doi:10.1007/s00406-005-0624-4

Andersen, S. L., Rutstein, M., Benzo, J. M., Hostetter, J. C., & Teicher, M. H. (1997). Sex differences in dopamine receptor overproduction and elimination. *NeuroReport, 8*(6).

Andersen, S. L., & Teicher, M. H. (2000). Sex differences in dopamine receptors and their relevance to ADHD. *Neuroscience & Biobehavioral Reviews, 24*(1), 137-141. doi:https://doi.org/10.1016/S0149-7634(99)00044-5

Andrade, L. F., & Petry, N. M. (2012). Delay and probability discounting in pathological gamblers with and without a history of substance use problems. *Psychopharmacology, 219*(2), 491-499. doi:10.1007/s00213-011-2508-9

Andrews, S. (2015). FastQC: A quality control tool for high-throughput sequence data (2015). Retrieved from www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Arata, C. M., Langhinrichsen-Rohling, J., Bowers, D., & O’Brien, N. (2007). Differential correlates of multi-type maltreatment among urban youth. *Child Abuse & Neglect, 31*(4), 393-415. doi:https://doi.org/10.1016/j.chiabu.2006.09.006

Ashok, A. H., Marques, T. R., Jauhar, S., Nour, M. M., Goodwin, G. M., Young, A. H., & Howes, O. D. (2017). The dopamine hypothesis of bipolar affective disorder: the state of the art and implications for treatment. *Molecular Psychiatry, 22*(5), 666-679. doi:10.1038/mp.2017.16

Ayada, C., Toru, Ü., & Korkut, Y. (2015). The relationship of stress and blood pressure effectors. *Hippokratia, 19*(2), 99-108.

Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2012). Dissociable effects of monoamine reuptake inhibitors on distinct forms of impulsive behavior in rats. *Psychopharmacology, 219*(2), 313-326. doi:10.1007/s00213-011-2576-x

Badgaiyan, R. D., Sinha, S., Sajjad, M., & Wack, D. S. (2015). Attenuated Tonic and Enhanced Phasic Release of Dopamine in Attention Deficit Hyperactivity Disorder. *PLoS One, 10*(9), e0137326. doi:10.1371/journal.pone.0137326

Baharlouei, N., Sarihi, A., Moradi, M., Zarrabian, S., & Haghparast, A. (2018). Microinjection of the mGluR2/3 agonist, LY379268, into the nucleus accumbens attenuates extinction latencies and the reinstatement of morphine-induced conditioned place preference in rats. *Behavioural pharmacology, 29*(5), 385-392. doi:10.1097/fbp.0000000000000375

Baier, C. J., Katunar, M. R., Adrover, E., Pallarés, M. E., & Antonelli, M. C. (2012). Gestational Restraint Stress and the Developing Dopaminergic System: An Overview. *Neurotoxicity Research, 22*(1), 16-32. doi:10.1007/s12640-011-9305-4

Banqueri, M., Méndez, M., & Arias, J. L. (2017). Behavioral effects in adolescence and early adulthood in two length models of maternal separation in male rats. *Behav Brain Res, 324,* 77-86. doi:10.1016/j.bbr.2017.02.006

Bari, A., & Robbins, T. W. (2013). Inhibition and impulsivity: behavioral and neural basis of response control. *Prog Neurobiol, 108,* 44-79. doi:10.1016/j.pneurobio.2013.06.005

Barlow, R. L., Gorges, M., Wearn, A., Niessen, H. G., Kassubek, J., Dalley, J. W., & Pekcec, A. (2018). Ventral Striatal D2/3 Receptor Availability Is Associated with Impulsive Choice Behavior As Well As Limbic Corticostriatal Connectivity. *Int J Neuropsychopharmacol, 21*(7), 705-715. doi:10.1093/ijnp/pyy030

Bath, K. G. (2020). Synthesizing Views to Understand Sex Differences in Response to Early Life Adversity. *Trends in Neurosciences, 43*(5), 300-310. doi:https://doi.org/10.1016/j.tins.2020.02.004

Bauer, J., Werner, A., Kohl, W., Kugel, H., Shushakova, A., Pedersen, A., & Ohrmann, P. (2018). Hyperactivity and impulsivity in adult attention-deficit/hyperactivity disorder is related to glutamatergic dysfunction in the anterior cingulate cortex. *The World Journal of Biological Psychiatry, 19*(7), 538-546. doi:10.1080/15622975.2016.1262060

Bauer, J., Werner, A., Kohl, W., Kugel, H., Shushakova, A., Pedersen, A., & Ohrmann, P. (2018). Hyperactivity and impulsivity in adult attention-deficit/hyperactivity disorder is related to glutamatergic dysfunction in the anterior cingulate cortex. *World J Biol Psychiatry, 19*(7), 538-546. doi:10.1080/15622975.2016.1262060

Baunez, C., Nieoullon, A., & Amalric, M. (1995). In a rat model of parkinsonism, lesions of the subthalamic nucleus reverse increases of reaction time but induce a dramatic premature responding deficit. *The Journal of Neuroscience, 15*(10), 6531. doi:10.1523/JNEUROSCI.15-10-06531.1995

Beatty, W. W. (1973). Effects of gonadectomy on sex differences in DRL behavior. *Physiology & Behavior, 10*(1), 177-178. doi:https://doi.org/10.1016/0031-9384(73)90108-X

Beaulieu, J.-M., & Gainetdinov, R. R. (2011). The Physiology, Signaling, and Pharmacology of Dopamine Receptors. *Pharmacological Reviews, 63*(1), 182. doi:10.1124/pr.110.002642

Bechara, A., Dolan, S., Denburg, N., Hindes, A., Anderson, S. W., & Nathan, P. E. (2001). Decision-making deficits, linked to a dysfunctional ventromedial prefrontal cortex, revealed in alcohol and stimulant abusers. *Neuropsychologia, 39*(4), 376-389. doi:https://doi.org/10.1016/S0028-3932(00)00136-6

Bechara, A., Tranel, D., & Damasio, H. (2000). Characterization of the decision-making deficit of patients with ventromedial prefrontal cortex lesions. *Brain, 123*(11), 2189-2202. doi:10.1093/brain/123.11.2189

Becker, J. B., & Chartoff, E. (2019). Sex differences in neural mechanisms mediating reward and addiction. *Neuropsychopharmacology, 44*(1), 166-183. doi:10.1038/s41386-018-0125-6

Berger, M. A., Barros, V. G., Sarchi, M. I., Tarazi, F. I., & Antonelli, M. C. (2002). Long-Term Effects of Prenatal Stress on Dopamine and Glutamate Receptors in Adult Rat Brain. *Neurochemical Research, 27*(11), 1525-1533. doi:10.1023/A:1021656607278

Berk, M., Dodd, S., Kauer-Sant'anna, M., Malhi, G. S., Bourin, M., Kapczinski, F., & Norman, T. (2007). Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. *Acta Psychiatr Scand Suppl*(434), 41-49. doi:10.1111/j.1600-0447.2007.01058.x

Bernal, J. (2007). Thyroid hormone receptors in brain development and function. *Nat Clin Pract Endocrinol Metab, 3*(3), 249-259. doi:10.1038/ncpendmet0424

Bernosky-Smith, K. A., Qiu, Y. Y., Feja, M., Lee, Y. B., Loughlin, B., Li, J. X., & Bass, C. E. (2018). Ventral tegmental area D2 receptor knockdown enhances choice impulsivity in a delay-discounting task in rats. *Behav Brain Res, 341*, 129-134. doi:10.1016/j.bbr.2017.12.029

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev, 28*(3), 309-369. doi:10.1016/s0165-0173(98)00019-8

Biederman, J., Milberger, S., Faraone, S. V., Kiely, K., Guite, J., Mick, E., . . . Davis, S. G. (1995). Impact of adversity on functioning and comorbidity in children with attention-deficit hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry, 34*(11), 1495-1503. doi:10.1097/00004583-199511000-00017

Biggio, F., Pisu, M. G., Garau, A., Boero, G., Locci, V., Mostallino, M. C., . . . Serra, M. (2014). Maternal separation attenuates the effect of adolescent social isolation on HPA axis responsiveness in adult rats. *European Neuropsychopharmacology, 24(7)*, 1152-1161. doi:https://doi.org/10.1016/j.euroneuro.2014.03.009

Birn, R. M., Roeber, B. J., & Pollak, S. D. (2017). Early childhood stress exposure, reward pathways, and adult decision making. *Proceedings of the National Academy of Sciences, 114*(51), 13549. doi:10.1073/pnas.1708791114

Blum, K., Chen, A. L.-C., Braverman, E. R., Comings, D. E., Chen, T. J. H., Arcuri, V., . . . Oscar-Berman, M. (2008). Attention-deficit-hyperactivity disorder and reward deficiency syndrome. *Neuropsychiatric disease and treatment, 4*(5), 893-918. doi:10.2147/ndt.s2627

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics, 30*(15), 2114-2120. doi:10.1093/bioinformatics/btu170

Bossert, J. M., Gray, S. M., Lu, L., & Shaham, Y. (2006). Activation of group II metabotropic glutamate receptors in the nucleus accumbens shell attenuates context-induced relapse to heroin seeking. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 31*(10), 2197-2209. doi:10.1038/sj.npp.1300977

Boutros, N., Der-Avakian, A., Markou, A., & Semenova, S. (2017). Effects of early life stress and adolescent ethanol exposure on adult cognitive performance in the 5-choice serial reaction time task in Wistar male rats. *Psychopharmacology (Berl), 234*(9-10), 1549-1556. doi:10.1007/s00213-017-4555-3

Boutros, N., Der-Avakian, A., Semenova, S., Lee, S., & Markou, A. (2016). Risky choice and brain CRF after adolescent ethanol vapor exposure and social stress in adulthood. *Behavioural Brain Research, 311*, 160-166. doi:https://doi.org/10.1016/j.bbr.2016.05.038

Brake, W. G., Zhang, T. Y., Diorio, J., Meaney, M. J., & Gratton, A. (2004). Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *European Journal of Neuroscience, 19*(7), 1863-1874. doi:https://doi.org/10.1111/j.1460-9568.2004.03286.x

Brand, M., Roth-Bauer, M., Driessen, M., & Markowitsch, H. J. (2008). Executive functions and risky decision-making in patients with opiate dependence. *Drug and Alcohol Dependence, 97*(1), 64-72. doi:https://doi.org/10.1016/j.drugalcdep.2008.03.017

Brenhouse, H. C., Lukkes, J. L., & Andersen, S. L. (2013). Early life adversity alters the developmental profiles of addiction-related prefrontal cortex circuitry. (2076-3425 (Print)).

