

SEX DIFFERENCES IN SYNAPTIC PLASTICITY WITHIN
THE REWARD SYSTEM: THE ROLE OF PKM ζ AND
IMPLICATIONS FOR OPIOID USE DISORDER

A Dissertation
Submitted to
the Temple University Graduate Board

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

by
Melissa C. Knouse
Diploma Date May 2023

Examining Committee Members:

Dr. Lisa Briand, Advisory Chair, Psychology

Dr. Mathieu Wimmer, Psychology

Dr. Vinay Parikh, Psychology

Dr. Ames Sutton Hickey, Psychology

Dr. Lynn Kirby, Lewis Katz School of Medicine, Neural Sciences

Dr. Debra Bangasser, Georgia State University, Neuroscience Institute

ABSTRACT

Despite the fact that more men are diagnosed with substance use disorder, women escalate their drug consumption faster, exhibit higher craving during withdrawal, and have poorer treatment outcomes. Furthermore, as our cultural expectations of men and women have changed, there has been an increase in drug use in women and this increase is likely to persist. Preclinically, female rodents show stronger behavioral responses to drugs of abuse during initiation, escalation, and reinstatement of drug seeking. These behavioral differences are accompanied by alterations in structural plasticity within the mesocorticolimbic reward system. However, little is known about what functional sex differences exist in glutamate transmission in these circuits. The goal of these experiments was to determine functional sex differences in reward circuitry that may underlie behavioral sex differences in substance use disorder. We found heightened glutamate transmission in both the medial prefrontal cortex and nucleus accumbens in females compared to males. These findings corresponded with the nucleus accumbens being less plastic in females. We then investigated the role of PKM ζ , a glutamatergic AMPA receptor trafficking protein, in plasticity and opioid-taking. We found PKM ζ plays a role in synaptic plasticity within the nucleus accumbens and it works to blunt oxycodone-taking and motivation in a dose-dependent manner. Taken together these findings suggest there are functional sex differences at many levels within the reward system and gaining a better understanding of these differences could provide insight into improved treatments for substance use disorder.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Lisa Briand, for her mentorship during my time in graduate school. Despite learning electrophysiology during a pandemic, graduate school was an incredible experience for me. I owe this to her positive feedback and continuous understanding. I would also like to thank the rest of my committee: Drs. Debra Bangasser, Ames Sutton Hickey, Lynn Kirby, Vinay Parikh, and Mathieu Wimmer. I am so grateful to have had the opportunity to learn from all of you during my time at Temple. Special thank you to Dr. Wimmer, alongside Drs. Chris Pierce, Sarah Swinford-Jackson, and Matthew Matell for seeing me through my first experiments and still supporting me today.

Thank you to the entire Briand lab for the endless support and laughs. Special shoutout to Dr. Andre Deutschmann, Lizzie Birmingham, and Troy Houser. Andre: I am so grateful for your constant guidance despite being located across the Atlantic. Lizzie and Troy: thank for either getting me a margarita or humbling me every day.

Lastly, I would like to thank my family and my husband. Mom and dad: you have always done everything in your power to make sure I can accomplish all of my dreams. I am the woman I am today because of you. Kristin and Bryan: despite the childhood character development program you put me through I have looked up to both of you my entire life. Impressive is an understatement for your combined drive and creativity. To my husband, Matt: thank you for listening to every talk and reading every manuscript despite the fact that you work in finance. I am so grateful to have someone so supportive and kind in my life.

TABLE OF CONTENTS

	Page
ABSTRACT.....	ii
ACKNOWLEDGMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
CHAPTER	
1. BEHAVIORAL SEX DIFFERENCES IN COCAINE AND OPIOID USE DISORDERS: THE ROLE OF GONADAL HORMONES	1
Introduction.....	1
Behavioral Sex Differences in Substance Use Disorder.....	2
The Influence of Gonadal Hormones on Behavior	5
Gonadal Hormones and Substance Use	6
Cocaine	6
Opioids.....	10
Neural Mechanisms Underlying the Reinforcing Properties of Cocaine and Opioids.....	14
Effects of Gonadal Hormones on Dendritic Spine Density	19
Conclusion	24
2. SEX DIFFERENCES IN THE MEDIAL PREFRONTAL CORTICAL GLUTAMATE SYSTEM.....	27
Introduction.....	27
Methods.....	30
Results.....	33

Discussion.....	36
3. SEX DIFFERENCES IN PRE- AND POSTSYNAPTIC GLUTAMATE SIGNALING IN THE NUCLEUS ACCUMBENS CORE.....	41
Introduction.....	41
Methods.....	43
Results.....	46
Discussion.....	56
4. PKM ζ ALTERS OXYCODONE-TAKING.....	64
Introduction.....	64
Methods.....	66
Results.....	68
Discussion.....	74
5. CONCLUSION.....	79
REFERENCES.....	89

LIST OF TABLES

Table	Page
1. The role of ovarian hormones in substance use disorder	14

LIST OF FIGURES

Figure	Page
1. Female mice exhibit higher levels of synaptosomal GluA1 and GluA2 expression in the mPFC compared to male mice	34
2. Female mice have higher levels of glutamate transmission in the mPFC compared to male mice	35
3. AMPA/NMDA ratio in the nucleus accumbens core is higher in female Long-Evans rats compared to male rats	48
4. AMPA/NMDA ratio in the nucleus accumbens core is higher in female C57Bl6/J mice compared to male mice	49
5. Female mice have a larger readily releasable pool of glutamate and lower release probability in the nucleus accumbens core than male mice.....	51
6. Longer induction protocols are needed to induce LTD in the nucleus accumbens core of female mice compared to male mice	53
7. PKM ζ knockout alters LTD in a sex-specific manner	55
8. PKM ζ knockout potentiates 0.25 mg/kg/inf oxycodone self-administration in both sexes	70
9. PKM ζ knockout potentiates 0.125 mg/kg/inf oxycodone self-administration exclusively in female animals.....	72
10. PKM ζ knockout potentiates the final breakpoint in a progressive ratio paradigm exclusively in female animals	73
11. PKM ζ knockout does not functionally alter presynaptic activity in the nucleus accumbens core.....	84
12. Sex differences in glutamate transmission within the reward system	88

CHAPTER 1

Behavioral Sex Differences in Cocaine and Opioid Use Disorders: The Role of Gonadal Hormones

Introduction

Substance use disorder (SUD) is a growing public health crisis in the United States, with overdose deaths steadily increasing through 1999-2018 (Hedegaard et al., 2020). While the ongoing opioid epidemic is a large driver of this, overdose deaths involving cocaine are also rising. Rates of age-adjusted overdose deaths involving synthetic opioids other than methadone increased by 10% from 2017-2018 and overdose deaths involving cocaine more than tripled from 2012-2018 (Hedegaard et al., 2020). While there are FDA-approved pharmacotherapies to treat opioid use disorder, many of them are limited in efficacy and none are currently approved to treat cocaine use disorder. Furthermore, the available pharmacotherapies for opioid use disorder do not yield the same treatment outcomes in males and females. After 2 weeks of treatment with buprenorphine, a larger percentage of males remain abstinent from illicit opioids than females (K. P. Johnson et al., 1995). This eventually reverses, however, and more females remain abstinent from illicit opioids than males at 17 and 24 weeks of treatment (Jones et al., 2005; Schottenfeld et al., 1998). This is possibly due to sex differences in the development and experience of substance use disorder. The information on opioid use disorder is less extensive, though existing research shows that males and females also do not develop and experience opioid use disorder in the same manner. Across the board, it appears that females are more vulnerable than males to cocaine and opioid use disorders.

While the behavioral sex differences in opioid and cocaine use disorder overlap, the neural mechanisms underlying these differences are less understood. Cocaine and

opioids produce their rewarding effects through similar mechanisms, though their effects on the brain diverge in numerous ways. This makes it difficult to determine whether behavioral sex differences in SUD develop through the same mechanisms for different drugs of abuse. While various mechanisms have been implicated, ovarian hormones are likely the primary driver of the behavioral sex differences seen in cocaine use disorder. It is unclear, however, whether ovarian hormones also drive the behavioral sex differences seen in opioid use disorder. It seems plausible, given that cocaine and opioids have many overlapping effects on the brain. The opioid literature is limited, however, and paints a slightly different story. Overall, it seems overlapping behavioral sex differences in drug abuse might not be driven by the same biological mechanisms. This review highlights the need for further exploration of the mechanisms underlying sex differences in opioid use disorder.

Behavioral Sex Differences in Substance Use Disorder

Both preclinical and clinical research have traditionally ignored sex as a biological variable. The majority of research was conducted in males, leading to heavily biased results (Greenfield et al., 2007). Unfortunately, male biases in biomedical research have led to economic loss and unintended fatalities (S. K. Lee, 2018). These consequences led to NIH mandates for the inclusion of female subjects in research, which has exponentially increased the number of published reports about substance abuse in women (Greenfield et al., 2007). As substance abuse research involving female subjects increases, new trends are emerging.

According to the 2018 National Survey on Drug Use and Health, the rate of illicit drug use other than marijuana in those aged 12+ is higher in males than in females

(SAMHSA, 2018). However, this gap is steadily closing, likely due to attitude changes towards women in the home and workplace (Brady & Randall, 1999). Despite the fact that the rate of abuse and dependence is currently higher in males, females are more vulnerable to many aspects of SUD. While the breadth of research on sex differences in cocaine abuse is extensive, less is known about sex differences in opioid abuse. However, with the ongoing opioid epidemic, new research has indicated that opioids likely have similar effects in females as cocaine. Both lines of research point to the same trend: females are more vulnerable than males to many aspects of cocaine and opioid use disorders.

Clinical research indicates that women are more sensitive to many aspects of cocaine use. Women report higher cocaine craving following exposure to drug-related cues, more frequently having used more cocaine than they meant to, and more frequently having used despite trying not to than men (Kennedy et al., 2013). Women also report a higher desire to use cocaine, lower desires to not use cocaine, and lesser highs than men (Elman et al., 2001; Sofuoglu et al., 1999). Together, these data indicate that men and women do not experience cocaine use disorder in the same way. Biological sex therefore appears to influence responses to cocaine, likely increasing female vulnerability to cocaine use disorder.

Preclinical work generally recapitulates the clinical findings, with female rodents exhibiting increased behavioral responses to cocaine. It is important to note that these differences are not always seen and the effect of sex in preclinical models of substance use disorder often depends on paradigm (Larson et al., 2005). Nonetheless, a breadth of studies indicate that female rodents acquire cocaine self-administration more rapidly, self-administer more cocaine, and exhibit higher responding during reinstatement and on

progressive ratio (PR) schedules than males (Bechard et al., 2018; Carroll et al., 2002; Festa & Quinones-Jenab, 2004; Kerstetter et al., 2008; Lynch & Carroll, 2000; Lynch & Taylor, 2004; Ramoa et al., 2013; Roberts et al., 1989). Additionally, females are more sensitive to factors that contribute to the escalation of cocaine-taking and they acquire cocaine conditioned place preference quicker and at lower doses than males (Roth & Carroll, 2004; Russo et al., 2003). These differences are present from an early age, as adolescent females also acquire self-administration more readily and respond at higher levels in a PR paradigm than males (Lynch, 2008). Together, these data indicate that females are more vulnerable than males to the behavioral effects of cocaine. Preclinical models of cocaine taking, seeking, and relapse indicate that cocaine interacts with biological sex in some manner to alter addiction-like behaviors.

These effects do not appear to be specific to cocaine, as the emerging opioid literature also indicates that females are more susceptible to the addiction-like effects of opioids. Clinically, women report higher baseline opioid craving and higher heroin craving following exposure to drug-paired cues than men (Back et al., 2011; Yu et al., 2007). Preclinically, females acquire heroin self-administration more rapidly and take more heroin, fentanyl, oxycodone, and morphine than males (Carroll et al., 2002; Cicero et al., 2003; Kimbrough et al., 2020; Klein et al., 1997; Lynch & Carroll, 1999; Zanni et al., 2020). Additionally, females respond more than males during cue-induced heroin reinstatement and for morphine on a PR schedule (Cicero et al., 2003; Vazquez et al., 2020). Females also exhibit higher morphine withdrawal scores compared to males (Reiss et al., 2020).

Given these findings, it seems the existing literature on sex differences in opioid abuse points to a similar trend: females are more susceptible to many aspects of opioid use disorder. The available pharmacotherapies to treat opioid use disorder are unfortunately limited by side effects and efficacy. Through understanding how sex influences SUD, we might be able to create more targeted pharmacotherapies that would limit side effects and increase efficacy. While the behavioral sex differences in opioid and cocaine use disorders seem to overlap, it is unclear whether they are driven by the same mechanisms.

The Influence of Gonadal Hormones on Behavior

Biological sex can influence behavior via actions of the different complement of genes on sex chromosomes and via circulating gonadal hormones (Arnold, 2009). Gonadal hormones are capable of modulating behavior in two ways: through organizational and activational effects. The organizational-activational hypothesis of gonadal hormones was first proposed by Phoenix and colleagues (Phoenix et al., 1959). Organizational effects of hormones permanently shape the brain. These effects can occur throughout life during critical periods such as puberty (Romeo, 2003). A crucial time point for the organizational effects of hormones is around the time of birth. During the perinatal period, testosterone masculinizes the male brain (Phoenix et al., 1959). Importantly, perinatal masculinization alters excitatory synaptic input in the nucleus accumbens (Cao et al., 2016). Therefore, it is possible that organizational effects of gonadal hormones during the perinatal period shape the brain in a way that primes females to be more vulnerable to SUD than males. Activational effects of hormones are transient, due to circulating levels of gonadal hormones, and usually reversible. The activational effects of gonadal hormones occur when they interact with the masculinized or feminized brain to influence behavior. Once

onboard, drugs of abuse can interact with circulating ovarian hormones in a manner that makes females more susceptible than males to subsequent use (Baker et al., 2003; Becker & Hu, 2008; Festa & Quinones-Jenab, 2004; Jackson et al., 2006; A. N. Perry et al., 2013).

Understanding the role of both organizational and activational effects of gonadal hormones on reward signaling and the response to drugs of abuse could provide us with insight into mechanisms underlying behavioral sex differences. It is possible that the masculinization (or lack thereof) of the brain affects vulnerability to drug use in adulthood. Furthermore, interactions amongst circulating gonadal hormones and drugs of abuse also likely influence how males and females respond in the context of SUD. Understanding how gonadal hormones might affect vulnerability to SUD will ideally lead to better treatment plans and subsequent outcomes.

Gonadal Hormones and Substance Use

Cocaine

It is well-established that ovarian hormones play a key role in sex differences in cocaine abuse. The human menstrual cycle lasts approximately 28 days and is divided into luteal and follicular phases. Estrogen levels peak about halfway through the cycle, followed by a peak in progesterone (Staley & Scharfman, 2005). In rodents, the estrous cycle lasts approximately 4 days and is divided into 4 stages: proestrous, estrous, metestrous, and diestrous. Estrogen levels peak during the proestrous phase and progesterone levels peak in between the proestrous and estrous phases (Staley & Scharfman, 2005). Changes in ovarian hormone levels throughout both the human and rodent menstrual and estrous cycles are known to influence substance use behaviors, as circulating estradiol levels significantly contribute to the enhanced sensitivity to the reinforcing effects of cocaine seen in females

(Lynch, 2008). Clinically, the subjective effects of cocaine vary throughout the menstrual cycle (Evans & Foltin, 2010; Sofuoglu et al., 1999; Terner & de Wit, 2006). Preclinically, female rats in estrus exhibit higher breakpoints in a PR paradigm but remain stable in males (Lacy et al., 2016; Roberts et al., 1989). Additionally, female rats in estrus exhibit greater responding during self-administration, extinction, and reinstatement than either males or non-estrous females (Feltenstein et al., 2009; Feltenstein & See, 2007; Kippin et al., 2005; Lynch, 2008). Together, these data indicate the reinforcing efficacy of cocaine is dramatically altered by fluctuating levels of ovarian hormones throughout the estrous cycle.

Further confirming this association, studies ovariectomizing (OVX) female rats have shown that ovarian hormones increase vulnerability to cocaine abuse. Blocking the activational effects of ovarian hormones via OVX in adulthood affects behavioral responses to cocaine (Russo et al., 2003; Sircar & Kim, 1999; Walker et al., 2001). OVX decreases the magnitude of cocaine conditioned place preference, the magnitude of reinstatement, and the percentage of animals acquiring self-administration (Larson et al., 2005; Lynch et al., 2001; Russo et al., 2003). Furthermore, estradiol replacement to OVX females facilitates cocaine self-administration and conditioned place preference (Hu et al., 2004; A. N. Perry et al., 2013; Ramoa et al., 2013; Segarra et al., 2010; Twining et al., 2013). This effect is specific to females, as a dose of estradiol that enhances self-administration in OVX females has no effect on behavior in castrated males (Jackson et al., 2006). Estradiol replacement also increases cocaine reinstatement and breakpoints on a PR schedule (Becker & Hu, 2008; Doncheck et al., 2018; Larson et al., 2005; Larson & Carroll, 2007; A. N. Perry et al., 2013). Thus, removal of ovarian hormones seems to

decrease behavioral responses to cocaine in female rats, while hormonal replacement facilitates addiction-like behaviors. However, there are multiple studies that demonstrate differing effects of sex and gonadal hormones on behavioral responses to cocaine.

While some studies show differences in cocaine progressive ratio breakpoint throughout the menstrual cycle (Mello et al., 2007), other studies do not (Cooper et al., 2013). Furthermore, sex and gonadal hormone status do not always influence cocaine self-administration in cynomolgus monkeys or rats (Baptista et al., 2004; Kerstetter et al., 2012; Mello et al., 2007). There are many possibilities for these discrepancies in the literature. Methodological differences are a likely explanation, as increases in progressive ratio during the estrous phase in cynomolgus monkeys are seen at a low dose of cocaine (Mello et al., 2007). Furthermore, the paradigm used influences the effect of estrus on cocaine-seeking (Kerstetter et al., 2008). It is proposed that duration of cocaine exposure influences estrogen levels and duration of estradiol replacement can modulate dopamine release in the striatum (Becker, 1990; Larson et al., 2005; Lynch & Taylor, 2005). This indicates that the paradigm used in estradiol replacement studies could significantly impact behavioral responses to cocaine. Indeed, the length of estradiol replacement significantly alters cocaine reinstatement and the dose of cocaine used significantly modulates the effect of estradiol replacement on cocaine conditioned place preference scores (S. A. Bobzean et al., 2014; Larson et al., 2005).

Overall, the literature indicates a strong, but nuanced, role of ovarian hormones in cocaine abuse. Factors such as reinforcement schedule, dose, and prior behavioral experience are frequent explanations for discrepancies in this literature. However, there is a significant role of progesterone in behavioral responses to cocaine that is often ignored.

Clinically, progesterone attenuates the “good drug effect” of smoked cocaine in women and decreases cocaine use in post-partum women (Evans & Foltin, 2010; Yonkers et al., 2014). Preclinically, progesterone decreases cocaine self-administration in non-human primates and decreases escalation of cocaine taking in rodents (Larson & Carroll, 2007; Mello et al., 2011). Furthermore, cocaine seeking is at its lowest when progesterone is at its highest, and cocaine seeking is at its highest when progesterone levels are at their lowest (Feltenstein & See, 2007). These studies exemplify the need for further consideration of progesterone in the behavioral sex differences seen in cocaine abuse.

While there is a demonstrated role for estrogen in these differences, progesterone has a consistent effect on dampening behavioral responses to cocaine in females.

Less is known about the perinatal organizational effects of gonadal hormones on cocaine abuse, as prepubertal animals are less commonly studied. While female rats exhibit higher cocaine-induced locomotor activity than males in adulthood, there do not appear to be sex differences in this measure in prepubertal rats (Cailhol & Mormede, 1999; Kuhn et al., 2001; Segarra et al., 2010; Ujike et al., 1995). However, prepubertal gonadectomy has opposing effects on locomotor activity in males and females. Male rats gonadectomized prepubertally exhibit increased behavioral responsiveness to cocaine while female rats gonadectomized prepubertally exhibit decreased behavioral responsiveness to cocaine (Parylak et al., 2008). This suggests that there are multiple organizational and activational effects of gonadal hormones that modulate responsiveness to cocaine. Furthermore, it also demonstrates that male gonadal hormones might be protective against some aspects of SUD, while female gonadal hormones increase vulnerability to SUD.

Testosterone appears to play a smaller, but still significant, role in the sex differences seen in cocaine use. Testosterone replacement to castrated male rats reduces cocaine-induced focused stereotypy sensitization and partially restores cocaine-induced reductions of dopamine uptake in the striatum, indicating that testosterone modulates cocaine-induced alterations of dopamine homeostasis (R. Chen et al., 2003). Furthermore, intact male rats display sensitization to cocaine-induced stereotypic activity over the course of a week while castrated male rats display sensitization only following a cocaine challenge (Chin et al., 2002). This shows that behavioral stereotypy can sensitize to cocaine without testosterone, albeit in a different manner. It is plausible that testosterone is protective against cocaine use, whereas estrogen is detrimental to cocaine use.

Overall, there is a breadth of evidence indicating that gonadal hormones modulate behavioral responses to cocaine. Both clinical and preclinical literature indicate that ovarian hormones are a major driver of female vulnerability to cocaine use and abuse. Removal of ovarian hormones blocks behavioral responses to cocaine and estradiol replacement rescues these effects. What is unknown, however, is whether gonadal hormones are also the predominant driver of behavioral sex differences in opioid abuse.

Opioids

Unfortunately, the work examining the effects of gonadal hormones on opioid abuse is limited. Clinically, little is known about subjective responses to opioids throughout the menstrual cycle in patients with SUD. While some pain literature indicates the analgesic efficacy of opioids may vary throughout the human menstrual cycle, the results are conflicting (Ribeiro-Dasilva et al., 2011; Turner & de Wit, 2006). While there are gaps in the clinical literature, there are behavioral differences seen in preclinical models of

opioid use disorder throughout the estrous cycle. Female rats take a similar number of heroin infusions during the estrous, metestrous, and diestrous phases but self-administer significantly less during the proestrous phase (Lacy et al., 2016). Together, these data highlight the possibility that opioid use disorder is similarly influenced by ovarian hormones as cocaine.

Just as with cocaine, behavioral responses to opioids can vary following OVX. OVX in female mice decreases the magnitude of morphine conditioned place preference similarly to cocaine, an effect that can be reversed with estradiol replacement (Mirbaha et al., 2009). Additionally, estradiol replacement following OVX in rats increases acquisition of heroin self-administration and infusions during the last 5 days of acquisition compared to OVX conspecifics treated with vehicle (Roth et al., 2002). Preclinical pain literature indicates an organizational effect of perinatal gonadal hormones on opioid-induced analgesia, as gonadectomy shortly after birth, but not in adulthood, significantly alters morphine analgesia in both male and female rats (Borzan & Fuchs, 2006; Cataldo et al., 2005; Cicero et al., 2002; Krzanowska et al., 2002). These results indicate significant organizational and activational effects of gonadal hormones in responses to opioids. While this literature would lead to the conclusion that gonadal hormones drive cocaine and opioid use disorders similarly, many studies have found opposing results.

For example, OVX followed by estradiol replacement in rats has no effect on heroin self-administration and responding on a PR schedule (Stewart et al., 1996). Furthering this, multiple studies have found no effect of OVX at all in rat models of opioid use disorder. OVX has no effect on heroin self-administration, seeking, or responding on a PR schedule (Sedki et al., 2015; Stewart et al., 1996). Additionally, while estradiol replacement to OVX

animals increases the number of heroin infusions earned, sham controls do not differ from OVX controls (Roth et al., 2002). This makes the role of ovarian hormones in opioid use disorder difficult to understand, as blocking circulating ovarian hormones in rats does not always affect taking and seeking behavior on its own. This calls into question whether the activational effects of ovarian hormones play the same role in cocaine and opioid abuse.

Adding to the confusion, OVX in mice actually increases conditioned place preference at a dose of 10 mg/kg morphine, an effect that is then decreased by estradiol replacement (Mirbaha et al., 2009). Additionally, estradiol replacement blocks increased heroin seeking seen in OVX female rats following food restriction (Sedki et al., 2015). However, it should be noted that this effect may be mediated, in part, by the anorexic properties of estradiol (Sedki et al., 2015). While the role of progesterone in opioid abuse is largely unknown, there is evidence it does not affect motivation for heroin in rats (Stewart et al., 1996). Thus, while the role of ovarian hormones in cocaine taking and seeking is well-established, the role in opioid taking and seeking is not. Ovarian hormones have been shown to promote, block, or not affect the behavioral effects of opioids, making it difficult to understand their role in these behaviors. This leads to the possibility that ovarian hormones do not play the same role in the sex differences seen in cocaine and opioid abuse.

Although there is little work examining the role of testosterone in opioid use disorder, there is evidence that testosterone and opioids interact. Both morphine and heroin reduce testosterone levels in rats and humans, indicating some form of interaction between them (Cicero et al., 1976; Mendelson & Mello, 1975; Yilmaz et al., 1999). The majority of the literature on this topic focuses on how testosterone modulates opioid antinociception.

Female and castrated male rats develop morphine tolerance slower than intact male or testosterone-treated female rats (South et al., 2001). Additionally, naloxone can increase pain threshold and enhance morphine-induced analgesia in castrated rats. This can be abolished with testosterone treatment (Rao & Saifi, 1985). There appears to be a strong organizational effect of neonatal testosterone on morphine antinociception, as males gonadectomized neonatally exhibit reduced morphine analgesia in adulthood and female rats treated neonatally with testosterone exhibit increased morphine analgesia (Borzan & Fuchs, 2006; Cataldo et al., 2005; Cicero et al., 2002; Krzanowska et al., 2002). Overall, this indicates there are organizational and activational effects of testosterone that alter the analgesic efficacy of opioids. This provides evidence that testosterone might also modulate behavioral responses in opioid use disorder.

The role of circulating gonadal hormones in opioid use disorder remains unclear. While ovarian hormones exhibit clear organizational and activational effects in cocaine abuse, there are inconsistent results in opioid abuse. OVX blunts behavioral effects of cocaine, an effect that can be reversed with estradiol replacement. However, OVX does not consistently affect behavioral effects of opioids. Furthermore, estradiol replacement can decrease, increase, or not affect these responses. These studies indicate that the role of the activational and organizational effects of ovarian hormones in SUD might differ by drug class (see table 1). It is possible that there are activational effects of ovarian hormones that are not the same following cocaine or opioid exposure. The role of testosterone in SUD is less clear. It appears that testosterone may be protective against SUD, but a lack of data prevents any definitive conclusions. Overall, it seems that ovarian hormones might not play the same role in opioid use disorder as they do in cocaine use disorder. Behavioral sex

differences themselves might be similar across drug classes, but the underlying mechanisms might not. Given the lack of available studies, further research examining how gonadal hormones and opioids interact are crucial to understand behavioral sex differences in opioid use disorder.

Table 1				
OVX				
	CPP	Self-administration	Seeking	PR Responding
Cocaine	↓	↓	↓	–
Opioids	↓, ↑	No effect	No effect	No effect
OVX + estradiol replacement				
	CPP	Self-administration	Seeking	PR Responding
Cocaine	↑	↑	↑	↑
Opioids	↑, No effect	↑, No effect	↓, No effect	No effect

This table summarizes the studies that have examined the role of ovarian hormones in preclinical models of cocaine and opioid use disorder. The rows for each drug reflect changes to behavioral phenotypes following OVX and OVX + estradiol replacement. The line symbolizes that no studies have examined this directly.

Neural Mechanisms Underlying the Reinforcing Properties of Cocaine and Opioids

Given the existing literature on gonadal hormones and substance use disorder, it seems behavioral sex differences in cocaine and opioid abuse might be driven by different mechanisms. This seems in direct contradiction to the fact that cocaine and opioids both produce their rewarding effects through activation of the mesolimbic dopamine system. Put simply, both drugs increase dopamine release from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), albeit through slightly different mechanisms. Cocaine, a

dopamine, serotonin, and norepinephrine transporter blocker, prevents neurotransmitter clearance from the synapse. Blocking the dopamine transporter (DAT) results in a flood of dopamine in the NAc, producing the rewarding effects of the drug (Hummel and Unterwald, 2002). Opioids primarily bind to μ opioid receptors on GABAergic neurons in the VTA, leading to disinhibition of dopamine projections to the NAc. Overall, this induces an increase in dopaminergic signaling to the NAc (Fields and Margolis, 2015). While they work through slightly different mechanisms, the end result is a flood of dopamine in the NAc.

The mechanisms driving relapse to opioids and cocaine overlap as well. Specifically, it has been proposed that the glutamatergic effects of the drugs overlap (Hearing et al., 2018). In basal conditions, the cystine-glutamate antiporter (system xc-) increases extracellular glutamate levels which in turn stimulates presynaptic group II metabotropic glutamate receptors (mGluR2/3), resulting in a decrease of excitatory transmission (Moran et al., 2005). Both opioids and cocaine disrupt glutamate homeostasis during withdrawal. This is in part due to decreased function of system xc- and mGluR2/3 (Bossert et al., 2006; Knackstedt, Melendez, et al., 2010; Xi et al., 2002; Zhou & Kalivas, 2008). Restoring function to system xc-, usually by administration of N-acetylcysteine, reverses cocaine-mediated alterations of long-term potentiation (LTP) and long-term depression (LTD), prevents both cue- and drug-primed cocaine and heroin reinstatement, and induces a lasting reduction in cue- and heroin-induced seeking (Baker et al., 2003; Kau et al., 2008; Madayag et al., 2007; Moussawi et al., 2009, 2011; Zhou & Kalivas, 2008). This effect appears to be at least partially dependent on mGluR2/3, as antagonists block the ability of N-acetylcysteine to restore LTP following cocaine self-administration

(Moussawi et al., 2009). Additionally, cocaine conditioned reinstatement, cocaine-primed reinstatement, and context-induced heroin seeking can be attenuated with mGluR2/3 agonists (Baptista et al., 2004; Bossert et al., 2006; Peters & Kalivas, 2006). This indicates that both cocaine and opioid exposure produce alterations to system xc- and mGluR2/3 in the NAc that disrupt glutamate homeostasis and set the stage for relapse.

Overall, the literature points to common mechanisms underlying cocaine and opioid relapse: altered glutamate homeostasis in the NAc via decreased function of system xc- and mGluR2/3. The mechanistic overlap seen in cocaine and opioid use disorders leads to the hypothesis that similar mechanisms might actually drive female vulnerability to both opioid and cocaine abuse. Unfortunately, the issue is not so clear cut. While there are overlapping mechanisms underlying cocaine and opioid abuse, there is strong evidence indicating that cocaine and opioids have differing effects on synaptic plasticity.

LTP and LTD are two forms of synaptic plasticity that can be altered by exposure to drugs of abuse. Cocaine consistently disrupts LTD in the NAc. A single exposure can abolish LTD (Fourgeaud et al., 2004) and various models of chronic exposure and withdrawal can induce persistent inhibition of LTD in the core and shell regions (Knackstedt, Melendez, et al., 2010; Martin et al., 2006; Moussawi et al., 2009; Thomas et al., 2001). This impairment is likely due to downregulated mGluR5 and subsequently disrupted system xc- function (Fourgeaud et al., 2004; Knackstedt, Melendez, et al., 2010; Moussawi et al., 2009). N-acetylcysteine administration restores the deficits, an effect that is dependent on mGluR5 but not mGluR2/3 (Moussawi et al., 2009). Impairment also appears to depend on subregion, as it persists in the core region during abstinence but does not persist in the shell (Martin et al., 2006).

