The Impact of Perinatal Morphine Exposure on Alcohol Reward & Anhedonia in Rats

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by

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ABSTRACT

Every fifteen minutes, a baby is born in the U.S. experiencing neonatal opioid withdrawal syndrome. Clinical studies have established intrauterine exposure to drugs of abuse as a risk factor for adverse health outcomes in adolescence, including future drug use and depression. Although substantial evidence supports shared neural mechanisms underlying opioid and alcohol reward, there is limited data on the risks of alcohol use later in life for infants exposed to opioids. Additionally, ongoing research on the long-term consequences of perinatal opioid exposure (POE) has been limited to analyses of school performance and early life sociability. Our laboratory has developed a clinically relevant model for morphine exposure spanning pre-conception to the first postnatal week. Using this model, we report that perinatal opioid exposure increases alcohol consumption in female rats under noncontingent conditions, and inversely, reduces alcohol consumption in both sexes during operant conditioning sessions. Operant responding was also reduced for sucrose, suggesting that the impact of POE on reward-seeking behaviors is not limited to drugs of abuse. Parallel studies examined the impact of perinatal morphine exposure on central expression of μ opioid receptors (MOR) at postnatal and adolescent ages. We report age and region-dependent changes in MOR within the nucleus accumbens and medial habenula, regions previously shown to play a significant role in reward/aversion circuitry. In extending our assessment of POE-induced changes in natural reward behaviors, we find reduced sucrose preference during adolescence. Juvenile and adult POE rats also spend less time socially interacting with familiar conspecifics. Changes in adult social behaviors were linked to increased nondopaminergic mesolimbic reward brain activity.

We suggest that changes in reward outcomes may be linked to comparatively fragmented and unpredictable maternal behavior in opioid-dependent dams that disrupts pup development.

INDEX WORDS: Morphine, Perinatal, Alcohol, Sucrose, Reward, Anhedonia

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DEDICATION

I dedicate this work to my family and friends, who have endlessly supported me through this process. Above all to my wife, Hannah, who stood by every moment and believed in me. Lastly, to those who doubt themselves.

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1 INTRODUCTION

1.1 Neonatal Opioid Withdrawal Syndrome (NOWS)

Opioid use in the United States has steadily increased in the past 30 years. By 2015, 37% of American adults were estimated to have been prescribed an opioid pain reliever (Jansson & Patrick, 2019). As nationwide opioid use rises, so does usage amongst pregnant women (Patrick et al., 2012). This has produced an epidemic of newborns with neonatal opioid withdrawal syndrome, or NOWS. NOWS is a condition produced by intrauterine exposure to opioids that presents a broad spectrum of acute and chronic symptoms detrimental to infant health, including deficits in cognitive, behavioral, and motor development (Weller et al., 2021). The rate of NOWS diagnoses grew from 1.2 to 8.8 per 1000 births between 2000-2017 (Patrick et al., 2012, 2015, 2020; Winkelman et al., 2018). Amongst 42 states with data, at least 25 doubled their incidence rates of NOWS (Hirai et al., 2021). In Arizona, Arkansas, New Mexico, Oklahoma, Tennessee, and West Virginia, NOWS per 1000 births more than tripled (Hirai et al., 2021). Although this rapid escalation has brought considerable attention to this demographic of the opioid epidemic, maternal opioid-related issues continue to rise, especially in rural and smaller metro areas of the country (Hirai et al., 2021). Increased NOWS also disproportionately impacts rural or suburban areas with higher rates of unemployment and mental health assistance (Patrick et al., 2019; Sanlorenzo et al., 2018). Additionally, states may vary in opioid use disorder treatment accessibility, which is statistically more difficult for pregnant people and minorities (Bulgin et al., 2023). Unsurprisingly, escalation in NOWS diagnoses has driven hospital fees to over $500 million every year (Winkelman et al., 2018).

1.1.1 Clinical outcomes of NOWS/NAS

The clinical presentation of NOWS is characterized by a wide spectrum of acute symptoms beginning shortly after birth, including irritability, hypertonicity, tremors, and hypo- or hypersensitivity to ordinary stimuli. Autonomic signs of stress such as gagging, fever, and tachypnea that create imbalances in state control (adaptability and expression of behavioral states) can disrupt basic functions like feeding, sleeping, alertness, and communication (Jansson & Patrick, 2019; Velez & Jansson, 2008). Loss of state control is reflective of reduced system maturity and neuroregulatory competence (Thoman et al., 1981; Vandenberg, 2007). Early neural dysregulation contributes to long-term differences between opioid-exposed neonates and healthy individuals and is associated with decreased brain volume across several regions and morphology (Nygaard et al., 2018; Sirnes et al., 2017; Walhovd et al., 2007, 2012). For example, reduced cortical thickness was linked to poor cognitive performance in children aged 9 or older (Walhovd et al., 2007). Opioid-exposed infants also exhibit increased neural tract mean diffusivity, suggesting delayed maturity and dysmyelination (Walhovd et al., 2012).