Brevers, D., Bechara, A., Cleeremans, A., Kornreich, C., Verbanck, P., & Noël, X. (2014). Impaired Decision-Making Under Risk in Individuals with Alcohol Dependence. *Alcoholism: Clinical and Experimental Research, 38*(7), 1924-1931. doi:https://doi.org/10.1111/acer.12447

Brevers, D., Cleeremans, A., Verbruggen, F., Bechara, A., Kornreich, C., Verbanck, P., & Noël, X. (2012). Impulsive Action but Not Impulsive Choice Determines Problem Gambling Severity. *PLoS One, 7*(11), e50647. doi:10.1371/journal.pone.0050647

Breyer, J. L., Botzet, A. M., Winters, K. C., Stinchfield, R. D., August, G., & Realmuto, G. (2009). Young Adult Gambling Behaviors and their Relationship with the Persistence of ADHD. *Journal of Gambling Studies, 25*(2), 227-238. doi:10.1007/s10899-009-9126-z

Britton, K. T., & Koob, G. F. (1989). Effects of corticotropin releasing factor, desipramine and haloperidol on a DRL schedule of reinforcement. *Pharmacol Biochem Behav, 32*(4), 967-970. doi:10.1016/0091-3057(89)90067-1

Brog, J. S., Salyapongse, A., Deutch, A. Y., & Zahm, D. S. (1993). The patterns of afferent innervation of the core and shell in the "accumbens" part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol, 338*(2), 255-278. doi:10.1002/cne.903380209

Broos, N., Schmaal, L., Wiskerke, J., Kostelijk, L., Lam, T., Stoop, N., . . . Goudriaan, A. E. (2012). The Relationship between Impulsive Choice and Impulsive Action: A Cross-Species Translational Study. *PLoS One, 7*(5), e36781. doi:10.1371/journal.pone.0036781

Brunson, K. L., Kramár, E., Lin, B., Chen, Y., Colgin, L. L., Yanagihara, T. K., . . . Baram, T. Z. (2005). Mechanisms of Late-Onset Cognitive Decline after Early-Life Stress. *The Journal of Neuroscience, 25*(41), 9328. doi:10.1523/JNEUROSCI.2281-05.2005

Bryden, D. W., Johnson, E. E., Tobia, S. C., Kashtelyan, V., & Roesch, M. R. (2011). Attention for Learning Signals in Anterior Cingulate Cortex. *The Journal of Neuroscience, 31*(50), 18266. doi:10.1523/JNEUROSCI.4715-11.2011

Burton, C. L., Chatterjee, D., Chatterjee-Chakraborty, M., Lovic, V., Grella, S. L., Steiner, M., & Fleming, A. S. (2007). Prenatal restraint stress and motherless rearing disrupts expression of plasticity markers and stress-induced corticosterone release in adult female Sprague-Dawley rats. *Brain Res, 1158*, 28-38. doi:10.1016/j.brainres.2007.05.003

Bussey, T. J., Everitt, B. J., & Robbins, T. W. (1997). Dissociable effects of cingulate and medial frontal cortex lesions on stimulus-reward learning using a novel Pavlovian autoshaping procedure for the rat: implications for the neurobiology of emotion. *Behav Neurosci, 111*(5), 908-919. doi:10.1037//0735-7044.111.5.908

Caballero-Puntiverio, M., Fitzpatrick, C. M., Woldbye, D. P., & Andreasen, J. T. (2017). Effects of amphetamine and methylphenidate on attentional performance and impulsivity in the mouse 5-Choice Serial Reaction Time Task. *J Psychopharmacol, 31*(2), 272-283. doi:10.1177/0269881116684339

Cahill, K. M., Huo, Z., Tseng, G. C., Logan, R. W., & Seney, M. L. (2018). Improved identification of concordant and discordant gene expression signatures using an updated rank-rank hypergeometric overlap approach. *Scientific Reports, 8*(1), 9588. doi:10.1038/s41598-018-27903-2

Calipari, E. S., Juarez, B., Morel, C., Walker, D. M., Cahill, M. E., Ribeiro, E., . . . Nestler, E. J. (2017). Dopaminergic dynamics underlying sex-specific cocaine reward. *Nature Communications, 8*(1), 13877. doi:10.1038/ncomms13877

Caprioli, D., Jupp, B., Hong, Y. T., Sawiak, S. J., Ferrari, V., Wharton, L., . . . Dalley, J. W. (2015). Dissociable rate-dependent effects of oral methylphenidate on impulsivity and D2/3 receptor availability in the striatum. *J Neurosci, 35*(9), 3747-3755. doi:10.1523/jneurosci.3890-14.2015

Cardinal, R. N., Pennicott, D. R., Sugathapala, C. L., Robbins, T. W., & Everitt, B. J. (2001). Impulsive choice induced in rats by lesions of the nucleus accumbens core. *Science, 292*(5526), 2499-2501. doi:10.1126/science.1060818

Cardinal, R. N., Robbins, T. W., & Everitt, B. J. (2000). The effects of d-amphetamine, chlordiazepoxide, alpha-flupenthixol and behavioural manipulations on choice of signalled and unsignalled delayed reinforcement in rats. *Psychopharmacology (Berl), 152*(4), 362-375. doi:10.1007/s002130000536

Carr, C. P., Martins, C. M., Stingel, A. M., Lemgruber, V. B., & Juruena, M. F. (2013). The role of early life stress in adult psychiatric disorders: a systematic review according to childhood trauma subtypes. *J Nerv Ment Dis, 201*(12), 1007-1020. doi:10.1097/NMD.0000000000000049

Casey, J. L., & Cservenka, A. (2020). Effects of frequent marijuana use on risky decision-making in young adult college students. *Addictive Behaviors Reports, 11*, 100253. doi:https://doi.org/10.1016/j.abrep.2020.100253

Chen, Y., & Baram, T. Z. (2016). Toward Understanding How Early-Life Stress Reprograms Cognitive and Emotional Brain Networks. *Neuropsychopharmacology, 41*(1), 197-206. doi:10.1038/npp.2015.181

Cheng, R.-K., & Liao, R.-M. (2017). Regional differences in dopamine receptor blockade affect timing impulsivity that is altered by d-amphetamine on differential reinforcement of low-rate responding (DRL) behavior in rats. *Behavioural Brain Research, 331*, 177-187. doi:https://doi.org/10.1016/j.bbr.2017.05.020

Cho, Y. H., & Jeantet, Y. (2010). Differential involvement of prefrontal cortex, striatum, and hippocampus in DRL performance in mice. *Neurobiol Learn Mem, 93*(1), 85-91. doi:10.1016/j.nlm.2009.08.007

Chocyk, A., Przyborowska, A., Dudys, D., Majcher, I., Maćkowiak, M., & Wędzony, K. (2011). The impact of maternal separation on the number of tyrosine hydroxylase-expressing midbrain neurons during different stages of ontogenesis. *Neuroscience, 182*, 43-61. doi:https://doi.org/10.1016/j.neuroscience.2011.03.008

Christakou, A., Robbins, T. W., & Everitt, B. J. (2004). Prefrontal cortical-ventral striatal interactions involved in affective modulation of attentional performance: implications for corticostriatal circuit function. *J Neurosci, 24*(4), 773-780. doi:10.1523/JNEUROSCI.0949-03.2004

Chudasama, Y., Passetti, F., Rhodes, S. E., Lopian, D., Desai, A., & Robbins, T. W. (2003). Dissociable aspects of performance on the 5-choice serial reaction time task following lesions of the dorsal anterior cingulate, infralimbic and orbitofrontal cortex in the rat: differential effects on selectivity, impulsivity and compulsivity. *Behav Brain Res, 146*(1-2), 105-119. doi:10.1016/j.bbr.2003.09.020

Corbit, L. H., & Balleine, B. W. (2000). The role of the hippocampus in instrumental conditioning. *J Neurosci, 20*(11), 4233-4239. doi:10.1523/jneurosci.20-11-04233.2000

Cory-Slechta, D. A., Virgolini, M. B., Thiruchelvam, M., Weston, D. D., & Bauter, M. R. (2004). Maternal stress modulates the effects of developmental lead exposure. *Environmental Health Perspectives, 112*(6), 717-730. doi:10.1289/ehp.6481

Cousins, D. A., Butts, K., & Young, A. H. (2009). The role of dopamine in bipolar disorder. *Bipolar Disord, 11*(8), 787-806. doi:10.1111/j.1399-5618.2009.00760.x

Cousins, M. S., Wei, W., & Salamone, J. D. (1994). Pharmacological characterization of performance on a concurrent lever pressing/feeding choice procedure: effects of dopamine antagonist, cholinomimetic, sedative and stimulant drugs. *Psychopharmacology, 116*(4), 529-537. doi:10.1007/BF02247489

Cropley, V. L., Fujita, M., Innis, R. B., & Nathan, P. J. (2006). Molecular imaging of the dopaminergic system and its association with human cognitive function. *Biol Psychiatry, 59*(10), 898-907. doi:10.1016/j.biopsych.2006.03.004

Dalley, & Ersche, K. D. (2019). Neural circuitry and mechanisms of waiting impulsivity: relevance to addiction. *Philos Trans R Soc Lond B Biol Sci, 374*(1766), 20180145. doi:10.1098/rstb.2018.0145

Dalley, Everitt, B. J., & Robbins, T. W. (2011). Impulsivity, Compulsivity, and Top-Down Cognitive Control. *Neuron, 69*(4), 680-694. doi:10.1016/j.neuron.2011.01.020

Dalley, Fryer, T. D., Brichard, L., Robinson, E. S., Theobald, D. E., Lääne, K., . . . Robbins, T. W. (2007). Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. *Science, 315*(5816), 1267-1270. doi:10.1126/science.1137073

Dalley, J. W., & Robbins, T. W. (2017). Fractionating impulsivity: neuropsychiatric implications. *Nat Rev Neurosci, 18*(3), 158-171. doi:10.1038/nrn.2017.8

Dalley, J. W., & Roiser, J. P. (2012). Dopamine, serotonin and impulsivity. *Neuroscience, 215*, 42-58. doi:10.1016/j.neuroscience.2012.03.065

Dawe, S., & Loxton, N. J. (2004). The role of impulsivity in the development of substance use and eating disorders. *Neuroscience & Biobehavioral Reviews, 28*(3), 343-351. doi:https://doi.org/10.1016/j.neubiorev.2004.03.007

Day, J. J., Jones, J. L., Wightman, R. M., & Carelli, R. M. (2010). Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs. *Biol Psychiatry, 68*(3), 306-309. doi:10.1016/j.biopsych.2010.03.026

de Wit, H., Crean, J., & Richards, J. B. (2000). Effects of d-amphetamine and ethanol on a measure of behavioral inhibition in humans. *Behav Neurosci, 114*(4), 830-837. doi:10.1037//0735-7044.114.4.830

Deykin, E. Y., & Buka, S. L. (1997). Prevalence and risk factors for posttraumatic stress disorder among chemically dependent adolescents. *Am J Psychiatry, 154*(6), 752-757. doi:10.1176/ajp.154.6.752

Diergaarde, L., Pattij, T., Poortvliet, I., Hogenboom, F., de Vries, W., Schoffelmeer, A. N. M., & De Vries, T. J. (2008). Impulsive Choice and Impulsive Action Predict Vulnerability to Distinct Stages of Nicotine Seeking in Rats. *Biological Psychiatry, 63*(3), 301-308. doi:10.1016/j.biopsych.2007.07.011

Donohew, L., Zimmerman, R., Cupp, P. S., Novak, S., Colon, S., & Abell, R. (2000). Sensation seeking, impulsive decision-making, and risky sex: implications for risk-taking and design of interventions. *Personality and Individual Differences, 28*(6), 1079-1091. doi:https://doi.org/10.1016/S0191-8869(99)00158-0

Dubé, C. M., Molet, J., Singh-Taylor, A., Ivy, A., Maras, P. M., & Baram, T. Z. (2015). Hyper-excitability and epilepsy generated by chronic early-life stress. *Neurobiol Stress, 2,* 10-19. doi:10.1016/j.ynstr.2015.03.001

Duckworth, A., Kim, B., & Tsukayama, E. (2013). Life Stress Impairs Self-Control in Early Adolescence. *Frontiers in Psychology, 3*, 608.