Opioids also exert long-lasting impairments in LTD in the NAc. Prolonged withdrawal from opioids impairs LTD in both the NAc core and shell (Z. Dong et al., 2007; Qian et al., 2019; Shen & Kalivas, 2013). Interestingly, this effect appears to be due to downregulation of mGluR2/3 (Z. Dong et al., 2007; Robbe et al., 2002). This is inconsistent with the prior results showing cocaine-mediated disruptions in LTD are due to mGluR5, not mGluR2/3. This leads to the possibility that while the net results are similar, different mechanisms might drive the impaired LTD seen in the NAc following cocaine and opioid exposure. Furthering this, there are also differences seen in synaptic potentiation following drug exposure.

Chronic cocaine, regardless of whether it is followed by extinction, induces a LTP-like state in the NAc. Frequently, this state prevents the induction of further LTP (Moussawi et al., 2009). Thus, many studies show that cocaine actually prevents the induction of LTP in the NAc (Knackstedt, Moussawi, et al., 2010; Moussawi et al., 2009). Similarly, acute morphine withdrawal impairs induction of LTP in the NAc shell, an effect that is restored over prolonged withdrawal (Z. Dong et al., 2007). Within the NAc core, heroin self-administration followed by extinction also impairs induction of LTP (Shen & Kalivas, 2013). While this leads to the possibility that cocaine and opioids exert similar effects on synaptic plasticity, the NAc actually exhibits a LTD-like state during heroin withdrawal (Shen et al., 2011). There is a decrease in the AMPA/NMDA ratio in the NAc core during heroin extinction which is due to increases in NR2B and NMDA current decay time (Shen et al., 2011). Overall, this indicates the NAc core is likely in a depotentiated state following heroin exposure. Therefore, it appears that while both opioids and cocaine can impair LTP induction, they lead to opposing states in the NAc.

Overall, this indicates there are opposing changes to synaptic plasticity in the NAc following cocaine and opioid exposure (Shen & Kalivas, 2013). This can lead to similar outcomes such as blunted LTD and difficulty inducing LTP even though the underlying mechanisms are not the same. Synaptic plasticity is an important modulator of SUD (Kauer & Malenka, 2007), therefore changes in synaptic plasticity can drive behavioral responses to drugs of abuse. These differences in synaptic plasticity within the NAc represent the possibility that different mechanisms might drive the overlapping behavioral sex differences in SUD.

Differences in synaptic plasticity following exposure to drugs of abuse can also be examined using dendritic spine density as changes to spine density underlie drug use (Kalivas, 2009). Interestingly, different classes of drugs alter spine density in different ways. Specifically, cocaine and opioids have opposing effects on spine density within the mesolimbic reward system. Cocaine self-administration increases dendritic branching and density of dendritic spines in NAc shell medium spiny neurons and prefrontal and parietal pyramidal cells (Robinson et al., 2001). Repeated cocaine increases spine density in the NAc shell (Dumitriu et al., 2012) and core (Norrholm et al., 2003). Interestingly, the effect of cocaine on spine density in the core appears to be time-dependent, with density actually decreasing at certain time points following exposure (Dumitriu et al., 2012; Siemsen et al., 2019). Nonetheless, cocaine overall induces increases in spine density in the NAc. The opposite effect is generally seen following opioid exposure.

Both self-administered and experimenter-administered morphine decrease dendritic spine density in the NAc shell (Robinson et al., 2002). Along these lines, repeated morphine exposure followed by a withdrawal paradigm decreases dendritic spine density

on medium spiny neurons (MSNs) in the NAc shell (Diana et al., 2006; Kasture et al., 2009; Robinson & Kolb, 1999; Spiga et al., 2005), core (Leite-Morris et al., 2014), and on prefrontal pyramidal cells (Robinson & Kolb, 1999). Together, these data indicate that opioids decrease dendritic spine density within the NAc. As cocaine generally does the opposite, it seems the behavioral outcomes in opioid and cocaine use disorders might be driven by different mechanisms.

Taken together, these studies indicate that opioids and cocaine might have functionally distinct effects on synaptic plasticity. Both drugs are capable of blunting LTD and impairing LTP in the NAc, which alludes to overlapping effects on synaptic plasticity. However, cocaine induces a LTP-like state in the NAc whereas opioids induce a LTD-like state. Furthermore, cocaine increases dendritic spine density in the NAc. Opioids, however, most often decrease spine density within the NAc. Opposing alterations of synaptic and structural plasticity in the NAc indicate that opioids and cocaine might not drive SUD in the same manner. This information makes it unclear whether the overlapping behavioral sex differences seen in opioid and cocaine abuse stem from the same neural mechanisms. The interactions between biological sex and drugs of abuse could differ by drug class, even though they lead to similar behavioral outcomes.

Effects of Gonadal Hormones on Dendritic Spine Density

In addition to drugs of abuse, gonadal hormones also modulate spine density in the brain. It is possible drugs of abuse interact with gonadal hormones to modulate synaptic plasticity. While the behavioral outcomes of these interactions may be similar, different drugs of abuse might interact with gonadal hormones to modulate synaptic plasticity in different manners. The predominant portion of studies investigating gonadal hormones and

spine density examine their effects on hippocampal circuits involved in learning and memory. These studies give us a glimpse into how gonadal hormones can differentially affect synaptic plasticity in the brain, which might be a mechanism that makes females more vulnerable to the effects of drugs of abuse.

Within the hippocampus, dendritic spine density fluctuates throughout the estrous cycle in females with the lowest density seen during the estrous phase (Brusco et al., 2008). OVX decreases spine density in pyramidal CA1 neurons in the hippocampus (Wallace et al., 2006). Estradiol administration increases CA1 apical spine density, but 10 weeks following OVX the ability of estradiol to increase CA1 apical spine density is decreased. In contrast, a different protocol of estradiol administration failed to alter apical spine density and actually decreased basal spine density (McLaughlin et al., 2008). Thus, the effect of estradiol on spine density appears to be time course and paradigm dependent. OVX decreases spine density in the hippocampus over the course of a few days, an effect that can be reversed with estradiol treatment. Within 24 hours of estradiol treatment, spine density begins to increase. Over the next week, spine density gradually decreases (Woolley & McEwen, 1994). Therefore, it seems estradiol indeed modulates spine density in the hippocampus, but the effect depends on when spines are examined following treatment. As estradiol also modulates responses to drugs of abuse, it is possible that an interaction exists between gonadal hormones and spine density that alters how the body responds to drug exposure.

As estrogens are not the only circulating ovarian hormones, many studies have also examined the role of progesterone. Progesterone treatment following estradiol initially increases spine density but then induces a decrease in density that is stronger than the effect

of estradiol alone (Woolley & McEwen, 1994). Additionally, blocking progesterone receptors inhibits the drop in spine density seen as animals progress from the proestrous to estrous phase of their cycle (Woolley & McEwen, 1994). Overall, the work in the hippocampus indicates that ovarian hormones significantly alter dendritic spine density. In basal conditions, their overall effect fluctuates with estrous cycle stage. Given these results, it seems plausible that the reward system is affected by ovarian hormones, potentially affecting how the brain interacts with drugs of abuse in the future.

Similar results are seen in the medial amygdala, with dendritic spine density also varying throughout the estrous cycle. Decreases are seen during the proestrous, estrous, and metestrous phases. Additionally, males have more dendritic spines than females at baseline in this region (Rasia-Filho et al., 2004). These results also appear to translate to the reward system. OVX females exhibit lower spine densities in pyramidal neurons of the medial prefrontal cortex (mPFC) (Wallace et al., 2006). Estradiol actually decreases spine density in the NAc core and causes deconstruction of spines from more to less mature subtypes in both the NAc core and shell (Peterson et al., 2015; Staffend et al., 2011). Thus, circulating ovarian hormones likely play a large role in how drugs of abuse affect synaptic plasticity. Through altering baseline spine density, ovarian hormones can modulate how the reward system responds to drugs of abuse.

Studies of gonadal hormones and spine density in male animals show a similar trend. Similar to OVX females, castrated males have decreased spine density within the hippocampus. Specifically, evidence consistently indicates that castration decreases spine density in the CA1 region of the hippocampus and the medial preoptic area, an effect that can be reversed with testosterone or dihydrotestosterone (DHT) replacement (Garelick &

Swann, 2014; Harley et al., 2000; Hatanaka et al., 2015; Kovacs et al., 2003; Leranth et al., 2003). Again, these results indicate a baseline effect of gonadal hormones on synaptic plasticity in the brain. Within the reward system specifically, it seems that testosterone also influences spine density.

The research on spine density in the NAc also shows striking similarities in males and females. Testosterone treatment to adolescents significantly decreases spine density in the NAc shell, as does DHT treatment to castrated adults (Gross et al., 2018; Wallin-Miller et al., 2016). Though the work on gonadal hormones and spine density in the mesolimbic reward system is limited, the existing research shows that testosterone and estradiol likely decrease spine density in the NAc. As changes to neuronal morphology influence drug use (Kalivas, 2009), it is plausible that gonadal hormone-induced adjustments to spine density underlie sex differences in SUD. Interactions between gonadal hormones and drugs of abuse likely exist to modulate synaptic plasticity in various manners depending on the hormones and drugs that are onboard.

The influence of ovarian hormones on spine density appears to be dependent on glutamate activity. The estradiol-induced alteration of spine density in CA1 pyramidal cells occurs through a NMDA-dependent mechanism (Woolley & McEwen, 1994). Additionally, ovarian hormones alter glutamate receptor binding in the frontal cortex, hippocampus, striatum, and NAc (Cyr et al., 2001). Further, estradiol-induced decreases in spine density in the NAc core can be blocked with mGluR5 antagonists (Peterson et al., 2015). Estrogen receptor alpha ($ER\alpha$) functionally couples with mGluR5, which mediates the effects of estradiol on dendritic spine plasticity in the striatum (Grove-Strawser et al., 2010). Cocaine-induced impairments of LTD are likely due to downregulated mGluR5

(Fourgeaud et al., 2004; Knackstedt, Melendez, et al., 2010; Moussawi et al., 2009) and mGluR5 activation is essential for the actions of estradiol on cocaine-induced behavioral sensitization and self-administration (Martinez et al., 2014, 2016).

Together, these data indicate that estradiol interacts with glutamate to produce the behavioral effects of cocaine. mGluR5 is also involved in opioid reward to some degree, as mGluR5 antagonists inhibit the acquisition and expression of morphine conditioned place preference (Popik & Wrobel, 2002). However, downregulated mGluR2/3 drives opioid-induced impairments to LTD, not mGluR5 (Z. Dong et al., 2007; Robbe et al., 2002). This indicates that interactions amongst opioids, synaptic plasticity, and gonadal hormones likely take a different form than that of cocaine. Given this, it seems likely that differing interactions exist amongst different drugs of abuse, gonadal hormones, and synaptic plasticity that may nonetheless create overlapping behavioral sex differences in SUD.

The role of glutamate in testicular hormone-induced changes in spine density is less clear. It is possible the effects are also glutamate-dependent, as DHT-induced decreases in spine density in the NAc shell similarly depend on mGluR5 activity (Gross et al., 2018). However, developmental increases in hypothalamic spinophilin, a protein that positively correlates with number of dendritic spines, are blocked with an AMPA/kainate antagonist in females but not males (Todd et al., 2007). Therefore, gonadal hormones may similarly modulate spine density in males and females, but through different mechanisms. Given the capability of opioids, cocaine, and gonadal hormones to affect spine density in reward-related brain regions, estradiol and testosterone could modulate synaptic plasticity in a way that affects responses to drugs of abuse. An interaction between drugs of abuse and gonadal

hormones might induce sex-specific plasticity in response to cocaine or opioids that might underlie the behavioral sex differences seen in SUD.

Conclusion

Though there are FDA approved treatments for opioid use disorder, many are limited in efficacy and there are none currently approved to treat cocaine use disorder. As there are sex differences in treatment outcomes, these therapies are likely limited by factors related to biological sex. Males and females do not develop and experience cocaine and opioid use disorder in the same manner. As females are more vulnerable to many aspects of SUD, biological sex is an important factor to consider in the creation of new pharmacotherapies. Better understanding of the mechanisms driving behavioral sex differences in SUD will ideally lead to more targeted, and thus more effective, pharmaceutical treatments for SUD.

Female vulnerability to cocaine abuse appears to be driven heavily by both organizational and activational effects of ovarian hormones. Fluctuating ovarian hormones throughout the estrous cycle contribute to the enhanced sensitivity to the reinforcing effects of cocaine seen in females (Kippin et al., 2005; Lacy et al., 2016; Lynch, 2008). OVX blunts behavioral responses to cocaine which can be reversed with estradiol replacement (Hu et al., 2004; Jackson et al., 2006; Lynch et al., 2001; Parylak et al., 2008; Russo et al., 2003; Sircar & Kim, 1999; Walker et al., 2001). Additionally, testosterone appears to be somewhat protective against the behavioral effects of cocaine in males (R. Chen et al., 2003; Chin et al., 2002). Overall, the behavioral sex differences in cocaine use and abuse appear to be driven predominately by gonadal hormones.

Though similar results have been shown in preclinical models of opioid use disorder (Lacy et al., 2016), OVX studies show a spectrum of results which clouds the role of ovarian hormones in opioid use disorder (Mirbaha et al., 2009; Roth et al., 2002; Sedki et al., 2015; Stewart et al., 1996). Additionally, the role of testosterone in opioid use disorder is unknown. Overall, the existing research indicates that sex differences in opioid abuse might not be driven directly by organizational and activational effects of gonadal hormones. However, the research is limited, and much more work needs to be done in order to draw definitive conclusions.

Cocaine and opioids have divergent effects on synaptic plasticity in the form of dendritic spine density. As gonadal hormones also modulate spine density, it is likely there is an interaction amongst gonadal hormones, drugs of abuse, and synaptic plasticity that may take different forms but similarly drive female vulnerability to SUD. Estradiol replacement can decrease spine density in the NAc core (Sanchez et al., 2012). The decrease in spine density depends mGluR5, which functionally couples with ER α (Grove-Strawser et al., 2010). This coupling mediates the effects of estradiol on dendritic spine plasticity in the striatum (Grove-Strawser et al., 2010; Peterson et al., 2015). As mentioned, cocaine-induced impairments to LTD are likely due to downregulated mGluR5 (Fourgeaud et al., 2004; Knackstedt, Melendez, et al., 2010; Moussawi et al., 2009). Furthermore, activation of mGluR5 is essential for the actions of estradiol on cocaine-induced behavioral sensitization and self-administration (Martinez et al., 2014, 2016). This provides a mechanism by which estradiol and cocaine might interact with functionally coupled ER α and mGluR5 to produce the behavioral sex differences in SUD. As opioids and cocaine induce opposing effects on synaptic plasticity, the interactions underlying behavioral sex

differences likely do not take the same form. As evidence of this, opioid-induced impairments to LTD are not driven by downregulated mGluR5, but by downregulated mGluR2/3 (Z. Dong et al., 2007; Robbe et al., 2002). This leads to the possibility that opioids act through a separate mGluR2/3 mechanism to drive the behavioral sex differences in SUD.

Differences in synaptic plasticity following cocaine and opioid exposure indicate that the overlapping behavioral effects of cocaine and opioids are likely driven by different mechanisms, possibly through differing glutamate receptors. The lack of effect of ovariectomy in multiple preclinical studies of opioid abuse makes it unlikely that ovarian hormones predominantly drive sex differences in opioid use disorder. Therefore, it seems that behavioral sex differences in SUD are potentially driven by different mechanisms depending on drug class. Ultimately, more work is needed in order to fully elucidate the mechanisms driving behavioral sex differences in opioid use disorder.

CHAPTER 2

Sex Differences in the Medial Prefrontal Cortical Glutamate System

Introduction

The prefrontal cortex (PFC) consists predominantly of pyramidal glutamatergic neurons (Steketee, 2003) and acts as a driver of goal-directed behavior (Szczepanski & Knight, 2014). The medial prefrontal cortex (mPFC) in particular is crucial for reward processing, attention, and memory (Riga et al., 2014). The nature of its role in these processes has made it an interesting target for studies on psychiatric diseases involving dysregulated cognitive processing and motivation. Indeed, dysregulation in the mPFC is consistently implicated in illnesses including anxiety, depression, and substance use disorder (SUD) (Hare & Duman, 2020; Jasinska et al., 2015; Klenowski, 2018; Peters et al., 2009; Riga et al., 2014; Xu et al., 2019). While the specific mechanisms driving various disease states differ, the mPFC is an important contributor to the presentation of these illnesses.

Imaging studies indicate that depressed patients have reduced mPFC volume compared to healthy control subjects (Belleau et al., 2019; Kang et al., 2012). Further, it is proposed that individuals with generalized anxiety disorder may have elevated activation in the mPFC (Shin & Liberzon, 2010). Additionally, smokers exposed to smoking-related cues exhibit increased activation in mPFC subregions, an effect that is modulated by smoking expectancy (Bolla et al., 2003; McBride et al., 2006). There is also evidence that altering mPFC activity can impact symptomology in clinical populations. Continuous theta burst stimulation delivered to portions of the mPFC decreases drug cue reactivity in cocaine and heavy alcohol users and reduces craving in cocaine users (Hanlon et al., 2015;

Kearney-Ramos et al., 2018). Altogether, these data indicate that the mPFC is an important contributor to the clinical presentation of psychiatric illnesses such as depression, anxiety, and SUD.

Biological sex was traditionally ignored as a variable in these illnesses (Greenfield et al., 2007; Mamlouk et al., 2020). Despite this fact, there are notable sex differences emerging in the prevalence and presentation of disorders associated with mPFC dysfunction. Rates of depression and anxiety are higher in women than men (Altemus et al., 2014; Bangasser & Cuarenta, 2021; Donner & Lowry, 2013). The age of depression and anxiety onset is lower in females, and depressive episodes last longer and occur more frequently in women than men (Simon et al., 2006; Sramek et al., 2016). There are established sex differences in SUD as well, with men being diagnosed more frequently but women being more prone to drug craving (Back et al., 2011; McHugh et al., 2018). Additionally, women relapse to drug use more readily than men, and men have longer periods of abstinence than women (Becker et al., 2017).

There are also sex differences in treatment efficacy for these illnesses. While there is still no clear consensus, clinical studies show that men and women likely do not respond in the same manner to the different classes of antidepressants (Sramek et al., 2016). For example, some studies show better therapeutic outcomes in women taking selective serotonin reuptake inhibitors (SSRIs) for depression but men have better therapeutic outcomes with the tricyclic antidepressant imipramine (Sramek et al., 2016). However, there is also evidence fluoxetine, an SSRI, can be less effective in treating generalized anxiety disorder in women than men (Simon et al., 2006). There are also emerging sex differences in the treatment outcomes of men and women undergoing buprenorphine

maintenance for opioid use disorder, though more studies are needed (S. Ling et al., 2019). Overall, these data indicate that biological sex likely influences treatment outcomes of psychiatric diseases that involve dysregulation in the PFC.

At baseline, biological sex and estrous cycle can influence electrophysiological properties of neurons within brain regions such as the striatum and PFC (Krentzel & Meitzen, 2018; Pena-Bravo et al., 2019; Proano et al., 2018; Willett et al., 2019). Alterations in glutamate signaling specifically may contribute to these sex differences in psychiatric disorders such as depression, anxiety and SUD (Wickens et al., 2018). Sex differences in levels of glutamate, the brain's most prevalent excitatory neurotransmitter, are seen in several brain regions (Frankfurt et al., 1984). Several sex differences in the glutamatergic system have been observed, including differences in AMPA and NMDA receptor signaling, and differences in long-term potentiation (C. J. Perry et al., 2021; Wickens et al., 2018). However, less is known about baseline sex differences in glutamatergic transmission specifically in the mPFC.

Glutamatergic transmission between the mPFC and other reward structures is implicated in a spectrum of psychiatric illnesses (Xu et al., 2019). As there are known sex differences in psychiatric diseases involving the mPFC, we hypothesized there may be sex differences in glutamatergic transmission within this region that could drive these differences seen clinically. To determine this, we examined baseline sex differences in mPFC glutamate receptor expression and function. Our data indicate there are baseline sex differences in glutamatergic transmission within this region, with females exhibiting enhanced glutamatergic transmission in the mPFC compared to males.

Methods

Subjects. 33 male and female C57Bl/6J mice were bred in house for all experiments. Animals (8 weeks old) were group housed throughout the experiments with food and water available ad libitum. All animals were housed in a temperature- and humidity-controlled animal care facility. Mice had a 12hr light/dark cycle (lights on at 7:00 A.M.). Estrous cycle was not monitored in female animals during the course of these experiments. All procedures were approved by the Temple University Animal Care and Use Committee.

Tissue processing and fractionation. Tissue samples were processed as previously published (Burgdorf et al., 2017). Briefly, bilateral mPFC tissue including the infralimbic and prelimbic regions (anterior-posterior 2.0, lateral +/- 0.5, dorso-vental -1.5 to -3.2) was dissected from 13 animals (7 females, 6 males). Tissue was then homogenized with a Teflon pestle (Pyrex) in 150 μ l ice cold sucrose buffer containing protease and phosphatase inhibitors. Homogenates were spun at 1000 \times g for 10 minutes at 4 $^{\circ}$ C. 40 μ l of supernatant was saved for the total protein lysate fraction and the remainder was spun at 1000 x g for 5 minutes 4 $^{\circ}$ C. The supernatant was then spun at 12000 x g for 20 minutes at 4 $^{\circ}$ C. The pellet was resuspended in 100 μ l ice cold HEPES/EDTA buffer containing protease and phosphatase inhibitors and spun at 12000 x g for 20 minutes at 4 $^{\circ}$ C. The pellet was then resuspended in 100 μ l of HEPES/EDTA buffer containing protease and phosphatase inhibitors and saved as the synaptosomal protein lysate fraction. Protein concentration was measured using a BCA protein assay kit (Thermo Fischer Scientific).

Western blot analysis. 20-30 μ g of protein was run on a 10% SDS-PAGE gel electrophoresis (constant 200 V, 50 minutes). Proteins were transferred to a PDVF membrane (constant 0.3 mA, 3 hours) and transfer efficacy was verified with Ponceau S

staining. Membranes were probed with primary antibodies against GluA1 (Abcam, ab140739, 1:1000), GluA2 (EMD, 07-261, 1:250), and GAPDH (Abcam, ab22555, 1:20000), and a peroxidase-labeled anti-rabbit secondary antibody (Vector, PI-1000, 1:5000). Signal was quantified using ImageJ analysis software (NIH). Protein quantities were normalized to GAPDH as a protein loading control.

Slice preparation. 20 animals (10 females, 10 males) were used for electrophysiology experiments. Mice were decapitated following cervical dislocation. The brain was removed and coronal slices (250 μm) containing the PFC were cut with a Vibratome (VT1000S, Leica Microsystems) in an ice-cold artificial cerebrospinal fluid solution (ACSF), as described previously (Briand et al., 2014). 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 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 \times water-immersion objective.

Electrophysiology. The recording chamber was continuously perfused (1–2 ml/min) with oxygenated ACSF heated to 32 \pm 1°C using an automatic temperature controller (Warner 278 Instruments). Picrotoxin (100 μM) was added to the solution to block GABA receptor mediated currents. Recording pipettes were pulled from borosilicate glass capillaries (World Precision Instruments) to a resistance of 4–7 M Ω when filled with the intracellular solution. All recordings were conducted with a MultiClamp700B amplifier (Molecular Devices). Intracellular solution contained (in mM): 100 CsCH₃O₃S, 50 CsCl, 3 KCl, 0.2 BAPTA, 10 HEPES, 1 MgCl₂, 2.5 phosphocreatine-2Na, 2 Mg-ATP, 0.25 GTP-Tris (pH 7.2–7.3 with CsOH, osmolarity 280–290 mOsm). For rectification experiments,

dl-AP5 (50 μM) was present in the bath and spermine (100 μM) was added to the intracellular solution. 11 cells from 5 female animals and 9 cells from 3 male animals were used to calculate the rectification index. All sEPSC recordings were conducted in whole-cell voltage-clamp mode ($V_h = -70$ mV). Currents were low-pass filtered at 2 kHz and digitized at 20 kHz using a Digidata 1440A acquisition board and pClamp10 software (both from Molecular Devices). Access resistance (10–32 $\text{M}\Omega$) 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. Cell health and viability was determined through the microscope and recording quality by monitoring the leak current. Recordings with an increase in leak currents more than 20% of the initial target currents were discarded. sEPSCs were detected using an automated sliding-template-based algorithm in pClamp10. This method compares the shape of the detected current to that of a template and has been shown to detect events with amplitude of at least 3 times the square deviation of the noise (Clements & Bekkers, 1997). All detected events were verified by visual confirmation of a fast rise time and slower exponential decay to baseline. Mean sEPSC amplitude was analyzed from an average sEPSCs trace computed from a minimum of 100 individual sEPSCs. Mean sEPSC frequencies and inter-event intervals were analyzed from 180-s long trace segments. Evoked responses were triggered by 300 μs constant-current pulses generated by an A310 Accupulser (World Precision Instruments) and delivered at 0.1 Hz via a glass capillary electrode filled with ACSF 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 μ A). 9 cells from 5 female animals and 14 cells from 7 male animals were used for analysis of sEPSC frequency and amplitude. Recordings were taken from cells within layer V of the infralimbic and prelimbic mPFC.

Data Analysis. All analyses were performed using GraphPad Prism 9 software (GraphPad Software). Data were analyzed using two-tailed Student's t-test, two-way ANOVA with Sidak's post hoc tests, or Kolmogorov-Smirnov (K-S) as appropriate. Statistical significance for all tests was set at $\alpha=0.05$. Experimenters were blind to group conditions when analyzing data for all experiments.

Results

Female mice exhibit higher levels of synaptosomal GluA1 and GluA2 expression in the mPFC compared to male mice.

Baseline levels of synaptosomal and total GluA1 and GluA2 in the mPFC were examined using western blotting. We found females have significantly higher synaptosomal expression of GluA1 than males [Fig. 1A; $t(10)=3.237$, $p<0.01$]. This does not extend to total expression of GluA1, as we did not see any significant differences between males and females in this measure [Fig 1B; $t(15)=1.50$, $p=0.15$]. Females also exhibit significantly higher synaptosomal expression of GluA2 than males [Fig 1C; $t(10)=2.351$, $p=0.04$], an effect that does not translate to any significant sex differences in total levels of GluA2 [Fig 1D; $t(11)=2.026$; $p=0.06$].

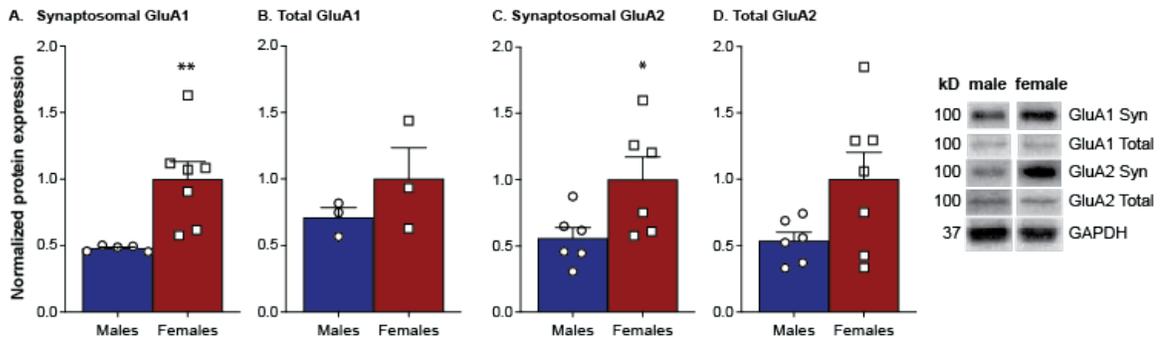


Figure 1. Female mice exhibit higher levels of synaptosomal GluA1 and GluA2 expression in the mPFC compared to male mice. Western blotting revealed higher levels of synaptosomal GluA1 in the mPFC of females compared to males (A; n=5-7/group). However, there are no significant differences between females and males in total levels of GluA1 (B; n=3/group). We also found females exhibit higher levels of synaptosomal GluA2 in the mPFC compared to males (C; n=6/group). Again, these differences are not present in total GluA2, where males and females do not exhibit significant differences (D; n=6-7/group).

Female mice have enhanced glutamatergic transmission in the mPFC compared to male mice.

Baseline glutamate transmission within the mPFC was examined using whole-cell patch clamp recordings. Recordings from female mice revealed significantly higher sEPSC amplitude than males which is further reflected in a rightward shift of the cumulative probability curve [Fig. 2A; $t(21)=2.39$, $p=0.027$; Fig. 2B; $p < 0.001$, K-S test]. Females also exhibit significantly higher sEPSC frequency than males, further reflected by a leftward shift in the cumulative probability of inter-event intervals (IEIs) [Fig. 2C; $t(21)=4.49$, $p=0.0002$; Fig. 2D; $p < 0.0001$, K-S test]. Females also exhibit a significantly larger rectification index than males, indicating females have more inward rectification in the mPFC than males [Fig 2E; $t(18)=2.375$, $p=0.03$].