Given the volatility of homes with opioid misuse, tracking NOWS newborns can be challenging. Thus, there are only a few studies that have successfully tracked long-term outcomes associated with intrauterine opioid exposure. Cognitive and language skills are reduced in infants with prenatal exposure to heroin, methadone, or other opioids (Beckwith & Burke, 2015). Similarly, young children with gestational opioid exposure exhibit cognitive deficits, reduced socialization, and increased anxiety (Azuine et al., 2019; Larson et al., 2019). Educational delays have also been reported amongst children with a history of NOWS aged 3-8. These children were also more likely to require classroom therapy or services even when controlling for confounding factors such as maternal tobacco use, maternal education, and NICU admission (Fill et al., 2018). In those with a history of NOWS, early signs of Attention Deficit Hyperactivity Disorder, specifically poor performance on attention-based tasks and increased impulsivity are consistently reported (Nygaard et al., 2016; Sirnes et al., 2018). Poor school performance may also extend into high school age (Oei et al., 2017). Educational difficulties may be considerably worsened by disorders affecting visual acuity, such as strabismus and ocular muscle disorders, that are more common amongst children with prenatal opioid exposure (Auger et al., 2020; R. Hamilton et al., 2024; Rees et al., 2020; Yoo et al., 2017). Generally, studies of NOWS outcomes focus on infants with clinical diagnoses, likely following severe or multi-symptom presentation at birth. Importantly, subclinical opioid exposure, as defined as those born without the need for post-birth pharmacological maintenance, also reported increased incidence of behavioral/emotional, developmental, and ocular disorders shown in other studies (Hall et al., 2019). Additionally, infant mortality is significantly increased with or without NOWS diagnosis accompanying maternal opioid use disorder (OUD) (Grossarth et al., 2023).

As NOWS patients age, it becomes more difficult to attribute specific outcomes to their mother’s opioid use during pregnancy. Homes with substance use disorders, depressed parents, or NOWS diagnosis are strongly associated with child maltreatment (Chaffin et al., 1996; Rees et al., 2020). One study interviewed 30 adults born with NOWS (mean age 22.23 years) and reported one third were diagnosed with a psychiatric disorder, a third had been previously arrested, and more than a third were unemployed (Herranz et al., 2014). These participants also reported a high rate of emotional or physical abuse in their upbringing, making it impossible to discern which aspects of these long-term negative outcomes are due specifically to opioids. Some data suggests that cognitive changes related to prenatal opioid exposure are more closely related to mother-child interactions, where suboptimal or negative rearing is strongly associated with cognitive changes in NOWS children (Konijnenberg et al., 2016). Indeed, maternal distress exhibited by women undergoing opioid maintenance therapy (OMT) largely contributes to changes in childhood behavior (Konijnenberg et al., 2015; Sarfi et al., 2011). Infants with NOWS that have been adopted also present cognitive deficits suggesting that both intrauterine opioids and suboptimal rearing scenarios drive these outcomes (Nygaard et al., 2015; Ornoy et al., 2010; Walhovd et al., 2007).

1.1.2 NOWS treatment

American hospitals significantly vary in their treatment of NOWS (Bogen et al., 2017; Patrick et al., 2014). To improve patient outcomes, the American Academy of Pediatrics recommends standardization of neonatal intensive care treatment and implementation of evidence-based “potentially better practices” (PBPs) to reduce nationwide average hospital length of stay (LOS) and treatment costs (Hudak et al., 2012; Patrick et al., 2016). Chiefly, pregnant opioid-dependent women are advised to undergo medication-assisted treatment (MAT) with long-acting opioid agonists, typically methadone or buprenorphine, to avoid overdose and reduce overall opioid use (ACOG Committee on Health Care for Underserved Women & American Society of Addiction Medicine, 2012). Most hospitals also employ direct pharmacological treatments to reduce post-birth withdrawal and improve infant health (Milliren et al., 2018). Morphine and methadone are the first and second most common choices for pharmacological treatment (Milliren et al., 2018). Buprenorphine has also been widely adopted as a morphine alternative for pharmacological treatment (Kraft et al., 2017). Use of morphine alternatives correlates with reduced LOS compared to morphine, but overall, pharmacological treatment significantly increases LOS due to slower weaning (J. M. Davis et al., 2018; Hudak et al., 2012; Kraft et al., 2017; Milliren et al., 2018).