Eck, S. R., Ardekani, C. S., Salvatore, M., Luz, S., Kim, E. D., Rogers, C. M., . . . Bangasser, D. A. (2019). The effects of early life adversity on growth, maturation, and steroid hormones in male and female rats. *Eur J Neurosci*. doi:10.1111/ejn.14609

Eck, S. R., & Bangasser, D. A. (2020). The effects of early life stress on motivated behaviors: A role for gonadal hormones. *Neuroscience & Biobehavioral Reviews, 119*, 86-100. doi:https://doi.org/10.1016/j.neubiorev.2020.09.014

Evenden, J. L., & Ryan, C. N. (1996). The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement. *Psychopharmacology (Berl), 128*(2), 161-170. doi:10.1007/s002130050121

Everitt, B. J., Belin, D., Economidou, D., Pelloux, Y., Dalley, J. W., & Robbins, T. W. (2008). Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. *Philos Trans R Soc Lond B Biol Sci, 363*(1507), 3125-3135. doi:10.1098/rstb.2008.0089

Fattore, L., & Diana, M. (2016). Drug addiction: An affective-cognitive disorder in need of a cure. *Neuroscience & Biobehavioral Reviews, 65*, 341-361. doi:https://doi.org/10.1016/j.neubiorev.2016.04.006

Filipkowski, K. B., Heron, K. E., & Smyth, J. M. (2016). Early Adverse Experiences and Health: The Transition to College. *Am J Health Behav, 40*(6), 717-728. doi:10.5993/ajhb.40.6.4

Fineberg, N. A., Chamberlain, S. R., Goudriaan, A. E., Stein, D. J., Vanderschuren, L. J., Gillan, C. M., . . . Potenza, M. N. (2014). New developments in human neurocognition: clinical, genetic, and brain imaging correlates of impulsivity and compulsivity. *CNS Spectr, 19*(1), 69-89. doi:10.1017/s1092852913000801

Fitzpatrick, C. M., Runegaard, A. H., Christiansen, S. H., Hansen, N. W., Jørgensen, S. H., McGirr, J. C., . . . Andreasen, J. T. (2019). Differential effects of chemogenetic inhibition of dopamine and norepinephrine neurons in the mouse 5-choice serial reaction time task. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 90*, 264-276. doi:https://doi.org/10.1016/j.pnpbp.2018.12.004

Floresco, S. B., Tse, M. T., & Ghods-Sharifi, S. (2008). Dopaminergic and glutamatergic regulation of effort- and delay-based decision making. *Neuropsychopharmacology, 33*(8), 1966-1979. doi:10.1038/sj.npp.1301565

Floresco, S. B., Tse, M. T. L., & Ghods-Sharifi, S. (2008). Dopaminergic and Glutamatergic Regulation of Effort- and Delay-Based Decision Making. *Neuropsychopharmacology, 33*(8), 1966-1979. doi:10.1038/sj.npp.1301565

Fuentes, S., Carrasco, J., Hatto, A., Navarro, J., Armario, A., Monsonet, M., . . . Nadal, R. (2018). Sex-dependent impact of early-life stress and adult immobilization in the attribution of incentive salience in rats. *PLoS One, 13*(1), e0190044. doi:10.1371/journal.pone.0190044

Fuentes, S., Daviu, N., Gagliano, H., Garrido, P., Zelena, D., Monasterio, N., . . . Nadal, R. (2014). Sex-dependent effects of an early life treatment in rats that increases maternal care: vulnerability or resilience? *Front Behav Neurosci, 8*, 56. doi:10.3389/fnbeh.2014.00056

Funk, R. R., McDermeit, M., Godley, S. H., & Adams, L. (2003). Maltreatment issues by level of adolescent substance abuse treatment: the extent of the problem at intake and relationship to early outcomes. *Child Maltreat, 8*(1), 36-45. doi:10.1177/1077559502239607

Gallo, M., Shleifer, D. G., Godoy, L. D., Ofray, D., Olaniyan, A., Campbell, T., & Bath, K. G. (2019). Limited Bedding and Nesting Induces Maternal Behavior Resembling Both Hypervigilance and Abuse. *Frontiers in Behavioral Neuroscience, 13 (167)*. doi:doi:10.3389/fnbeh.2019.00167

Ganguly, P., Honeycutt, J. A., Rowe, J. R., Demaestri, C., & Brenhouse, H. C. (2019). Effects of early life stress on cocaine conditioning and AMPA receptor composition are sex-specific and driven by TNF. *Brain Behav Immun., 78*, 41-51. doi:doi:10.1016/j.bbi.2019.01.006

Gatta, E., Mairesse, J., Deruyter, L., Marrocco, J., Van Camp, G., Bouwalerh, H., . . . Maccari, S. (2018). Reduced maternal behavior caused by gestational stress is predictive of life span changes in risk-taking behavior and gene expression due to altering of the stress/anti-stress balance. *Neurotoxicology, 66*, 138-149. doi:https://doi.org/10.1016/j.neuro.2018.04.005

Ghods-Sharifi, S., St. Onge, J. R., & Floresco, S. B. (2009). Fundamental Contribution by the Basolateral Amygdala to Different Forms of Decision Making. *The Journal of Neuroscience, 29*(16), 5251. doi:10.1523/JNEUROSCI.0315-09.2009

Gilles, E. E., Schultz, L., & Baram, T. Z. (1996). Abnormal corticosterone regulation in an immature rat model of continuous chronic stress. *Pediatr Neurol, 15*(2), 114-119. doi:10.1016/0887-8994(96)00153-1

Gingrich, J. A., & Caron, M. G. (1993). Recent advances in the molecular biology of dopamine receptors. *Annu Rev Neurosci, 16*, 299-321. doi:10.1146/annurev.ne.16.030193.001503

Gold, M. S., Blum, K., Oscar-Berman, M., & Braverman, E. R. (2014). Low dopamine function in attention deficit/hyperactivity disorder: should genotyping signify early diagnosis in children? *Postgraduate medicine, 126*(1), 153-177. doi:10.3810/pgm.2014.01.2735

Gondré-Lewis, M. C., Warnock, K. T., Wang, H., June, H. L., Bell, K. A., Rabe, H., . . . Aurelian, L. (2016). Early life stress is a risk factor for excessive alcohol drinking and impulsivity in adults and is mediated via a CRF/GABAA mechanism. *Stress (Amsterdam, Netherlands), 19*(2), 235-247. doi:10.3109/10253890.2016.1160280

Goodwill, H. L., Manzano-Nieves, G., LaChance, P., Teramoto, S., Lin, S., Lopez, C., . . . Bath, K. G. (2018). Early Life Stress Drives Sex-Selective Impairment in Reversal Learning by Affecting Parvalbumin Interneurons in Orbitofrontal Cortex of Mice. *Cell Reports, 25*(9), 2299-2307.e2294. doi:https://doi.org/10.1016/j.celrep.2018.11.010

Gordon, R. D., Küchel, O., Liddle, G. W., & Island, D. P. (1967). Role of the sympathetic nervous system in regulating renin and aldosterone production in man. *The Journal of clinical investigation, 46*(4), 599-605. doi:10.1172/JCI105561

Gracia-Rubio, I., Martinez-Laorden, E., Moscoso-Castro, M., Milanés, M. V., Laorden, M. L., & Valverde, O. (2016). Maternal Separation Impairs Cocaine-Induced Behavioural Sensitization in Adolescent Mice. *PLoS One, 11*(12), e0167483. doi:10.1371/journal.pone.0167483

Grant, J. E., Redden, S. A., Lust, K., & Chamberlain, S. R. (2018). Nonmedical Use of Stimulants Is Associated With Riskier Sexual Practices and Other Forms of Impulsivity. *J Addict Med, 12*(6), 474-480. doi:10.1097/adm.0000000000000448

Grassi-Oliveira, R., Honeycutt, J. A., Holland, F. H., Ganguly, P., & Brenhouse, H. C. (2016). Cognitive impairment effects of early life stress in adolescents can be predicted with early biomarkers: Impacts of sex, experience, and cytokines. *Psychoneuroendocrinology, 71*, 19-30. doi:https://doi.org/10.1016/j.psyneuen.2016.04.016

Green, J. G., McLaughlin, K. A., Berglund, P. A., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., & Kessler, R. C. (2010). Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: associations with first onset of DSM-IV disorders. *Archives of General Psychiatry, 67*(2), 113-123. doi:10.1001/archgenpsychiatry.2009.186

Green, N. F. O., Maniam, J., Riese, J., Morris, M. J., & Voineagu, I. (2021). Transcriptomic signature of early life stress in male rat prefrontal cortex. *Neurobiology of Stress, 14*, 100316. doi:https://doi.org/10.1016/j.ynstr.2021.100316

Groenewegen, H. J., der Zee, E. V.-V., te Kortschot, A., & Witter, M. P. (1987). Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. *Neuroscience, 23*(1), 103-120. doi:https://doi.org/10.1016/0306-4522(87)90275-2

Groenewegen, H. J., & Russchen, F. T. (1984). Organization of the efferent projections of the nucleus accumbens to pallidal, hypothalamic, and mesencephalic structures: A tracing and immunohistochemical study in the cat. *Journal of Comparative Neurology, 223*(3), 347-367. doi:https://doi.org/10.1002/cne.902230303

Groenewegen, H. J., Wright, C. I., Beijer, A. V., & Voorn, P. (1999). Convergence and segregation of ventral striatal inputs and outputs. *Ann N Y Acad Sci, 877*, 49-63. doi:10.1111/j.1749-6632.1999.tb09260.x

Gu, J., Fan, Y. Q., Zhang, H. L., Pan, J. A., Yu, J. Y., Zhang, J. F., & Wang, C. Q. (2018). Resveratrol suppresses doxorubicin-induced cardiotoxicity by disrupting E2F1 mediated autophagy inhibition and apoptosis promotion. *Biochem Pharmacol, 150*, 202-213. doi:10.1016/j.bcp.2018.02.025

Guillaumin, A., Serra, G. P., Georges, F., & Wallén-Mackenzie, Å. (2021). Experimental investigation into the role of the subthalamic nucleus (STN) in motor control using optogenetics in mice. (1872-6240 (Electronic)).