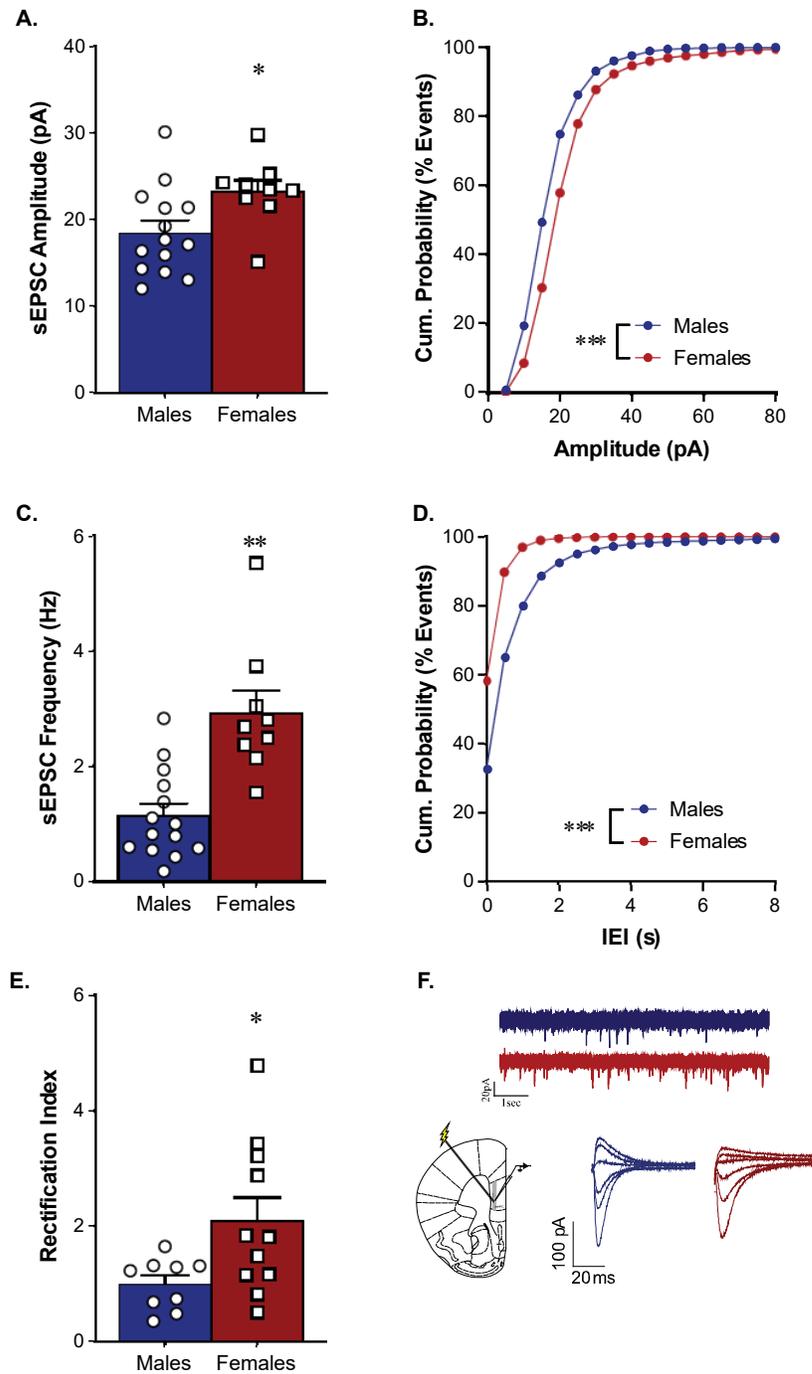


Figure 2. Female mice have enhanced glutamatergic transmission in the mPFC compared to male mice. Whole-cell recordings demonstrate females have heightened sEPSC amplitude (A; n=9-14/group) and a rightward shift in the cumulative probability distribution (B) compared to males. Females also exhibit heightened sEPSC frequency (C; n=9-14/group) and a leftward shift in the cumulative probability distribution of inter-event intervals (D) compared to males. Females also exhibit a larger rectification index compared to males (E; n=9-11/group). Example electrode placement in the mPFC and representative traces for sEPSC and rectification recordings (F).

Discussion

Despite established sex differences in the prevalence and presentation of various psychiatric disorders, little is known about the mechanisms driving these differences. The mPFC is an important contributor to psychiatric diseases such as depression, anxiety, and SUD (Xu et al., 2019), all of which have significant sex differences in clinical presentation. The majority of neurons in the PFC are pyramidal glutamatergic projections (Steketee, 2003). As glutamatergic transmission within the mPFC is implicated in these diseases (Xu et al., 2019), we investigated whether there could be baseline sex differences in glutamatergic transmission in this region that may underline sex differences in psychiatric disease. Our results demonstrate there is indeed a sex difference in the mPFC, where females exhibit heightened glutamatergic transmission compared to males.

AMPA receptors are the main source of fast excitatory transmission in the central nervous system. There are 4 AMPAR subunits (GluA1-4) that form homo- or heteromers (Henley & Wilkinson, 2013; Traynelis et al., 2010). The various AMPAR subunits are involved in many of the diseases that involve glutamate dysregulation (Chang et al., 2012; Henley & Wilkinson, 2013; Kamalova & Nakagawa, 2021; Loweth et al., 2014). We found females exhibit higher levels of synaptosomal GluA1 and GluA2 in the mPFC compared to males. We do not see any statistically significant sex differences in total GluA1 or GluA2 expression. This would suggest that rather than an overall difference in expression of these subunits, there is greater synaptic AMPA subunit expression. However, there were trends towards higher total protein levels in females suggesting the effects may not be isolated to the synaptosome. Greater synaptic expression of AMPAR subunits highlights the

possibility that females have enhanced glutamatergic transmission within the mPFC compared to males.

Functional differences between GluA1-4 are well-established, with the subunits exhibiting different kinetic properties and distinct roles in synaptic plasticity (Derkach et al., 2007; Toyoda et al., 2009; Wright & Vissel, 2012). GluA1 homomers are inwardly rectifying and are proposed to have greater conductance than GluA2-containing heteromers (Benke & Traynelis, 2019; Guire et al., 2008; Oh & Derkach, 2005; Song & Huganir, 2002; Wolf & Ferrario, 2010). We found that females exhibit a larger rectification index in the mPFC than males. The calculated rectification index in females is also greater than 1, indicating there is more inward rectification in females compared to males. Under our recording conditions, this change in rectification indicates a change in CP-AMPARs. Combined with the kinetic properties and heightened synaptosomal expression of GluA1, we propose this indicates there are more synaptic CP-AMPARs in the mPFC of females compared to males. However, we do see increases in synaptosomal GluA2 along with GluA1, which may suggest overall increases in AMPARs rather than specific increases in CP-AMPARs. As synaptosomal preparations include both membrane bound receptors and intracellular pools, the rectification index measurements more accurately reflect functional differences at the synapse. Overall, these data show there are baseline sex differences in AMPAR distribution within the mPFC. As the calcium-permeable, GluA1 homomers have even higher conductance, this further supports that female mice exhibit greater AMPA transmission than male mice in within this region.

An increased contribution of GluA2-lacking AMPARs is indicative of increased excitatory synaptic strength (S. R. Chen et al., 2013). Therefore, we investigated whether

there are sex differences in excitatory transmission as measured by sEPSC frequency and amplitude. We found that females have a higher sEPSC frequency and larger amplitude in the mPFC compared to males. sEPSC frequency is generally regarded as a measure of presynaptic glutamatergic transmission and amplitude as a measure of postsynaptic glutamatergic transmission. Therefore, the heightened sEPSC frequency and amplitude values we see in females compared to males suggest sex differences in both pre- and postsynaptic glutamate transmission with the mPFC. Overall, our data indicate females have heightened excitatory AMPA transmission in this region that may underlie sex differences in psychiatric disease.

While we uncovered sex differences in excitatory transmission in layer V of the PFC, previously published data demonstrate conflicting findings. In layers V and VI of the prelimbic PFC, males exhibit higher sEPSC amplitudes than females and there were no sex differences seen in sEPSC frequency (Pena-Bravo et al., 2019). The medial PFC is sometimes subdivided into the prelimbic and infralimbic portions and the current study did not differentiate between the prelimbic and infralimbic portions of the mPFC. Therefore, it is possible methodological differences explain this discrepancy. Nonetheless, there are reported aspects of transmission in this region that do not differ between males and females. Maturational trajectories of current-voltage curves, resting membrane potentials, rheobases, mGluR2/3-mediated LTD, and paired pulse ratios in layer V of the PFC are similar between the sexes in rats (Bernabeu et al., 2020). Additionally, field excitatory postsynaptic potentials are similar between the sexes across multiple age groups (Bernabeu et al., 2020). Together, these data indicate that males and females mature similarly in many aspects of synaptic plasticity within the PFC. As our data indicate female mice have

heightened AMPA transmission in this region compared to males, it is likely there are compensatory mechanisms to counteract this difference in transmission.

In line with this hypothesis, the number of action potentials in response to depolarizing steps is lower in adult females than pubescent or juvenile females, an effect of age that is not seen in males (Bernabeu et al., 2020). This recapitulates previously published data demonstrating prepubescent females have enhanced excitability in medium spiny neurons within the striatum compared to males (Dorris et al., 2015). Together, these suggest there may be enhanced excitability in certain regions in the reward system in younger females that decreases over time. As we see heightened AMPAR transmission in the PFC of adult females compared to males, it is possible that age-related decreases in cell excitability in the PFC of females serves to balance these changes in AMPAR transmission. Additionally, we focused on AMPAR expression and function in the current studies, however there may be sex differences in other glutamate receptor subtypes, such as metabotropic glutamate receptors (mGluRs) or N-methyl-D-aspartate receptors (NMDARs). Previous work demonstrated females and males exhibit similar levels of mGluR2/3, mGluR1, and NR2B in the PFC but females exhibit higher levels of mGluR5 and NR1 than males (Wang et al., 2015). Further work could investigate possible sex-specific roles of these receptors in glutamate transmission and cell excitability within the PFC.

Our data indicate there are sex differences in AMPAR expression and function within the mPFC. Aberrant AMPAR expression is thought to underlie a multitude of neuropsychiatric diseases (H. Zhang & Bramham, 2020). For example, enhanced AMPAR transmission in the nucleus accumbens is proposed to drive cocaine reinstatement and

incubation of cocaine craving (Briand et al., 2016; Conrad et al., 2008). As diseases such as SUD have known sex differences in presentation (Agabio et al., 2016; Back et al., 2011; McHugh et al., 2018; Rehm & Imtiaz, 2016), it is possible the sex differences we see in excitatory transmission within the mPFC underlie some of the sex differences seen in diseases such as depression, anxiety, and SUD. Gonadal hormones in both sexes modulate synaptic plasticity in the reward system (Hyer et al., 2018; Knouse & Briand, 2021; Krentzel et al., 2022; Meitzen et al., 2018). As we did not track estrous cycle stage in females in these studies, it is possible the effects we see on excitatory transmission may change with natural fluctuations in gonadal hormone levels. Overall, our data indicate there are baseline sex differences in glutamate transmission that may influence the effectiveness of pharmacotherapies aimed at treating a variety of psychiatric disorders.

CHAPTER 3

Sex Differences in Pre- and Postsynaptic Glutamate Signaling in the Nucleus Accumbens Core

Introduction

Glutamate, the main excitatory neurotransmitter in the brain, is involved in many aspects of the substance use disorder (SUD) cycle (Chiamulera et al., 2021; Duncan & Lawrence, 2012; Kalivas, 2000, 2004, 2009; Kalivas et al., 2005, 2009; Kenny & Markou, 2004; Lapish et al., 2006; Loweth et al., 2013; Márquez et al., 2017; Reissner & Kalivas, 2010; Schmidt & Pierce, 2010; Scofield et al., 2016; Spencer et al., 2016; Wolf & Ferrario, 2010). While sex differences in substance use disorder are well-established (Becker et al., 2017; Becker & Hu, 2008; Becker & Koob, 2016; S. A. M. Bobzean et al., 2014; Fattore et al., 2008; Quigley et al., 2021), less is known about how excitatory signaling differs between the sexes. Sex differences in glutamatergic transmission do exist, with region-specific expression of glutamate receptors and glutamate levels (L. Giacometti & Barker, 2020; C. J. Perry et al., 2021; Wickens et al., 2018). Further, we previously demonstrated females exhibit heightened glutamatergic transmission in the medial prefrontal cortex (mPFC) compared to males (Knouse et al., 2022). Given the established sex differences in SUD, it is plausible that baseline differences in glutamatergic transmission between males and females underlie behavioral differences seen during the SUD cycle.

The nucleus accumbens (NAc), a brain region within the striatum, plays a critical role in drug-taking and seeking. The NAc is part of the mesocorticolimbic reward system, receiving glutamatergic input from the prefrontal cortex, amygdala, medial thalamus, and hippocampus. There are sex differences in excitatory transmission in the NAc, with prior work showing that distal dendritic spine density and the proportion of large spines on

medium spiny neurons (MSNs) in the NAc are greater in females (Forlano & Woolley, 2010; Wissman et al., 2011) and miniature excitatory postsynaptic current (mEPSC) frequency is increased in prepubertal female MSNs in the core region of the NAc (Cao et al., 2016). Overall, these data indicate that sex differences in glutamate signaling exist within reward circuitry and females may have heightened glutamatergic transmission in the NAc compared to males.

Trafficking of glutamatergic AMPA receptors (AMPA) specifically underlies reward-driven behaviors such as drug use (Malenka, 2003; Malinow & Malenka, 2002). PKM ζ , a constitutively active isoform of Protein Kinase C, is an AMPAR trafficking protein that potentiates NSF-mediated insertion of GluA2-containing AMPARs to the cell membrane (Yao et al., 2008). This makes PKM ζ an interesting target for studies on the synaptic plasticity underlying learning, memory, and drug use. Previously published data demonstrate PKM ζ facilitates memory formation and preservation, potentially through an involvement in long-term potentiation (LTP). PKM ζ levels increase during LTP maintenance and it proposed to be sufficient to maintain LTP (D. S. F. Ling et al., 2002; Sacktor et al., 1993). Further, PKM ζ works to dampen drug-taking behavior. PKM ζ knockout enhances ethanol consumption in male mice and cocaine-taking and seeking in both sexes (A. M. Lee et al., 2014; McGrath et al., 2018). Interestingly, site-specific PKM ζ knockout in the NAc enhances cocaine-taking and seeking exclusively in male animals (McGrath et al., 2018). Together, these studies indicate PKM ζ influences reward-driven behaviors and may play a sex-specific role in the NAc. Therefore, further characterization of the role of PKM ζ in synaptic plasticity within the reward system will aid in our understanding in the mechanisms that may drive behavioral sex differences in SUD.

The aim of these experiments was to better characterize baseline sex differences in glutamatergic signaling in the NAc. We found significant sex differences in multiple electrophysiological measures indicating excitatory transmission works differently in males and females. In combination with our previous findings in the medial prefrontal cortex (Knouse et al., 2022), these data suggest there are functional sex differences at many levels within the mesocorticolimbic reward system. Gaining a better understanding of these differences could provide insight into sex-specific treatments for disorders involving dysregulated motivation and reward behavior.

Methods

Subjects. Wildtype studies: male and female Long-Evans rats and C57Bl/6J mice were bred in house for electrophysiology experiments. Constitutive PKM ζ deletion: the current study used PKM ζ knockout mice as described previously (Volk et al., 2013). Heterozygous PKM ζ KO mice on a C57BL/6J background were mated resulting in mutant and wildtype littermates. Animals (8 weeks old) were group housed throughout the experiments with food and water available *ad libitum*. All animals were housed in a temperature- and humidity-controlled animal care facility. Mice had a 12hr light/dark cycle (lights on at 7:00 A.M.) and rats had a 12hr light/dark cycle (lights off at 8:30 A.M.). All procedures were approved by the Temple University Animal Care and Use Committee.

Slice preparation. Mice were decapitated following cervical dislocation and rats were decapitated following isoflurane anesthesia. The brain was removed and coronal slices (250 μ m for mice, 300 μ m for rats) containing the nucleus accumbens 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 (in mM): 128.2 NaCl, 2.9 KCl, 1.2 MgSO₄·7H₂O, 1.25 NaH₂PO₄, 28.8 NaHCO₃, 2 CaCl₂, 10 glucose (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 transferred to the recording chamber. The osmolarity of all 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.

Electrophysiology. The recording chamber was continuously perfused (1–2 ml/min) with oxygenated ACSF heated to 32±1°C using an automatic temperature controller (Warner 278 Instruments). Picrotoxin (100 µM) was added to all solutions to block the GABA_A 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 1–2 MΩ when filled with extracellular solution (field recordings). All recordings were conducted with a MultiClamp700B amplifier (Molecular Devices). *Whole-cell recordings.* Intracellular solution contained (in mM): 100 CsCH₃O₃S, 50 CsCl, 3 KCl, 0.2 BAPTA, 10 HEPES, 1 MgCl₂, 2.5 phosphocreatine-2Na, 2 Mg-ATP, 0.25 GTP-Tris pH 7.2–7.3 with CsOH, osmolarity 280–290 mOsm). For all measures, cells from at least 3 animals, within each group, were used. Recordings were taken from cells within the nucleus accumbens core. *sEPSCs.* Recordings were conducted in whole-cell voltage-clamp mode (V_h = –70 mV). Currents were low-pass filtered at 2 kHz and digitized at 20 kHz using a Digidata 1440A acquisition board and pClamp10 software (both from Molecular Devices). Access resistance (10–32 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. This method compares the shape of the detected current to that of a template and has been shown to detect events with amplitude of at least 3 times the square deviation of the noise (Clements & Bekkers, 1997). All detected events were verified by visual confirmation of a fast rise time and slower exponential decay to baseline. Mean sEPSC amplitude was analyzed from an average sEPSCs trace computed from a minimum of 150 individual sEPSCs. Mean sEPSC frequencies were analyzed from 180-s long trace segments. Evoked responses were triggered by 300 μ s constant-current pulses generated by an A310 Accupulser (World Precision Instruments) and delivered at 0.1 Hz via a glass capillary electrode filled with ACSF. The stimulation electrode was positioned within 100 μ m 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 μ A). *AMPA/NMDA ratio*. 1 mM QX-314 was added to intracellular solution for these recordings. Current ratios were computed by dividing the mean peak sEPSC at -70 mV (AMPA-mediated) by the mean amplitude at +40 mV, 35 ms after the peak over a 2 ms window (NMDA-mediated). *Readily releasable pool*. After obtaining a stable baseline at -70 mV, a 100 Hz train was applied for 10 s. EPSC amplitudes were measured by subtracting the baseline current just preceding an EPSC from the subsequent peak of the EPSC. For the RRPtrain technique, EPSC amplitudes were then summed throughout the train stimulus to give a cumulative EPSC curve. A straight line was fitted to the final 15 points of the cumulative EPSC and back-extrapolated to the y-axis. The y-intercept corresponds to RRPtrain, and $p_{train} = EPSC_0/RRP_{train}$. *Field Recordings*. A

glass capillary electrode filled with ACSF was placed within 100–300 μm from the recording electrode and used to stimulate excitatory afferents at 0.1 Hz. The field recordings were performed within the nucleus accumbens core. *Long-term depression.* The amplitude of current pulses was set at the intensity required to evoke a 70% maximal response. After 10 minutes of stable responding, LTD was induced using a paired-pulse protocol (50 ms inter-pulse interval) consisting of a 1 Hz train of paired stimuli for 5 or 10 min. Both the field EPSP (fEPSP) slope (calculated over 1 ms after peak) and fEPSP amplitude were measured (graphs depict slope) from fEPSPs recorded at 0.05 Hz for 60 minutes following the pairing protocol.

Data Analysis. All analyses were performed using GraphPad Prism 9.0 software (GraphPad Software). Data were analyzed using two-tailed Student's t-test or two-way ANOVA with Sidak's post hoc tests as appropriate. Statistical significance for all tests was set at $\alpha = 0.05$.

Results

Females have a heightened AMPA/NMDA ratio within the nucleus accumbens core compared to males.

We examined sEPSC amplitude and frequency and AMPA/NMDA ratio within the NAc core of naïve adult male and female Long-Evans rats. We did not see any significant differences between male and female rats in sEPSC amplitude or frequency ($t(21) = 0.2223$, $p = .8262$, $n = 11-12/\text{group}$, Fig. 3A; $t(20) = .8497$, $p = .4055$, $n = 11/\text{group}$, Fig. 3B). There was, however, a significant difference in AMPA/NMDA ratio between females and males, with females exhibiting a higher ratio ($t(28) = 2.814$, $p = .0088$, $n = 13-17/\text{group}$; Fig. 3C;). To determine whether these differences in glutamate signaling were present across multiple

species, we examined sEPSCs and AMPA/NMDA ratio in naïve male and female C57BL/6J mice. We found similar results, with no significant differences between males and females in sEPSC amplitude or frequency ($t(41) = .5078$, $p = .6143$, $n = 19-24/\text{group}$, Fig. 4A; $t(39) = .2768$, $p = .7834$, $n = 19-22/\text{group}$, Fig. 4B) but a significant difference in AMPA/NMDA ratio ($t(27) = 2.210$, $p = .0358$, $n = 12-17/\text{group}$; Fig. 4C).

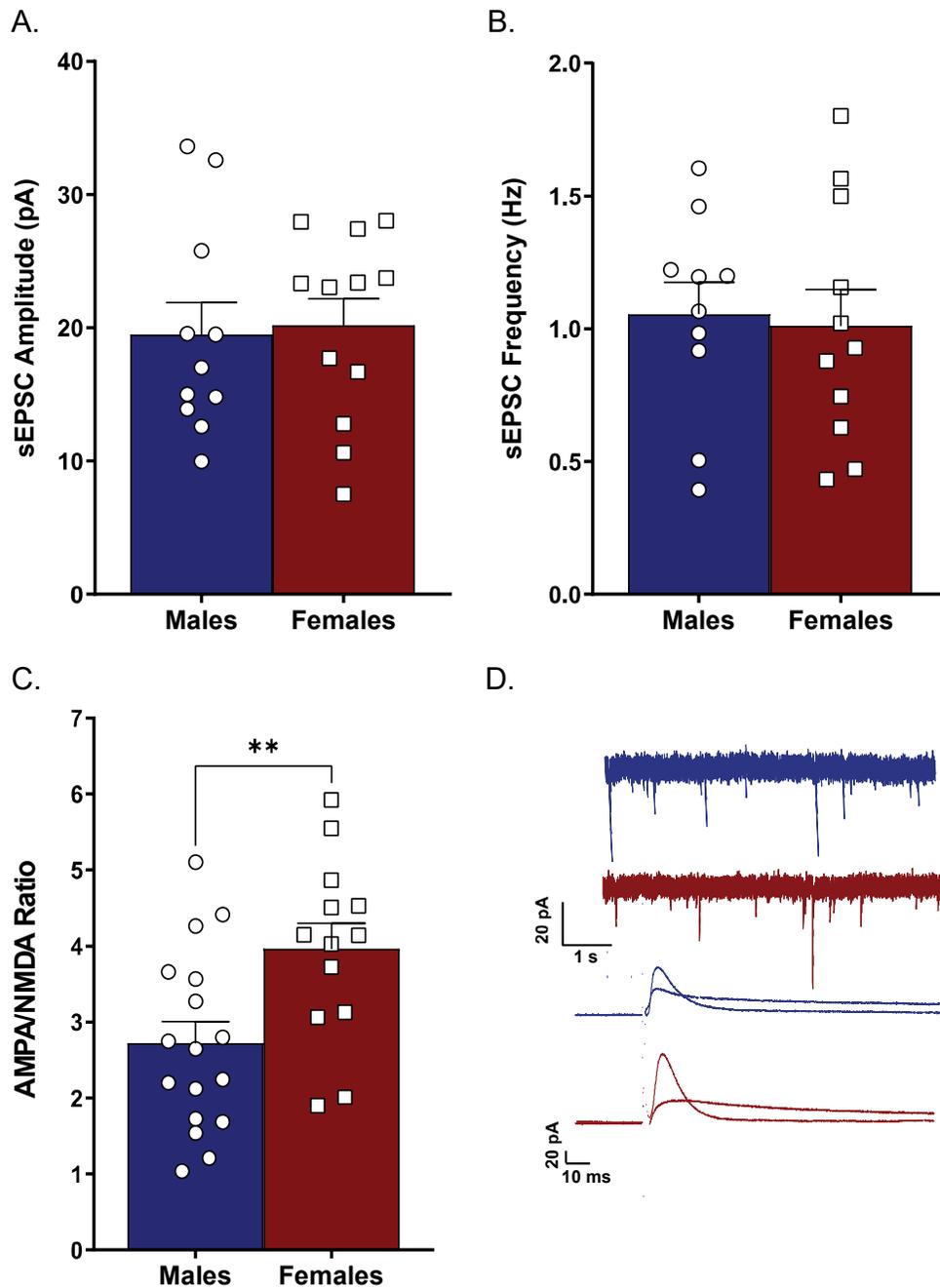


Figure 3. AMPA/NMDA ratio in the nucleus accumbens core is higher in female Long-Evans rats compared to male rats.

Whole-cell recordings demonstrated no significant effect of sex on sEPSC amplitude (A; n=11-12/group) or frequency (B; n=10-11/group). Female rats have a significantly higher AMPA/NMDA ratio in this region than male rats (C; n=13-17/group). Representative traces for sEPSC recordings and AMPA and NMDA currents (D). **p<.01 effect of biological sex.

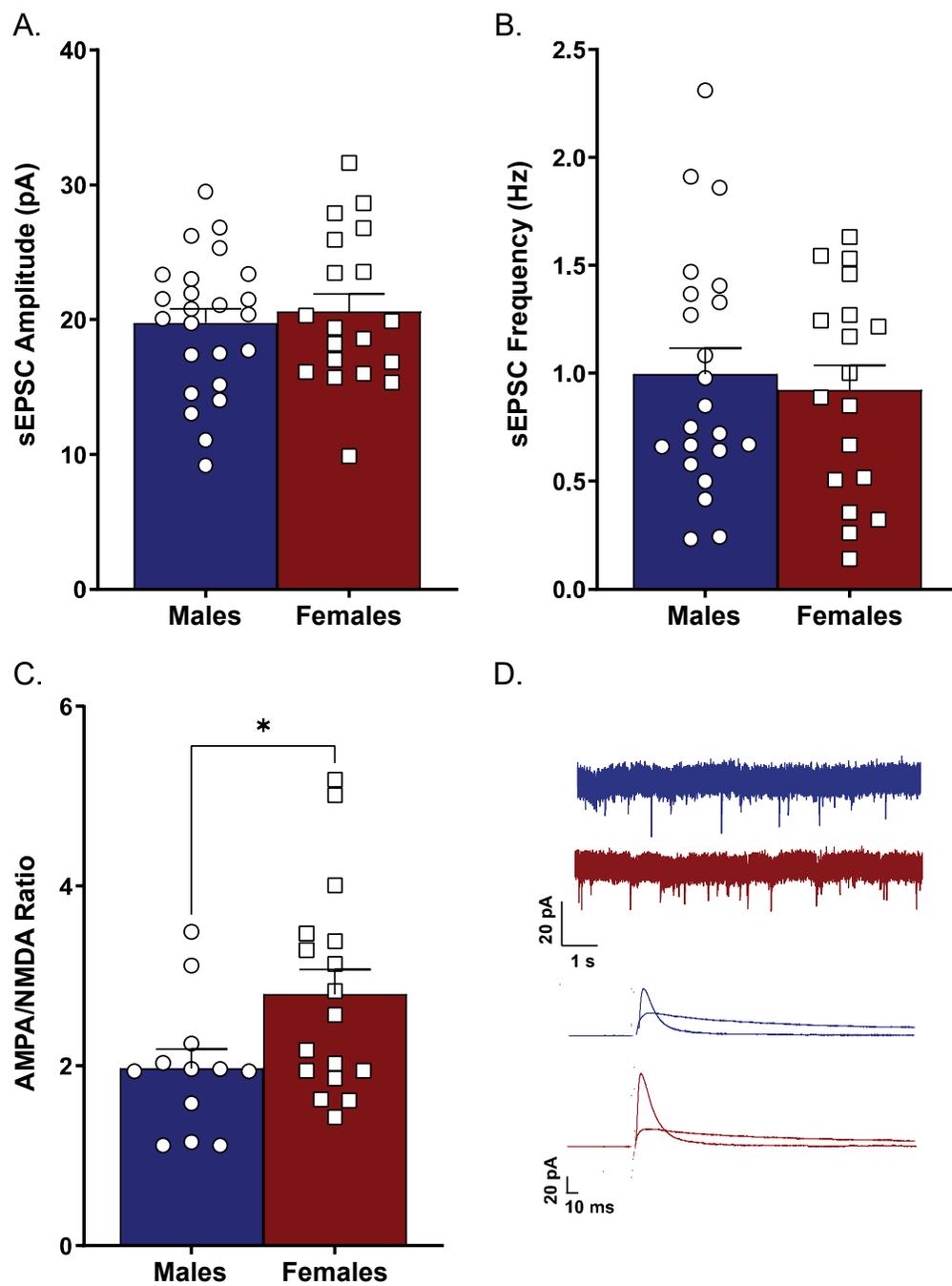


Figure 4. AMPA/NMDA ratio in the nucleus accumbens core is higher in female C57Bl6/J mice compared to male mice.

Whole-cell recordings demonstrated no significant effect of sex on sEPSC amplitude (A; n=19-24/group) or frequency (B; n=18-22/group). Female mice have a significantly higher AMPA/NMDA ratio in this region than male mice (C; n=12-17/group). Representative traces for sEPSC recordings and AMPA and NMDA currents (D). * $p < .05$ effect of biological sex.

Females have a larger readily releasable pool of glutamate in the nucleus accumbens core compared to males.

To determine whether the postsynaptic alterations in glutamate transmission were accompanied by differences in presynaptic glutamate transmission we examined the size of the readily releasable pool (RRP) of glutamate in naïve male and female mice. Cumulative EPSC data following the 100 Hz stimulation train reveal a greater response in females compared to males [main effect of sex, $F(1,11)=5.97$, $p=.033$, $n=6-7$ /group, Fig. 5A]. An analysis of the RRP size revealed that females have a significantly larger readily releasable pool of glutamate compared to males [$t(10)=2.394$, $p=.0377$, $n=5-7$ /group, Fig. 5B]. This increase is accompanied by a decrease in release probability, as evidenced by the significantly lower PTrain value seen in females [$t(10)=2.61$, $p=.026$, $n=5-7$ /group, Fig. 5C].

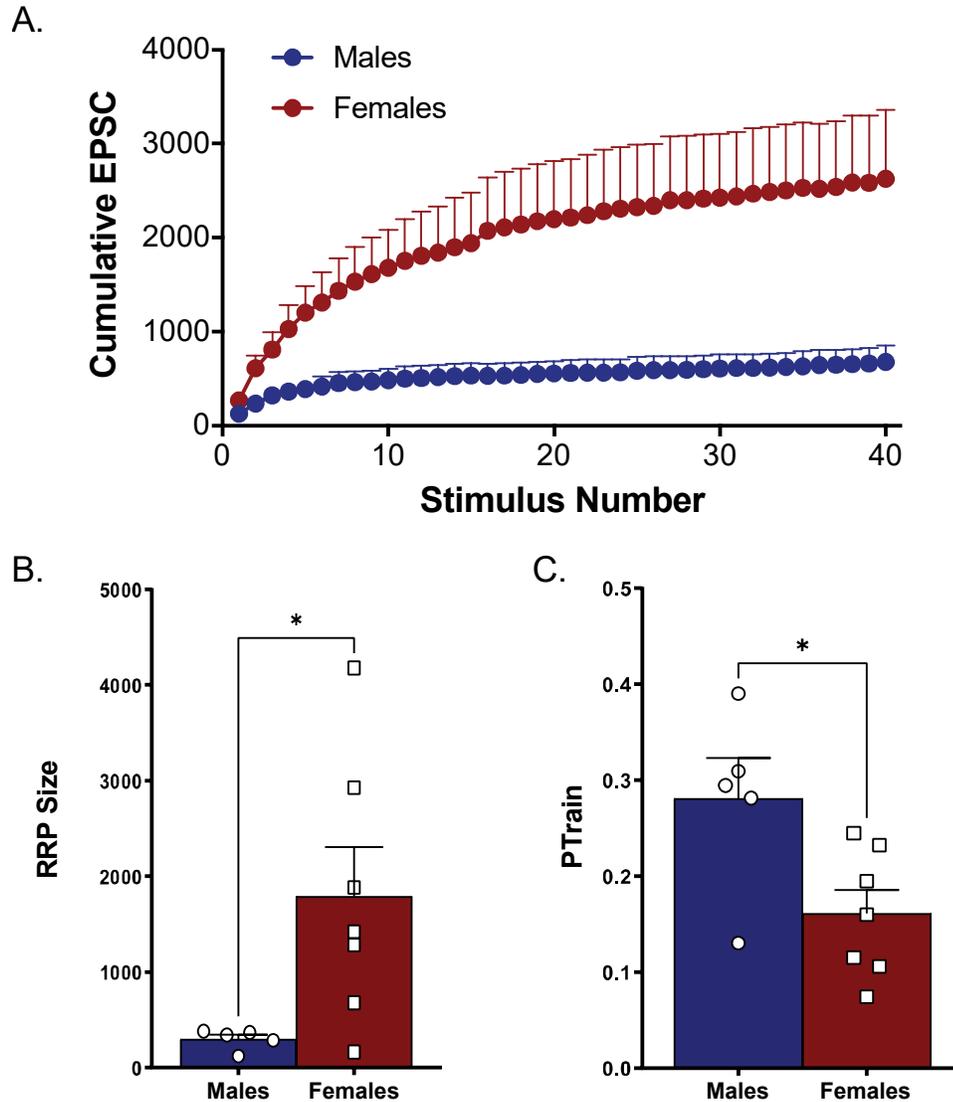


Figure 5. Female mice have a larger readily releasable pool of glutamate and lower release probability in the nucleus accumbens core than male mice.