Nonpharmacological treatments have been recently identified as an improvement on traditional NOWS treatment protocols with evidence of reduced LOS and decreased rates of pharmacological intervention (MacMillan et al., 2018). The "Eat, Sleep, Console" non-pharmacological treatment method has shown success in reducing LOS (Blount et al., 2019). This method includes rooming-in (mother-child dyad hospital stays), increased skin-to-skin time, swaddling, and limited stimulation (Atwood et al., 2016; Holmes et al., 2016; MacMillan et al., 2018; Patrick et al., 2016). Together these protocol components are designed to promote consistent maternal care, destigmatize NOWS, and rebalance neonatal loss of control symptoms. Breastfeeding, specifically, has been associated with improved infant health and decreases in LOS and the need for pharmacological treatment (Abdel-Latif et al., 2006; Demirci et al., 2015; Kocherlakota, 2014; Short et al., 2016; Welle-Strand et al., 2013). It is suggested that breastfeeding's reducing effect on additional opioid agonist treatment is due to the transfer of methadone or buprenorphine through breast milk (Pritham, 2013). Importantly, nonpharmacological treatments are best used outside of high-stress ICU environments (Wachman et al., 2018).

1.1.3 NOWS pathophysiology

Opioids are highly lipophilic and transfer rapidly from mother to fetus across the placenta (Gerdin et al., 1990; Kocherlakota, 2014). This rate of transfer increases with gestational age (Nanovskaya et al., 2008). Increased free fraction of opioids, defined as the portion exerting pharmacological effects, is found in fetal blood, thus increasing the amount of opioids that can cross the infant blood-brain barrier (Bhat et al., 1990; Farrington et al., 1993; Scott et al., 1999). Clearance of opioids is lower in early development and increases with gestational age, leading to increased accumulation and half-life of opioids in a fetus (Anand et al., 2008; Lu & Rosenbaum, 2014; Pacifici, 2016; Scott et al., 1999). Interestingly, premature birth is associated with a lower risk of withdrawal, likely due to reduced overall opioid exposure time (Doberczak et al., 1991; Kocherlakota, 2014). Indeed, thirty days or more of opioid exposure, or any third-trimester use of opioids during pregnancy, strongly predicts NOWS diagnosis (Wang et al., 2023). Unfortunately, prenatal opioid exposure therefore creates a paradoxical high-risk situation where preterm birth is itself associated with a spectrum of complications but also protective against withdrawal profiles.

Opioids, such as morphine, bind to the μ-opioid receptor (MOR), a G-coupled protein receptor that is expressed extensively throughout the central nervous system. Opioid withdrawal in infants generates a cascading mechanism of increased adenyl cyclase, cAMP, protein kinase, transcription factors, and neurotransmitter activity that together underlie the acute physiological profile of NOWS (Kocherlakota, 2014). Initially, reversible receptor desensitization due to acute opioid exposure may result from uncoupling of G protein structures required for their function (Allouche et al., 2014). Sustained opioid receptor activation leads to a pronounced reduction in Gα subunit coupling and reduced inhibitory opioid action (Christie, 2008; K. Rehni et al., 2013). The combination of compensatory increases in neural signaling and reductions in opioid's inhibitory effects stems from increases in adenylyl cyclase/cAMP activity and neurotransmission (Adhikary & Williams, 2022; K. Rehni et al., 2013). Together, these adaptations are major components of tolerance that develop to combat drug effects.

The absence of opioids during a period of withdrawal results in increased noradrenergic activity in the locus coeruleus (LC) that can lead to multiple symptoms of NOWS, including tremors, poor feeding, and altered sleep patterns (Kocherlakota, 2014). The LC contains high levels of MOR and is associated with alertness, breathing, and cognitive flexibility. Neurotransmission in other brain regions is also impacted by opioid withdrawal in infants and contributes to NOWS phenotypes including changes in central levels of serotonin (sleep disturbances), corticotrophin (stress), dopamine (anxiety), and acetylcholine (autonomic deficits) (Kocherlakota, 2014).

Some evidence suggests that genetics play a role in NOWS severity as single nucleotide polymorphisms (SNPs) in genes coding for components of opioid use can be linked to both neonatal withdrawal and increased risk of drug use in adults. SNPs in mu, delta, and kappa opioid receptors genes (*OPRM1*, *OPRD1*, & *OPRK1*) are linked to increased LOS and pharmacological treatment (Kreek et al., 2005; Mague & Blendy, 2010; Wachman et al., 2013, 2015). SNP variants in *PNOC*, an opioid peptide gene, are associated with both NOWS severity and increased risk for alcohol abuse disorder (Wachman et al., 2017; Xuei et al., 2008). It is suggested that SNPs are also strongly linked to the psychological maternal stress experienced by pregnant opioid users (Kocherlakota, 2014). Variants in the gene that codes for catechol-O-methyltransferase (*COMT*) are also associated with both NOWS severity and increased risk for depression and stress disorders in adults (Pap et al., 2012; Wachman et al., 2013, 2017).