Gut-Fayand, A., Dervaux, A., Olié, J.-P., Lôo, H., Poirier, M.-F., & Krebs, M.-O. (2001). Substance abuse and suicidality in schizophrenia: a common risk factor linked to impulsivity. *Psychiatry Research, 102*(1), 65-72. doi:https://doi.org/10.1016/S0165-1781(01)00250-5

Halevi, G., Djalovski, A., Vengrober, A., & Feldman, R. (2016). Risk and resilience trajectories in war-exposed children across the first decade of life. *J Child Psychol Psychiatry, 57*(10), 1183-1193. doi:10.1111/jcpp.12622

Hamidovic, A., Dlugos, A., Skol, A., Palmer, A. A., & de Wit, H. (2009). Evaluation of genetic variability in the dopamine receptor D2 in relation to behavioral inhibition and impulsivity/sensation seeking: an exploratory study with d-amphetamine in healthy participants. *Exp Clin Psychopharmacol, 17*(6), 374-383. doi:10.1037/a0017840

Hayton, S. J., Maracle, A. C., & Olmstead, M. C. (2012). Opposite effects of amphetamine on impulsive action with fixed and variable delays to respond. *Neuropsychopharmacology, 37*(3), 651-659. doi:10.1038/npp.2011.236

Hearing, M. C., Jedynak, J., Ebner, S. R., Ingebretson, A., Asp, A. J., Fischer, R. A., . . . Thomas, M. J. (2016). Reversal of morphine-induced cell-type-specific synaptic plasticity in the nucleus accumbens shell blocks reinstatement. *Proc Natl Acad Sci U S A, 113*(3), 757-762. doi:10.1073/pnas.1519248113

Hedges, D. W., & Woon, F. L. (2011). Early-life stress and cognitive outcome. *Psychopharmacology, 214*(1), 121-130. doi:10.1007/s00213-010-2090-6

Heithoff, B. P., George, K. K., Phares, A. N., Zuidhoek, I. A., Munoz-Ballester, C., & Robel, S. (2020). Astrocytes are necessary for blood-brain barrier maintenance in the adult mouse brain. *bioRxiv*, 2020.2003.2016.993691. doi:10.1101/2020.03.16.993691

Helms, C. M., Reeves, J. M., & Mitchell, S. H. (2006). Impact of strain and D-amphetamine on impulsivity (delay discounting) in inbred mice. *Psychopharmacology (Berl), 188*(2), 144-151. doi:10.1007/s00213-006-0478-0

Hensleigh, E., & Pritchard, L. M. (2015). Maternal separation increases methamphetamine-induced damage in the striatum in male, but not female rats. *Behavioural Brain Research*, *295*, 3-8. doi:10.1016/j.bbr.2014.12.008

Herman, A. M., Critchley, H. D., & Duka, T. (2018). Risk-Taking and Impulsivity: The Role of Mood States and Interoception. *Frontiers in Psychology*, *9*, 1625.

Herzberg, M. P., Hodel, A. S., Cowell, R. A., Hunt, R. H., Gunnar, M. R., & Thomas, K. M. (2018). Risk taking, decision-making, and brain volume in youth adopted internationally from institutional care. *Neuropsychologia*, *119*, 262-270. doi:10.1016/j.neuropsychologia.2018.08.022

Hodes, G. E., & Epperson, C. N. (2019). Sex Differences in Vulnerability and Resilience to Stress Across the Life Span. *Biological Psychiatry*, *86*(6), 421-432. doi:https://doi.org/10.1016/j.biopsych.2019.04.028

Hodes, G. E., Pfau, M. L., Purushothaman, I., Ahn, H. F., Golden, S. A., Christoffel, D. J., . . . Russo, S. J. (2015). Sex Differences in Nucleus Accumbens Transcriptome Profiles Associated with Susceptibility versus Resilience to Subchronic Variable Stress. *J Neurosci*, *35*(50), 16362-16376. doi:10.1523/jneurosci.1392-15.2015

Holland, F. H., Ganguly, P., Potter, D. N., Chartoff, E. H., & Brenhouse, H. C. (2014). Early life stress disrupts social behavior and prefrontal cortex parvalbumin interneurons at an earlier time-point in females than in males. *Neuroscience Letters*, *566*, 131-136. doi:https://doi.org/10.1016/j.neulet.2014.02.023

Holt, D. D., Green, L., & Myerson, J. (2003). Is discounting impulsive?: Evidence from temporal and probability discounting in gambling and non-gambling college students. *Behavioural Processes*, *64*(3), 355-367. doi:https://doi.org/10.1016/S0376-6357(03)00141-4

Hopf, F. W., Cascini, M. G., Gordon, A. S., Diamond, I., & Bonci, A. (2003). Cooperative Activation of Dopamine D₁ and D₂ Receptors Increases Spike Firing of Nucleus Accumbens Neurons via G-Protein βγ Subunits. *The Journal of Neuroscience*, *23*(12), 5079. doi:10.1523/JNEUROSCI.23-12-05079.2003

Hostinar, C. E., Stellern, S. A., Schaefer, C., Carlson, S. M., & Gunnar, M. R. (2012). Associations between early life adversity and executive function in children adopted internationally from orphanages. *Proceedings of the National Academy of Sciences, 109*(Supplement 2), 17208. doi:10.1073/pnas.1121246109

Hsiao, Y.-M., Tsai, T.-C., Lin, Y.-T., Chen, C.-C., Huang, C.-C., & Hsu, K.-S. (2016). Early life stress dampens stress responsiveness in adolescence: Evaluation of neuroendocrine reactivity and coping behavior. *Psychoneuroendocrinology, 67*, 86-99.

Huang, L. T. (2014). Early-life stress impacts the developing hippocampus and primes seizure occurrence: cellular, molecular, and epigenetic mechanisms. *Front Mol Neurosci, 7*, 8. doi:10.3389/fnmol.2014.00008

Islas-Preciado, D., Wainwright, S. R., Sniegocki, J., Lieblich, S. E., Yagi, S., Floresco, S. B., & Galea, L. A. M. (2020). Risk-based Decision Making in Rats: Modulation by Sex and Amphetamine. *bioRxiv*, 2020.2003.2009.981563. doi:10.1101/2020.03.09.981563

Ito, R., Robbins, T. W., & Everitt, B. J. (2004). Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. *Nature Neuroscience, 7*(4), 389-397. doi:10.1038/nn1217

Ivy, A. S., Brunson, K. L., Sandman, C., & Baram, T. Z. (2008). Dysfunctional nurturing behavior in rat dams with limited access to nesting material: a clinically relevant model for early-life stress. *Neuroscience, 154*(3), 1132-1142. doi:10.1016/j.neuroscience.2008.04.019

Iyer, E. S., Kairiss, M. A., Liu, A., Otto, A. R., & Bagot, R. C. (2020). Probing relationships between reinforcement learning and simple behavioral strategies to understand probabilistic reward learning. *Journal of Neuroscience Methods, 341*, 108777. doi:https://doi.org/10.1016/j.jneumeth.2020.108777

Jupp, B., Caprioli, D., Saigal, N., Reverte, I., Shrestha, S., Cumming, P., . . . Dalley, J. W. (2013). Dopaminergic and GABA-ergic markers of impulsivity in rats: evidence for anatomical localisation in ventral striatum and prefrontal cortex. *Eur J Neurosci, 37*(9), 1519-1528. doi:10.1111/ejn.12146

Kapor, S., Aksić, M., Puškaš, L., Jukić, M., Poleksić, J., Milosavljević, F., . . . Filipović, B. (2020). Long-Term Effects of Maternal Deprivation on the Volume of Dopaminergic Nuclei and Number of Dopaminergic Neurons in Substantia Nigra and Ventral Tegmental Area in Rats. *Front Neuroanat, 14*, 578900. doi:10.3389/fnana.2020.578900

Katunar, M. R., Saez, T., Brusco, A., & Antonelli, M. C. (2009). Immunocytochemical expression of dopamine-related transcription factors Pitx3 and Nurr1 in prenatally stressed adult rats. *Journal of Neuroscience Research, 87*(4), 1014-1022. doi:https://doi.org/10.1002/jnr.21911

Kelley, A. E., & Domesick, V. B. (1982). The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: an anterograde- and retrograde-horseradish peroxidase study. *Neuroscience, 7*(10), 2321-2335. doi:10.1016/0306-4522(82)90198-1

Kentrop, J., van der Tas, L., Loi, M., van IJzendoorn, M. H., Bakermans-Kranenburg, M. J., Joëls, M., & van der Veen, R. (2016). Mifepristone Treatment during Early Adolescence Fails to Restore Maternal Deprivation-Induced Deficits in Behavioral Inhibition of Adult Male Rats. *Front Behav Neurosci, 10*, 122. doi:10.3389/fnbeh.2016.00122

Kessler, R. C., McLaughlin, K. A., Green, J. G., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., . . . Williams, D. R. (2010). Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *The British journal of psychiatry : the journal of mental science, 197*(5), 378-385. doi:10.1192/bjp.bp.110.080499

Kheramin, S., Body, S., Ho, M. Y., Velázquez-Martinez, D. N., Bradshaw, C. M., Szabadi, E., . . . Anderson, I. M. (2004). Effects of orbital prefrontal cortex dopamine depletion on inter-temporal choice: a quantitative analysis. *Psychopharmacology (Berl), 175*(2), 206-214. doi:10.1007/s00213-004-1813-y

Khibnik, L. A., Beaumont, M., Doyle, M., Heshmati, M., Slesinger, P. A., Nestler, E. J., & Russo, S. J. (2016). Stress and Cocaine Trigger Divergent and Cell Type-Specific Regulation of Synaptic Transmission at Single Spines in Nucleus Accumbens. *Biol Psychiatry, 79*(11), 898-905. doi:10.1016/j.biopsych.2015.05.022

Kim, H. (2018). Associations between cognitive function and striatal DAT loss in Parkinson’s disease with mild cognitive impairment. *Journal of Nuclear Medicine, 59*(supplement 1), 1650.

Kim, S. T., Hwang, S. S., Kim, H. W., Hwang, E. H., Cho, J., Kang, J. I., & Kim, S. J. (2018). Multidimensional impulsivity as a mediator of early life stress and alcohol dependence. *Scientific Reports, 8*(1), 4104. doi:10.1038/s41598-018-22474-8

Kohno, M., Okita, K., Morales, A. M., Robertson, C. L., Dean, A. C., Ghahremani, D. G., . . . London, E. D. (2016). Midbrain functional connectivity and ventral striatal dopamine D2-type receptors: link to impulsivity in methamphetamine users. *Molecular Psychiatry, 21*(11), 1554-1560. doi:10.1038/mp.2015.223

Kokane, S. S., & Perrotti, L. I. (2020). Sex Differences and the Role of Estradiol in Mesolimbic Reward Circuits and Vulnerability to Cocaine and Opiate Addiction. *Front Behav Neurosci, 14*, 74. doi:10.3389/fnbeh.2020.00074

Krugers, H. J., & Joëls, M. (2014). Long-lasting Consequences of Early Life Stress on Brain Structure, Emotion and Cognition. In C. M. Pariante & M. D. Lapiz-Bluhm (Eds.), *Behavioral Neurobiology of Stress-related Disorders* (pp. 81-92). Berlin, Heidelberg: Springer Berlin Heidelberg.