Cumulative EPSC obtained following a 100 Hz train demonstrates a larger response in females compared to males (A; n=6-7/group). Analysis of the size of the readily releasable pool (RRP) reveals that female mice have a larger RRP of glutamate compared to male mice (B; n=5-7/group). Female mice also exhibit a lower release probability than male mice (C; n=5-7/group). *p<.05 effect of biological sex.

Biological sex affects LTD induction in the nucleus accumbens core.

We next explored whether LTD in the nucleus accumbens core functions similarly in both sexes. Following a 5 minute paired-pulse protocol there is successful LTD induction in male, but not female, mice [$t(22)=3.404$, $p=.003$, $n=12/\text{group}$, Fig. 6A & B]. It is proposed increasing the number of pulses in a LTD protocol can increase the magnitude of depression (Dudek & Bear, 1992). Therefore, we doubled the number of pulses to examine whether a longer protocol induces LTD more effectively in female animals. Following a 10 minute paired-pulse protocol this difference is abolished as we see a similar magnitude of LTD induction in both sexes [$t(40)=.9152$, $p=.366$, $n=20-22/\text{group}$, Fig. 6D & E].

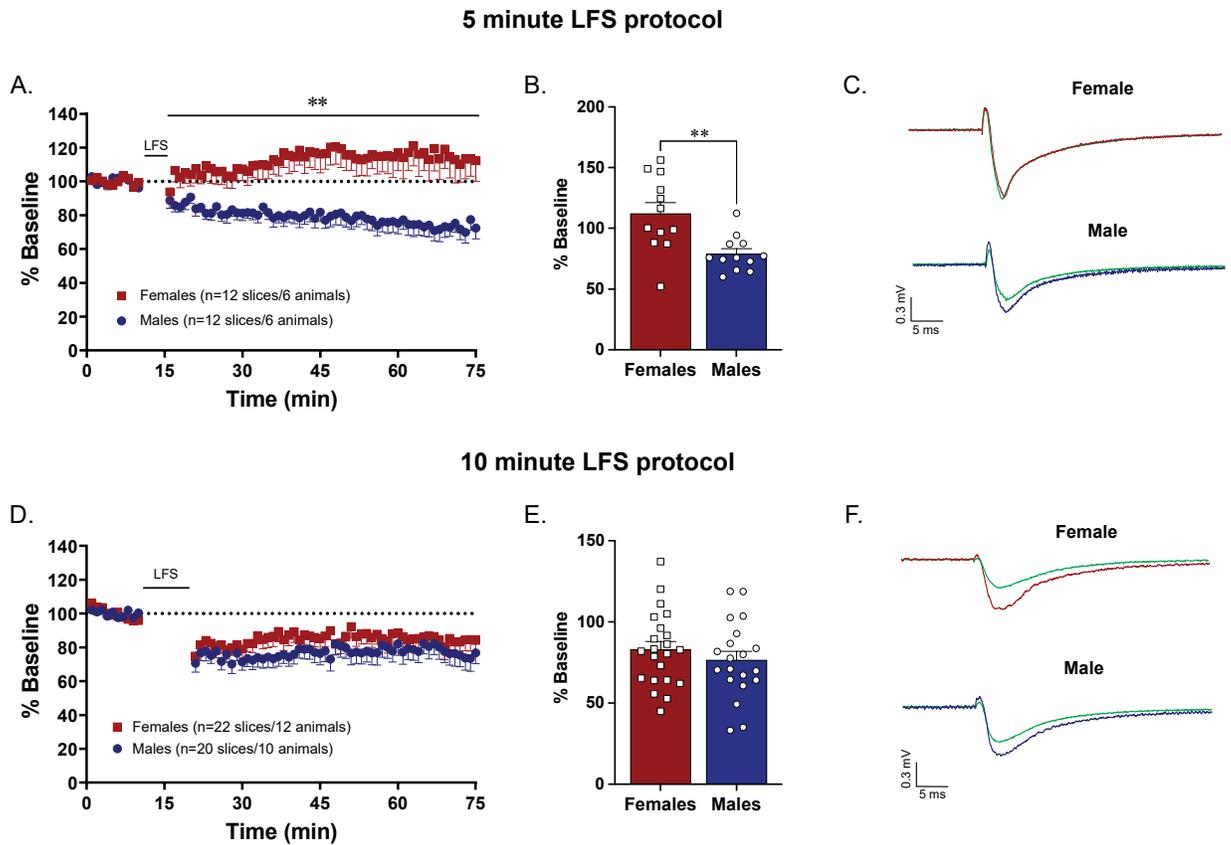


Figure 6. Longer induction protocols are needed to induce LTD in the nucleus accumbens core of female mice compared to male mice.

While a shorter low frequency stimulation protocol (1 Hz train of paired stimuli for 5 minutes) is sufficient to induce LTD in males, it does not induce LTD in females (A; n=12/group). Change in fEPSP slope over 1-hour post-LFS shows a significant blunting of LTD in females compared to males in this protocol (B; n=12/group). In contrast, a longer low frequency stimulation protocol (1 Hz train of paired stimuli for 10 minutes) is sufficient to induce LTD in both sexes (A; n=20-22/group). There is no effect of sex on fEPSP slope over 1-hour post-LFS in this protocol (B; n=20-22/group). Representative pre- and post-LFS traces (C & F). **p<.01 effect of biological sex.

PKM ζ knockout alters LTD in a sex- and protocol-specific manner.

Lastly, we aimed to understand whether PKM ζ plays a role in LTD within the nucleus accumbens. While our 5 minute paired-pulse protocol does not induce LTD in female wildtype animals, it does in female PKM ζ knockout animals [t(23)=2.764, p=.011, n=12-13/group, Fig. 7A & B]. In males we see the opposite effect where LTD induction is blunted in PKM ζ knockout animals [t(20)=2.856, p=.009, n=10-12/group Fig. 7D & E]. We found the differences between genotypes are abolished in both sexes with a 10 minute paired-pulse protocol. There are no significant differences in the magnitude of LTD induction between female wildtype and PKM ζ knockout animals [t(22)=.4305, p=.671, n=10-14/group, Fig. 7G & H] or between male wildtype and PKM ζ knockout animals [t(19)=1.016, p=.323, n=9-12/group, Fig. 7J & K].

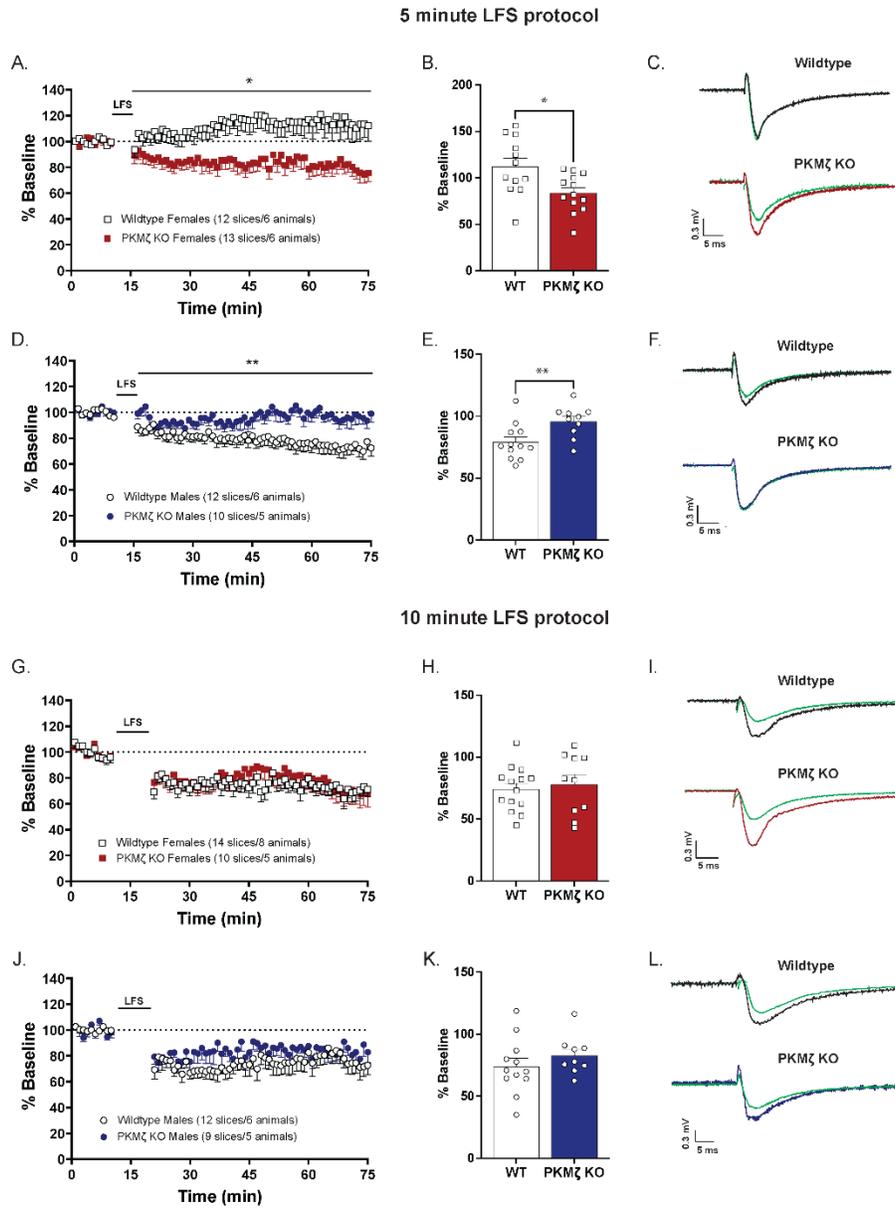


Figure 7. PKM ζ knockout alters LTD in a sex-specific manner.

LTD induction in female mice by a shorter protocol of LFS (1 Hz train of paired stimuli for 5 minutes) is facilitated in PKM ζ knockout animals compared to wildtype controls (A; n=12-13/group). Change in fEPSP slope over 1-hour post-LFS shows PKM ζ knockout facilitates LTD in female mice compared to wildtype controls (B; n=12-13/group). LTD induction in male mice by a shorter protocol of LFS (1 Hz train of paired stimuli for 5 minutes) is blunted in PKM ζ knockout animals compared to wildtype controls (D; n=10-12/group). Change in fEPSP slope over 1-hour post-LFS shows PKM ζ knockout blunts LTD in male mice compared to wildtype controls (E; n=10-12/group). Following the longer LFS induction protocol (1 Hz train of paired stimuli for 10 minutes), there is no

effect of PKM ζ knockout on LTD induction in either male or female mice (G; n=10-14/group; J; n=9-12/group). Change in fEPSP slope over 1-hour post-LFS reveals robust LTD in both wildtype and PKM ζ knockout mice (females: H; n=10-14/group; males: K; n=9-12/group). Representative pre- and post-LFS traces (D, F, I, L). *p<.05; **p<.01 effect of genotype.

Discussion

Sex differences in the prevalence and presentation of substance use disorder are well-established. Despite this fact, the neural mechanisms driving behavioral sex differences in SUD are not fully understood. As glutamate transmission influences the development and presentation of SUD (Chiamulera et al., 2021; Duncan & Lawrence, 2012; Kalivas, 2000, 2004, 2009; Kalivas et al., 2005, 2009; Kenny & Markou, 2004; Lapish et al., 2006; Loweth et al., 2013; Márquez et al., 2017; Reissner & Kalivas, 2010; Schmidt & Pierce, 2010; Scofield et al., 2016; Spencer et al., 2016; Wolf & Ferrario, 2010), we aimed to understand how glutamatergic transmission within the reward system may differ between males and females. Here, we found baseline sex differences in excitatory transmission at multiple levels within the nucleus accumbens. These differences may, in part, underlie some of the well-established behavioral sex differences in SUD.

Sex differences in postsynaptic glutamatergic transmission.

We found in both rats and mice females have a significantly higher AMPA/NMDA ratio in the NAc than males. These data indicate heightened synaptic strength in females, an effect that is replicable across multiple species. AMPAR subunits display different kinetic properties with GluA1 homomers having greater conductance than GluA2-containing heteromers (Benke & Traynelis, 2019; Derkach et al., 2007; Guire et al., 2008; Oh & Derkach, 2005; Song & Huganir, 2002; Toyoda et al., 2009; Wolf & Ferrario, 2010; Wright & Vissel, 2012). As increased excitatory synaptic strength is associated with an

increased contribution of GluA2-lacking AMPARs (S. R. Chen et al., 2013), we propose females may have a heightened contribution of GluA1 AMPARs in the NAc compared to males. While we did not investigate receptor distribution here, there are known sex differences in glutamate receptor expression in the PFC (Ganguly et al., 2019; Knouse et al., 2022; Wang et al., 2015) and surface expression of GluA1 fluctuates during the estrous cycle in the NAc (Bechard et al., 2018). An increased AMPAR contribution in females would drive the heightened AMPA/NMDA ratio we see in both species.

With regards to SUD, the AMPA/NMDA ratio in the NAc is altered following drug use. Chronic cocaine exposure and cocaine and nicotine reinstatement cause an increase in the AMPA/NMDA ratio in the NAc core (Gipson et al., 2013; Moussawi et al., 2011; Spencer et al., 2017). This effect of chronic cocaine is reversed with N-acetylcysteine, a compound that helps restore disrupted glutamate homeostasis (Moussawi et al., 2011). N-acetylcysteine administration also induces lasting reductions in cocaine and heroin reinstatement and seeking (Baker et al., 2003; Kau et al., 2008; Madayag et al., 2007; Moussawi et al., 2011; Zhou & Kalivas, 2008). Therefore, heightened synaptic strength as evidenced by the AMPA/NMDA ratio may drive vulnerability to substance use and relapse. As females are more vulnerable than males to many aspects of SUD (Becker et al., 2017; Becker & Hu, 2008; Becker & Koob, 2016; S. A. M. Bobzean et al., 2014; Fattore et al., 2008; Knouse & Briand, 2021; Quigley et al., 2021), we propose the heightened synaptic strength we found in the NAc of females may, in part, drive female vulnerability to SUD.

While we might expect to see a corresponding sex difference in sEPSC amplitude, we do not. Others have found effects of sex on mEPSC amplitude in adult animals, an effect that is not present in prepubertal animals and is dependent on estrous cycle stage

(Cao et al., 2016; Proano et al., 2018). It is possible there would be effects of estrous cycle stage on sEPSC amplitude that we are not capturing in these studies as we did not track estrus in our animals. Nonetheless, our results are not the first to find an effect on the AMPA/NMDA ratio without a corresponding effect on sEPSC amplitude. In the basolateral amygdala, pubescent male rats have a higher AMPA/NMDA ratio than pubescent females but there is no significant difference in sEPSC amplitude (Guily et al., 2022). In the dorsolateral striatum, high alcohol preference mice exhibit a lower AMPA/NMDA ratio than low alcohol preference mice but there is also no change in sEPSC amplitude (Fritz et al., 2019).

Spontaneous activity is proposed to rely on different mechanisms than evoked activity. As sEPSCs are spontaneous and the AMPA/NMDA ratio is evoked, it is plausible NAc inputs are less active spontaneously and this is why we do not see an effect of sex on sEPSC amplitude. Despite the lack of effect of sex on sEPSC amplitude, our AMPA/NMDA ratio data indicate females have heightened postsynaptic strength in the NAc. This could, in part, underlie some of the behavioral sex differences seen in SUD. In order to further investigate sex differences in the reward system, we also examined measures of presynaptic transmission within the NAc.

Sex differences in presynaptic glutamatergic transmission.

We found significant sex differences in presynaptic glutamatergic transmission across two species in the NAc. First, we found females have a larger readily releasable pool of glutamate than males. Differences in the size of the RRP would lead to a variety of changes in presynaptic transmission, including possible differences in release probability. Therefore, we also examined release probability and found it is lower in females within

this region compared to males. We did not see a corresponding effect on sEPSC frequency. Despite this, our RRP data indicate females may have heightened presynaptic glutamatergic transmission in the NAc.

There are a few explanations for why we see a significant effect of sex on the size of the RRP and release probability but not on sEPSC frequency. First, the size of the RRP in the NAc is projection-specific as the effect of sex we see on overall RRP size is not present in projections specifically from the ventral hippocampus (Deutschmann et al., 2022). While we found an overall sex difference in the size of the RRP, we are not able to determine whether this effect is driven by specific glutamatergic inputs. This indicates another input region to the NAc likely drives the large sex difference we see. Input-specificity may explain why there is an effect of sex on the RRP but not on sEPSC frequency.

It is possible our sEPSC frequency data are also modulated by circulating ovarian hormones, as mEPSC frequency in the NAc core is altered by sex and estrous cycle stage (Cao et al., 2016; Proano et al., 2018). Lastly, sEPSC frequency is also cell-type specific within the NAc. Frequency is significantly higher in D2-containing neurons compared to D1-containing neurons (Ma et al., 2012). Differentiating by D1 vs D2 neurons may elicit effects of sex that we did not capture in these studies. Therefore, our effect of sex on the RRP and release probability but not on sEPSC frequency could be due to the estrous cycle, the specific inputs and cell-types involved, or a combination of these. Nonetheless, our RRP and release probability data indicate there are biological sex differences in presynaptic glutamate transmission within the NAc. These differences may have functional consequences that alter behavioral responses in SUD.

Sex Differences in LTD.

Along with these differences in baseline post- and presynaptic glutamate transmission, we also found sex differences in synaptic plasticity within the NAc. The current study demonstrates that the induction threshold for LTD is higher in females than males, with a shorter paired pulse protocol inducing LTD in males but not females. LTD was induced in females following a longer paired pulse protocol and the extent of the LTD in females was similar to that in males at this stimulation duration. LTD is therefore harder to induce in the NAc of females and requires a more intense stimulation protocol than in males. LTD is a result of depleting the RRP (Bailey & Chen, 1988; Gottmann, 2008; Stanton et al., 2003). Therefore, the larger RRP we found in females likely prevents the induction of LTD at shorter protocols. Longer protocols are required to release enough vesicles to induce LTD. These data indicate heightened glutamatergic activity in the NAc of females also makes it harder to induce alterations to synaptic plasticity.

While sex differences in LTD are largely unexplored, it is established LTD in the hippocampus is modulated by estradiol (Zamani et al., 2000). Most work has focused on long-term potentiation (LTP), however. The mechanisms and expression of LTP are modulated by biological sex. LTP in the hippocampus is significantly influenced by fluctuating hormone levels during the estrous cycle (Gall et al., 2021; Koss & Frick, 2017; Simpson & Kelly, 2012; Yagi & Galea, 2019) and male rats exhibit LTP to a broader range of tetani than females (Yang et al., 2004). Our data further the existing evidence that LTD is susceptible to alterations by biological sex as well. Altogether, our data indicate increased excitatory activity in females blunts LTD induction.

PKM ζ has a sex-specific role in LTD.

Our findings from both the whole-cell and LTD studies clearly suggest sex differences in the glutamate system. We further investigated these differences by examining the effect of PKM ζ knockout on LTD. We found male knockout mice exhibit blunted LTD compared to wildtype controls. The effect of genotype was abolished following a more intense LTD induction protocol. This suggests that PKM ζ knockout increased the induction threshold for LTD in male mice rather than eliminating this form of plasticity altogether.

Trafficking of GluA2 AMPA receptor subunits plays a critical role in LFS-induced LTD (Anwyl, 2006; Malinow & Malenka, 2002; Purkey & Dell'Acqua, 2020). Within the NAc, altering GluA2 trafficking via glutamate receptor-interacting protein (GRIP) knockout abolishes LTD (Briand et al., 2014). GRIP works to stabilize GluA2-containing AMPARs to the synapse, similarly to PKM ζ . PKM ζ potentiates NSF-mediated insertion of GluA2-containing AMPARs into the cell membrane (Yao et al., 2008) and cytosolic levels are decreased in the hippocampus following LTD induction (Hrabetova & Sacktor, 1996). The results found here demonstrate a definitive role for PKM ζ in LTD within the NAc. Our data further the evidence that AMPAR trafficking proteins play a crucial role in LTD.

In contrast to the effect of PKM ζ knockout in males, we found that PKM ζ knockout had the opposite effect in female mice, decreasing the threshold for LTD induction. While the shorter induction protocol did not elicit LTD in wildtype females, it did successfully induce LTD in PKM ζ knockout females. This is the first study to examine LTD in constitutive PKM ζ knockout mice in both sexes. However, previous studies have explored

the effect of constitutive PKM ζ knockout on LTP. Within the hippocampus, PKM ζ knockout animals exhibit normal LTP and there are no effects of sex (Volk et al., 2013). While this suggests that PKM ζ is not necessary for hippocampal LTP, it is not known whether this reflects regional differences in the role of PKM ζ or differences in the mechanisms driving these different forms of plasticity.

We found that PKM ζ knockout alters LTD in a sex-specific manner. There is other evidence PKM ζ activity and expression is sex-specific. Behaviorally, constitutive PKM ζ knockout reduces anxiety-like behavior in males but not females (A. M. Lee et al., 2013) and site-specific deletion of PKM ζ in the NAc potentiates cocaine-taking exclusively in male mice (McGrath et al., 2018). Following exposure to cocaine and methamphetamine there are also sex-specific alterations to PKM ζ expression (Avila et al., 2021; McGrath et al., 2018). Our data indicate PKM ζ also plays a sex-specific role in LTD within the NAc. In addition to sex-specificity, we also found the effect of PKM ζ knockout on LTD to be “dose” specific as the effect of genotype is not apparent following the stronger LTD induction protocol. This further highlights the need to examine multiple induction protocols as sex differences in plasticity may be quantitative rather than qualitative.

Altogether, our data demonstrate there are sex differences in synaptic plasticity at multiple levels within the reward system. As glutamate transmission drives many aspects of SUD (Chiamulera et al., 2021; Duncan & Lawrence, 2012; Kalivas, 2000, 2004, 2009; Kalivas et al., 2005, 2009; Kenny & Markou, 2004; Lapish et al., 2006; Loweth et al., 2013; Márquez et al., 2017; Reissner & Kalivas, 2010; Schmidt & Pierce, 2010; Scofield et al., 2016; Spencer et al., 2016; Wolf & Ferrario, 2010), we propose baseline sex differences in synaptic plasticity within this region may drive some of the behavioral sex

differences in drug use. It is established that gonadal hormones in both sexes can influence both synaptic plasticity and behaviors associated with SUD (Hyer et al., 2018; Knouse & Briand, 2021; Krentzel et al., 2022; Meitzen et al., 2018). Though we did not track estrous cycle in female animals here, it is possible many of the effects we see are mediated by natural hormonal fluctuations. Nonetheless, these data highlight the need for further exploration of baseline sex differences in the mechanisms driving disorders such as SUD. Greater understanding of these mechanisms will allow for the development of more targeted, and ideally more effective, pharmacotherapies to treat diseases such as SUD.

CHAPTER 4

PKM ζ Alters Oxycodone-Taking

Introduction

Altered glutamate transmission underlies many aspects of the substance use disorder (SUD) cycle, with disruptions to glutamate homeostasis driving relapse (Gardner, 2011; Hearing, 2019; Hearing et al., 2018; Heinsbroek et al., 2021; Knackstedt & Kalivas, 2009; Knouse & Briand, 2021; Koob & Volkow, 2016; Kruyer et al., 2020; Roberts-Wolfe & Kalivas, 2015; Ross & Peselow, 2009; Tzschentke & Schmidt, 2003). Trafficking of glutamatergic AMPA receptors (AMPA receptors) specifically underlies learning and drug use (Malenka, 2003; Malinow & Malenka, 2002). PKM ζ , an atypical isoform of Protein Kinase C (PKC), is an AMPAR trafficking protein. It potentiates NSF-mediated insertion of GluA2-containing AMPARs to the cell membrane (Yao et al., 2008). This makes PKM ζ an interesting target for studies on the synaptic plasticity underlying learning, memory, and drug use.

Early studies demonstrated a role for PKM ζ in long-term potentiation (LTP). PKM ζ levels increase during and after LTP induction and it was proposed to be sufficient to maintain LTP (Hsieh et al., 2021; D. S. F. Ling et al., 2002; Sacktor et al., 1993). In learning and memory, PKM ζ levels increase following spatial conditioning and during memory reconsolidation (Bernabo et al., 2021; Hsieh et al., 2021). Further, an infusion of zeta inhibitory peptide (ZIP), a proposed PKM ζ inhibitor, into the hippocampus prevented rats from exhibiting a previously-learned fear response (Pastalkova et al., 2006). Relevant to substance use, ZIP was also shown to block morphine conditioned place preference (Li et

al., 2011). Altogether, these studies demonstrated a role for PKM ζ in memory formation, memory preservation, and learning.

Recently, the specificity of ZIP has been called into question. It likely targets both PKM ζ and another atypical PKC isoform, PKC δ/λ . PKM ζ knockout mice exhibit normal learning, memory, and LTP and ZIP still induces learning and memory deficits in these animals (A. M. Lee et al., 2013; Volk et al., 2013). This makes studies that utilized ZIP to block PKM ζ difficult to interpret. These findings could indicate PKM ζ is not involved in LTP, learning, and memory. Another explanation, however, is that there are compensatory mechanisms at play in PKM ζ knockout mice. As evidence of this, PKC δ/λ is recruited for LTP in animals that lack PKM ζ (Tsokas et al., 2016). Regardless of the controversy surrounding ZIP, PKM ζ does alter spine density.

PKM ζ expression is widespread throughout postsynaptic neurons, including in dendritic spines (Hernández et al., 2014). Overexpression of PKM ζ in cultured cortical neurons reduces spine length and increases the fraction of stubby heads (Ron et al., 2012). This switch in morphology is indicative of stronger, more mature spines. Drug exposure can also alter spine density. Multiple paradigms of morphine exposure decrease dendritic spine density in the nucleus accumbens (NAc) shell and core (Diana et al., 2006; Kasture et al., 2009; Leite-Morris et al., 2014; Robinson et al., 2002; Robinson & Kolb, 1999; Spiga et al., 2005). Cocaine exposure, in contrast, generally increases spine density in the NAc (Dumitriu et al., 2012; Norrholm et al., 2003; Robinson et al., 2001). As PKM ζ can enhance dendritic spine density, we aimed to examine whether PKM ζ knockout would alter drug-taking behaviors.

There is evidence for this as PKM ζ knockout increases ethanol consumption in an intermittent access paradigm in male mice (A. M. Lee et al., 2014). We previously extended these findings to cocaine where we demonstrated PKM ζ knockout potentiates cocaine-taking and seeking in both male and female mice (McGrath et al., 2018). Together, these studies indicate PKM ζ works to dampen drug reward. Alterations to spine density underlie both opioid and cocaine use (Kalivas, 2009). As opioids and cocaine generally have opposing effects on spine density in the NAc, we were interested in exploring how PKM ζ knockout may alter opioid-taking behaviors.

Here, we examined the role of PKM ζ in oxycodone-taking and motivation. We tested two doses of oxycodone and used a progressive ratio paradigm to further our understanding of PKM ζ 's role in opioid use. We found that PKM ζ dampens these oxycodone-taking in both sexes at a moderate dose of oxycodone, but only significantly affects oxycodone-taking in females at a low dose of oxycodone. These data indicate that while PKM ζ does work to blunt opioid-taking and seeking, its role in these processes may be more important for females at lower doses of oxycodone. This highlights the possibility that while PKM ζ does dampen drug reward across multiple drug classes, it does not function in the same manner in both sexes.

Methods

Subjects. The current study utilized male and female PKM ζ knockout (KO) mice as described previously (Volk et al., 2013). Heterozygous PKM ζ KO mice on a C57BL/6J background were mated resulting in mutant and wildtype littermates. Mice (2–6 months old, age matched across group) were group housed until the start of the behavioral experiments at which point they were individually housed. 90 mice were used for these

experiments (49 females and 41 males). All animals were housed in a temperature and humidity controlled animal care facility with a 12 h reverse light/dark cycle (lights on at 7:00 p.m.). All procedures were approved by the Temple University Animal Care and Use Committee.

Drugs. Oxycodone was obtained from the National Institutes of Drug Abuse Drug Supply Program (Bethesda, MD) and dissolved in sterile 0.9% saline.

Jugular Catheterization Surgery. Prior to surgery, mice were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine. An indwelling silastic catheter was placed into the right jugular vein and sutured in place. The catheter was then threaded subcutaneously over the shoulder blade and was routed to a backmount platform (Instech Laboratories, Inc.) that secured the placement. Catheters were flushed daily with 0.1 ml of an antibiotic (Timentin, 0.93 mg/ml) dissolved in heparinized saline. Mice recovered in their home cage for 3 days prior to the start of our experiments.

Operant Food Training. Following catheterization, mice were trained to perform an operant response for sucrose pellets. The mice were placed in operant chambers (Med-Associates) and trained to press a lever to receive a sucrose pellet. Mice performed 2 days of fixed ratio 1 (FR1) responding where only the active lever was available. A compound cue stimulus consisting of a cue light above the active lever, a 2900-Hz tone, and house light off was concurrent with each pellet administration, followed by an additional 8 s time-out when responding had no programmed consequences and the house light remained off. Mice were allowed to self-administer a maximum of 50 pellets per 60 min operant session. The mice were food restricted to approximately 90% of their free feeding weight

throughout the course of the operant training. They returned to ad libitum feeding 6 days into oxycodone self-administration.

Oxycodone Self-Administration. Oxycodone self-administration was measured over 2 h sessions (7 days per week) in the same chamber used for operant food training. Mice were trained to self-administer oxycodone for 3 days using an active and inactive wheel. Following the training phase, the active and inactive wheels were replaced with levers and mice continued oxycodone self-administration for 12 days. Throughout all of oxycodone self-administration, responding on the active manipulandum delivered an intravenous oxycodone injection (0.25 or 0.125 mg/kg/infusion) paired with the same cues as food training. Following the self-administration phase, mice were tested for 1 day on a progressive ratio (PR) schedule, where the response requirement for each infusion increased until the subject did not fulfill the requirement. The response requirement was defined as $R(i)=[5e0.15i-5]$ and the session ended if the animal took longer than 30 min to meet the requirement. The breakpoint is defined as the final ratio completed.