Epigenetic changes are also associated with NOWS, including methylation of *OPRM1* that may contribute to changes in μ-opioid receptor expression or viability in preclinical NOWS models (Grecco & Atwood, 2020; McLaughlin et al., 2017; Wachman et al., 2014).

1.1.4 Modeling NOWS in rodents

Preclinical models that control for early life trauma, polysubstance use, and other common risk factors are critical for delineating the contribution of opioids to long-term consequences of NOWS. However, differences in postnatal treatment, cross-fostering, exposure length, and drug choice across decades of prenatal opioid animal models have produced considerable variability in results (Grecco & Atwood, 2020). Debate over the biological relevance of some models is evident, especially when truncated exposure time or withdrawal precipitated by naloxone administration is employed (Byrnes & Vassoler, 2018). For example, postnatal treatment with naloxone produces an aggressive withdrawal profile that is in direct contrast to clinical pharmacological treatment with opioids or recommended breastfeeding practices (Abdel-Latif et al., 2006; Bogen & Whalen, 2019; Kocherlakota, 2014; Welle-Strand et al., 2013). Use of cross-fostering to isolate the effects of prenatal opioid exposure from postnatal maternal behavior may be considered, but rates of foster or out-of-home care of children born with NOW range from 20% to 72% (Yeoh et al., 2019).

Previous preclinical studies frequently utilized opioid dosing paradigms that begin around gestational age 7-14 days (G7) that, although coincides with fetal opioid and endocrine system development, does not reflect preconception or first-trimester opioid use (Byrnes & Vassoler, 2018; Harder & Murphy, 2019; I. U. Vathy et al., 1985). A recent epidemiological study of more than two million women who had given birth found that adverse events that occurred during the first trimester were most predictive of psychiatric disorders in their children (Kang et al., 2024). It is also estimated that more women use drugs earlier in their pregnancy, most often during the first trimester (SAMHSA, 2014). This is unsurprising given that nearly half of all pregnancies are unplanned and MAT is increasingly available for, and used by, pregnant women (Krans et al., 2019; Rossen et al., 2023). Therefore, preclinical studies that fail to recapitulate the use of opioids during the first trimester do not accurately model a majority of NOWS cases.

Opioid choice for preclinical study is also critical for modeling human use. Morphine, a prototypical opioid, is the most common drug used in the field, but increasing focus has centered on alternatives in MAT such as methadone and buprenorphine (Grecco & Atwood, 2020). The urgency for these long-acting opioid alternatives in treating drug dependence has driven their increased use in pregnant women, leading many researchers to model MAT in rodents (Krans et al., 2019; Simmons et al., 2023). Most studies suggest that despite encouraging NICU outcomes of MAT, rodent models of perinatal methadone or buprenorphine exposure result in a wide spectrum of adverse effects on fetal development and behavior later in life (H.-H. Chen et al., 2015; Grecco, Haggerty, et al., 2022; Grecco, Muñoz, et al., 2022; Kongstorp, Bogen, Stiris, et al., 2020; C.-Y. Wu et al., 2023).

1.2 Reward, hedonics, and anhedonia

Motivation is an evolutionary drive to focus on stimuli that promote survival. Neurological processes tuned to increase engagement with these stimuli manifest as liking (hedonics/instinct) and wanting (motivation) (Kringelbach & Berridge, 2016). Learning and executive function brain networks interact with hedonic brain circuits to facilitate future interactions with rewarding stimuli. As a result, the neurobiology of reward is a multi-network process with several homeostatic feedback loops tied to positive and negative outcomes of reward seeking and use. Disruption of functions and inability to feel pleasure normally is referred to as anhedonia. Anhedonia, often expressed as reduced engagement with rewarding stimuli, is theorized to be the result of dysfunction of rewarding circuitry or hyperactivity in brain regions that inhibit monoaminergic signaling (Der-Avakian & Markou, 2012).

1.2.1 Overview of the reward system

Traditionally, reward processing in the central nervous system is categorized into circuits that respond to specific neurotransmitters or circuits that respond to stimulus valence. Olds & Milner famously were the first to report that using region-specific direct brain stimulation in rodents generated motivation and positive reinforcement (J. Olds & Milner, 1954). These regions were later identified as the medial forebrain, ventral tegmental area (VTA), and pons, and elicited the strongest reinforcing responses to self-administered stimulation (M. E. Olds & Olds, 1963; Simon et al., 1975). With *in vivo* electrophysiology, researchers also determined that brain activity in other regions coincided with stimulation avoidance (Bielajew & Shizgal, 1980).