Kuczenski, R., & Segal, D. S. (1997). Effects of Methylphenidate on Extracellular Dopamine, Serotonin, and Norepinephrine: Comparison with Amphetamine. *Journal of Neurochemistry, 68*(5), 2032-2037. doi:https://doi.org/10.1046/j.1471-4159.1997.68052032.x

Kunzler, J., Braun, K., & Bock, J. (2015). Early life stress and sex-specific sensitivity of the catecholaminergic systems in prefrontal and limbic regions of Octodon degus. *Brain Structure and Function, 220*(2), 861-868. doi:10.1007/s00429-013-0688-2

Labonté, B., Engmann, O., Purushothaman, I., Menard, C., Wang, J., Tan, C., . . . Nestler, E. J. (2017). Sex-specific transcriptional signatures in human depression. *Nature Medicine, 23*(9), 1102-1111. doi:10.1038/nm.4386

Lacey, R. E., Zilanawala, A., Webb, E., Abell, J., & Bell, S. (2018). Parental absence in early childhood and onset of smoking and alcohol consumption before adolescence. *Arch Dis Child, 103*(7), 691-694. doi:10.1136/archdischild-2016-310444

Lee, B., London, E. D., Poldrack, R. A., Farahi, J., Nacca, A., Monterosso, J. R., . . . Mandelkern, M. A. (2009). Striatal dopamine d2/d3 receptor availability is reduced in methamphetamine dependence and is linked to impulsivity. *J Neurosci, 29*(47), 14734-14740. doi:10.1523/JNEUROSCI.3765-09.2009

Leong, K.-C., Cox, S., King, C., Becker, H., & Reichel, C. M. (2018). Oxytocin and Rodent Models of Addiction. *International review of neurobiology, 140*, 201-247. doi:10.1016/bs.irn.2018.07.007

Levis, S. C., Bentzley, B. S., Molet, J., Bolton, J. L., Perrone, C. R., Baram, T. Z., & Mahler, S. V. (2019). On the early life origins of vulnerability to opioid addiction. *Molecular Psychiatry*. doi:10.1038/s41380-019-0628-5

Li, B., Nguyen, T. P., Ma, C., & Dan, Y. (2020). Inhibition of impulsive action by projection-defined prefrontal pyramidal neurons. *Proceedings of the National Academy of Sciences, 117*(29), 17278. doi:10.1073/pnas.2000523117

Li, J.-N., Liu, X.-L., & Li, L. (2020). Prefrontal GABA and glutamate levels correlate with impulsivity and cognitive function of prescription opioid addicts: A 1H-magnetic resonance spectroscopy study. *Psychiatry and Clinical Neurosciences, 74*(1), 77-83. doi:https://doi.org/10.1111/pcn.12940

Liao, Y., Wang, J., Jaehnig, E. J., Shi, Z., & Zhang, B. (2019). WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Res, 47*(W1), W199-w205. doi:10.1093/nar/gkz401

Lichtenberg, N. T., Sepe-Forrest, L., Pennington, Z. T., Lamparelli, A. C., Greenfield, V. Y., & Wassum, K. M. (2021). The Medial Orbitofrontal Cortex–Basolateral Amygdala Circuit Regulates the Influence of Reward Cues on Adaptive Behavior and Choice. *The Journal of Neuroscience, 41*(34), 7267. doi:10.1523/JNEUROSCI.0901-21.2021

Locklear, M. N., Michaelos, M., Collins, W. F., & Kritzer, M. F. (2017). Gonadectomy but not biological sex affects burst-firing in dopamine neurons of the ventral tegmental area and in prefrontal cortical neurons projecting to the ventral tegmentum in adult rats. *Eur J Neurosci, 45*(1), 106-120. doi:10.1111/ejn.13380

London, E. D. (2020). Human Brain Imaging Links Dopaminergic Systems to Impulsivity. In H. de Wit & J. D. Jentsch (Eds.), *Recent Advances in Research on Impulsivity and Impulsive Behaviors* (pp. 53-71). Cham: Springer International Publishing.

Loos, M., Pattij, T., Janssen, M. C., Counotte, D. S., Schoffelmeer, A. N., Smit, A. B., . . . van Gaalen, M. M. (2010). Dopamine receptor D1/D5 gene expression in the medial prefrontal cortex predicts impulsive choice in rats. *Cereb Cortex, 20*(5), 1064-1070. doi:10.1093/cercor/bhp167

Lovallo, W. R., Farag, N. H., Sorocco, K. H., Acheson, A., Cohoon, A. J., & Vincent, A. S. (2013). Early life adversity contributes to impaired cognition and impulsive behavior: studies from the Oklahoma Family Health Patterns Project. *Alcohol Clin Exp Res, 37*(4), 616-623. doi:10.1111/acer.12016

Lovic, V., Keen, D., Fletcher, P. J., & Fleming, A. S. (2011). Early-life maternal separation and social isolation produce an increase in impulsive action but not impulsive choice. *Behav Neurosci, 125*(4), 481-491. doi:10.1037/a0024367

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience, 10*(6), 434-445. doi:10.1038/nrn2639

Lyons, D. M., Parker, K. J., & Schatzberg, A. F. (2010). Animal models of early life stress: Implications for understanding resilience. *Developmental psychobiology, 52*(5), 402-410. doi:10.1002/dev.20429

Lévesque, D., Gagnon, S., & Di Paolo, T. (1989). Striatal D1 dopamine receptor density fluctuates during the rat estrous cycle. *Neuroscience Letters, 98*(3), 345-350. doi:https://doi.org/10.1016/0304-3940(89)90426-6

Madden, G. J., Petry, N. M., & Johnson, P. S. (2009). Pathological gamblers discount probabilistic rewards less steeply than matched controls. *Experimental and clinical psychopharmacology, 17*(5), 283-290. doi:10.1037/a0016806

Majcher-Maślanka, I., Solarz, A., Wędzony, K., & Chocyk, A. (2017). The effects of early-life stress on dopamine system function in adolescent female rats. *Int J Dev Neurosci, 57*, 24-33. doi:10.1016/j.ijdevneu.2017.01.001

Mar, A. C., Walker, A. L., Theobald, D. E., Eagle, D. M., & Robbins, T. W. (2011). Dissociable effects of lesions to orbitofrontal cortex subregions on impulsive choice in the rat. *J Neurosci, 31*(17), 6398-6404. doi:10.1523/JNEUROSCI.6620-10.2011

Marino, E. N., Rosen, K. D., Gutierrez, A., Eckmann, M., Ramamurthy, S., & Potter, J. S. (2013). Impulsivity but not sensation seeking is associated with opioid analgesic misuse risk in patients with chronic pain. *Addictive behaviors, 38*(5), 2154-2157. doi:10.1016/j.addbeh.2013.01.020

Martina, J. A., Diab, H. I., Lishu, L., Jeong, A. L., Patange, S., Raben, N., & Puertollano, R. (2014). The nutrient-responsive transcription factor TFE3 promotes autophagy, lysosomal biogenesis, and clearance of cellular debris. *Sci Signal, 7*(309), ra9. doi:10.1126/scisignal.2004754

Masten, A. S. (2001). Ordinary magic: Resilience processes in development. *American Psychologist, 56(3)*, 227-238.

Mathew, S. J., Shungu, D. C., Mao, X., Smith, E. L. P., Perera, G. M., Kegeles, L. S., . . . Coplan, J. D. (2003). A magnetic resonance spectroscopic imaging study of adult nonhuman primates exposed to early-life stressors. *Biological Psychiatry, 54*(7), 727-735. doi:https://doi.org/10.1016/S0006-3223(03)00004-0

Matisz, C. E., Badenhorst, C. A., & Gruber, A. J. (2021). Chronic unpredictable stress shifts rat behavior from exploration to exploitation. *Stress (Amsterdam, Netherlands), 24*(5), 635-644. doi:10.1080/10253890.2021.1947235

McLaughlin, K. A., Green, J. G., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., & Kessler, R. C. (2010). Childhood Adversities and Adult Psychiatric Disorders in the National Comorbidity Survey Replication II: Associations With Persistence of DSM-IV Disorders. *Archives of General Psychiatry, 67*(2), 124-132. doi:10.1001/archgenpsychiatry.2009.187

McLaughlin, K. A., Greif Green, J., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., & Kessler, R. C. (2012). Childhood adversities and first onset of psychiatric disorders in a national sample of US adolescents. *Arch Gen Psychiatry, 69*(11), 1151-1160. doi:10.1001/archgenpsychiatry.2011.2277

Meaney, M. J., Brake, W., & Gratton, A. (2002). Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology, 27*(1-2), 127-138. doi:10.1016/s0306-4530(01)00040-3

Messina, B. G., Silvestri, M. M., Diulio, A. R., Murphy, J. G., Garza, K. B., & Correia, C. J. (2014). Alcohol use, impulsivity, and the non-medical use of prescription stimulants among college students. *Addictive behaviors, 39*(12), 1798-1803. doi:https://doi.org/10.1016/j.addbeh.2014.07.012

Meyer, H. C., & Bucci, D. J. (2016). Imbalanced Activity in the Orbitofrontal Cortex and Nucleus Accumbens Impairs Behavioral Inhibition. *Current biology : CB, 26*(20), 2834-2839. doi:10.1016/j.cub.2016.08.034

Millstein, R. A., & Holmes, A. (2007). Effects of repeated maternal separation on anxiety- and depression-related phenotypes in different mouse strains. *Neurosci Biobehav Rev, 31*(1), 3-17. doi:10.1016/j.neubiorev.2006.05.003

Mobini, S., Body, S., Ho, M. Y., Bradshaw, C. M., Szabadi, E., Deakin, J. F., & Anderson, I. M. (2002). Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. *Psychopharmacology (Berl), 160*(3), 290-298. doi:10.1007/s00213-001-0983-0

Molet, J., Maras, P. M., Avishai-Eliner, S., & Baram, T. Z. (2014). Naturalistic rodent models of chronic early-life stress. *Dev Psychobiol, 56*(8), 1675-1688. doi:10.1002/dev.21230

Monroy, E., Hernández-Torres, E., & Flores, G. (2010). Maternal separation disrupts dendritic morphology of neurons in prefrontal cortex, hippocampus, and nucleus accumbens in male rat offspring. *J Chem Neuroanat, 40*(2), 93-101. doi:10.1016/j.jchemneu.2010.05.005

Mueller, B. R., & Bale, T. L. (2008). Sex-specific programming of offspring emotionality after stress early in pregnancy. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 28*(36), 9055-9065. doi:28/36/9055 [pii]
10.1523/JNEUROSCI.1424-08.2008

Muhammad, A., Carroll, C., & Kolb, B. (2012). Stress during development alters dendritic morphology in the nucleus accumbens and prefrontal cortex. *Neuroscience, 216*, 103-109. doi:https://doi.org/10.1016/j.neuroscience.2012.04.041

Muir, J., Tse, Y. C., Iyer, E. S., Biris, J., Cvetkovska, V., Lopez, J., & Bagot, R. C. (2020). Ventral Hippocampal Afferents to Nucleus Accumbens Encode Both

Latent Vulnerability and Stress-Induced Susceptibility. *Biological Psychiatry, 88*(11), 843-854. doi:https://doi.org/10.1016/j.biopsych.2020.05.021

Muir, J. L., Everitt, B. J., & Robbins, T. W. (1996). The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. *Cereb Cortex, 6*(3), 470-481. doi:10.1093/cercor/6.3.470

Murphy, E. R., Fernando, A. B. P., Urcelay, G. P., Robinson, E. S. J., Mar, A. C., Theobald, D. E. H., . . . Robbins, T. W. (2012). Impulsive behaviour induced by both NMDA receptor antagonism and GABAA receptor activation in rat ventromedial prefrontal cortex. *Psychopharmacology, 219*(2), 401-410. doi:10.1007/s00213-011-2572-1

Murphy, E. R., Robinson, E. S., Theobald, D. E., Dalley, J. W., & Robbins, T. W. (2008). Contrasting effects of selective lesions of nucleus accumbens core or shell on inhibitory control and amphetamine-induced impulsive behaviour. *Eur J Neurosci, 28*(2), 353-363. doi:10.1111/j.1460-9568.2008.06309.x