Data Analysis. All analyses were performed using GraphPad Prism 9 software (GraphPad Software). Data were analyzed using two-way ANOVA with Sidak's post hoc tests or linear mixed-effects models (Verbeke & Molenberghs, 2000) as appropriate. Statistical significance for all tests was set at $\alpha=0.05$.

Results

PKM ζ knockout potentiates 0.25 mg/kg/inf oxycodone self-administration in both sexes.

Wildtype and PKM ζ knockout mice self-administered 0.25 mg/kg/infusion oxycodone for 12 days. Linear mixed-effects models revealed male and female PKM ζ knockout mice earned more infusions [main effect of genotype; females: $F(1,29) = 4.778$,

$p = .037$, Fig. 8A; males: $F(1,24) = 6.797$, $p = .015$, Fig. 8B] and responded more for oxycodone [main effect of genotype; females: $F(1,29) = 5.587$, $p = .025$, Fig. 8C; males: $F(1,24) = 6.04$, $p = .02$, Fig. 8D] than wildtype conspecifics. We found no significant effect of genotype on the number of inactive lever presses over the 12 days [females: $F(1,29) = 1.028$, $p = .319$, Fig. 8E; males: $F(1,24) = 1.75$, $p = .198$, Fig 8F).

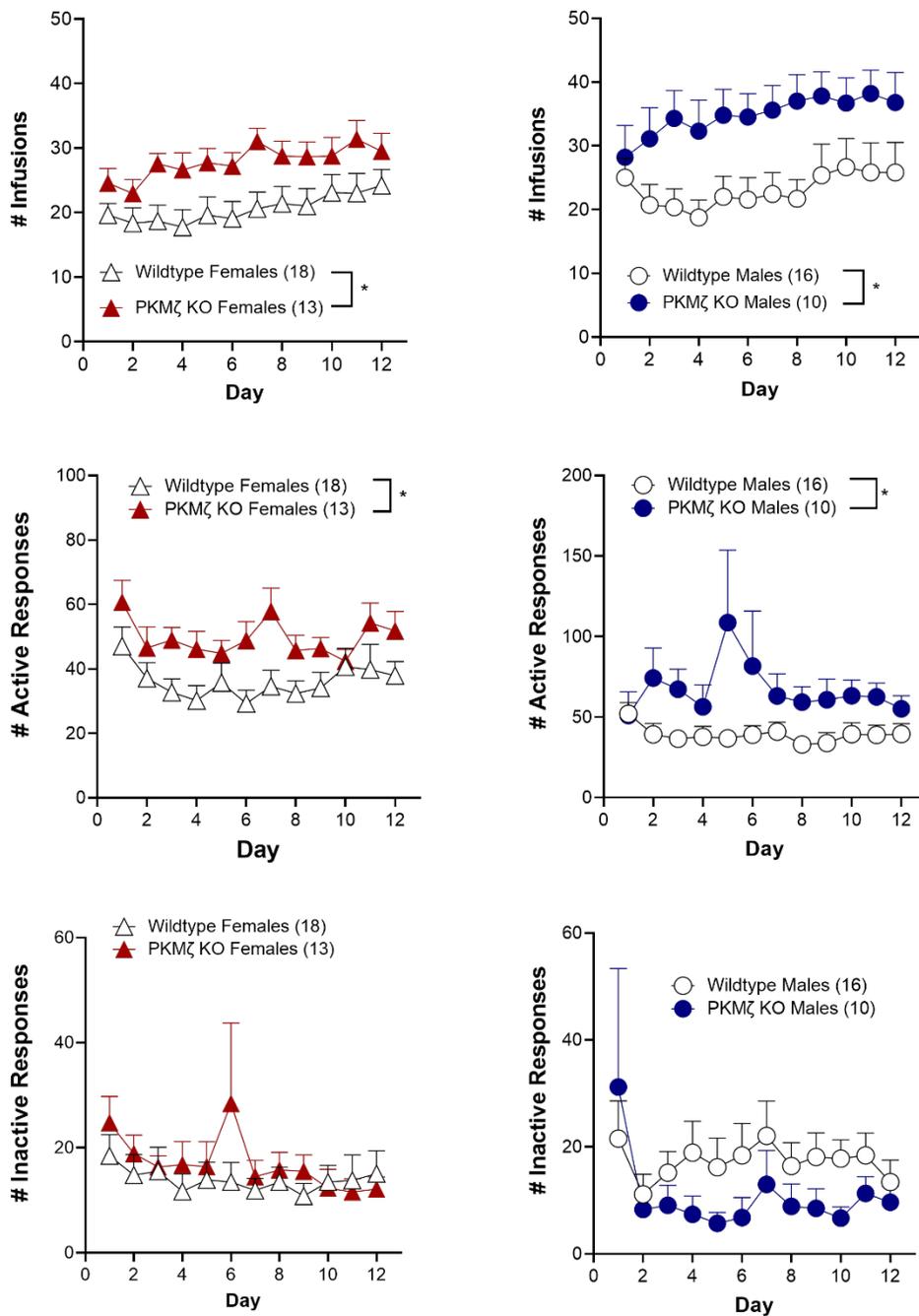


Figure 8. PKM ζ knockout potentiates 0.25 mg/kg/inf oxycodone self-administration in both sexes.

Constitutive PKM ζ deletion leads to an increase in the number of infusions earned (females: Fig. 8A; males: Fig. 8B) and the number of active responses (females: Fig 8C; males: Fig. 8D) across 12 days of oxycodone self-administration. There were no significant effects of PKM ζ knockout on inactive responding (females: Fig. 8E; males: Fig. 8F).

PKM ζ knockout potentiates 0.125 mg/kg/inf oxycodone self-administration exclusively in female animals.

Wildtype and PKM ζ knockout mice self-administered 0.125 mg/kg/inf oxycodone for 12 days. Two way ANOVA revealed female PKM ζ knockout animals earned more infusions [main effect of genotype; $F(1,16) = 7.23, p = .016$, Fig. 9A] and responded significantly more for oxycodone [main effect of genotype; $F(1,16) = 9.039, p = .008$, Fig. 9C] than wildtype conspecifics. In contrast, male PKM ζ knockout animals earned a statistically similar number of infusions [$F(1,13) = 3.579, p = .08$, Fig. 9B] and responded a similar amount for oxycodone [$F(1,13) = .4776, p = .50$, Fig. 9D] as wildtype conspecifics. There was no significant effect of genotype on inactive lever responding [females: $F(1,16) = 4.182, p = .057$, Fig. 9E; males: $F(1,13) = 2.031, p = .177$, Fig. 9F].

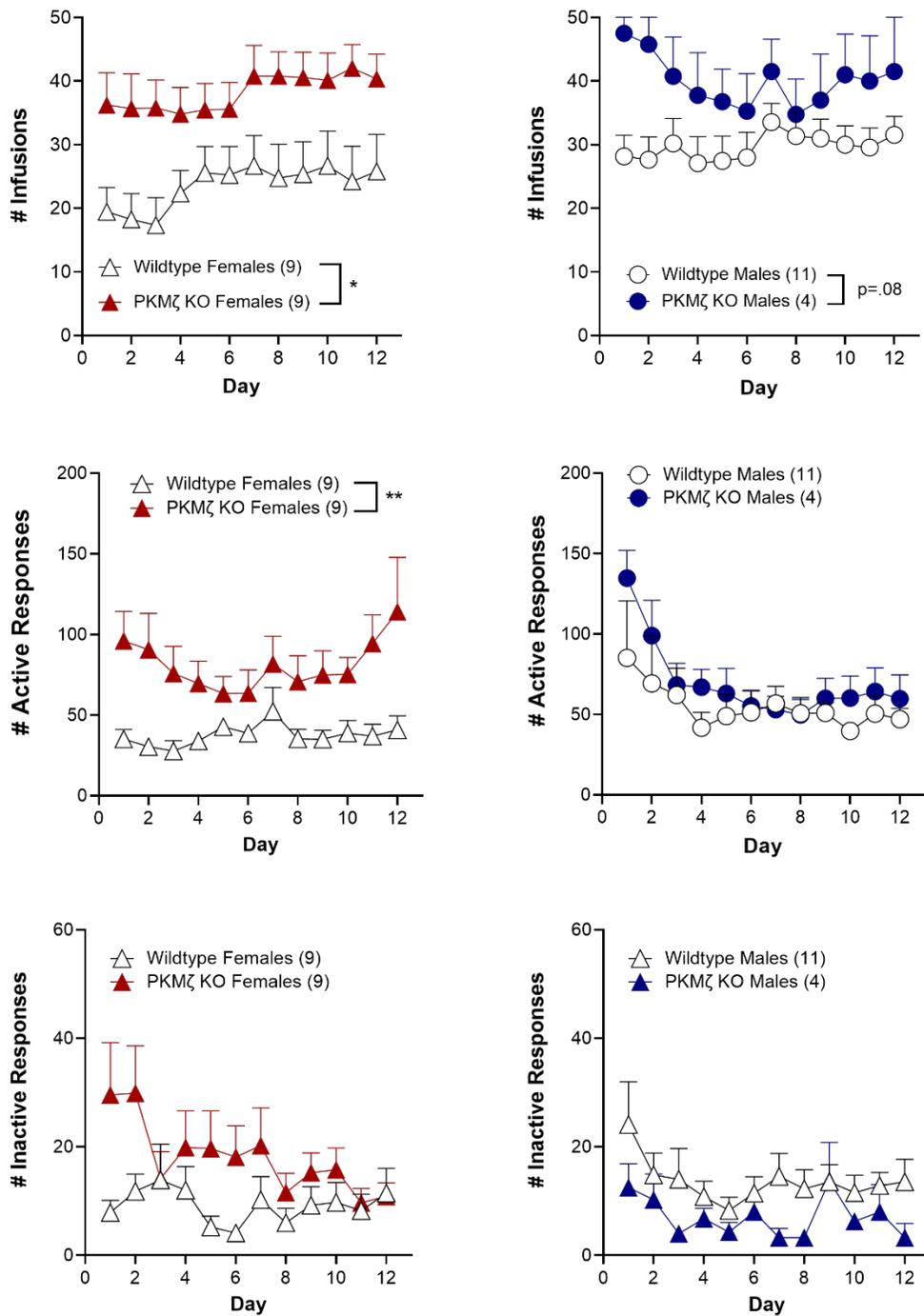


Figure 9. PKMζ knockout potentiates 0.125 mg/kg/inf oxycodone self-administration exclusively in female animals.

Constitutive PKMζ deletion leads to an increase in the number of infusions earned and the number of active responses in female (Fig. 9A; 9C), but not male (Fig. 9B; 9D) mice. There were no significant effects of PKMζ knockout on inactive responding in either sex (females: Fig. 9E; males: Fig. 9F).

PKMζ knockout alters motivation for 0.125 mg/kg/inf oxycodone exclusively in female animals.

We lastly examined whether PKMζ knockout affects motivation to obtain oxycodone as measured by a progressive ratio paradigm. Two way ANOVA revealed PKMζ deletion leads to an increase in breakpoint in female mice but not males [effect of genotype: $F(1,29) = 6.039, p = .02$; effect of sex: $F(1,29) = 2.368, p = .135$; interaction: $F(1,29) = 5.963, p = .021$, Fig. 10; post-hoc tests, females: $t(29) = 3.9, p = .001$, Fig. 10; males: $t(29) = .009, p > .99$, Fig. 10].

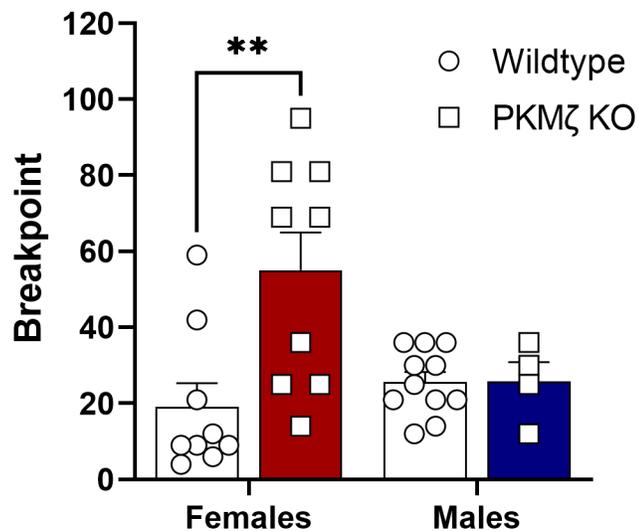


Figure 10. PKMζ knockout potentiates the final breakpoint in a progressive ratio paradigm exclusively in female animals. Constitutive PKMζ deletion leads to an increase in the breakpoint for oxycodone in female, but not male, mice.

Discussion

Glutamatergic AMPAR activity underlies much of the SUD cycle (Malenka, 2003; Malinow & Malenka, 2002). PKM ζ , an atypical isoform of Protein Kinase C, potentiates NSF-mediated insertion of GluA2-containing AMPARs to the cell membrane (Yao et al., 2008). This made PKM ζ a popular target for studies on synaptic plasticity, mainly LTP (Hsieh et al., 2021; D. S. F. Ling et al., 2002; Sacktor et al., 1993). Despite some controversy surrounding the role of PKM ζ in synaptic plasticity and behavior (A. M. Lee et al., 2013; Tsokas et al., 2016; Volk et al., 2013), PKM ζ can alter dendritic spine density and is involved in cocaine and ethanol-taking behaviors (A. M. Lee et al., 2014; McGrath et al., 2018; Ron et al., 2012). As cocaine and opioids alter spine density in opposing manners, we were interested in whether PKM ζ knockout would affect opioid self-administration in a different manner than we previously found with cocaine (Robinson et al., 2002). In these experiments we found biological sex can influence the effect of PKM ζ knockout on oxycodone-taking and motivation. Our results indicate PKM ζ works to dampen drug reward across multiple drug classes in females but these effects may be dose-dependent in males.

PKM ζ blunts oxycodone-taking in both sexes at a moderate dose of oxycodone.

In our first experiment, we found PKM ζ knockout potentiates oxycodone self-administration in both sexes at a dose of 0.25 mg/kg/inf. We first examined this dose as it is a moderate dose of oxycodone that is readily self-administered by mice (Y. Zhang et al., 2009). Our data show significant potentiation in both the number of infusions earned and the number of active responses in PKM ζ knockout animals compared to wildtype controls across 12 days of self-administration. This is in line with previous data showing PKM ζ

works to dampen ethanol- and cocaine consumption (A. M. Lee et al., 2014; McGrath et al., 2018). We do not see any significant difference in the number of responses on the inactive lever, indicating potentiated responding for oxycodone is not due to increases in perseverative responding.

PKM ζ induces a maturation of dendritic spines in cultured cortical neurons (Ron et al., 2012). Though the role of PKM ζ in spine density is still being explored, this highlights the possibility knocking out PKM ζ would lead to less stable spines. Drug use involves alterations to dendritic spine density (Kalivas, 2009). Therefore, the effect of PKM ζ knockout on drug-taking could be due to disruptions in spine morphology. However, cocaine and opioids affect spine density in the NAc in opposing manners (Diana et al., 2006; Dumitriu et al., 2012; Kasture et al., 2009; Leite-Morris et al., 2014; Norrholm et al., 2003; Robinson et al., 2001, 2002; Robinson & Kolb, 1999; Spiga et al., 2005) and we found the same behavioral phenotype in PKM ζ knockout animals across both drugs. This indicates that while PKM ζ may play a role in spine maturation, its role in drug-taking may not be due explicitly to its role in spine density.

In addition to a possible role in dendritic spine density, PKM ζ potentiates insertion of GluA2 AMPARs to the cell membrane. Previous data show disrupting GluA2 trafficking through mechanisms other than PKM ζ alters cocaine reinstatement (Briand et al., 2014; Famous et al., 2008; Wickens et al., 2019, 2021). Though there are fewer studies examining opioid self-administration, opioid exposure does alter GluA2 trafficking (Hearing et al., 2018). Here, our findings further the data that disrupting GluA2 trafficking alters drug-taking behaviors by demonstrating a role for PKM ζ in opioid self-administration. Further, we found the effect of PKM ζ knockout on opioid-taking is present in both sexes. The

results from our first experiment highlight the ubiquitous role of GluA2 trafficking in drug use in both males and females. In combination with previous studies, these data specifically further the finding that GluA2 trafficking via PKM ζ broadly works to dampen drug reward in both sexes.

PKM ζ alters oxycodone-taking in females at a low dose of oxycodone.

In our next experiment we found PKM ζ knockout significantly potentiates oxycodone self-administration exclusively in female animals at a dose of 0.125 mg/kg/inf. While we saw the same effect of PKM ζ knockout on oxycodone-taking in both sexes at the moderate dose, there are known dose-dependent sex differences in drug-taking behaviors (Becker & Hu, 2008). Therefore, we were interested in whether PKM ζ plays the same role in both sexes at another dose of oxycodone. We chose this lower dose as it may elicit more responding and capture group differences better than higher doses (Y. Zhang et al., 2009). While there was no significant effect of genotype in male animals, we do not have a large sample size in the PKM ζ knockout group. It remains possible that there may be an effect of genotype in male animals that we have not captured with this sample size. If that is the case, we could conclude PKM ζ knockout blunts opioid reward in both sexes at multiple doses. Nonetheless, we do not see any effects of genotype on either the number of infusions earned or active responding in the male animals. Currently, our data indicate PKM ζ may be more important for females when self-administering low doses of oxycodone.

To investigate whether PKM ζ influences motivation to obtain oxycodone, we tested the animals on a progressive ratio schedule of responding using the low dose. We saw a similar effect with PKM ζ knockout significantly potentiating the final breakpoint exclusively in female animals. Our current data indicate PKM ζ also plays a larger role in

females in motivation to acquire low dose oxycodone. However, our data set for the male animals in this paradigm is also underpowered. As we also have a low sample size for PKM ζ knockout males in the progressive ratio paradigm, it is still possible there may be effects of genotype in male animals that we do not see at this time. If we do see effects of genotype as studies continue, it would indicate PKM ζ knockout enhances motivation to acquire opioids similarly in both sexes at a low dose of oxycodone.

Biological sex can modulate behavioral responses to different doses of the same drug. Females acquire drug-taking at lower doses and when given a choice will choose a higher dose of cocaine than males (Becker & Hu, 2008). Thus, varying the dose may elicit effects of sex not previously seen at other doses. PKM ζ can also be modulated by biological sex. There are sex differences in expression within the hippocampus and NAc following drug exposure (Avila et al., 2021; McGrath et al., 2018). This indicates that while many behaviors involving PKM ζ may present in a similar manner, PKM ζ activity may not explicitly be the same between the sexes. Previous studies demonstrate this, where constitutive PKM ζ knockout potentiates cocaine-taking in both sexes but site-specific knockout in the NAc potentiates cocaine-taking exclusively in males (McGrath et al., 2018). Altogether, these data and ours indicate that PKM ζ does work to dampen drug reward but its role is not explicitly the same in males and females.

Here, we found PKM ζ knockout potentiates oxycodone-taking though its role in these behaviors may be greater in females than males. This adds to the literature demonstrating PKM ζ works to dampen drug-taking across multiple drug classes. Additionally, these studies suggest that PKM ζ may function differently in males and females when low doses of opioids are involved. These experiments highlight the

importance of including both sexes in biomedical research. Recent evidence indicates men and women do not always respond to SUD treatments in the same manner (Agabio et al., 2016; Huhn et al., 2019; S. Ling et al., 2019; Seguí et al., 2020). Further investigation into the neural mechanisms driving SUD in both sexes could aid in the development of more effective pharmacotherapies for males and females.

CHAPTER 5

Conclusion

Behavioral sex differences are present in many aspects of the SUD cycle (Becker et al., 2017; Becker & Hu, 2008; Becker & Koob, 2016; S. A. M. Bobzean et al., 2014; Fattore et al., 2008; Knouse & Briand, 2021; Quigley et al., 2021). Despite this fact the mechanisms driving behavioral sex differences in drug-taking and relapse are largely unexplored. Glutamate transmission underlies many components of the SUD cycle (Chiamulera et al., 2021; Duncan & Lawrence, 2012; Kalivas, 2000, 2004, 2009; Kalivas et al., 2005, 2009; Kenny & Markou, 2004; Lapish et al., 2006; Loweth et al., 2013; Márquez et al., 2017; Reissner & Kalivas, 2010; Schmidt & Pierce, 2010; Scofield et al., 2016; Spencer et al., 2016; Wolf & Ferrario, 2010). There are also known sex differences in glutamate transmission (L. L. Giacometti & Barker, 2020; C. J. Perry et al., 2021; Tonn Eisinger et al., 2018; Wickens et al., 2018), highlighting the possibility sex differences in excitatory transmission may underlie some of the behavioral sex differences in drug reward. As males and females do not always respond similarly to pharmacotherapies for SUD (R. E. Johnson et al., 1995; Jones et al., 2005; Schottenfeld et al., 1998), it is crucial to understand the mechanisms driving sex differences in SUD to better treat the clinical population.

Behavioral sex differences in cocaine use disorder are well-established. Women experience higher desires to use cocaine and more intense craving following exposure to cocaine-related cues than men (Elman et al., 2001; Kennedy et al., 2013; Sofuoglu et al., 1999). Preclinically, female rodents take more cocaine, reinstate at higher rates, and are more motivated to work for cocaine than males (Bechard et al., 2018; Carroll et al., 2002;

Hu et al., 2004; Lynch & Carroll, 1999, 2000; Lynch & Taylor, 2004; Ramôa et al., 2013; Roberts et al., 1989). Though less is known about opioid use disorder, the emerging data point to the same trend where females are more vulnerable to many aspects of opioid use disorder. Clinically, women experience higher opioid craving at baseline and following exposure to drug paired cues than men (Back et al., 2011; Yu et al., 2007). Preclinically, female animals acquire opioid self-administration more rapidly, take more opioids, and are more motivated to acquire opioids than males (Carroll et al., 2002; Cicero et al., 2003; Klein et al., 1997; Lynch & Carroll, 1999; Vazquez et al., 2020). Altogether, the literature points to a common trend between cocaine and opioid use disorders: females are more vulnerable than males.

Glutamate homeostasis is disrupted following drug use and these disruptions drive relapse (Hearing et al., 2018). While overall less is known about sex differences in excitatory transmission, there are known sex differences in receptor expression and glutamate levels that may underlie drug reward (L. L. Giacometti & Barker, 2020; C. J. Perry et al., 2021; Tonn Eisinger et al., 2018; Wickens et al., 2018). In our first study, we found multiple sex differences in glutamate signaling within the mPFC. Females exhibit heightened AMPAR expression compared to males as evidenced by higher synaptosomal expression of GluA1 and GluA2 and a higher rectification index. Together, these data specifically found females have a larger contribution of GluA1-containing CP-AMPARs. As CP-AMPARs have higher conductance than GluA2-containing AMPARs they are proposed to underlie heightened synaptic strength (S.-R. Chen et al., 2013). We found a corresponding increase in both sEPSC frequency and amplitude in the mPFC, indicating both pre- and postsynaptic glutamatergic transmission is heightened in females. Altogether,

our results in the mPFC demonstrate females have heightened excitatory transmission and synaptic strength within this region.

Glutamatergic projections from the mPFC to NAc drive drug use and relapse (Hearing, 2019). As we found significantly heightened glutamatergic transmission in the mPFC of females compared to males, we were interested in exploring whether these effects translate to alterations in glutamate activity within the NAc. Presynaptically, we found females have a larger readily releasable pool of glutamate and a lower release probability in this region than males. The lower release probability likely works to counteract heightened glutamatergic input in this region. We did not see any effects of sex on sEPSC frequency. While we may have expected to see this based on our RRP data, it is not entirely surprising as we did not look at this measure in a cell-specific manner and it can vary by cell-type (Ma et al., 2012). Together, these data indicate the sex differences we found in glutamate transmission within the mPFC translate to presynaptic sex differences within the NAc as well. We then examined measures of postsynaptic glutamate transmission to determine whether these differences translate to an overall sex difference in transmission within this region.

We found females have a significantly larger AMPA/NMDA ratio in the NAc compared to males. This difference was present in both rats and mice, indicating this effect is conserved across two species. While we did not see any significant difference in sEPSC amplitude, this is again likely due to cell specificity we did not examine here (Deroche et al., 2020). As AMPAR endocytosis is involved in LTD, we next examined whether these differences in AMPA-mediated transmission correspond to sex differences in LTD. We found LTD was blunted in the NAc of female animals when using a LFS protocol that

successfully induces LTD in males. This sex difference was abolished with a more intense stimulation protocol. These data indicate the threshold for LTD induction in naïve females is higher than that in males.

While less is known about sex difference in LTD, LTP is influenced by biological sex with males exhibiting LTP to a broader range of induction protocols than females (Gall et al., 2021; Koss & Frick, 2017; Simpson & Kelly, 2012; Yagi & Galea, 2019; Yang et al., 2004). Our data extend these findings to LTD in the NAc. The larger RRP we found in females could explain why we need a longer LFS protocol to induce LTD. LTD induction reduces the size of the RRP and synaptic depression is a result of RRP depletion (Bailey & Chen, 1988; Gottmann, 2008; Stanton et al., 2003). In females, the larger RRP likely makes it more difficult to induce synaptic depression. The short protocol may not cause enough vesicle release to induce depression but longer protocols could. These data indicate heightened glutamatergic activity in the NAc of females also makes it harder to induce alterations to synaptic plasticity. Altogether, these studies indicate there is potentiated excitatory transmission in the mPFC of females that translates to potentiated excitatory transmission in the NAc. These differences are seen both pre- and postsynaptically and could drive female vulnerability to drug use.

Our data from these studies indicated there are sex differences in AMPA-mediated transmission within the reward system. Therefore, we were interested in examining the role of AMPAR trafficking proteins in synaptic plasticity in both sexes. GRIP, an AMPAR trafficking protein, stabilizes GluA2 at the cell membrane (H. Dong et al., 1997). Intra-accumbal GRIP knockout blunts LTD and potentiates cue-induced cocaine reinstatement in male mice (Briand et al., 2014). PKM ζ , another AMPAR trafficking protein, is proposed

to work similarly to GRIP by potentiating NSF-mediated insertion of GluA2 at the cell membrane (Yao et al., 2008). Relevant to substance use, PKM ζ knockout potentiates ethanol-taking in male mice and cocaine-taking in both sexes (A. M. Lee et al., 2014; McGrath et al., 2018). We were interested in exploring whether PKM ζ plays a similar role in LTD as GRIP and whether this effect is the same in both sexes.

These studies revealed a sex-specific role of PKM ζ in LTD. In females, PKM ζ knockout facilitated LTD whereas in males, PKM ζ knockout blunted it. Our data complement the emerging literature that PKM ζ does not function exactly the same in males and females. PKM ζ alters anxiety in males but not females and there are sex differences in expression following drug use (Avila et al., 2021; A. M. Lee et al., 2013). In the NAc, PKM ζ knockout potentiates cocaine-taking in male, but not female, mice (McGrath et al., 2018). Therefore, PKM ζ functions in a sex-specific manner in synaptic plasticity and perhaps behavior.

We found a more intense LFS protocol that better induces LTD in wildtype females abolishes the effect of PKM ζ knockout on LTD in both sexes. As previously mentioned, the larger RRP we found in wildtype females explains the sex difference we see in LTD. A larger RRP requires more stimulation to successfully weaken synapses, therefore we see LTD in females with a longer LFS protocol but not a shorter one. Though the role of PKM ζ is thought to be exclusively postsynaptic, the majority of studies examining PKM ζ activity have been conducted in males. It is possible that at least in females, PKM ζ could alter presynaptic activity. We found PKM ζ knockout abolishes the blunted LTD we see in wildtype females due to the larger RRP. Therefore, PKM ζ may be acting presynaptically in females in a manner that blunts synaptic plasticity.

While a presynaptic role for PKM ζ is possible, this is likely not the case. In a preliminary study, we found no significant differences in the paired pulse ratio between PKM ζ knockout animals and wildtype controls [females: $F(1,25) = .016, p = .9$, Fig. 11A; males: $F(1,22) = .182, p = .674$, Fig. 11B]. The paired pulse ratio is a broad electrophysiological measure of presynaptic activity. These results indicate PKM ζ is not functionally affecting presynaptic activity in the nucleus accumbens core in either sex. This is consistent with data showing no effect of PKM ζ overexpression on the paired pulse ratio in the hippocampus of male rats (Schuette et al., 2016). Rather, our LTD data indicate PKM ζ has a postsynaptic role in synaptic plasticity in females. While its role may be different from that of males, our paired pulse data rule out the possibility of a presynaptic mechanism for PKM ζ in females. This is in line with published data demonstrating PKM ζ acts exclusively in a postsynaptic manner in males (Hernández et al., 2014; D. S. Ling et al., 2006; Schuette et al., 2016).

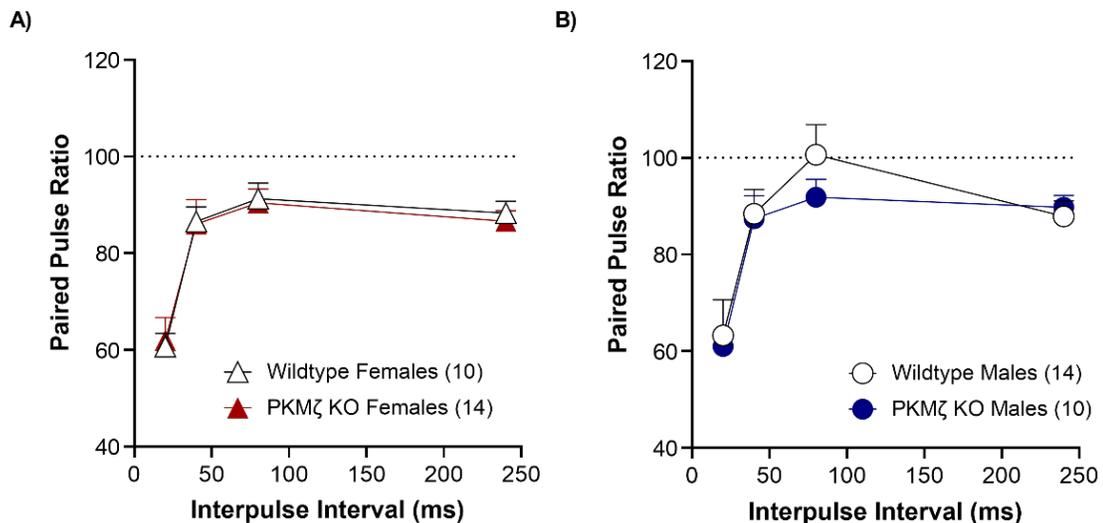


Figure 11. PKM ζ knockout does not functionally alter presynaptic activity in the nucleus accumbens core.

PKM ζ knockout does not significantly affect the paired pulse ratio in behaviorally naïve female (A) or male (B) mice compared to wildtype controls.