1.2.2 Reward system development

Meso-diencephalic dopamine neurons (mdDA) are formed around E12 in rats and begin extending on E13 to form three distinct pathways: mesolimbic, mesocortical, and mesostriatal (Carlsson et al., 1962; Dahlstroem & Fuxe, 1964; Smidt & Burbach, 2007). Axon formation is guided initially by Slit3 expression in the brainstem and later by a rostrocaudal gradient of Slit1 and Sema3C/F (Gates et al., 2004; Hernández-Montiel et al., 2008; Nakamura et al., 2000).

Recent evidence in mice has suggested that functional separation of the nigrostriatal and mesolimbic pathways relies on expression of bone morphogenetic protein (BMP) 2 and 6 and transforming growth factor (TGF)-β2 (Terauchi et al., 2023). Functional dopaminergic systems develop rapidly across early life, as shown by mRNA and autoradiographical evidence of D1 and D2 receptor expression between P0 and P30 (Y. I. Chen et al., 2010). In rodents, development of the reward system continues across adolescence with D1/D2 expression peaking during mid-adolescence and stabilizing in adulthood (Reynolds & Flores, 2021). The guidance cue, Netrin-1, and its receptor, DCC, in dopaminergic neurons are critical to maintaining axonal plasticity during adolescence (Vosberg et al., 2020). Genetic variation in *Netrin*-1 and *DCC* leads to altered dopamine concentration and connectivity across the mesolimbic system and is linked to psychiatric disorders, including depression (Vosberg et al., 2020).

During early development, microglia play a crucial role in the outgrowth of dopamine axons (Squarzoni et al., 2014). In adolescent rats, synaptic pruning by microglia in the mesolimbic reward system generates sex-specific differences in adolescent social behaviors (Kopec et al., 2018). Interruption of mesolimbic microglial pruning alters these social behaviors in adulthood (Kirkland et al., 2024). Interestingly, prenatal opioid exposure in rats reduces microglia-mediated pruning of reward system dopamine receptors and is associated with changes in conditioned responses to drug administration (Smith et al., 2022). In humans, postmortem analysis shows that OUD alters synaptic remodeling by microglia in the nucleus accumbens (Seney et al., 2021). The role of microglia in reward-associated disorders in NOWS patients is, however, unexplored.

Opioidergic systems also develop before birth. Opioid receptor and peptide expression, particularly β-endorphin, is present in human and murine oocytes and contributes directly to follicular maturation (Agirregoitia et al., 2012; Olabarrieta et al., 2019). In rats, MOR expression is present at E15-17 in reward-associated regions, including the nucleus accumbens, striatum, frontal cortex, habenula, amygdala, and septum (Georges et al., 1998). Early life MOR expression is linked to hedonic feeding responses in rat pups and fetal growth in humans (Bischoff et al., 2024). Indeed, these opioid-dependent responses are present as early as the first day of life (D. C. Castro & Berridge, 2014; Steiner, 1973). During rewarding events, endogenous opioids are released within mesolimbic brain regions to modulate dopaminergic activity (S. W. Johnson & North, 1992; Pitchers et al., 2014).

Early life exogenous opioid use induces developmental changes in opioid circuitry within reward-associated brain regions, often reflected in decreased VTA and increased accumbal MOR expression (Gorio et al., 1996; Panerai et al., 1983; Tempel et al., 1988; Tempel, 1991a; Tempel & Espinoza, 1992; I. Vathy et al., 2003). Notably, POE-induced changes in MOR can last long into adulthood in rats (Buisman-Pijlman et al., 2009; Handelmann & Quirion, 1983; Slamberová et al., 2003). Early life is, therefore, a highly sensitive period for opioid system development.

1.2.3 Mesolimbic dopamine pathway

The mesolimbic dopamine pathway provides bidirectional signaling between the ventral tegmental area (VTA) and nucleus accumbens (NAc). Dopaminergic VTA neurons densely innervate GABAergic medium spiny neurons (MSNs) of the NAc. Dopamine-dependent reward signaling originating from the VTA and NAc projects to septal, amygdalar, hippocampal, and prefrontal cortical regions to create the mesocorticolimbic network. Most VTA activity is dopaminergic; the region is comprised of roughly 60% DA cells, 35% GABA cells, and 5% glutamatergic cells (Nair-Roberts et al., 2008). Extracellular dopamine produced during rewarding stimuli or associated cues strengthens reward-seeking behaviors. Spontaneous dopaminergic activity is inhibited by tonic GABAergic innervation from the lateral habenula and rostromedial tegmental nucleus preventing neurotransmission (Jhou et al., 2009; Ji & Shepard, 2007). During rewarding events this hyperpolarizing gate is disinhibited, promoting dopamine release from the VTA (Lecca et al., 2012; Matsumoto & Hikosaka, 2007). The NAc is 90-95% GABAergic MSNs that express D1 and D2 receptors (Lewis et al., 2021). These cells receive VTA dopaminergic signals and send reciprocal feedback data that regulates dopamine output to modulate reward behaviors (Xia et al., 2011; Yang et al., 2018). The strong connectivity between the VTA and NAc is thought to contribute to reward processing, learning, and prediction error.