Murthy, S., & Gould, E. (2018). Early Life Stress in Rodents: Animal Models of Illness or Resilience? *Frontiers in Behavioral Neuroscience, 12*, 157-157. doi:10.3389/fnbeh.2018.00157

Najt, P., Perez, J., Sanches, M., Peluso, M. A. M., Glahn, D., & Soares, J. C. (2007). Impulsivity and bipolar disorder. *European Neuropsychopharmacology, 17*(5), 313-320. doi:https://doi.org/10.1016/j.euroneuro.2006.10.002

Newman, L. A., Creer, D. J., & McGaughy, J. A. (2015). Cognitive control and the anterior cingulate cortex: how conflicting stimuli affect attentional control in the rat. *J Physiol Paris, 109*(1-3), 95-103. doi:10.1016/j.jphysparis.2014.06.004

Nishi, M., Horii-Hayashi, N., & Sasagawa, T. (2014). Effects of early life adverse experiences on the brain: implications from maternal separation models in rodents. *Frontiers in neuroscience, 8*, 166-166. doi:10.3389/fnins.2014.00166

Oades, R. D., & Halliday, G. M. (1987). Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. *Brain Res, 434*(2), 117-165. doi:10.1016/0165-0173(87)90011-7

Ohta, K.-i., Suzuki, S., Warita, K., Sumitani, K., Tenkumo, C., Ozawa, T., . . . Miki, T. (2020). The effects of early life stress on the excitatory/inhibitory balance of the medial prefrontal cortex. *Behavioural Brain Research, 379*, 112306. doi:https://doi.org/10.1016/j.bbr.2019.112306

Ordoñes Sanchez, E., Bavley, C. C., Deutschmann, A. U., Carpenter, R., Peterson, D. R., Karbalaei, R., . . . Bangasser, D. A. (2021). Early life adversity promotes resilience to opioid addiction-related phenotypes in male rats and sex-specific transcriptional changes. *Proceedings of the National Academy of Sciences, 118*(8), e2020173118. doi:10.1073/pnas.2020173118

Orsini, C. A., Hernandez, C. M., Singhal, S., Kelly, K. B., Frazier, C. J., Bizon, J. L., & Setlow, B. (2017). Optogenetic Inhibition Reveals Distinct Roles for Basolateral Amygdala Activity at Discrete Time Points during Risky Decision Making. *J Neurosci, 37*(48), 11537-11548. doi:10.1523/jneurosci.2344-17.2017

O'Callaghan, E. L., Bassi, J. K., Porrello, E. R., Delbridge, L. M. D., Thomas, W. G., & Allen, A. M. (2011). Regulation of angiotensinogen by angiotensin II in mouse primary astrocyte cultures. *Journal of Neurochemistry, 119*(1), 18-26. doi:https://doi.org/10.1111/j.1471-4159.2011.07406.x

Pan, W. X., Schmidt, R., Wickens, J. R., & Hyland, B. I. (2005). Dopamine cells respond to predicted events during classical conditioning: evidence for eligibility traces in the reward-learning network. *J Neurosci, 25*(26), 6235-6242. doi:10.1523/jneurosci.1478-05.2005

Parker, K. J., Buckmaster, C. L., Justus, K. R., Schatzberg, A. F., & Lyons, D. M. (2005). Mild early life stress enhances prefrontal-dependent response inhibition in monkeys. *Biol Psychiatry, 57*(8), 848-855. doi:10.1016/j.biopsych.2004.12.024

Pattij, T., Janssen, M. C., Vanderschuren, L. J., Schoffelmeer, A. N., & van Gaalen, M. M. (2007). Involvement of dopamine D1 and D2 receptors in the nucleus accumbens core and shell in inhibitory response control. *Psychopharmacology (Berl), 191*(3), 587-598. doi:10.1007/s00213-006-0533-x

Pattij, T., & Vanderschuren, L. J. M. J. (2008). The neuropharmacology of impulsive behaviour. *Trends in Pharmacological Sciences, 29*(4), 192-199. doi:10.1016/j.tips.2008.01.002

Pechtel, P., & Pizzagalli, D. A. (2011). Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology, 214*(1), 55-70. doi:10.1007/s00213-010-2009-2

Pertea, M., & *al., e.* (2015). StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. In (Vol. **33**, pp. 290).

Perugi, G., Vannucchi, G., Bedani, F., & Favaretto, E. (2017). Use of Stimulants in Bipolar Disorder. *Current Psychiatry Reports, 19*(1), 7. doi:10.1007/s11920-017-0758-x

Pettorruso, M., Martinotti, G., Cocciolillo, F., De Risio, L., Cinquino, A., Di Nicola, M., . . . Di Giuda, D. (2019). Striatal presynaptic dopaminergic dysfunction in gambling disorder: A 123I-FP-CIT SPECT study. *Addiction Biology, 24*(5), 1077-1086. doi:https://doi.org/10.1111/adb.12677

Petzold, J., Kienast, A., Lee, Y., Pooseh, S., London, E. D., Goschke, T., & Smolka, M. N. (2019). Baseline impulsivity may moderate L-DOPA effects on value-based decision-making. *Sci Rep, 9*(1), 5652. doi:10.1038/s41598-019-42124-x

Pezze, M. A., Dalley, J. W., & Robbins, T. W. (2009). Remediation of attentional dysfunction in rats with lesions of the medial prefrontal cortex by intra-accumbens administration of the dopamine D(2/3) receptor antagonist sulpiride. *Psychopharmacology (Berl), 202*(1-3), 307-313. doi:10.1007/s00213-008-1384-4

Porter, A. J., Pillidge, K., Grabowska, E. M., & Stanford, S. C. (2015). The angiotensin converting enzyme inhibitor, captopril, prevents the hyperactivity and impulsivity of neurokinin-1 receptor gene 'knockout' mice: Sex differences and implications for the treatment of attention deficit hyperactivity disorder. *European Neuropsychopharmacology, 25*(4), 512-521. doi:https://doi.org/10.1016/j.euroneuro.2015.01.013

Puumala, T., & Sirviö, J. (1998). Changes in activities of dopamine and serotonin systems in the frontal cortex underlie poor choice accuracy and impulsivity of rats in an attention task. *Neuroscience, 83*(2), 489-499. doi:10.1016/s0306-4522(97)00392-8

Quinn, K., Boone, L., Scheidell, J. D., Mateu-Gelabert, P., McGorray, S. P., Behave, N., . . . Khan, M. R. (2016). The Relationships of Childhood Trauma and Adulthood

Prescription Pain Reliever Misuse and Injection Drug Use. *Drug and Alcohol Dependence, 169*, 190-198. doi:https://doi.org/10.1016/j.drugalcdep.2016.09.021

Raben, N., & Puertollano, R. (2016). TFEB and TFE3: Linking Lysosomes to Cellular Adaptation to Stress. *Annu Rev Cell Dev Biol, 32*, 255-278. doi:10.1146/annurev-cellbio-111315-125407

Raghavendra, V., Chopra, K., & Kulkarni, S. K. (1999). Brain renin angiotensin system (RAS) in stress-induced analgesia and impaired retention. *Peptides, 20*(3), 335-342. doi:https://doi.org/10.1016/S0196-9781(99)00040-6

Reimand, J., Isserlin, R., Voisin, V., Kucera, M., Tannus-Lopes, C., Rostamianfar, A., . . . Bader, G. D. (2019). Pathway enrichment analysis and visualization of omics data using g:Profiler, GSEA, Cytoscape and EnrichmentMap. *Nature Protocols, 14*(2), 482-517. doi:10.1038/s41596-018-0103-9

Rice, C. J., Sandman, C. A., Lenjavi, M. R., & Baram, T. Z. (2008). A novel mouse model for acute and long-lasting consequences of early life stress. *Endocrinology, 149*(10), 4892-4900. doi:10.1210/en.2008-0633

Rincón-Cortés, M., & Grace, A. A. (2017). Sex-Dependent Effects of Stress on Immobility Behavior and VTA Dopamine Neuron Activity: Modulation by Ketamine. *Int J Neuropsychopharmacol, 20*(10), 823-832. doi:10.1093/ijnp/pyx048

Robbins, T. W., & Dalley, J. W. (2017). Dissecting Impulsivity: Brain Mechanisms and Neuropsychiatric Implications. *Nebr Symp Motiv, 64*, 201-226.

Roesch, Taylor, & Schoenbaum. (2006). Encoding of time-discounted rewards in orbitofrontal cortex is independent of value representation. *Neuron, 51*(4), 509-520. doi:10.1016/j.neuron.2006.06.027

Roesch, M. R., Calu, D. J., & Schoenbaum, G. (2007). Dopamine neurons encode the better option in rats deciding between differently delayed or sized rewards. *Nature Neuroscience, 10*(12), 1615-1624. doi:10.1038/nn2013

Romano-López, A., Méndez-Díaz, M., García, F. G., Regalado-Santiago, C., Ruiz-Contreras, A. E., & Prospéro-García, O. (2016). Maternal separation and early stress cause long-lasting effects on dopaminergic and endocannabinergic systems and alters dendritic morphology in the nucleus accumbens and frontal cortex in rats. *Dev Neurobiol, 76*(8), 819-831. doi:10.1002/dneu.22361

Rosa-Neto, P., Lou, H. C., Cumming, P., Pryds, O., Karrebaek, H., Lunding, J., & Gjedde, A. (2005). Methylphenidate-evoked changes in striatal dopamine correlate with inattention and impulsivity in adolescents with attention deficit hyperactivity disorder. *NeuroImage, 25*(3), 868-876. doi:https://doi.org/10.1016/j.neuroimage.2004.11.031

Rudd, R. A., Aleshire, N., Zibbell, J. E., & Matthew Gladden, R. (2016). Increases in Drug and Opioid Overdose Deaths—United States, 2000–2014. *American Journal of Transplantation, 16*(4), 1323-1327. doi:10.1111/ajt.13776

Rudebeck, P. H., Walton, M. E., Smyth, A. N., Bannerman, D. M., & Rushworth, M. F. S. (2006). Separate neural pathways process different decision costs. *Nature Neuroscience, 9*(9), 1161-1168. doi:10.1038/nn1756

Russell, S. E., Puttick, D. J., Sawyer, A. M., Potter, D. N., Mague, S., Carlezon, W. A., Jr., & Chartoff, E. H. (2016). Nucleus Accumbens AMPA Receptors Are Necessary for Morphine-Withdrawal-Induced Negative-Affective States in Rats. *J Neurosci, 36*(21), 5748-5762. doi:10.1523/jneurosci.2875-12.2016

Sachs, B. D., & Lumia, A. R. (1981). Is stress due to shipment of animals a confounding variable in developmental research? *Dev Psychobiol, 14*(2), 169-171. doi:10.1002/dev.420140211

Saddoris, M. P., Cacciapaglia, F., Wightman, R. M., & Carelli, R. M. (2015). Differential Dopamine Release Dynamics in the Nucleus Accumbens Core and Shell Reveal Complementary Signals for Error Prediction and Incentive Motivation. *The Journal of Neuroscience, 35*(33), 11572. doi:10.1523/JNEUROSCI.2344-15.2015

Saddoris, M. P., Sugam, J. A., Stuber, G. D., Witten, I. B., Deisseroth, K., & Carelli, R. M. (2015). Mesolimbic dopamine dynamically tracks, and is causally linked to, discrete aspects of value-based decision making. *Biological psychiatry, 77*(10), 903-911. doi:10.1016/j.biopsych.2014.10.024

Sahraeian, S. M. E., & *al., e.* (2017). Gaining comprehensive biological insight into the transcriptome by performing a broad-spectrum RNA-seq analysis. In (Vol. 8).