LTD is altered in the NAc following opioid exposure (Z. Dong et al., 2007; Qian et al., 2019; Shen & Kalivas, 2013). As we found a sex-specific role for PKM ζ in LTD, we next examined whether PKM ζ knockout also alters oxycodone-taking in both sexes. At a moderate dose, we found PKM ζ knockout potentiates oxycodone self-administration in both males and females. This is in line with previous work demonstrating potentiated cocaine-taking in both male and female constitutive PKM ζ knockout mice (McGrath et al., 2018). We found this effect is recapitulated in females at a low dose of oxycodone where again PKM ζ knockout potentiates oxycodone-taking. We also examined motivation to obtain oxycodone at this dose and found female PKM ζ knockout mice had higher progressive ratio breakpoints than wildtype females.

The effect in males seen at the moderate dose is not recapitulated at a low dose, however. We found no significant effect of genotype on oxycodone-taking or progressive ratio breakpoints at this dose in male animals. While our group size for PKM ζ knockout males is low, our current data demonstrate PKM ζ knockout may only affect oxycodone-taking in males at higher doses. This would mean PKM ζ is more important as a protective mechanism against substance use in females. While still involved in drug-taking in males, PKM ζ could be a target mechanism for dampening opioid reward particularly in females. As females are more vulnerable to opioid use, PKM ζ may be more active to counteract some of this vulnerability.

Different doses of drugs can elicit effects of sex not seen at other doses. Female offspring of morphine-exposed mothers exhibit decreased acquisition of morphine self-administration and decreased progressive ratio breakpoints at 3 different doses. Male offspring, however, exhibit the same behaviors only at the highest dose tested (Vassoler et al., 2017). In our paradigm, we see a similar effect where our manipulation alters drug-taking in males only at certain doses. This effect is not entirely surprising as there are known sex differences in PKM ζ expression following drug exposure. Females have lower PKM ζ levels in the hippocampus and NAc following methamphetamine and cocaine (Avila et al., 2021; McGrath et al., 2018). These data indicate PKM ζ expression and function is likely not the same in males and females during substance use though the behavioral output may sometimes overlap.

It is possible the effect of PKM ζ knockout on LTD drives the effect we see in low dose oxycodone self-administration. The blunted LTD we see in male PKM ζ knockout animals could translate to the NAc being less plastic. Opioid exposure can alter LTD in the NAc (Z. Dong et al., 2007; Qian et al., 2019; Shen & Kalivas, 2013). Less plasticity in the NAc of PKM ζ knockout males could make the region less susceptible to drug-induced alterations to plasticity. This may be preventative against the development of drug-taking behaviors. In females, facilitations to LTD in PKM ζ knockout animals could indicate the NAc is more plastic than in wildtype females and is thus more susceptible to drug-induced alterations in plasticity. This would explain potentiated drug-taking behaviors in PKM ζ knockout females. As drug dosing regimen matters for alterations to synaptic plasticity (Cahill, 2020; Kenney & Gould, 2008; Kourrich et al., 2007), the effect of PKM ζ knockout on LTD in males may not continue to alter oxycodone-taking at higher doses. It is possible

the moderate dose of oxycodone used in these studies is high enough to overcome blunted plasticity in PKM ζ knockout males. A higher dose may facilitate oxycodone-induced alterations to synaptic plasticity that a lower dose cannot, therefore we see effects in males exclusively at the low dose.

These studies were completed to further investigate baseline sex differences in glutamate transmission and their potential implications for opioid use disorder. Figure 12 broadly shows the sex differences we found in excitatory transmission in both wildtype and PKM ζ knockout animals. We discovered heightened glutamate transmission in the mPFC of females that translates to heightened downstream glutamate transmission in the NAc. The increased excitation in the NAc caused the region to be less plastic in females, with LTD being harder to induce. These sex differences in the NAc are modulated by PKM ζ , as PKM ζ knockout alters LTD in a sex-specific manner. Together, these sex differences may, in part, drive behavioral sex differences in SUD. We found PKM ζ knockout potentiates opioid-taking in both sexes but its role in the development and expression of SUD may be more important for females. As males and females do not always respond to treatments for opioid use disorder in the same manner (R. E. Johnson et al., 1995; Jones et al., 2005; Schottenfeld et al., 1998), these studies highlight the need for further understanding of the mechanisms driving behavioral sex differences in opioid use and relapse.

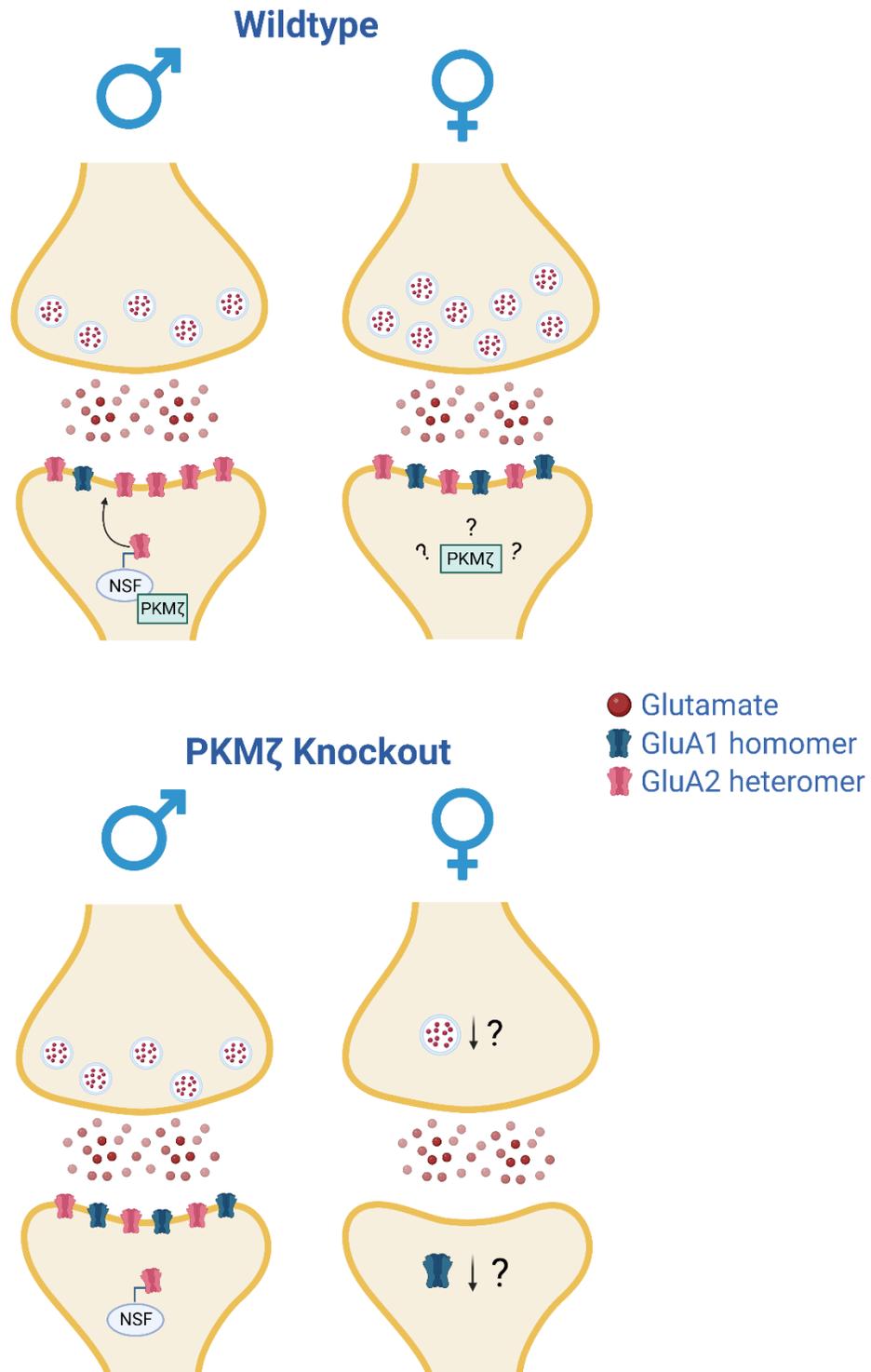


Figure 12. Sex differences in glutamate transmission within the reward system.

We found significant sex differences in excitatory transmission at multiple levels within the reward system. These differences are influenced by PKMζ which plays a sex-specific role in synaptic plasticity. The exact role of PKMζ in females remains unclear (*figure made with biorender.com*).

REFERENCES

- Agabio, R., Campesi, I., Pisanu, C., Gessa, G. L., & Franconi, F. (2016). Sex differences in substance use disorders: Focus on side effects. *Addict Biol*, *21*(5), 1030–1042. <https://doi.org/10.1111/adb.12395>
- Altemus, M., Sarvaiya, N., & Neill Epperson, C. (2014). Sex differences in anxiety and depression clinical perspectives. *Front Neuroendocrinol*, *35*(3), 320–330. <https://doi.org/10.1016/j.yfrne.2014.05.004>
- Anwyl, R. (2006). Induction and expression mechanisms of postsynaptic NMDA receptor-independent homosynaptic long-term depression. *Progress in Neurobiology*, *78*(1), 17–37. <https://doi.org/10.1016/j.pneurobio.2005.12.001>
- Arnold, A. P. (2009). The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. *Horm Behav*, *55*(5), 570–578. <https://doi.org/10.1016/j.yhbeh.2009.03.011>
- Avila, J. A., Memos, N., Aslan, A., Andrejewski, T., Luine, V. N., & Serrano, P. A. (2021). Voluntary oral methamphetamine increases memory deficits and contextual sensitization during abstinence associated with decreased PKM ζ and increased κ OR in the hippocampus of female mice. *Journal of Psychopharmacology (Oxford, England)*, *35*(10), 1240–1252. <https://doi.org/10.1177/02698811211048285>
- Back, S. E., Payne, R. L., Wahlquist, A. H., Carter, R. E., Stroud, Z., Haynes, L., Hillhouse, M., Brady, K. T., & Ling, W. (2011). Comparative profiles of men and women with opioid dependence: Results from a national multisite effectiveness trial. *The American Journal of Drug and Alcohol Abuse*, *37*(5), 313–323. <https://doi.org/10.3109/00952990.2011.596982>
- Bailey, C. H., & Chen, M. (1988). Morphological basis of short-term habituation in *Aplysia*. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *8*(7), 2452–2459. <https://doi.org/10.1523/JNEUROSCI.08-07-02452.1988>
- Baker, D. A., McFarland, K., Lake, R. W., Shen, H., Tang, X.-C., Toda, S., & Kalivas, P. W. (2003). Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nature Neuroscience*, *6*(7), 743–749. <https://doi.org/10.1038/nn1069>
- Bangasser, D. A., & Cuarenta, A. (2021). Sex differences in anxiety and depression: Circuits and mechanisms. *Nat Rev Neurosci*, *22*(11), 674–684. <https://doi.org/10.1038/s41583-021-00513-0>

- Baptista, M. A., Martin-Fardon, R., & Weiss, F. (2004). Preferential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on conditioned reinstatement versus primary reinforcement: Comparison between cocaine and a potent conventional reinforcer. *J Neurosci*, *24*(20), 4723–4727. <https://doi.org/10.1523/JNEUROSCI.0176-04.2004>
- Bechard, A. R., Hamor, P. U., Schwendt, M., & Knackstedt, L. A. (2018). The effects of ceftriaxone on cue-primed reinstatement of cocaine-seeking in male and female rats: Estrous cycle effects on behavior and protein expression in the nucleus accumbens. *Psychopharmacology*, *235*(3), 837–848. <https://doi.org/10.1007/s00213-017-4802-7>
- Becker, J. B. (1990). Direct effect of 17 beta-estradiol on striatum: Sex differences in dopamine release. *Synapse*, *5*(2), 157–164. <https://doi.org/10.1002/syn.890050211>
- Becker, J. B., & Hu, M. (2008). Sex differences in drug abuse. *Frontiers in Neuroendocrinology*, *29*(1), 36–47. <https://doi.org/10.1016/j.yfrne.2007.07.003>
- Becker, J. B., & Koob, G. F. (2016). Sex Differences in Animal Models: Focus on Addiction. *Pharmacol Rev*, *68*(2), 242–263. <https://doi.org/10.1124/pr.115.011163>
- Becker, J. B., McClellan, M. L., & Reed, B. G. (2017). Sex differences, gender and addiction. *Journal of Neuroscience Research*, *95*(1–2), 136–147. <https://doi.org/10.1002/jnr.23963>
- Belleau, E. L., Treadway, M. T., & Pizzagalli, D. A. (2019). The Impact of Stress and Major Depressive Disorder on Hippocampal and Medial Prefrontal Cortex Morphology. *Biol Psychiatry*, *85*(6), 443–453. <https://doi.org/10.1016/j.biopsych.2018.09.031>
- Benke, T., & Traynelis, S. F. (2019). AMPA-Type Glutamate Receptor Conductance Changes and Plasticity: Still a Lot of Noise. *Neurochemical Research*, *44*(3), 539–548. <https://doi.org/10.1007/s11064-018-2491-1>
- Bernabeu, A., Bara, A., Manduca, A., Borsoi, M., Lassalle, O., Pelissier-Alicot, A.-L., & Manzoni, O. J. (2020). Sex-specific maturational trajectory of endocannabinoid plasticity in the rat prefrontal cortex. *BioRxiv*, 2020.10.09.332965. <https://doi.org/10.1101/2020.10.09.332965>
- Bernabo, M., Haubrich, J., Gamache, K., & Nader, K. (2021). Memory Destabilization and Reconsolidation Dynamically Regulate the PKM ζ Maintenance Mechanism. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *41*(22), 4880–4888. <https://doi.org/10.1523/JNEUROSCI.2093-20.2021>

- Bobzean, S. A., Dennis, T. S., & Perrotti, L. I. (2014). Acute estradiol treatment affects the expression of cocaine-induced conditioned place preference in ovariectomized female rats. *Brain Res Bull*, *103*, 49–53. <https://doi.org/10.1016/j.brainresbull.2014.02.002>
- Bobzean, S. A. M., DeNobrega, A. K., & Perrotti, L. I. (2014). Sex differences in the neurobiology of drug addiction. *Experimental Neurology*, *259*, 64–74. <https://doi.org/10.1016/j.expneurol.2014.01.022>
- Bolla, K. I., Eldreth, D. A., London, E. D., Kiehl, K. A., Mouratidis, M., Contoreggi, C., Matochik, J. A., Kurian, V., Cadet, J. L., Kimes, A. S., Funderburk, F. R., & Ernst, M. (2003). Orbitofrontal cortex dysfunction in abstinent cocaine abusers performing a decision-making task. *Neuroimage*, *19*(3), 1085–1094.
- Borzan, J., & Fuchs, P. N. (2006). Organizational and activational effects of testosterone on carrageenan-induced inflammatory pain and morphine analgesia. *Neuroscience*, *143*(3), 885–893. <https://doi.org/10.1016/j.neuroscience.2006.08.034>
- 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*, *31*(10), 2197–2209. <https://doi.org/10.1038/sj.npp.1300977>
- Brady, K. T., & Randall, C. L. (1999). Gender differences in substance use disorders. *Psychiatr Clin North Am*, *22*(2), 241–252. [https://doi.org/10.1016/s0193-953x\(05\)70074-5](https://doi.org/10.1016/s0193-953x(05)70074-5)
- Briand, L. A., Deutschmann, A. U., Ellis, A. S., & Fosnocht, A. Q. (2016). Disrupting GluA2 phosphorylation potentiates reinstatement of cocaine seeking. *Neuropharmacology*, *111*, 231–241. <https://doi.org/10.1016/j.neuropharm.2016.09.010>
- Briand, L. A., Kimmey, B. A., Ortinski, P. I., Haganir, R. L., & Pierce, R. C. (2014). Disruption of glutamate receptor-interacting protein in nucleus accumbens enhances vulnerability to cocaine relapse. *Neuropsychopharmacology*, *39*(3), 759–769. <https://doi.org/10.1038/npp.2013.265>
- Brusco, J., Wittmann, R., de Azevedo, M. S., Lucion, A. B., Franci, C. R., Giovenardi, M., & Rasia-Filho, A. A. (2008). Plasma hormonal profiles and dendritic spine density and morphology in the hippocampal CA1 stratum radiatum, evidenced by light microscopy, of virgin and postpartum female rats. *Neurosci Lett*, *438*(3), 346–350. <https://doi.org/10.1016/j.neulet.2008.04.063>

- Burgdorf, C. E., Schierberl, K. C., Lee, A. S., Fischer, D. K., Van Kempen, T. A., Mudragel, V., Haganir, R. L., Milner, T. A., Glass, M. J., & Rajadhyaksha, A. M. (2017). Extinction of Contextual Cocaine Memories Requires Cav1.2 within D1R-Expressing Cells and Recruits Hippocampal Cav1.2-Dependent Signaling Mechanisms. *J Neurosci*, *37*(49), 11894–11911. <https://doi.org/10.1523/JNEUROSCI.2397-17.2017>
- Cahill, C. M. (2020). Opioid dose regimen shapes mesolimbic adaptations. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *45*(11), 1777–1778. <https://doi.org/10.1038/s41386-020-0679-y>
- Cailhol, S., & Mormede, P. (1999). Strain and sex differences in the locomotor response and behavioral sensitization to cocaine in hyperactive rats. *Brain Res*, *842*(1), 200–205. [https://doi.org/10.1016/s0006-8993\(99\)01742-4](https://doi.org/10.1016/s0006-8993(99)01742-4)
- Cao, J., Dorris, D. M., & Meitzen, J. (2016). Neonatal Masculinization Blocks Increased Excitatory Synaptic Input in Female Rat Nucleus Accumbens Core. *Endocrinology*, *157*(8), 3181–3196. <https://doi.org/10.1210/en.2016-1160>
- Carroll, M. E., Morgan, A. D., Lynch, W. J., Campbell, U. C., & Dess, N. K. (2002). Intravenous cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: Phenotype and sex differences. *Psychopharmacology*, *161*(3), 304–313. <https://doi.org/10.1007/s00213-002-1030-5>
- Cataldo, G., Bernal, S., Markowitz, A., Ogawa, S., Ragnauth, A., Pfaff, D. W., & Bodnar, R. J. (2005). Organizational manipulation of gonadal hormones and systemic morphine analgesia in female rats: Effects of adult ovariectomy and estradiol replacement. *Brain Res*, *1059*(1), 13–19. <https://doi.org/10.1016/j.brainres.2005.08.003>
- Chang, P. K., Verbich, D., & McKinney, R. A. (2012). AMPA receptors as drug targets in neurological disease—Advantages, caveats, and future outlook. *Eur J Neurosci*, *35*(12), 1908–1916. <https://doi.org/10.1111/j.1460-9568.2012.08165.x>
- Chen, R., Osterhaus, G., McKerchar, T., & Fowler, S. C. (2003). The role of exogenous testosterone in cocaine-induced behavioral sensitization and plasmalemmal or vesicular dopamine uptake in castrated rats. *Neurosci Lett*, *351*(3), 161–164. <https://doi.org/10.1016/j.neulet.2003.07.018>
- Chen, S. R., Zhou, H. Y., Byun, H. S., & Pan, H. L. (2013). Nerve injury increases GluA2-lacking AMPA receptor prevalence in spinal cords: Functional significance and signaling mechanisms. *J Pharmacol Exp Ther*, *347*(3), 765–772. <https://doi.org/10.1124/jpet.113.208363>

- Chen, S.-R., Zhou, H.-Y., Byun, H. S., & Pan, H.-L. (2013). Nerve injury increases GluA2-lacking AMPA receptor prevalence in spinal cords: Functional significance and signaling mechanisms. *The Journal of Pharmacology and Experimental Therapeutics*, *347*(3), 765–772. <https://doi.org/10.1124/jpet.113.208363>
- Chiamulera, C., Piva, A., & Abraham, W. C. (2021). Glutamate receptors and metaplasticity in addiction. *Current Opinion in Pharmacology*, *56*, 39–45. <https://doi.org/10.1016/j.coph.2020.09.005>
- Chin, J., Sternin, O., Wu, H. B., Burrell, S., Lu, D., Jenab, S., Perrotti, L. I., & Quinones-Jenab, V. (2002). Endogenous gonadal hormones modulate behavioral and neurochemical responses to acute and chronic cocaine administration. *Brain Res*, *945*(1), 123–130. [https://doi.org/10.1016/s0006-8993\(02\)02807-x](https://doi.org/10.1016/s0006-8993(02)02807-x)
- Cicero, T. J., Aylward, S. C., & Meyer, E. R. (2003). Gender differences in the intravenous self-administration of mu opiate agonists. *Pharmacology, Biochemistry, and Behavior*, *74*(3), 541–549. [https://doi.org/10.1016/s0091-3057\(02\)01039-0](https://doi.org/10.1016/s0091-3057(02)01039-0)
- Cicero, T. J., Meyer, E. R., Bell, R. D., & Koch, G. A. (1976). Effects of morphine and methadone on serum testosterone and luteinizing hormone levels and on the secondary sex organs of the male rat. *Endocrinology*, *98*(2), 367–372. <https://doi.org/10.1210/endo-98-2-367>
- Cicero, T. J., Nock, B., O'Connor, L., & Meyer, E. R. (2002). Role of steroids in sex differences in morphine-induced analgesia: Activational and organizational effects. *J Pharmacol Exp Ther*, *300*(2), 695–701. <https://doi.org/10.1124/jpet.300.2.695>
- Clements, J. D., & Bekkers, J. M. (1997). Detection of spontaneous synaptic events with an optimally scaled template. *Biophysical Journal*, *73*(1), 220–229. [https://doi.org/10.1016/S0006-3495\(97\)78062-7](https://doi.org/10.1016/S0006-3495(97)78062-7)
- Conrad, K. L., Tseng, K. Y., Uejima, J. L., Reimers, J. M., Heng, L. J., Shaham, Y., Marinelli, M., & Wolf, M. E. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*, *454*(7200), 118–121. <https://doi.org/10.1038/nature06995>
- Cooper, Z. D., Foltin, R. W., & Evans, S. M. (2013). Effects of menstrual cycle phase on cocaine self-administration in rhesus macaques. *Horm Behav*, *63*(1), 105–113. <https://doi.org/10.1016/j.yhbeh.2012.10.008>
- Cyr, M., Ghribi, O., Thibault, C., Morissette, M., Landry, M., & Di Paolo, T. (2001). Ovarian steroids and selective estrogen receptor modulators activity on rat brain NMDA and AMPA receptors. *Brain Res Brain Res Rev*, *37*(1–3), 153–161. [https://doi.org/10.1016/s0165-0173\(01\)00115-1](https://doi.org/10.1016/s0165-0173(01)00115-1)

- Derkach, V. A., Oh, M. C., Guire, E. S., & Soderling, T. R. (2007). Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat Rev Neurosci*, 8(2), 101–113. <https://doi.org/10.1038/nrn2055>
- Deroche, M. A., Lassalle, O., Castell, L., Valjent, E., & Manzoni, O. J. (2020). Cell-Type- and Endocannabinoid-Specific Synapse Connectivity in the Adult Nucleus Accumbens Core. *J Neurosci*, 40(5), 1028–1041. <https://doi.org/10.1523/JNEUROSCI.1100-19.2019>
- Deutschmann, A. U., Kirkland, J. M., & Briand, L. A. (2022). Adolescent social isolation induced alterations in nucleus accumbens glutamate signalling. *Addict Biol*, 27(1), e13077. <https://doi.org/10.1111/adb.13077>
- Diana, M., Spiga, S., & Acquas, E. (2006). Persistent and reversible morphine withdrawal-induced morphological changes in the nucleus accumbens. *Ann N Y Acad Sci*, 1074, 446–457. <https://doi.org/10.1196/annals.1369.045>
- Doncheck, E. M., Urbanik, L. A., DeBaker, M. C., Barron, L. M., Liddiard, G. T., Tuscher, J. J., Frick, K. M., Hillard, C. J., & Mantsch, J. R. (2018). 17beta-Estradiol Potentiates the Reinstatement of Cocaine Seeking in Female Rats: Role of the Prelimbic Prefrontal Cortex and Cannabinoid Type-1 Receptors. *Neuropsychopharmacology*, 43(4), 781–790. <https://doi.org/10.1038/npp.2017.170>
- Dong, H., O'Brien, R. J., Fung, E. T., Lanahan, A. A., Worley, P. F., & Huganir, R. L. (1997). GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature*, 386(6622), 279–284. <https://doi.org/10.1038/386279a0>
- Dong, Z., Cao, J., & Xu, L. (2007). Opiate withdrawal modifies synaptic plasticity in subicular-nucleus accumbens pathway in vivo. *Neuroscience*, 144(3), 845–854. <https://doi.org/10.1016/j.neuroscience.2006.10.018>
- Donner, N. C., & Lowry, C. A. (2013). Sex differences in anxiety and emotional behavior. *Pflugers Arch*, 465(5), 601–626. <https://doi.org/10.1007/s00424-013-1271-7>
- Dorris, D. M., Cao, J., Willett, J. A., Hauser, C. A., & Meitzen, J. (2015). Intrinsic excitability varies by sex in prepubertal striatal medium spiny neurons. *Journal of Neurophysiology*, 113(3), 720–729. <https://doi.org/10.1152/jn.00687.2014>
- Dudek, S. M., & Bear, M. F. (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proceedings of the National Academy of Sciences of the United States of America*, 89(10), 4363–4367. <https://doi.org/10.1073/pnas.89.10.4363>

- Dumitriu, D., Laplant, Q., Grossman, Y. S., Dias, C., Janssen, W. G., Russo, S. J., Morrison, J. H., & Nestler, E. J. (2012). Subregional, dendritic compartment, and spine subtype specificity in cocaine regulation of dendritic spines in the nucleus accumbens. *J Neurosci*, *32*(20), 6957–6966. <https://doi.org/10.1523/JNEUROSCI.5718-11.2012>
- Duncan, J. R., & Lawrence, A. J. (2012). The role of metabotropic glutamate receptors in addiction: Evidence from preclinical models. *Pharmacology, Biochemistry, and Behavior*, *100*(4), 811–824. <https://doi.org/10.1016/j.pbb.2011.03.015>
- Elman, I., Karlsgodt, K. H., & Gastfriend, D. R. (2001). Gender differences in cocaine craving among non-treatment-seeking individuals with cocaine dependence. *The American Journal of Drug and Alcohol Abuse*, *27*(2), 193–202. <https://doi.org/10.1081/ada-100103705>
- Evans, S. M., & Foltin, R. W. (2010). Does the response to cocaine differ as a function of sex or hormonal status in human and non-human primates? *Horm Behav*, *58*(1), 13–21. <https://doi.org/10.1016/j.yhbeh.2009.08.010>
- Famous, K. R., Kumaresan, V., Sadri-Vakili, G., Schmidt, H. D., Mierke, D. F., Cha, J. H., & Pierce, R. C. (2008). Phosphorylation-dependent trafficking of GluR2-containing AMPA receptors in the nucleus accumbens plays a critical role in the reinstatement of cocaine seeking. *J Neurosci*, *28*(43), 11061–11070. <https://doi.org/10.1523/JNEUROSCI.1221-08.2008>
- Fattore, L., Altea, S., & Fratta, W. (2008). Sex Differences in Drug Addiction: A Review of Animal and Human Studies. *Women's Health*, *4*(1), 51–65. <https://doi.org/10.2217/17455057.4.1.51>
- Feltenstein, M. W., Byrd, E. A., Henderson, A. R., & See, R. E. (2009). Attenuation of cocaine-seeking by progesterone treatment in female rats. *Psychoneuroendocrinology*, *34*(3), 343–352. <https://doi.org/10.1016/j.psyneuen.2008.09.014>
- Feltenstein, M. W., & See, R. E. (2007). Plasma progesterone levels and cocaine-seeking in freely cycling female rats across the estrous cycle. *Drug Alcohol Depend*, *89*(2–3), 183–189. <https://doi.org/10.1016/j.drugalcdep.2006.12.017>
- Festa, E. D., & Quinones-Jenab, V. (2004). Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine. *Horm Behav*, *46*(5), 509–519. <https://doi.org/10.1016/j.yhbeh.2004.04.009>
- Forlano, P. M., & Woolley, C. S. (2010). Quantitative analysis of pre- and postsynaptic sex differences in the nucleus accumbens. *J Comp Neurol*, *518*(8), 1330–1348. <https://doi.org/10.1002/cne.22279>

- Fourgeaud, L., Mato, S., Bouchet, D., Hemar, A., Worley, P. F., & Manzoni, O. J. (2004). A single in vivo exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. *J Neurosci*, *24*(31), 6939–6945. <https://doi.org/10.1523/JNEUROSCI.0671-04.2004>
- Frankfurt, M., Fuchs, E., & Wuttke, W. (1984). Sex differences in gamma-aminobutyric acid and glutamate concentrations in discrete rat brain nuclei. *Neurosci Lett*, *50*(1–3), 245–250. [https://doi.org/10.1016/0304-3940\(84\)90493-2](https://doi.org/10.1016/0304-3940(84)90493-2)
- Fritz, B. M., Muñoz, B., & Atwood, B. K. (2019). Genetic Selection for Alcohol Preference in Mice Alters Dorsal Striatum Neurotransmission. *Alcoholism, Clinical and Experimental Research*, *43*(11), 2312–2321. <https://doi.org/10.1111/acer.14187>
- Gall, C. M., Le, A. A., & Lynch, G. (2021). Sex differences in synaptic plasticity underlying learning. *Journal of Neuroscience Research*. <https://doi.org/10.1002/jnr.24844>
- 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*. <https://doi.org/10.1016/j.bbi.2019.01.006>
- Gardner, E. L. (2011). Addiction and brain reward and antireward pathways. *Advances in Psychosomatic Medicine*, *30*, 22–60. <https://doi.org/10.1159/000324065>
- Garelick, T., & Swann, J. (2014). Testosterone regulates the density of dendritic spines in the male preoptic area. *Horm Behav*, *65*(3), 249–253. <https://doi.org/10.1016/j.yhbeh.2014.01.008>
- Giacometti, L., & Barker, J. (2020). Sex differences in the glutamate system: Implications for addiction. *Neuroscience & Biobehavioral Reviews*, *113*, 157–168. <https://doi.org/10.1016/j.neubiorev.2020.03.010>
- Giacometti, L. L., & Barker, J. M. (2020). Sex differences in the glutamate system: Implications for addiction. *Neuroscience and Biobehavioral Reviews*, *113*, 157–168. <https://doi.org/10.1016/j.neubiorev.2020.03.010>
- Gipson, C. D., Reissner, K. J., Kupchik, Y. M., Smith, A. C. W., Stankeviciute, N., Hensley-Simon, M. E., & Kalivas, P. W. (2013). Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(22), 9124–9129. <https://doi.org/10.1073/pnas.1220591110>
- Gottmann, K. (2008). Transsynaptic modulation of the synaptic vesicle cycle by cell-adhesion molecules. *Journal of Neuroscience Research*, *86*(2). <https://doi.org/10.1002/jnr.21484>