Tonic dopamine promotes arousal, while phasic dopamine builds reinforcement, or “stamping-in” of associations between rewards and their cues (Volkow et al., 2017). While VTA dopaminergic cells are considered to promote reward behavior and learning throughout the brain, GABAergic and glutamatergic neurons that create local connections within the VTA modulate dopaminergic activity and contribute to aversion, depression, stress, and wakefulness (Barbano et al., 2020; Cai & Tong, 2022; M. Morales & Margolis, 2017; Root et al., 2014; Tan et al., 2012; van Zessen et al., 2012). Nondopaminergic information is also projected to the lateral habenula and nucleus accumbens, generally contributing to aversion (Qi et al., 2016; Root et al., 2014).

Mesolimbic pathway control over appetitive and reward learning processes has been debated for decades, leading to multiple theories regarding hedonics and incentive motivation (Alcaro et al., 2007). Dopaminergic control over initial and lasting responses to rewards is facilitated by the widespread connectivity of the VTA (Alcaro et al., 2007). Dopaminergic neuronal lesions reduce reward-seeking, instrumental learning, and rewarding effects of electrical stimulation and drugs of abuse (Wise, 2004). Dopamine also contributes to post-reward reinforcement, or reward-stimulus associations, as demonstrated by lasting reward-associated memories. Blockade of dopamine signaling leads to reward devaluation over time (Wise, 2004). Pre-reward behavior and anticipation, as demonstrated through enhanced responding in repeated trials of operant behavior, are also mediated by dopamine (Wise, 2004).

Dopamine-dependent reward associations depend on incentive motivation of a given stimulus. Lack of sufficient strength of a stimulus may fail to maintain association-driven behaviors (Wise, 2004). Dopamine is proposed to primarily underlie formation of incentive motivation, given that blockade of dopamine across several trials is required to devalue established motivational associations. Use of and close proximity to reward-associated stimuli triggers dopamine activity, suggesting a predictive role of the mesolimbic pathway that contributes to reinforcement (Fouriezos et al., 1978). It is suggested that mesolimbic dopamine response patterns tune the strength of a reward predictor.

Dopaminergic signaling relies on D1-like, Gs-coupled receptors, and D2-like, Gi-coupled receptors. Both D1 and D2 receptors are essential for reward processing, but postsynaptic D1 receptors maintain a lower affinity for dopamine and require phasic burst firing that is often associated with rewarding activity (Grace et al., 2007). Presynaptic D2 autoreceptors generally act to reduce neuronal activity (Baik, 2013). Blockade or deletion of either receptor type disrupts instrumental learning and reward responding (reviewed in El-Ghundi et al., 2007). Pharmacological manipulation of D1 or D2 receptors in several mesolimbic-associated regions (e.g. hippocampus, amygdala, dorsal striatum, frontal cortex, and VTA) has also been shown to potentiate or block reward-related long-term potentiation (LTP) and depression (LTD) (Wise, 2004). Dopamine is therefore important for behavioral and cellular components of reward learning and memory.

Recent evidence highlights the complexity of D1 and D2-like receptor expression and their simultaneous activation, sometimes presenting as heteromers (Ferré, 2015). Additionally, elucidation of heterogenous mesolimbic cell populations has led to discussion of distinct subregional pathways for governing characteristics of reward behavior, including reinforcement, reward learning, and aversion. This heterogeneity is also caused by diversity in neurotransmitter release in mesolimbic neurons, which are often capable of co-releasing dopamine, GABA, and/or glutamate (M. Morales & Margolis, 2017).

1.2.4 Hedonics

Generally, brain activity associated with drug-induced reward signaling relies on dopaminergic transmission in the mesolimbic pathway, originating from the VTA (Wise & Rompre, 1989). Dopamine-specific lesions in the mesolimbic circuit dysregulate or abolish the rewarding properties of drugs of abuse (Wise, 1996). Similarly, repeated sucrose reinforcement requires dopamine signaling. Initial orofacial responses to sucrose, however, are present in dopamine-disrupted subjects (Wise, 2004). These dopamine-independent, unconditioned responses to reward are thought to represent “liking” behavior, as opposed to “wanting” behavior, and are modulated by opioidergic signaling in "hedonic hotspots" of the NAc (Berridge, 1996; D. C. Castro & Berridge, 2014; Kringelbach & Berridge, 2016).