Salamone, J. D. (1994). The involvement of nucleus accumbens dopamine in appetitive and aversive motivation. *Behav Brain Res, 61*(2), 117-133. doi:10.1016/0166-4328(94)90153-8

Sanna, A., Fattore, L., Badas, P., Corona, G., & Diana, M. (2021). The hypodopaminergic state ten years after: transcranial magnetic stimulation as a tool to test the dopamine hypothesis of drug addiction. *Current Opinion in Pharmacology, 56*, 61-67. doi:https://doi.org/10.1016/j.coph.2020.11.001

Santarelli, S., Zimmermann, C., Kalideri, G., Less, S. L., Arloth, J., Uribe, A., . . . Schmidt, M. V. (2017). An adverse early life environment can enhance stress resilience in adulthood. *Psychoneuroendocrinology, 78*, 213-221. doi:https://doi.org/10.1016/j.psyneuen.2017.01.021

Sasagawa, T., Horii-Hayashi, N., Okuda, A., Hashimoto, T., Azuma, C., & Nishi, M. (2017). Long-term effects of maternal separation coupled with social isolation on reward seeking and changes in dopamine D1 receptor expression in the nucleus accumbens via DNA methylation in mice. *Neurosci Lett, 641*, 33-39. doi:10.1016/j.neulet.2017.01.025

Saunders, A., Macosko, E. Z., Wysoker, A., Goldman, M., Krienen, F. M., de Rivera, H., . . . McCarroll, S. A. (2018). Molecular Diversity and Specializations among the Cells of the Adult Mouse Brain. *Cell, 174*(4), 1015-1030.e1016. doi:https://doi.org/10.1016/j.cell.2018.07.028

Schultz, W., Dayan, P., & Montague, P. R. (1997). A Neural Substrate of Prediction and Reward. *Science, 275*(5306), 1593. doi:10.1126/science.275.5306.1593

Sekiguchi, H., Pavey, G., & Dean, B. (2019). Altered levels of dopamine transporter in the frontal pole and dorsal striatum in schizophrenia. *npj Schizophrenia, 5*(1), 20. doi:10.1038/s41537-019-0087-7

Seney, M. L., Huo, Z., Cahill, K., French, L., Puralewski, R., Zhang, J., . . . Sibille, E. (2018). Opposite Molecular Signatures of Depression in Men and Women. *Biological Psychiatry, 84*(1), 18-27. doi:https://doi.org/10.1016/j.biopsych.2018.01.017

Sesack, S. R., Deutch, A. Y., Roth, R. H., & Bunney, B. S. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat:

an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *J Comp Neurol*, *290*(2), 213-242. doi:10.1002/cne.902900205

Shafiei, N., Gray, M., Viau, V., & Floresco, S. B. (2012). Acute Stress Induces Selective Alterations in Cost/Benefit Decision-Making. *Neuropsychopharmacology, 37*(10), 2194-2209. doi:10.1038/npp.2012.69

Sherrod, M., Liu, X., Zhang, X., & Sigmund, C. D. (2005). Nuclear localization of angiotensinogen in astrocytes. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *288*(2), R539-R546. doi:10.1152/ajpregu.00594.2004

Sibley, D. R., & Monsma, F. J., Jr. (1992). Molecular biology of dopamine receptors. *Trends Pharmacol Sci, 13*(2), 61-69. doi:10.1016/0165-6147(92)90025-2

Silvagni, A., Vg., B., Mura, C., Antonelli, M. C., Mc., A., & Carboni, E. (2008). Prenatal restraint stress differentially modifies basal and stimulated dopamine and noradrenaline release in the nucleus accumbens shell: an 'in vivo' microdialysis study in adolescent and young adult rats. (1460-9568 (Electronic)).

Simon, N. W., Beas, B. S., Montgomery, K. S., Haberman, R. P., Bizon, J. L., & Setlow, B. (2013). Prefrontal cortical-striatal dopamine receptor mRNA expression predicts distinct forms of impulsivity. *The European journal of neuroscience, 37*(11), 1779-1788. doi:10.1111/ejn.12191

Sirén, J., Välimäki, N., & Mäkinen, V. (2014). Indexing graphs for path queries with applications in genome research. *IEEE/ACM Trans. Comput. Biol. Bioinformatics, 11*(2), 375–388. doi:10.1109/tcbb.2013.2297101

Smaragdi, A., Chavez, S., Lobaugh, N. J., Meyer, J. H., & Kolla, N. J. (2019). Differential levels of prefrontal cortex glutamate+glutamine in adults with antisocial personality disorder and bipolar disorder: A proton magnetic resonance spectroscopy study. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 93*, 250-255. doi:https://doi.org/10.1016/j.pnpbp.2019.04.002

Smith, C. T., San Juan, M. D., Dang, L. C., Katz, D. T., Perkins, S. F., Burgess, L. L., . . . Zald, D. H. (2018). Ventral striatal dopamine transporter availability is associated with lower trait motor impulsivity in healthy adults. *Translational Psychiatry, 8*(1), 269. doi:10.1038/s41398-018-0328-y

Smith, R., Schwartenbeck, P., Stewart, J. L., Kuplicki, R., Ekhtiari, H., & Paulus, M. P. (2020). Imprecise action selection in substance use disorder: Evidence for active learning impairments when solving the explore-exploit dilemma. *Drug and Alcohol Dependence, 215*, 108208. doi:https://doi.org/10.1016/j.drugalcdep.2020.108208

Soares-Cunha, C., Coimbra, B., Borges, S., Domingues, A. V., Silva, D., Sousa, N., & Rodrigues, A. J. (2018). Mild Prenatal Stress Causes Emotional and Brain Structural Modifications in Rats of Both Sexes. *Frontiers in Behavioral Neuroscience, 12*, 129.

Sohal, V. S., Zhang, F., Yizhar, O., & Deisseroth, K. (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature, 459*(7247), 698-702. doi:10.1038/nature07991

Solanto, M. V., Abikoff, H., Sonuga-Barke, E., Schachar, R., Logan, G. D., Wigal, T., . . . Turkel, E. (2001). The ecological validity of delay aversion and response inhibition as measures of impulsivity in AD/HD: a supplement to the NIMH multimodal treatment study of AD/HD. *J Abnorm Child Psychol, 29*(3), 215-228. doi:10.1023/a:1010329714819

Spooren, W. P., Veening, J. G., Groenewegen, H. J., & Cools, A. R. (1991). Efferent connections of the striatopallidal and amygdaloid components of the substantia innominata in the cat: projections to the nucleus accumbens and caudate nucleus. *Neuroscience, 44*(2), 431-447. doi:10.1016/0306-4522(91)90067-x

Spyrka, J., Gugula, A., Rak, A., Tylko, G., Hess, G., & Blasiak, A. (2020). Early life stress-induced alterations in the activity and morphology of ventral tegmental area neurons in female rats. (2352-2895 (Print)).

St. Onge, J. R., Abhari, H., & Floresco, S. B. (2011). Dissociable Contributions by Prefrontal D₁ and D₂ Receptors to Risk-Based Decision Making. *The Journal of Neuroscience, 31*(23), 8625. doi:10.1523/JNEUROSCI.1020-11.2011

St. Onge, J. R., & Floresco, S. B. (2010). Prefrontal Cortical Contribution to Risk-Based Decision Making. *Cerebral Cortex, 20*(8), 1816-1828. doi:10.1093/cercor/bhp250

St. Onge, J. R., Stopper, C. M., Zahm, D. S., & Floresco, S. B. (2012). Separate Prefrontal-Subcortical Circuits Mediate Different Components of Risk-Based Decision Making. *The Journal of Neuroscience, 32*(8), 2886. doi:10.1523/JNEUROSCI.5625-11.2012

Steinberg, E. E., Keiflin, R., Boivin, J. R., Witten, I. B., Deisseroth, K., & Janak, P. H. (2013). A causal link between prediction errors, dopamine neurons and learning. *Nature Neuroscience, 16*(7), 966-973. doi:10.1038/nn.3413

Stopper, C. M., & Floresco, S. B. (2011). Contributions of the nucleus accumbens and its subregions to different aspects of risk-based decision making. *Cognitive, Affective, & Behavioral Neuroscience, 11*(1), 97-112. doi:10.3758/s13415-010-0015-9

Stopper, C. M., Green, E. B., & Floresco, S. B. (2014). Selective involvement by the medial orbitofrontal cortex in biasing risky, but not impulsive, choice. *Cereb Cortex, 24*(1), 154-162. doi:10.1093/cercor/bhs297

Stopper, C. M., Khayambashi, S., & Floresco, S. B. (2013). Receptor-Specific Modulation of Risk-Based Decision Making by Nucleus Accumbens Dopamine. *Neuropsychopharmacology, 38*(5), 715-728. doi:10.1038/npp.2012.240

Stopper, Colin M., Tse, Maric T. L., Montes, David R., Wiedman, Candice R., & Floresco, Stan B. (2014). Overriding Phasic Dopamine Signals Redirects Action Selection during Risk/Reward Decision Making. *Neuron, 84*(1), 177-189. doi:10.1016/j.neuron.2014.08.033

Stornetta, R. L., Hawelu-Johnson, C. L., Guyenet, P. G., & Lynch, K. R. (1988). Astrocytes Synthesize Angiotensinogen in Brain. *Science, 242*(4884), 1444-1446.

Sugam, J. A., Day, J. J., Wightman, R. M., & Carelli, R. M. (2012). Phasic Nucleus Accumbens Dopamine Encodes Risk-Based Decision-Making Behavior. *Biological Psychiatry, 71*(3), 199-205. doi:https://doi.org/10.1016/j.biopsych.2011.09.029

Sugaya, L., Hasin, D. S., Olfson, M., Lin, K. H., Grant, B. F., & Blanco, C. (2012). Child physical abuse and adult mental health: a national study. *J Trauma Stress, 25*(4), 384-392. doi:10.1002/jts.21719

Surmeier, D. J., Ding, J., Day, M., Wang, Z., & Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends in Neurosciences, 30*(5), 228-235. doi:https://doi.org/10.1016/j.tins.2007.03.008

Swain, Y., Muelken, P., LeSage, M. G., Gewirtz, J. C., & Harris, A. C. (2018). Locomotor activity does not predict individual differences in morphine self-administration in rats. *Pharmacology, biochemistry, and behavior, 166*, 48-56. doi:10.1016/j.pbb.2018.01.008

Sweeney, M. D., Ayyadurai, S., & Zlokovic, B. V. (2016). Pericytes of the neurovascular unit: key functions and signaling pathways. *Nature Neuroscience, 19*(6), 771-783. doi:10.1038/nn.4288

Syed, S. A., & Nemeroff, C. B. (2017). Early Life Stress, Mood, and Anxiety Disorders. *Chronic stress (Thousand Oaks, Calif.), 1*, 10.1177/2470547017694461. doi:10.1177/2470547017694461

Thompson, A. L., Molina, B. S. G., Pelham, W., Jr., & Gnagy, E. M. (2007). Risky Driving in Adolescents and Young Adults with Childhood ADHD. *Journal of Pediatric Psychology, 32*(7), 745-759. doi:10.1093/jpepsy/jsm002