- Greenfield, S. F., Brooks, A. J., Gordon, S. M., Green, C. A., Kropp, F., McHugh, R. K., Lincoln, M., Hien, D., & Miele, G. M. (2007). Substance abuse treatment entry, retention, and outcome in women: A review of the literature. *Drug Alcohol Depend*, *86*(1), 1–21. <https://doi.org/10.1016/j.drugalcdep.2006.05.012>
- Gross, K. S., Moore, K. M., Meisel, R. L., & Mermelstein, P. G. (2018). MGluR5 Mediates Dihydrotestosterone-Induced Nucleus Accumbens Structural Plasticity, but Not Conditioned Reward. *Front Neurosci*, *12*, 855. <https://doi.org/10.3389/fnins.2018.00855>
- Grove-Strawser, D., Boulware, M. I., & Mermelstein, P. G. (2010). Membrane estrogen receptors activate the metabotropic glutamate receptors mGluR5 and mGluR3 to bidirectionally regulate CREB phosphorylation in female rat striatal neurons. *Neuroscience*, *170*(4), 1045–1055. <https://doi.org/10.1016/j.neuroscience.2010.08.012>
- Guily, P., Lassalle, O., Chavis, P., & Manzoni, O. J. (2022). Sex-specific divergent maturational trajectories in the postnatal rat basolateral amygdala. *IScience*, *25*(2), 103815. <https://doi.org/10.1016/j.isci.2022.103815>
- Guire, E. S., Oh, M. C., Soderling, T. R., & Derkach, V. A. (2008). Recruitment of calcium-permeable AMPA receptors during synaptic potentiation is regulated by CaM-kinase I. *J Neurosci*, *28*(23), 6000–6009. <https://doi.org/10.1523/JNEUROSCI.0384-08.2008>
- Hanlon, C. A., Dowdle, L. T., Austelle, C. W., DeVries, W., Mithoefer, O., Badran, B. W., & George, M. S. (2015). What goes up, can come down: Novel brain stimulation paradigms may attenuate craving and craving-related neural circuitry in substance dependent individuals. *Brain Res*, *1628*(Pt A), 199–209. <https://doi.org/10.1016/j.brainres.2015.02.053>
- Hare, B. D., & Duman, R. S. (2020). Prefrontal cortex circuits in depression and anxiety: Contribution of discrete neuronal populations and target regions. *Mol Psychiatry*, *25*(11), 2742–2758. <https://doi.org/10.1038/s41380-020-0685-9>
- Harley, C. W., Malsbury, C. W., Squires, A., & Brown, R. A. (2000). Testosterone decreases CA1 plasticity in vivo in gonadectomized male rats. *Hippocampus*, *10*(6), 693–697. [https://doi.org/10.1002/1098-1063\(2000\)10:6<693::AID-HIPO1007>3.0.CO;2-G](https://doi.org/10.1002/1098-1063(2000)10:6<693::AID-HIPO1007>3.0.CO;2-G)
- Hatanaka, Y., Hojo, Y., Mukai, H., Murakami, G., Komatsuzaki, Y., Kim, J., Ikeda, M., Hiragushi, A., Kimoto, T., & Kawato, S. (2015). Rapid increase of spines by dihydrotestosterone and testosterone in hippocampal neurons: Dependence on synaptic androgen receptor and kinase networks. *Brain Res*, *1621*, 121–132. <https://doi.org/10.1016/j.brainres.2014.12.011>

- Hearing, M. (2019). Prefrontal-Accumbens Opioid Plasticity: Implications for relapse and dependence. *Pharmacological Research*, *139*, 158–165. <https://doi.org/10.1016/j.phrs.2018.11.012>
- Hearing, M., Graziane, N., Dong, Y., & Thomas, M. J. (2018). Opioid and Psychostimulant Plasticity: Targeting Overlap in Nucleus Accumbens Glutamate Signaling. *Trends in Pharmacological Sciences*, *39*(3), 276–294. <https://doi.org/10.1016/j.tips.2017.12.004>
- Hedegaard, H., Minino, A. M., & Warner, M. (2020). Drug Overdose Deaths in the United States, 1999-2019. *NCHS Data Brief*, *394*, 1–8.
- Heinsbroek, J. A., De Vries, T. J., & Peters, J. (2021). Glutamatergic Systems and Memory Mechanisms Underlying Opioid Addiction. *Cold Spring Harbor Perspectives in Medicine*, *11*(3), a039602. <https://doi.org/10.1101/cshperspect.a039602>
- Henley, J. M., & Wilkinson, K. A. (2013). AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging. *Dialogues Clin Neurosci*, *15*(1), 11–27.
- Hernández, A. I., Oxberry, W. C., Crary, J. F., Mirra, S. S., & Sacktor, T. C. (2014). Cellular and subcellular localization of PKM ζ . *Philosophical Transactions of the Royal Society B: Biological Sciences*, *369*(1633), 20130140. <https://doi.org/10.1098/rstb.2013.0140>
- Hrabetova, S., & Sacktor, T. C. (1996). Bidirectional regulation of protein kinase M zeta in the maintenance of long-term potentiation and long-term depression. *J Neurosci*, *16*(17), 5324–5333.
- Hsieh, C., Tsokas, P., Grau-Perales, A., Lesburguères, E., Bukai, J., Khanna, K., Chorny, J., Chung, A., Jou, C., Burghardt, N. S., Denny, C. A., Flores-Obando, R. E., Hartley, B. R., Rodríguez Valencia, L. M., Hernández, A. I., Bergold, P. J., Cottrell, J. E., Alarcon, J. M., Fenton, A. A., & Sacktor, T. C. (2021). Persistent increases of PKM ζ in memory-activated neurons trace LTP maintenance during spatial long-term memory storage. *The European Journal of Neuroscience*, *54*(8), 6795–6814. <https://doi.org/10.1111/ejn.15137>
- Hu, M., Crombag, H. S., Robinson, T. E., & Becker, J. B. (2004). Biological basis of sex differences in the propensity to self-administer cocaine. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *29*(1), 81–85. <https://doi.org/10.1038/sj.npp.1300301>
- Huhn, A. S., Berry, M. S., & Dunn, K. E. (2019). Review: Sex-Based Differences in Treatment Outcomes for Persons With Opioid Use Disorder. *The American Journal on Addictions*, *28*(4), 246–261. <https://doi.org/10.1111/ajad.12921>

- Hyer, M. M., Phillips, L. L., & Neigh, G. N. (2018). Sex Differences in Synaptic Plasticity: Hormones and Beyond. *Frontiers in Molecular Neuroscience, 11*, 266. <https://doi.org/10.3389/fnmol.2018.00266>
- Jackson, L. R., Robinson, T. E., & Becker, J. B. (2006). Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. *Neuropsychopharmacology, 31*(1), 129–138. <https://doi.org/10.1038/sj.npp.1300778>
- Jasinska, A. J., Chen, B. T., Bonci, A., & Stein, E. A. (2015). Dorsal medial prefrontal cortex (MPFC) circuitry in rodent models of cocaine use: Implications for drug addiction therapies. *Addict Biol, 20*(2), 215–226. <https://doi.org/10.1111/adb.12132>
- Johnson, K. P., Brooks, B. R., Cohen, J. A., Ford, C. C., Goldstein, J., Lisak, R. P., Myers, L. W., Panitch, H. S., Rose, J. W., & Schiffer, R. B. (1995). Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: Results of a phase III multicenter, double-blind placebo-controlled trial. The Copolymer 1 Multiple Sclerosis Study Group. *Neurology, 45*(7), 1268–1276. <https://doi.org/10.1212/wnl.45.7.1268>
- Johnson, R. E., Eissenberg, T., Stitzer, M. L., Strain, E. C., Liebson, I. A., & Bigelow, G. E. (1995). A placebo controlled clinical trial of buprenorphine as a treatment for opioid dependence. *Drug Alcohol Depend, 40*(1), 17–25. [https://doi.org/10.1016/0376-8716\(95\)01186-2](https://doi.org/10.1016/0376-8716(95)01186-2)
- Jones, H. E., Fitzgerald, H., & Johnson, R. E. (2005). Males and females differ in response to opioid agonist medications. *Am J Addict, 14*(3), 223–233. <https://doi.org/10.1080/10550490590949569>
- Kalivas, P. W. (2000). A role for glutamate transmission in addiction to psychostimulants. *Addiction Biology, 5*(3), 325–329. <https://doi.org/10.1111/j.1369-1600.2000.tb00199.x>
- Kalivas, P. W. (2004). Glutamate systems in cocaine addiction. *Current Opinion in Pharmacology, 4*(1), 23–29. <https://doi.org/10.1016/j.coph.2003.11.002>
- Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci, 10*(8), 561–572. <https://doi.org/10.1038/nrn2515>
- Kalivas, P. W., LaLumiere, R. T., Knackstedt, L., & Shen, H. (2009). Glutamate transmission in addiction. *Neuropharmacology, 56*, 169–173. <https://doi.org/10.1016/j.neuropharm.2008.07.011>
- Kalivas, P. W., Volkow, N., & Seamans, J. (2005). Unmanageable motivation in addiction: A pathology in prefrontal-accumbens glutamate transmission. *Neuron, 45*(5), 647–650. <https://doi.org/10.1016/j.neuron.2005.02.005>

- Kamalova, A., & Nakagawa, T. (2021). AMPA receptor structure and auxiliary subunits. *J Physiol*, *599*(2), 453–469. <https://doi.org/10.1113/JP278701>
- Kang, H. J., Voleti, B., Hajszan, T., Rajkowska, G., Stockmeier, C. A., Licznernski, P., Lepack, A., Majik, M. S., Jeong, L. S., Banasr, M., Son, H., & Duman, R. S. (2012). Decreased expression of synapse-related genes and loss of synapses in major depressive disorder. *Nature Medicine*, *18*(9), 1413–1417. <https://doi.org/10.1038/nm.2886>
- Kasture, S., Vinci, S., Ibba, F., Puddu, A., Marongiu, M., Murali, B., Pisanu, A., Lecca, D., Zernig, G., & Acquas, E. (2009). Withania somnifera prevents morphine withdrawal-induced decrease in spine density in nucleus accumbens shell of rats: A confocal laser scanning microscopy study. *Neurotox Res*, *16*(4), 343–355. <https://doi.org/10.1007/s12640-009-9069-2>
- Kau, K. S., Madayag, A., Mantsch, J. R., Grier, M. D., Abdulhameed, O., & Baker, D. A. (2008). Blunted cystine-glutamate antiporter function in the nucleus accumbens promotes cocaine-induced drug seeking. *Neuroscience*, *155*(2), 530–537. <https://doi.org/10.1016/j.neuroscience.2008.06.010>
- Kauer, J. A., & Malenka, R. C. (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci*, *8*(11), 844–858. <https://doi.org/10.1038/nrn2234>
- Kearney-Ramos, T. E., Dowdle, L. T., Lench, D. H., Mithoefer, O. J., Devries, W. H., George, M. S., Anton, R. F., & Hanlon, C. A. (2018). Transdiagnostic Effects of Ventromedial Prefrontal Cortex Transcranial Magnetic Stimulation on Cue Reactivity. *Biol Psychiatry Cogn Neurosci Neuroimaging*, *3*(7), 599–609. <https://doi.org/10.1016/j.bpsc.2018.03.016>
- Kennedy, A. P., Epstein, D. H., Phillips, K. A., & Preston, K. L. (2013). Sex differences in cocaine/heroin users: Drug-use triggers and craving in daily life. *Drug and Alcohol Dependence*, *132*(1–2), 29–37. <https://doi.org/10.1016/j.drugalcdep.2012.12.025>
- Kenney, J. W., & Gould, T. J. (2008). Modulation of hippocampus-dependent learning and synaptic plasticity by nicotine. *Molecular Neurobiology*, *38*(1), 101–121. <https://doi.org/10.1007/s12035-008-8037-9>
- Kenny, P. J., & Markou, A. (2004). The ups and downs of addiction: Role of metabotropic glutamate receptors. *Trends in Pharmacological Sciences*, *25*(5), 265–272. <https://doi.org/10.1016/j.tips.2004.03.009>
- Kerstetter, K. A., Aguilar, V. R., Parrish, A. B., & Kippin, T. E. (2008). Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. *Psychopharmacology (Berl)*, *198*(1), 63–75. <https://doi.org/10.1007/s00213-008-1089-8>

- Kerstetter, K. A., Ballis, M. A., Duffin-Lutgen, S., Carr, A. E., Behrens, A. M., & Kippin, T. E. (2012). Sex differences in selecting between food and cocaine reinforcement are mediated by estrogen. *Neuropsychopharmacology*, *37*(12), 2605–2614. <https://doi.org/10.1038/npp.2012.99>
- Kimbrough, A., Kononoff, J., Simpson, S., Kallupi, M., Sedighim, S., Palomino, K., Conlisk, D., Momper, J. D., de Guglielmo, G., & George, O. (2020). Oxycodone self-administration and withdrawal behaviors in male and female Wistar rats. *Psychopharmacology (Berl)*, *237*(5), 1545–1555. <https://doi.org/10.1007/s00213-020-05479-y>
- Kippin, T. E., Fuchs, R. A., Mehta, R. H., Case, J. M., Parker, M. P., Bimonte-Nelson, H. A., & See, R. E. (2005). Potentiation of cocaine-primed reinstatement of drug seeking in female rats during estrus. *Psychopharmacology (Berl)*, *182*(2), 245–252. <https://doi.org/10.1007/s00213-005-0071-y>
- Klein, L. C., Popke, E. J., & Grunberg, N. E. (1997). Sex differences in effects of predictable and unpredictable footshock on fentanyl self-administration in rats. *Experimental and Clinical Psychopharmacology*, *5*(2), 99–106. <https://doi.org/10.1037//1064-1297.5.2.99>
- Klenowski, P. M. (2018). Emerging role for the medial prefrontal cortex in alcohol-seeking behaviors. *Addict Behav*, *77*, 102–106. <https://doi.org/10.1016/j.addbeh.2017.09.024>
- Knackstedt, L. A., & Kalivas, P. W. (2009). Glutamate and reinstatement. *Current Opinion in Pharmacology*, *9*(1), 59–64. <https://doi.org/10.1016/j.coph.2008.12.003>
- Knackstedt, L. A., Melendez, R. I., & Kalivas, P. W. (2010). Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking. *Biological Psychiatry*, *67*(1), 81–84. <https://doi.org/10.1016/j.biopsych.2009.07.018>
- Knackstedt, L. A., Moussawi, K., Lalumiere, R., Schwendt, M., Klugmann, M., & Kalivas, P. W. (2010). Extinction training after cocaine self-administration induces glutamatergic plasticity to inhibit cocaine seeking. *J Neurosci*, *30*(23), 7984–7992. <https://doi.org/10.1523/JNEUROSCI.1244-10.2010>
- Knouse, M. C., & Briand, L. A. (2021). Behavioral sex differences in cocaine and opioid use disorders: The role of gonadal hormones. *Neuroscience and Biobehavioral Reviews*, *128*, 358–366. <https://doi.org/10.1016/j.neubiorev.2021.06.038>
- Knouse, M. C., McGrath, A. G., Deutschmann, A. U., Rich, M. T., Zallar, L. J., Rajadhyaksha, A. M., & Briand, L. A. (2022). Sex differences in the medial prefrontal cortical glutamate system. *Biology of Sex Differences*, *13*(1), 66. <https://doi.org/10.1186/s13293-022-00468-6>

- Koob, G. F., & Volkow, N. D. (2016). Neurobiology of addiction: A neurocircuitry analysis. *The Lancet. Psychiatry*, 3(8), 760–773. [https://doi.org/10.1016/S2215-0366\(16\)00104-8](https://doi.org/10.1016/S2215-0366(16)00104-8)
- Koss, W. A., & Frick, K. M. (2017). Sex differences in hippocampal function. *Journal of Neuroscience Research*, 95(1–2), 539–562. <https://doi.org/10.1002/jnr.23864>
- Kourrich, S., Rothwell, P. E., Klug, J. R., & Thomas, M. J. (2007). Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, 27(30), 7921–7928. <https://doi.org/10.1523/JNEUROSCI.1859-07.2007>
- Kovacs, E. G., MacLusky, N. J., & Leranth, C. (2003). Effects of testosterone on hippocampal CA1 spine synaptic density in the male rat are inhibited by fimbria/fornix transection. *Neuroscience*, 122(3), 807–810. <https://doi.org/10.1016/j.neuroscience.2003.08.046>
- Krentzel, A. A., & Meitzen, J. (2018). Biological Sex, Estradiol and Striatal Medium Spiny Neuron Physiology: A Mini-Review. *Frontiers in Cellular Neuroscience*, 12, 492. <https://doi.org/10.3389/fncel.2018.00492>
- Krentzel, A. A., Proaño, S. B., Dorris, D. M., Setzer, B., & Meitzen, J. (2022). The estrous cycle and 17 β -estradiol modulate the electrophysiological properties of rat nucleus accumbens core medium spiny neurons. *Journal of Neuroendocrinology*, 34(6), e13122. <https://doi.org/10.1111/jne.13122>
- Krueyer, A., Chioma, V. C., & Kalivas, P. W. (2020). The Opioid-Addicted Tetrapartite Synapse. *Biological Psychiatry*, 87(1), 34–43. <https://doi.org/10.1016/j.biopsych.2019.05.025>
- Krzanowska, E. K., Ogawa, S., Pfaff, D. W., & Bodnar, R. J. (2002). Reversal of sex differences in morphine analgesia elicited from the ventrolateral periaqueductal gray in rats by neonatal hormone manipulations. *Brain Res*, 929(1), 1–9. [https://doi.org/10.1016/s0006-8993\(01\)03350-9](https://doi.org/10.1016/s0006-8993(01)03350-9)
- Kuhn, C. M., Walker, Q. D., Kaplan, K. A., & Li, S. T. (2001). Sex, steroids, and stimulant sensitivity. *Ann N Y Acad Sci*, 937, 188–201. <https://doi.org/10.1111/j.1749-6632.2001.tb03565.x>
- Lacy, R. T., Strickland, J. C., Feinstein, M. A., Robinson, A. M., & Smith, M. A. (2016). The effects of sex, estrous cycle, and social contact on cocaine and heroin self-administration in rats. *Psychopharmacology (Berl)*, 233(17), 3201–3210. <https://doi.org/10.1007/s00213-016-4368-9>
- Lapish, C. C., Seamans, J. K., & Judson Chandler, L. (2006). Glutamate-Dopamine Cotransmission and Reward Processing in Addiction. *Alcoholism: Clinical and Experimental Research*, 30(9), 1451–1465. <https://doi.org/10.1111/j.1530-0277.2006.00176.x>

- Larson, E. B., & Carroll, M. E. (2007). Estrogen receptor beta, but not alpha, mediates estrogen's effect on cocaine-induced reinstatement of extinguished cocaine-seeking behavior in ovariectomized female rats. *Neuropsychopharmacology*, *32*(6), 1334–1345. <https://doi.org/10.1038/sj.npp.1301249>
- Larson, E. B., Roth, M. E., Anker, J. J., & Carroll, M. E. (2005). Effect of short- vs. Long-term estrogen on reinstatement of cocaine-seeking behavior in female rats. *Pharmacol Biochem Behav*, *82*(1), 98–108. <https://doi.org/10.1016/j.pbb.2005.07.015>
- Lee, A. M., Kanter, B. R., Wang, D., Lim, J. P., Zou, M. E., Qiu, C., McMahon, T., Dadgar, J., Fischbach-Weiss, S. C., & Messing, R. O. (2013). Prkcz null mice show normal learning and memory. *Nature*, *493*(7432), 416–419. <https://doi.org/10.1038/nature11803>
- Lee, A. M., Zou, M. E., Lim, J. P., Stecher, J., McMahon, T., & Messing, R. O. (2014). Deletion of Prkcz increases intermittent ethanol consumption in mice. *Alcoholism, Clinical and Experimental Research*, *38*(1), 170–178. <https://doi.org/10.1111/acer.12211>
- Lee, S. K. (2018). Sex as an important biological variable in biomedical research. *BMB Rep*, *51*(4), 167–173. <https://doi.org/10.5483/bmbrep.2018.51.4.034>
- Leite-Morris, K. A., Kobrin, K. L., Guy, M. D., Young, A. J., Heinrichs, S. C., & Kaplan, G. B. (2014). Extinction of opiate reward reduces dendritic arborization and c-Fos expression in the nucleus accumbens core. *Behav Brain Res*, *263*, 51–59. <https://doi.org/10.1016/j.bbr.2013.12.041>
- Leranth, C., Petnehazy, O., & MacLusky, N. J. (2003). Gonadal hormones affect spine synaptic density in the CA1 hippocampal subfield of male rats. *J Neurosci*, *23*(5), 1588–1592.
- Li, Y., Xue, Y., He, Y., Li, F., Xue, L., Xu, C., Sacktor, T. C., Shaham, Y., & Lu, L. (2011). Inhibition of PKMzeta in nucleus accumbens core abolishes long-term drug reward memory. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *31*(14), 5436–5446. <https://doi.org/10.1523/JNEUROSCI.5884-10.2011>
- Ling, D. S., Benardo, L. S., & Sacktor, T. C. (2006). Protein kinase Mzeta enhances excitatory synaptic transmission by increasing the number of active postsynaptic AMPA receptors. *Hippocampus*, *16*(5), 443–452. <https://doi.org/10.1002/hipo.20171>
- Ling, D. S. F., Benardo, L. S., Serrano, P. A., Blace, N., Kelly, M. T., Crary, J. F., & Sacktor, T. C. (2002). Protein kinase Mzeta is necessary and sufficient for LTP maintenance. *Nature Neuroscience*, *5*(4), 295–296. <https://doi.org/10.1038/nn829>

- Ling, S., Mangaoil, R., Cleverley, K., Sproule, B., & Puts, M. (2019). A systematic review of sex differences in treatment outcomes among people with opioid use disorder receiving buprenorphine maintenance versus other treatment conditions. *Drug and Alcohol Dependence*, *197*, 168–182. <https://doi.org/10.1016/j.drugalcdep.2019.02.007>
- Loweth, J. A., Tseng, K. Y., & Wolf, M. E. (2013). Using metabotropic glutamate receptors to modulate cocaine's synaptic and behavioral effects: MGLuR1 finds a niche. *Current Opinion in Neurobiology*, *23*(4), 500–506. <https://doi.org/10.1016/j.conb.2013.01.009>
- Loweth, J. A., Tseng, K. Y., & Wolf, M. E. (2014). Adaptations in AMPA receptor transmission in the nucleus accumbens contributing to incubation of cocaine craving. *Neuropharmacology*, *76 Pt B*, 287–300. <https://doi.org/10.1016/j.neuropharm.2013.04.061>
- Lynch, W. J. (2008). Acquisition and maintenance of cocaine self-administration in adolescent rats: Effects of sex and gonadal hormones. *Psychopharmacology (Berl)*, *197*(2), 237–246. <https://doi.org/10.1007/s00213-007-1028-0>
- Lynch, W. J., & Carroll, M. E. (1999). Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. *Psychopharmacology*, *144*(1), 77–82. <https://doi.org/10.1007/s002130050979>
- Lynch, W. J., & Carroll, M. E. (2000). Reinstatement of cocaine self-administration in rats: Sex differences. *Psychopharmacology*, *148*(2), 196–200. <https://doi.org/10.1007/s002130050042>
- Lynch, W. J., Roth, M. E., Mickelberg, J. L., & Carroll, M. E. (2001). Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats. *Pharmacol Biochem Behav*, *68*(4), 641–646. [https://doi.org/10.1016/s0091-3057\(01\)00455-5](https://doi.org/10.1016/s0091-3057(01)00455-5)
- Lynch, W. J., & Taylor, J. R. (2004). Sex differences in the behavioral effects of 24-h/day access to cocaine under a discrete trial procedure. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *29*(5), 943–951. <https://doi.org/10.1038/sj.npp.1300389>
- Lynch, W. J., & Taylor, J. R. (2005). Decreased motivation following cocaine self-administration under extended access conditions: Effects of sex and ovarian hormones. *Neuropsychopharmacology*, *30*(5), 927–935. <https://doi.org/10.1038/sj.npp.1300656>
- Ma, Y. Y., Cepeda, C., Chatta, P., Franklin, L., Evans, C. J., & Levine, M. S. (2012). Regional and cell-type-specific effects of DAMGO on striatal D1 and D2 dopamine receptor-expressing medium-sized spiny neurons. *ASN Neuro*, *4*(2). <https://doi.org/10.1042/AN20110063>

- Madayag, A., Lobner, D., Kau, K. S., Mantsch, J. R., Abdulhameed, O., Hearing, M., Grier, M. D., & Baker, D. A. (2007). Repeated N-acetylcysteine administration alters plasticity-dependent effects of cocaine. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*(51), 13968–13976. <https://doi.org/10.1523/JNEUROSCI.2808-07.2007>
- Malenka, R. C. (2003). Synaptic Plasticity and AMPA Receptor Trafficking. *Annals of the New York Academy of Sciences*, *1003*(1), 1–11. <https://doi.org/10.1196/annals.1300.001>
- Malinow, R., & Malenka, R. C. (2002). AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci*, *25*, 103–126. <https://doi.org/10.1146/annurev.neuro.25.112701.142758>
- Mamlouk, G. M., Dorris, D. M., Barrett, L. R., & Meitzen, J. (2020). Sex bias and omission in neuroscience research is influenced by research model and journal, but not reported NIH funding. *Frontiers in Neuroendocrinology*, *57*, 100835. <https://doi.org/10.1016/j.yfrne.2020.100835>
- Márquez, J., Campos-Sandoval, J. A., Peñalver, A., Matés, J. M., Segura, J. A., Blanco, E., Alonso, F. J., & de Fonseca, F. R. (2017). Glutamate and Brain Glutaminases in Drug Addiction. *Neurochemical Research*, *42*(3), 846–857. <https://doi.org/10.1007/s11064-016-2137-0>
- Martin, M., Chen, B. T., Hopf, F. W., Bowers, M. S., & Bonci, A. (2006). Cocaine self-administration selectively abolishes LTD in the core of the nucleus accumbens. *Nature Neuroscience*, *9*(7), 868–869. <https://doi.org/10.1038/nn1713>
- Martinez, L. A., Gross, K. S., Himmler, B. T., Emmitt, N. L., Peterson, B. M., Zlebnik, N. E., Foster Olive, M., Carroll, M. E., Meisel, R. L., & Mermelstein, P. G. (2016). Estradiol Facilitation of Cocaine Self-Administration in Female Rats Requires Activation of mGluR5. *ENeuro*, *3*(5). <https://doi.org/10.1523/ENEURO.0140-16.2016>
- Martinez, L. A., Peterson, B. M., Meisel, R. L., & Mermelstein, P. G. (2014). Estradiol facilitation of cocaine-induced locomotor sensitization in female rats requires activation of mGluR5. *Behav Brain Res*, *271*, 39–42. <https://doi.org/10.1016/j.bbr.2014.05.052>
- McBride, D., Barrett, S. P., Kelly, J. T., Aw, A., & Dagher, A. (2006). Effects of expectancy and abstinence on the neural response to smoking cues in cigarette smokers: An fMRI study. *Neuropsychopharmacology*, *31*(12), 2728–2738. <https://doi.org/10.1038/sj.npp.1301075>
- McGrath, A. G., Lenz, J. D., & Briand, L. A. (2018). PKM ζ in the nucleus accumbens acts to dampen cocaine seeking. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *43*(12), 2390–2398. <https://doi.org/10.1038/s41386-018-0170-1>

- McHugh, R. K., Votaw, V. R., Sugarman, D. E., & Greenfield, S. F. (2018). Sex and gender differences in substance use disorders. *Clin Psychol Rev*, *66*, 12–23. <https://doi.org/10.1016/j.cpr.2017.10.012>
- McLaughlin, K. J., Bimonte-Nelson, H., Neisewander, J. L., & Conrad, C. D. (2008). Assessment of estradiol influence on spatial tasks and hippocampal CA1 spines: Evidence that the duration of hormone deprivation after ovariectomy compromises 17beta-estradiol effectiveness in altering CA1 spines. *Horm Behav*, *54*(3), 386–395. <https://doi.org/10.1016/j.yhbeh.2008.04.010>
- Meitzen, J., Meisel, R. L., & Mermelstein, P. G. (2018). Sex Differences and the Effects of Estradiol on Striatal Function. *Current Opinion in Behavioral Sciences*, *23*, 42–48. <https://doi.org/10.1016/j.cobeha.2018.03.007>
- Mello, N. K., Knudson, I. M., Kelly, M., Fivel, P. A., & Mendelson, J. H. (2011). Effects of progesterone and testosterone on cocaine self-administration and cocaine discrimination by female rhesus monkeys. *Neuropsychopharmacology*, *36*(11), 2187–2199. <https://doi.org/10.1038/npp.2011.130>
- Mello, N. K., Knudson, I. M., & Mendelson, J. H. (2007). Sex and menstrual cycle effects on progressive ratio measures of cocaine self-administration in cynomolgus monkeys. *Neuropsychopharmacology*, *32*(9), 1956–1966. <https://doi.org/10.1038/sj.npp.1301314>
- Mendelson, J. H., & Mello, N. K. (1975). Plasma testosterone levels during chronic heroin use and protracted abstinence. A study of Hong Kong addicts. *Clin Pharmacol Ther*, *17*(5), 529–533. <https://doi.org/10.1002/cpt1975175529>
- Mirbaha, H., Tabaeizadeh, M., Shaterian-Mohammadi, H., Tahsili-Fahadan, P., & Dehpour, A. R. (2009). Estrogen pretreatment modulates morphine-induced conditioned place preference in ovariectomized mice. *Pharmacol Biochem Behav*, *92*(3), 399–403. <https://doi.org/10.1016/j.pbb.2009.01.009>
- Moran, M. M., McFarland, K., Melendez, R. I., Kalivas, P. W., & Seamans, J. K. (2005). Cystine/glutamate exchange regulates metabotropic glutamate receptor presynaptic inhibition of excitatory transmission and vulnerability to cocaine seeking. *J Neurosci*, *25*(27), 6389–6393. <https://doi.org/10.1523/JNEUROSCI.1007-05.2005>
- Moussawi, K., Pacchioni, A., Moran, M., Olive, M. F., Gass, J. T., Lavin, A., & Kalivas, P. W. (2009). N-Acetylcysteine reverses cocaine-induced metaplasticity. *Nat Neurosci*, *12*(2), 182–189. <https://doi.org/10.1038/nn.2250>
- Moussawi, K., Zhou, W., Shen, H., Reichel, C. M., See, R. E., Carr, D. B., & Kalivas, P. W. (2011). Reversing cocaine-induced synaptic potentiation provides enduring protection from relapse. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(1), 385–390. <https://doi.org/10.1073/pnas.1011265108>