Although dopaminergic and opioidergic signaling independently contribute to hedonics and motivation, they can also influence one another in mesolimbic regions. For example, direct injection of the MOR agonist, DAMGO, into either the VTA or NAc increases feeding (Spanagel et al., 1992); these changes in feeding behavior reflect both hedonic and homeostatic control of the mesolimbic pathway (Hanlon et al., 2004). VTA-NAc dopaminergic and opioidergic connections have also been well-characterized as contributors to stress response and depression, both key to characterizing the manifestation of reward signaling deficits (Russo & Nestler, 2013).

1.2.5 Opioid reward

Endogenous and exogenous opioid agonists drive mesolimbic dopaminergic signaling by inhibiting tonic afferent and intra-VTA GABAergic innervation that blocks dopamine release (Di Chiara & Imperato, 1988; S. W. Johnson & North, 1992). Endogenous opioids that modulate mesolimbic dopamine release include β-endorphins from the arcuate nucleus and enkephalins from the ventral striatum and lateral hypothalamus (Haber et al., 1985; Khachaturian et al., 1984; Raynor et al., 1994). Local VTA injection of exogenous opioids, like morphine or DAMGO, creates conditioned place preference (CPP) (Bozarth, 1987). Injection directly into GABAergic medium spiny neurons of the nucleus accumbens also generates CPP, suggesting that opioid reward works dependently and independently of dopamine transmission (G. F. Koob, 1992; van der Kooy et al., 1982).

Chronic opioid use leads to tolerance, reflected in reduced opioid efficacy. Behaviorally, tolerance creates a need for increased use to reach the same desired effects due to repeated receptor activation, leading to desensitization or internalization (Bohn et al., 2000). Desensitization of opioid receptors involves uncoupling of G-protein components that are necessary for intracellular signaling cascades, while internalization of opioid receptors requires a higher degree of receptor phosphorylation, leading to endocytosis and long-term reduction in receptor function (Arttamangkul et al., 2018). Agonist efficacy influences the tendency to desensitize versus internalize opioid receptors; morphine, for example, is less likely to produce internalization of μ-opioid receptors than methadone or DAMGO (Borgland et al., 2003).

1.2.6 Alcohol reward

Alcohol is easily accessible and by age 18, nearly 60% of teens have had at least one drink, with many having participated in binge-level consumption (SAMHSA, 2014). Rodents will readily self-administer alcohol with females displaying higher response rates (Nieto & Kosten, 2017; Tabakoff & Hoffman, 2000).

Like opioids, ethanol induces dopamine release in the VTA-nucleus accumbens mesolimbic pathway, resulting in hyperpolarization of VTA GABAergic interneurons, either through direct action on $GABA_A$ receptors or indirect action on MOR via release of β-endorphin from arcuate nucleus pro-opiomelanocortin (POMC) neurons (Di Chiara & Imperato, 1988; Font et al., 2013; Fu et al., 2016; Jarjour et al., 2009; Oswald & Wand, 2004; see **Figure 1.1**). Hyperpolarization of GABAergic interneurons within the VTA increases dopamine release to the NAc via disinhibition (Davies, 2003; Herz, 1997; S. W. Johnson & North, 1992). Naltrexone, a potent μ-opioid receptor antagonist, is well-characterized for use in managing alcohol and opioid use disorders by diminishing rewarding properties and craving tendencies (Bienkowski et al., 1999; Volpicelli et al., 1986; Zalewska-Kaszubska et al., 2008). For alcohol, naltrexone produces this effect by 1) suppressing alcohol-mediated beta-endorphin stimulation of NAc dopaminergic neurons, and 2) attenuating beta-endorphin disinhibition of VTA dopamine neurons (B. A. Johnson & Ait-Daoud, 2000; Spanagel & Zieglgänsberger, 1997; Sudakin, 2016). Further, chronic administration of naltrexone (7 days or 4 weeks), in the absence of substance abuse, increases MOR binding in multiple mesolimbic brain regions associated with reward (Bartus et al., 2003; Zukin et al., 1982).

1.2.7 Sucrose reward

In behavioral studies, sucrose is often used as a natural or food-directed reward, depending on the concentration used. Generally, low concentrations of sucrose are used to measure incentive value and preference while higher concentrations that provide caloric weight can be used to measure binge feeding. Sucrose consumption increases dopaminergic signaling in the mesolimbic pathway in both acute and chronic exposure paradigms (Avena et al., 2006); these changes in extracellular dopamine mirror those that take place during acute and chronic drug administration, including morphine (Di Chiara & Imperato, 1988; Pothos et al., 1991).

Mu-opioid receptors are also required for food reinforcement (Papaleo et al., 2007). Rats that undergo high sucrose consumption exhibit increased MOR binding in the NAc and hippocampus similar to those that consume heroin (Colantuoni et al., 2001; Fattore et al., 2007). Naloxone administration can reduce sucrose consumption and, in bingeing rodents, will even produce somatic symptoms of withdrawal (Cleary et al., 1996; Colantuoni et al., 2002).