Tremblay, M., Barrus, M. M., Cocker, P. J., Baunez, C., & Winstanley, C. A. (2019). Increased motor impulsivity in a rat gambling task during chronic ropinirole treatment: potentiation by win-paired audiovisual cues. *Psychopharmacology, 236*(6), 1901-1915. doi:10.1007/s00213-019-5173-z

Trifilieff, P., Feng, B., Urizar, E., Winiger, V., Ward, R. D., Taylor, K. M., . . . Javitch, J. A. (2013). Increasing dopamine D2 receptor expression in the adult nucleus accumbens enhances motivation. *Molecular Psychiatry, 18*(9), 1025-1033. doi:10.1038/mp.2013.57

Urošević, S., Halverson, T., Youngstrom, E. A., & Luciana, M. (2018). Probabilistic reinforcement learning abnormalities and their correlates in adolescent bipolar disorders. *Journal of abnormal psychology, 127*(8), 807-817. doi:10.1037/abn0000388

Uslaner, J. M., & Robinson, T. E. (2006). Subthalamic nucleus lesions increase impulsive action and decrease impulsive choice − mediation by enhanced incentive motivation? *European Journal of Neuroscience, 24*(8), 2345-2354. doi:https://doi.org/10.1111/j.1460-9568.2006.05117.x

Van den Hove, D. L. A., Steinbusch, H. W. M., Scheepens, A., Van de Berg, W. D. J., Kooiman, L. A. M., Boosten, B. J. G., . . . Blanco, C. E. (2006). Prenatal stress and neonatal rat brain development. *Neuroscience, 137*(1), 145-155. doi:https://doi.org/10.1016/j.neuroscience.2005.08.060

van der Veen, B., Kapanaiah, S. K. T., Kilonzo, K., Steele-Perkins, P., Jendryka, M. M., Schulz, S., . . . Kätzel, D. (2021). Control of impulsivity by Gi-protein signalling in layer-5 pyramidal neurons of the anterior cingulate cortex. *Communications Biology, 4*(1), 662. doi:10.1038/s42003-021-02188-w

van Gaalen, M. M., Brueggeman, R. J., Bronius, P. F. C., Schoffelmeer, A. N. M., & Vanderschuren, L. J. M. J. (2006). Behavioral disinhibition requires dopamine receptor activation. *Psychopharmacology, 187*(1), 73-85. doi:10.1007/s00213-006-0396-1

van Gaalen, M. M., van Koten, R., Schoffelmeer, A. N. M., & Vanderschuren, L. J. M. J. (2006). Critical Involvement of Dopaminergic Neurotransmission in Impulsive Decision Making. *Biological Psychiatry, 60*(1), 66-73. doi:https://doi.org/10.1016/j.biopsych.2005.06.005

van Hest, A., van Haaren, F., & van de Poll, N. E. (1987). Behavioral differences between male and female wistar rats on DRL schedules: Effect of stimuli promoting collateral activities. *Physiology & Behavior, 39*(2), 255-261. doi:https://doi.org/10.1016/0031-9384(87)90018-7

Vanlandewijck, M., He, L., Mäe, M. A., Andrae, J., Ando, K., Del Gaudio, F., . . . Betsholtz, C. (2018). A molecular atlas of cell types and zonation in the brain vasculature. *Nature, 554*(7693), 475-480. doi:10.1038/nature25739

Vest, N., Reynolds, C. J., & Tragesser, S. L. (2016). Impulsivity and risk for prescription opioid misuse in a chronic pain patient sample. *Addictive behaviors, 60*, 184-190. doi:10.1016/j.addbeh.2016.04.015

Viola, T. W., Wearick-Silva, L. E., Creutzberg, K. C., Kestering-Ferreira, É., Orso, R., Centeno-Silva, A., . . . Grassi-Oliveira, R. (2019). Postnatal impoverished housing impairs adolescent risk-assessment and increases risk-taking: A sex-specific effect associated with histone epigenetic regulation of Crfr1 in the medial prefrontal cortex. *Psychoneuroendocrinology, 99*, 8-19. doi:https://doi.org/10.1016/j.psyneuen.2018.08.032

Voorhees, J. L., Powell, N. D., Moldovan, L., Mo, X., Eubank, T. D., & Marsh, C. B. (2013). Chronic Restraint Stress Upregulates Erythropoiesis through Glucocorticoid Stimulation. *PLoS One, 8*(10), e77935. doi:10.1371/journal.pone.0077935

Vrijsen, J. N., Tendolkar, I., Onnink, M., Hoogman, M., Schene, A. H., Fernández, G., . . . Franke, B. (2018). ADHD symptoms in healthy adults are associated with stressful life events and negative memory bias. *Attention deficit and hyperactivity disorders, 10*(2), 151-160. doi:10.1007/s12402-017-0241-x

Wade, T. R., de Wit, H., & Richards, J. B. (2000). Effects of dopaminergic drugs on delayed reward as a measure of impulsive behavior in rats. *Psychopharmacology (Berl), 150*(1), 90-101. doi:10.1007/s002130000402

Walker, C.-D., Bath, K. G., Joels, M., Korosi, A., Larauche, M., Lucassen, P. J., . . . Baram, T. Z. (2017). Chronic early life stress induced by limited bedding and nesting (LBN) material in rodents: critical considerations of methodology, outcomes and translational potential. *Stress (Amsterdam, Netherlands), 20*(5), 421-448. doi:10.1080/10253890.2017.1343296

Weston, H. I., Weston, D. D., Allen, J. L., & Cory-Slechta, D. A. (2014). Sex-dependent impacts of low-level lead exposure and prenatal stress on impulsive choice behavior and associated biochemical and neurochemical manifestations. *Neurotoxicology, 44*, 169-183. doi:10.1016/j.neuro.2014.06.013

Whitton, A. E., Treadway, M. T., & Pizzagalli, D. A. (2015). Reward processing dysfunction in major depression, bipolar disorder and schizophrenia. *Curr Opin Psychiatry, 28*(1), 7-12. doi:10.1097/yco.0000000000000122

Williams, A. V., & Trainor, B. C. (2018). The impact of sex as a biological variable in the search for novel antidepressants. *Front Neuroendocrinol, 50*, 107-117. doi:10.1016/j.yfrne.2018.05.003

Williams, E. S., Manning, C. E., Eagle, A. L., Swift-Gallant, A., Duque-Wilckens, N., Chinnusamy, S., . . . Robison, A. J. (2020). Androgen-Dependent Excitability of

Mouse Ventral Hippocampal Afferents to Nucleus Accumbens Underlies Sex-Specific Susceptibility to Stress. *Biological Psychiatry, 87*(6), 492-501. doi:https://doi.org/10.1016/j.biopsych.2019.08.006

Williams, S. M., Goldman-Rakic, P. S., & Leranth, C. (1992). The synaptology of parvalbumin-immunoreactive neurons in the primate prefrontal cortex. *Journal of Comparative Neurology, 320*(3), 353-369. doi:https://doi.org/10.1002/cne.903200307

Wilson, C. A., Schade, R., & Terry, A. V. (2012). Variable prenatal stress results in impairments of sustained attention and inhibitory response control in a 5-choice serial reaction time task in rats. *Neuroscience, 218*, 126-137. doi:10.1016/j.neuroscience.2012.05.040

Winstanley, C. A., Dalley, J. W., Theobald, D. E. H., & Robbins, T. W. (2003). Global 5-HT depletion attenuates the ability of amphetamine to decrease impulsive choice on a delay-discounting task in rats. *Psychopharmacology, 170*(3), 320-331. doi:10.1007/s00213-003-1546-3

Winstanley, C. A., Theobald, D. E., Cardinal, R. N., & Robbins, T. W. (2004). Contrasting roles of basolateral amygdala and orbitofrontal cortex in impulsive choice. *J Neurosci, 24*(20), 4718-4722. doi:10.1523/JNEUROSCI.5606-03.2004

Winstanley, C. A., Theobald, D. E., Dalley, J. W., Cardinal, R. N., & Robbins, T. W. (2006). Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. *Cereb Cortex, 16*(1), 106-114. doi:10.1093/cercor/bhi088

Winstanley, C. A., Zeeb, F. D., Bedard, A., Fu, K., Lai, B., Steele, C., & Wong, A. C. (2010). Dopaminergic modulation of the orbitofrontal cortex affects attention, motivation and impulsive responding in rats performing the five-choice serial reaction time task. *Behav Brain Res, 210*(2), 263-272. doi:10.1016/j.bbr.2010.02.044

Wolraich, M. L., Hagan, J. F., Allan, C., Chan, E., Davison, D., Earls, M., . . . Adolescents With Attention-Deficit/Hyperactive, D. (2019). Clinical Practice Guideline for the Diagnosis, Evaluation, and Treatment of Attention-Deficit/Hyperactivity Disorder in Children and Adolescents. *Pediatrics, 144*(4), e20192528. doi:10.1542/peds.2019-2528

Womersley, J. S., Hsieh, J. H., Kellaway, L. A., Gerhardt, G. A., & Russell, V. A. (2011). Maternal separation affects dopamine transporter function in the spontaneously hypertensive rat: an in vivo electrochemical study. *Behav Brain Funct, 7*, 49. doi:10.1186/1744-9081-7-49

Yates, J. R., & Bardo, M. T. (2017). Effects of intra-accumbal administration of dopamine and ionotropic glutamate receptor drugs on delay discounting performance in rats. *Behavioral neuroscience, 131*(5), 392-405. doi:10.1037/bne0000214

Zachry, J. E., Nolan, S. O., Brady, L. J., Kelly, S. J., Siciliano, C. A., & Calipari, E. S. (2021). Sex differences in dopamine release regulation in the striatum. *Neuropsychopharmacology, 46*(3), 491-499. doi:10.1038/s41386-020-00915-1

Zack, M., Featherstone, R., Mathewson, S., & Fletcher, P. (2014). Chronic exposure to a gambling-like schedule of reward predictive stimuli can promote sensitization to amphetamine in rats. *Frontiers in Behavioral Neuroscience, 8*, 36.

Zahm, D. S., & Brog, J. S. (1992). On the significance of subterritories in the “accumbens” part of the rat ventral striatum. *Neuroscience*, *50*(4), 751-767. doi:https://doi.org/10.1016/0306-4522(92)90202-D

Zeeb, F. D., Floresco, S. B., & Winstanley, C. A. (2010). Contributions of the orbitofrontal cortex to impulsive choice: interactions with basal levels of impulsivity, dopamine signalling, and reward-related cues. *Psychopharmacology, 211*(1), 87-98. doi:10.1007/s00213-010-1871-2

Zeeb, F. D., Soko, A. D., Ji, X., & Fletcher, P. J. (2016). Low Impulsive Action, but not Impulsive Choice, Predicts Greater Conditioned Reinforcer Salience and Augmented Nucleus Accumbens Dopamine Release. *Neuropsychopharmacology, 41*(8), 2091-2100. doi:10.1038/npp.2016.9

Zeeb, F. D., & Winstanley, C. A. (2011). Lesions of the Basolateral Amygdala and Orbitofrontal Cortex Differentially Affect Acquisition and Performance of a Rodent Gambling Task. *The Journal of Neuroscience*, *31*(6), 2197. doi:10.1523/JNEUROSCI.5597-10.2011