- Norrholm, S. D., Bibb, J. A., Nestler, E. J., Ouimet, C. C., Taylor, J. R., & Greengard, P. (2003). Cocaine-induced proliferation of dendritic spines in nucleus accumbens is dependent on the activity of cyclin-dependent kinase-5. *Neuroscience*, *116*(1), 19–22. [https://doi.org/10.1016/s0306-4522\(02\)00560-2](https://doi.org/10.1016/s0306-4522(02)00560-2)
- Oh, M. C., & Derkach, V. A. (2005). Dominant role of the GluR2 subunit in regulation of AMPA receptors by CaMKII. *Nat Neurosci*, *8*(7), 853–854. <https://doi.org/10.1038/nn1476>
- Parylak, S. L., Caster, J. M., Walker, Q. D., & Kuhn, C. M. (2008). Gonadal steroids mediate the opposite changes in cocaine-induced locomotion across adolescence in male and female rats. *Pharmacol Biochem Behav*, *89*(3), 314–323. <https://doi.org/10.1016/j.pbb.2008.01.003>
- Pastalkova, E., Serrano, P., Pinkhasova, D., Wallace, E., Fenton, A. A., & Sacktor, T. C. (2006). Storage of spatial information by the maintenance mechanism of LTP. *Science (New York, N.Y.)*, *313*(5790), 1141–1144. <https://doi.org/10.1126/science.1128657>
- Pena-Bravo, J. I., Penrod, R., Reichel, C. M., & Lavin, A. (2019). Methamphetamine Self-Administration Elicits Sex-Related Changes in Postsynaptic Glutamate Transmission in the Prefrontal Cortex. *ENeuro*, *6*(1), ENEURO.0401-18.2018. <https://doi.org/10.1523/ENeuro.0401-18.2018>
- Perry, A. N., Westenbroek, C., & Becker, J. B. (2013). The development of a preference for cocaine over food identifies individual rats with addiction-like behaviors. *PLoS One*, *8*(11), e79465. <https://doi.org/10.1371/journal.pone.0079465>
- Perry, C. J., Campbell, E. J., Drummond, K. D., Lum, J. S., & Kim, J. H. (2021). Sex differences in the neurochemistry of frontal cortex: Impact of early life stress. *Journal of Neurochemistry*, *157*(4), 963–981. <https://doi.org/10.1111/jnc.15208>
- Peters, J., & Kalivas, P. W. (2006). The group II metabotropic glutamate receptor agonist, LY379268, inhibits both cocaine- and food-seeking behavior in rats. *Psychopharmacology (Berl)*, *186*(2), 143–149. <https://doi.org/10.1007/s00213-006-0372-9>
- Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learn Mem*, *16*(5), 279–288. <https://doi.org/10.1101/lm.1041309>
- Peterson, B. M., Mermelstein, P. G., & Meisel, R. L. (2015). Estradiol mediates dendritic spine plasticity in the nucleus accumbens core through activation of mGluR5. *Brain Struct Funct*, *220*(4), 2415–2422. <https://doi.org/10.1007/s00429-014-0794-9>

- Phoenix, C. H., Goy, R. W., Gerall, A. A., & Young, W. C. (1959). Organizing action of prenatally administered testosterone propionate on the tissues mediating mating behavior in the female guinea pig. *Endocrinology*, *65*, 369–382.
<https://doi.org/10.1210/endo-65-3-369>
- Popik, P., & Wrobel, M. (2002). Morphine conditioned reward is inhibited by MPEP, the mGluR5 antagonist. *Neuropharmacology*, *43*(8), 1210–1217.
[https://doi.org/10.1016/s0028-3908\(02\)00309-x](https://doi.org/10.1016/s0028-3908(02)00309-x)
- Proano, S. B., Morris, H. J., Kunz, L. M., Dorris, D. M., & Meitzen, J. (2018). Estrous cycle-induced sex differences in medium spiny neuron excitatory synaptic transmission and intrinsic excitability in adult rat nucleus accumbens core. *J Neurophysiol*, *120*(3), 1356–1373. <https://doi.org/10.1152/jn.00263.2018>
- Purkey, A. M., & Dell'Acqua, M. L. (2020). Phosphorylation-Dependent Regulation of Ca²⁺-Permeable AMPA Receptors During Hippocampal Synaptic Plasticity. *Frontiers in Synaptic Neuroscience*, *12*, 8.
<https://doi.org/10.3389/fnsyn.2020.00008>
- Qian, Z., Wu, X., Qiao, Y., Shi, M., Liu, Z., Ren, W., Han, J., & Zheng, Q. (2019). Downregulation of mGluR2/3 receptors during morphine withdrawal in rats impairs mGluR2/3- and NMDA receptor-dependent long-term depression in the nucleus accumbens. *Neurosci Lett*, *690*, 76–82.
<https://doi.org/10.1016/j.neulet.2018.10.018>
- Quigley, J. A., Logsdon, M. K., Turner, C. A., Gonzalez, I. L., Leonardo, N. B., & Becker, J. B. (2021). Sex differences in vulnerability to addiction. *Neuropharmacology*, *187*, 108491.
<https://doi.org/10.1016/j.neuropharm.2021.108491>
- Ramoia, C. P., Doyle, S. E., Naim, D. W., & Lynch, W. J. (2013). Estradiol as a mechanism for sex differences in the development of an addicted phenotype following extended access cocaine self-administration. *Neuropsychopharmacology*, *38*(9), 1698–1705.
<https://doi.org/10.1038/npp.2013.68>
- Ramôa, C. P., Doyle, S. E., Naim, D. W., & Lynch, W. J. (2013). Estradiol as a mechanism for sex differences in the development of an addicted phenotype following extended access cocaine self-administration. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *38*(9), 1698–1705.
<https://doi.org/10.1038/npp.2013.68>
- Rao, S. S., & Saifi, A. Q. (1985). Influence of testosterone on morphine analgesia in albino rats. *Indian J Physiol Pharmacol*, *29*(2), 103–106.

- Rasia-Filho, A. A., Fabian, C., Rigoti, K. M., & Achaval, M. (2004). Influence of sex, estrous cycle and motherhood on dendritic spine density in the rat medial amygdala revealed by the Golgi method. *Neuroscience*, *126*(4), 839–847. <https://doi.org/10.1016/j.neuroscience.2004.04.009>
- Rehm, J., & Imtiaz, S. (2016). A narrative review of alcohol consumption as a risk factor for global burden of disease. *Subst Abuse Treat Prev Policy*, *11*(1), 37. <https://doi.org/10.1186/s13011-016-0081-2>
- Reiss, D., Maduna, T., Maurin, H., Audouard, E., & Gaveriaux-Ruff, C. (2020). Mu opioid receptor in microglia contributes to morphine analgesic tolerance, hyperalgesia, and withdrawal in mice. *J Neurosci Res*. <https://doi.org/10.1002/jnr.24626>
- Reissner, K. J., & Kalivas, P. W. (2010). Using glutamate homeostasis as a target for treating addictive disorders. *Behavioural Pharmacology*, *21*(5–6), 514–522. <https://doi.org/10.1097/FBP.0b013e32833d41b2>
- Ribeiro-Dasilva, M. C., Shinal, R. M., Glover, T., Williams, R. S., Staud, R., Riley, J. L., 3rd, & Fillingim, R. B. (2011). Evaluation of menstrual cycle effects on morphine and pentazocine analgesia. *Pain*, *152*(3), 614–622. <https://doi.org/10.1016/j.pain.2010.11.033>
- Riga, D., Matos, M. R., Glas, A., Smit, A. B., Spijker, S., & Van den Oever, M. C. (2014). Optogenetic dissection of medial prefrontal cortex circuitry. *Front Syst Neurosci*, *8*, 230. <https://doi.org/10.3389/fnsys.2014.00230>
- Robbe, D., Bockaert, J., & Manzoni, O. J. (2002). Metabotropic glutamate receptor 2/3-dependent long-term depression in the nucleus accumbens is blocked in morphine withdrawn mice. *Eur J Neurosci*, *16*(11), 2231–2235. <https://doi.org/10.1046/j.1460-9568.2002.02273.x>
- Roberts, D. C., Bennett, S. A., & Vickers, G. J. (1989). The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. *Psychopharmacology*, *98*(3), 408–411. <https://doi.org/10.1007/BF00451696>
- Roberts-Wolfe, D. J., & Kalivas, P. W. (2015). Glutamate Transporter GLT-1 as a Therapeutic Target for Substance Use Disorders. *CNS & Neurological Disorders Drug Targets*, *14*(6), 745–756. <https://doi.org/10.2174/1871527314666150529144655>
- Robinson, T. E., Gorny, G., Mitton, E., & Kolb, B. (2001). Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. *Synapse*, *39*(3), 257–266. [https://doi.org/10.1002/1098-2396\(20010301\)39:3<257::AID-SYN1007>3.0.CO;2-1](https://doi.org/10.1002/1098-2396(20010301)39:3<257::AID-SYN1007>3.0.CO;2-1)

- Robinson, T. E., Gorny, G., Savage, V. R., & Kolb, B. (2002). Widespread but regionally specific effects of experimenter- versus self-administered morphine on dendritic spines in the nucleus accumbens, hippocampus, and neocortex of adult rats. *Synapse*, *46*(4), 271–279. <https://doi.org/10.1002/syn.10146>
- Robinson, T. E., & Kolb, B. (1999). Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur J Neurosci*, *11*(5), 1598–1604.
- Romeo, R. D. (2003). Puberty: A period of both organizational and activational effects of steroid hormones on neurobehavioural development. *J Neuroendocrinol*, *15*(12), 1185–1192. <https://doi.org/10.1111/j.1365-2826.2003.01106.x>
- Ron, S., Dudai, Y., & Segal, M. (2012). Overexpression of PKM ζ alters morphology and function of dendritic spines in cultured cortical neurons. *Cerebral Cortex (New York, N.Y.: 1991)*, *22*(11), 2519–2528. <https://doi.org/10.1093/cercor/bhr323>
- Ross, S., & Peselow, E. (2009). The neurobiology of addictive disorders. *Clinical Neuropharmacology*, *32*(5), 269–276. <https://doi.org/10.1097/wnf.0b013e3181a9163c>
- Roth, M. E., & Carroll, M. E. (2004). Sex differences in the escalation of intravenous cocaine intake following long- or short-access to cocaine self-administration. *Pharmacol Biochem Behav*, *78*(2), 199–207. <https://doi.org/10.1016/j.pbb.2004.03.018>
- Roth, M. E., Casimir, A. G., & Carroll, M. E. (2002). Influence of estrogen in the acquisition of intravenously self-administered heroin in female rats. *Pharmacol Biochem Behav*, *72*(1–2), 313–318. [https://doi.org/10.1016/s0091-3057\(01\)00777-8](https://doi.org/10.1016/s0091-3057(01)00777-8)
- Russo, S. J., Jenab, S., Fabian, S. J., Festa, E. D., Kemen, L. M., & Quinones-Jenab, V. (2003). Sex differences in the conditioned rewarding effects of cocaine. *Brain Res*, *970*(1–2), 214–220.
- Sacktor, T. C., Osten, P., Valsamis, H., Jiang, X., Naik, M. U., & Sublette, E. (1993). Persistent activation of the zeta isoform of protein kinase C in the maintenance of long-term potentiation. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(18), 8342–8346. <https://doi.org/10.1073/pnas.90.18.8342>
- Sanchez, M. G., Morissette, M., & Di Paolo, T. (2012). Effect of a chronic treatment with 17beta-estradiol on striatal dopamine neurotransmission and the Akt/GSK3 signaling pathway in the brain of ovariectomized monkeys. *Psychoneuroendocrinology*, *37*(2), 280–291. <https://doi.org/10.1016/j.psyneuen.2011.06.012>

- Schmidt, H. D., & Pierce, R. C. (2010). Cocaine-induced neuroadaptations in glutamate transmission: Potential therapeutic targets for craving and addiction. *Ann N Y Acad Sci*, *1187*, 35–75. <https://doi.org/10.1111/j.1749-6632.2009.05144.x>
- Schottenfeld, R. S., Pakes, J. R., & Kosten, T. R. (1998). Prognostic factors in Buprenorphine- versus methadone-maintained patients. *J Nerv Ment Dis*, *186*(1), 35–43. <https://doi.org/10.1097/00005053-199801000-00006>
- Schuette, S. R. M., Fernández-Fernández, D., Lamla, T., Rosenbrock, H., & Hobson, S. (2016). Overexpression of Protein Kinase M ζ in the Hippocampus Enhances Long-Term Potentiation and Long-Term Contextual But Not Cued Fear Memory in Rats. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *36*(15), 4313–4324. <https://doi.org/10.1523/JNEUROSCI.3600-15.2016>
- Scofield, M. D., Heinsbroek, J. A., Gipson, C. D., Kupchik, Y. M., Spencer, S., Smith, A. C., Roberts-Wolfe, D., & Kalivas, P. W. (2016). The Nucleus Accumbens: Mechanisms of Addiction across Drug Classes Reflect the Importance of Glutamate Homeostasis. *Pharmacol Rev*, *68*(3), 816–871. <https://doi.org/10.1124/pr.116.012484>
- Sedki, F., Gardner Gregory, J., Luminare, A., D’Cunha, T. M., & Shalev, U. (2015). Food restriction-induced augmentation of heroin seeking in female rats: Manipulations of ovarian hormones. *Psychopharmacology (Berl)*, *232*(20), 3773–3782. <https://doi.org/10.1007/s00213-015-4037-4>
- Segarra, A. C., Agosto-Rivera, J. L., Febo, M., Lugo-Escobar, N., Menendez-Delmestre, R., Puig-Ramos, A., & Torres-Diaz, Y. M. (2010). Estradiol: A key biological substrate mediating the response to cocaine in female rats. *Horm Behav*, *58*(1), 33–43. <https://doi.org/10.1016/j.yhbeh.2009.12.003>
- Seguí, H. A., Melin, K., Quiñones, D. S., & Duconge, J. (2020). A review of the pharmacogenomics of buprenorphine for the treatment of opioid use disorder. *Journal of Translational Genetics and Genomics*, *4*, 263–277. <https://doi.org/10.20517/jtgg.2020.35>
- Shen, H., & Kalivas, P. W. (2013). Reduced LTP and LTD in prefrontal cortex synapses in the nucleus accumbens after heroin self-administration. *Int J Neuropsychopharmacol*, *16*(5), 1165–1167. <https://doi.org/10.1017/S1461145712001071>
- Shen, H., Moussawi, K., Zhou, W., Toda, S., & Kalivas, P. W. (2011). Heroin relapse requires long-term potentiation-like plasticity mediated by NMDA2b-containing receptors. *Proc Natl Acad Sci U S A*, *108*(48), 19407–19412. <https://doi.org/10.1073/pnas.1112052108>

- Shin, L. M., & Liberzon, I. (2010). The neurocircuitry of fear, stress, and anxiety disorders. *Neuropsychopharmacology*, *35*(1), 169–191. <https://doi.org/10.1038/npp.2009.83>
- Siemsen, B. M., Giannotti, G., McFaddin, J. A., Scofield, M. D., & McGinty, J. F. (2019). Biphasic effect of abstinence duration following cocaine self-administration on spine morphology and plasticity-related proteins in prelimbic cortical neurons projecting to the nucleus accumbens core. *Brain Struct Funct*, *224*(2), 741–758. <https://doi.org/10.1007/s00429-018-1805-z>
- Simon, N. M., Zalta, A. K., Worthington, J. J., 3rd, Hoge, E. A., Christian, K. M., Stevens, J. C., & Pollack, M. H. (2006). Preliminary support for gender differences in response to fluoxetine for generalized anxiety disorder. *Depress Anxiety*, *23*(6), 373–376. <https://doi.org/10.1002/da.20184>
- Simpson, J., & Kelly, J. P. (2012). An investigation of whether there are sex differences in certain behavioural and neurochemical parameters in the rat. *Behavioural Brain Research*, *229*(1), 289–300. <https://doi.org/10.1016/j.bbr.2011.12.036>
- Sircar, R., & Kim, D. (1999). Female gonadal hormones differentially modulate cocaine-induced behavioral sensitization in Fischer, Lewis, and Sprague-Dawley rats. *J Pharmacol Exp Ther*, *289*(1), 54–65.
- Sofuoglu, M., Dudish-Poulsen, S., Nelson, D., Pentel, P. R., & Hatsukami, D. K. (1999). Sex and menstrual cycle differences in the subjective effects from smoked cocaine in humans. *Experimental and Clinical Psychopharmacology*, *7*(3), 274–283. <https://doi.org/10.1037//1064-1297.7.3.274>
- Song, I., & Huganir, R. L. (2002). Regulation of AMPA receptors during synaptic plasticity. *Trends Neurosci*, *25*(11), 578–588. [https://doi.org/10.1016/s0166-2236\(02\)02270-1](https://doi.org/10.1016/s0166-2236(02)02270-1)
- South, S. M., Wright, A. W., Lau, M., Mather, L. E., & Smith, M. T. (2001). Sex-related differences in antinociception and tolerance development following chronic intravenous infusion of morphine in the rat: Modulatory role of testosterone via morphine clearance. *J Pharmacol Exp Ther*, *297*(1), 446–457.
- Spencer, S., Garcia-Keller, C., Roberts-Wolfe, D., Heinsbroek, J. A., Mulvaney, M., Sorrell, A., & Kalivas, P. W. (2017). Cocaine Use Reverses Striatal Plasticity Produced During Cocaine Seeking. *Biological Psychiatry*, *81*(7), 616–624. <https://doi.org/10.1016/j.biopsych.2016.08.033>
- Spencer, S., Scofield, M., & Kalivas, P. W. (2016). The good and bad news about glutamate in drug addiction. *Journal of Psychopharmacology*, *30*(11), 1095–1098. <https://doi.org/10.1177/0269881116655248>

- Spiga, S., Puddu, M. C., Pisano, M., & Diana, M. (2005). Morphine withdrawal-induced morphological changes in the nucleus accumbens. *Eur J Neurosci*, *22*(9), 2332–2340. <https://doi.org/10.1111/j.1460-9568.2005.04416.x>
- Sramek, J. J., Murphy, M. F., & Cutler, N. R. (2016). Sex differences in the psychopharmacological treatment of depression. *Dialogues Clin Neurosci*, *18*(4), 447–457.
- Staffend, N. A., Loftus, C. M., & Meisel, R. L. (2011). Estradiol reduces dendritic spine density in the ventral striatum of female Syrian hamsters. *Brain Struct Funct*, *215*(3–4), 187–194. <https://doi.org/10.1007/s00429-010-0284-7>
- Staley, K., & Scharfman, H. (2005). A woman's prerogative. *Nat Neurosci*, *8*(6), 697–699. <https://doi.org/10.1038/nm0605-697>
- Stanton, P. K., Winterer, J., Bailey, C. P., Kyrozis, A., Raginov, I., Laube, G., Veh, R. W., Nguyen, C. Q., & Müller, W. (2003). Long-term depression of presynaptic release from the readily releasable vesicle pool induced by NMDA receptor-dependent retrograde nitric oxide. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *23*(13), 5936–5944. <https://doi.org/10.1523/JNEUROSCI.23-13-05936.2003>
- Steketee, J. D. (2003). Neurotransmitter systems of the medial prefrontal cortex: Potential role in sensitization to psychostimulants. *Brain Res Brain Res Rev*, *41*(2–3), 203–228. [https://doi.org/10.1016/s0165-0173\(02\)00233-3](https://doi.org/10.1016/s0165-0173(02)00233-3)
- Stewart, J., Woodside, B., & Shaham, Y. (1996). Ovarian hormones do not affect the initiation and maintenance of intravenous self-administration of heroin in the female rat. *Psychobiology*, *24*(2), 154–159.
- Szczepanski, S. M., & Knight, R. T. (2014). Insights into human behavior from lesions to the prefrontal cortex. *Neuron*, *83*(5), 1002–1018. <https://doi.org/10.1016/j.neuron.2014.08.011>
- Terner, J. M., & de Wit, H. (2006). Menstrual cycle phase and responses to drugs of abuse in humans. *Drug Alcohol Depend*, *84*(1), 1–13. <https://doi.org/10.1016/j.drugalcdep.2005.12.007>
- Thomas, M. J., Beurrier, C., Bonci, A., & Malenka, R. C. (2001). Long-term depression in the nucleus accumbens: A neural correlate of behavioral sensitization to cocaine. *Nat Neurosci*, *4*(12), 1217–1223. <https://doi.org/10.1038/nm757>
- Todd, B. J., Schwarz, J. M., Mong, J. A., & McCarthy, M. M. (2007). Glutamate AMPA/kainate receptors, not GABA(A) receptors, mediate estradiol-induced sex differences in the hypothalamus. *Dev Neurobiol*, *67*(3), 304–315. <https://doi.org/10.1002/dneu.20337>

- Tonn Eisinger, K. R., Gross, K. S., Head, B. P., & Mermelstein, P. G. (2018). Interactions between estrogen receptors and metabotropic glutamate receptors and their impact on drug addiction in females. *Hormones and Behavior*, *104*, 130–137. <https://doi.org/10.1016/j.yhbeh.2018.03.001>
- Toyoda, H., Zhao, M. G., Ulzhofer, B., Wu, L. J., Xu, H., Seeburg, P. H., Sprengel, R., Kuner, R., & Zhuo, M. (2009). Roles of the AMPA receptor subunit GluA1 but not GluA2 in synaptic potentiation and activation of ERK in the anterior cingulate cortex. *Mol Pain*, *5*, 46. <https://doi.org/10.1186/1744-8069-5-46>
- Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., & Dingledine, R. (2010). Glutamate receptor ion channels: Structure, regulation, and function. *Pharmacol Rev*, *62*(3), 405–496. <https://doi.org/10.1124/pr.109.002451>
- Tsokas, P., Hsieh, C., Yao, Y., Lesburguères, E., Wallace, E. J. C., Tcherepanov, A., Jothianandan, D., Hartley, B. R., Pan, L., Rivard, B., Farese, R. V., Sajan, M. P., Bergold, P. J., Hernández, A. I., Cottrell, J. E., Shouval, H. Z., Fenton, A. A., & Sacktor, T. C. (2016). Compensation for PKM ζ in long-term potentiation and spatial long-term memory in mutant mice. *ELife*, *5*, e14846. <https://doi.org/10.7554/eLife.14846>
- Twining, R. C., Tuscher, J. J., Doncheck, E. M., Frick, K. M., & Mueller, D. (2013). 17 β -estradiol is necessary for extinction of cocaine seeking in female rats. *Learn Mem*, *20*(6), 300–306. <https://doi.org/10.1101/lm.030304.113>
- Tzschentke, T. M., & Schmidt, W. J. (2003). Glutamatergic mechanisms in addiction. *Molecular Psychiatry*, *8*(4), 373–382. <https://doi.org/10.1038/sj.mp.4001269>
- Ujike, H., Tsuchida, K., Akiyama, K., Fujiwara, Y., & Kuroda, S. (1995). Ontogeny of behavioral sensitization to cocaine. *Pharmacol Biochem Behav*, *50*(4), 613–617. [https://doi.org/10.1016/0091-3057\(94\)00352-1](https://doi.org/10.1016/0091-3057(94)00352-1)
- Vassoler, F. M., Oliver, D. J., Wyse, C., Blau, A., Shtutman, M., Turner, J. R., & Byrnes, E. M. (2017). Transgenerational attenuation of opioid self-administration as a consequence of adolescent morphine exposure. *Neuropharmacology*, *113*(Pt A), 271–280. <https://doi.org/10.1016/j.neuropharm.2016.10.006>
- Vazquez, M., Frazier, J. H., Reichel, C. M., & Peters, J. (2020). Acute ovarian hormone treatment in freely cycling female rats regulates distinct aspects of heroin seeking. *Learning & Memory (Cold Spring Harbor, N.Y.)*, *27*(1), 6–11. <https://doi.org/10.1101/lm.050187.119>
- Verbeke, G., & Molenberghs, G. (2000). *Linear Mixed Models in Practice*. Springer.
- Volk, L. J., Bachman, J. L., Johnson, R., Yu, Y., & Huganir, R. L. (2013). PKM- ζ is not required for hippocampal synaptic plasticity, learning and memory. *Nature*, *493*(7432), 420–423. <https://doi.org/10.1038/nature11802>

- Walker, Q. D., Cabassa, J., Kaplan, K. A., Li, S. T., Haroon, J., Spohr, H. A., & Kuhn, C. M. (2001). Sex differences in cocaine-stimulated motor behavior: Disparate effects of gonadectomy. *Neuropsychopharmacology*, *25*(1), 118–130. [https://doi.org/10.1016/S0893-133X\(00\)00248-7](https://doi.org/10.1016/S0893-133X(00)00248-7)
- Wallace, M., Luine, V., Arellanos, A., & Frankfurt, M. (2006). Ovariectomized rats show decreased recognition memory and spine density in the hippocampus and prefrontal cortex. *Brain Res*, *1126*(1), 176–182. <https://doi.org/10.1016/j.brainres.2006.07.064>
- Wallin-Miller, K., Li, G., Kelishani, D., & Wood, R. I. (2016). Anabolic-androgenic steroids decrease dendritic spine density in the nucleus accumbens of male rats. *Neuroscience*, *330*, 72–78. <https://doi.org/10.1016/j.neuroscience.2016.05.045>
- Wang, Y., Ma, Y., Hu, J., Cheng, W., Jiang, H., Zhang, X., Li, M., Ren, J., & Li, X. (2015). Prenatal chronic mild stress induces depression-like behavior and sex-specific changes in regional glutamate receptor expression patterns in adult rats. *Neuroscience*, *301*, 363–374. <https://doi.org/10.1016/j.neuroscience.2015.06.008>
- Wickens, M. M., Bangasser, D. A., & Briand, L. A. (2018). Sex Differences in Psychiatric Disease: A Focus on the Glutamate System. *Frontiers in Molecular Neuroscience*, *11*, 197. <https://doi.org/10.3389/fnmol.2018.00197>
- Wickens, M. M., Deutschmann, A. U., McGrath, A. G., Parikh, V., & Briand, L. A. (2019). Glutamate receptor interacting protein acts within the prefrontal cortex to blunt cocaine seeking. *Neuropharmacology*, *157*, 107672. <https://doi.org/10.1016/j.neuropharm.2019.107672>
- Wickens, M. M., Kirkland, J. M., Knouse, M. C., McGrath, A. G., & Briand, L. A. (2021). Sex-specific role for prefrontal cortical protein interacting with C kinase 1 in cue-induced cocaine seeking. *Addict Biol*, *26*(5), e13051. <https://doi.org/10.1111/adb.13051>
- Willett, J. A., Cao, J., Johnson, A., Patel, O. H., Dorris, D. M., & Meitzen, J. (2019). The estrous cycle modulates rat caudate-putamen medium spiny neuron physiology. *Eur J Neurosci*. <https://doi.org/10.1111/ejn.14506>
- Wissman, A. M., McCollum, A. F., Huang, G. Z., Nikrodhanond, A. A., & Woolley, C. S. (2011). Sex differences and effects of cocaine on excitatory synapses in the nucleus accumbens. *Neuropharmacology*, *61*(1–2), 217–227. <https://doi.org/10.1016/j.neuropharm.2011.04.002>
- Wolf, M. E., & Ferrario, C. R. (2010). AMPA receptor plasticity in the nucleus accumbens after repeated exposure to cocaine. *Neurosci Biobehav Rev*, *35*(2), 185–211. <https://doi.org/10.1016/j.neubiorev.2010.01.013>

- Woolley, C. S., & McEwen, B. S. (1994). Estradiol regulates hippocampal dendritic spine density via an N-methyl-D-aspartate receptor-dependent mechanism. *J Neurosci*, *14*(12), 7680–7687.
- Wright, A., & Vissel, B. (2012). The essential role of AMPA receptor GluR2 subunit RNA editing in the normal and diseased brain. *Front Mol Neurosci*, *5*, 34. <https://doi.org/10.3389/fnmol.2012.00034>
- Xi, Z. X., Ramamoorthy, S., Baker, D. A., Shen, H., Samuvel, D. J., & Kalivas, P. W. (2002). Modulation of group II metabotropic glutamate receptor signaling by chronic cocaine. *J Pharmacol Exp Ther*, *303*(2), 608–615. <https://doi.org/10.1124/jpet.102.039735>
- Xu, P., Chen, A., Li, Y., Xing, X., & Lu, H. (2019). Medial prefrontal cortex in neurological diseases. *Physiol Genomics*, *51*(9), 432–442. <https://doi.org/10.1152/physiolgenomics.00006.2019>
- Yagi, S., & Galea, L. A. M. (2019). Sex differences in hippocampal cognition and neurogenesis. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *44*(1), 200–213. <https://doi.org/10.1038/s41386-018-0208-4>
- Yang, D.-W., Pan, B., Han, T.-Z., & Xie, W. (2004). Sexual dimorphism in the induction of LTP: Critical role of tetanizing stimulation. *Life Sciences*, *75*(1), 119–127. <https://doi.org/10.1016/j.lfs.2003.12.004>
- Yao, Y., Kelly, M. T., Sajikumar, S., Serrano, P., Tian, D., Bergold, P. J., Frey, J. U., & Sacktor, T. C. (2008). PKM zeta maintains late long-term potentiation by N-ethylmaleimide-sensitive factor/GluR2-dependent trafficking of postsynaptic AMPA receptors. *J Neurosci*, *28*(31), 7820–7827. <https://doi.org/10.1523/JNEUROSCI.0223-08.2008>
- Yilmaz, B., Konar, V., Kutlu, S., Sandal, S., Canpolat, S., Gezen, M. R., & Kelestimur, H. (1999). Influence of chronic morphine exposure on serum LH, FSH, testosterone levels, and body and testicular weights in the developing male rat. *Arch Androl*, *43*(3), 189–196. <https://doi.org/10.1080/014850199262481>
- Yonkers, K. A., Forray, A., Nich, C., Carroll, K. M., Hine, C., Merry, B. C., Shaw, H., Shaw, J., & Sofuoglu, M. (2014). Progesterone Reduces Cocaine Use in Postpartum Women with a Cocaine Use Disorder: A Randomized, Double-Blind Study. *Lancet Psychiatry*, *1*(5), 360–367. [https://doi.org/10.1016/S2215-0366\(14\)70333-5](https://doi.org/10.1016/S2215-0366(14)70333-5)
- Yu, J., Zhang, S., Epstein, D. H., Fang, Y., Shi, J., Qin, H., Yao, S., Le Foll, B., & Lu, L. (2007). Gender and stimulus difference in cue-induced responses in abstinent heroin users. *Pharmacology, Biochemistry, and Behavior*, *86*(3), 485–492. <https://doi.org/10.1016/j.pbb.2007.01.008>

- Zamani, M. R., Desmond, N. L., & Levy, W. B. (2000). Estradiol modulates long-term synaptic depression in female rat hippocampus. *Journal of Neurophysiology*, *84*(4), 1800–1808. <https://doi.org/10.1152/jn.2000.84.4.1800>
- Zanni, G., DeSalle, M. J., Deutsch, H. M., Barr, G. A., & Eisch, A. J. (2020). Female and male rats readily consume and prefer oxycodone to water in a chronic, continuous access, two-bottle oral voluntary paradigm. *Neuropharmacology*, *167*, 107978. <https://doi.org/10.1016/j.neuropharm.2020.107978>
- Zhang, H., & Bramham, C. R. (2020). Bidirectional Dysregulation of AMPA Receptor-Mediated Synaptic Transmission and Plasticity in Brain Disorders. *Front Synaptic Neurosci*, *12*, 26. <https://doi.org/10.3389/fnsyn.2020.00026>
- Zhang, Y., Picetti, R., Butelman, E. R., Schlussman, S. D., Ho, A., & Kreek, M. J. (2009). Behavioral and neurochemical changes induced by oxycodone differ between adolescent and adult mice. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *34*(4), 912–922. <https://doi.org/10.1038/npp.2008.134>
- Zhou, W., & Kalivas, P. W. (2008). N-acetylcysteine reduces extinction responding and induces enduring reductions in cue- and heroin-induced drug-seeking. *Biological Psychiatry*, *63*(3), 338–340. <https://doi.org/10.1016/j.biopsych.2007.06.008>