Sucrose consumption, like opioids and alcohol, is therefore tied to both dopaminergic and opioidergic signaling pathways of the brain's reward systems.

1.2.8 Mu-opioid-dependent reward disruption

Mesolimbic dopamine signaling is highly sensitive to opioidergic input. Endogenous opioids released during naturally pleasurable events, like mating, are enough to create long-term changes in dopamine-dependent reward behaviors (Pitchers et al., 2014). Manipulation and blockade of MOR in the VTA have been shown to disrupt both morphine and alcohol self-administration in rats (Heyser et al., 1999; Honkanen et al., 1996; Kawakami et al., 2016; Miranda-Morales et al., 2012; Pastor & Aragon, 2006). MOR in the NAc also contributes directly to alcohol and opioid reward (Richard & Fields, 2016; Vaccarino et al., 1985; Zito et al., 1985). Interestingly, baseline differences in MOR expression may also contribute to genetic predisposition to alcohol preference in rats (McBride et al., 1998).

Previous studies have reported that administration of morphine to pregnant rat dams during mid-gestation results in significant changes in MOR expression, specifically decreases in VTA and increases in NAc that persist into adulthood (Gorio et al., 1996; Panerai et al., 1983; Tempel et al., 1988; Tempel, 1991a; Tempel & Espinoza, 1992; I. Vathy et al., 2003). Changes in MOR due to early life opioid exposure and subsequent behavioral consequences will be reviewed later in this manuscript.

1.2.9 Anhedonia and the impact of early life stress

Regions of the mesolimbic reward system are activated by both rewarding and aversive stimuli (Alcaro et al., 2007). This is reportedly due to the reward system's evolutionary role in determining whether a given stimulus is life-promoting or life-threatening. Neural circuits important for stress and negative affect symptoms thus overlap with those for reward. Nondopaminergic VTA activity, primarily GABAergic, contributes to increased anxiety and aversion (Cohen et al., 2012). Glutamatergic innervation of nondopaminergic neurons promotes anxiety and aversion behaviors while GABAergic projections promote anxiolytic behaviors (Jennings et al., 2013). VTA GABAergic signaling has been shown to underlie defense behavior in response to a threat stimulus and conditioned place aversion (Tan et al., 2012; Zhou et al., 2019). VTA glutamatergic neurons may also contribute to defense behaviors (Barbano et al., 2020).

Extra-mesolimbic regions that receive reward information also play an important role in aversion signaling. The medial prefrontal cortex (mPFC) innervates both the NAc and amygdala to drive drug seeking and fear conditioning, respectfully. mPFC connections are divided into dorsal, prelimbic (PL), and ventral, infralimbic (IL), subregions with opposing command over reward and fear conditioning (J. Peters et al., 2009). PL projects to the NAc core and basolateral amygdala, promoting behavior and learning of both valences (Bonson et al., 2002). IL projects to the NAc shell and, indirectly, the central amygdala to inhibit reward/fear learning (Berretta et al., 2005).

These aversion signaling pathways are activated during periods of chronic stress or anxiety, producing multimodal expression of depression behaviors; this can include anhedonia, or inability to feel pleasure (Pizzagalli, 2014). In rodents, dopamine-dependent changes in reward prediction, reward learning, and motivation can all independently contribute to an anhedonic phenotype (Der-Avakian & Markou, 2012). Depression-like phenotypes can also present during cycles of drug abuse and withdrawal, commonly due to chronic physiological and psychological stress (Meye et al., 2015; Michaels et al., 2021; Zhu et al., 2016). Abstinence periods generate stress and depression that drive future drug use. Chronic drug use, including that of opioids, will also potentiate stress vulnerability (Blatchford et al., 2005).

Several assays are commonly employed to delineate the individual components of depression-like behavior in rodents. Perhaps most common is sucrose intake and preference which measures consummatory changes (Papp et al., 1991). Rats that undergo repeated stress, including chronic unpredictable stress or social defeat, consistently exhibit anhedonia, inferred by a reduction in sucrose preference (Katz, 1982). At the molecular level, anhedonic rats have reduced dopamine signaling during reward tests (Krishnan et al., 2007; Valenti et al., 2011). Anhedonia can also be reflected in dopamine-dependent motivation for a conditioned reward, as measured by progressive ratio during which rewards require increasing effort (Der-Avakian & Markou, 2012). Chronic stress reduces dopamine function during sucrose reward responding (Bergamini et al., 2018). These deficits are reversed by pharmacological excitation of dopamine-expressing neurons (L. Li et al., 2017).

Another historical behavioral measure for depression-like behavior is the Porsolt forced swim test, which measures latency to immobility during an inescapable swim. While the interpretation of immobility as “despair” has been challenged, the efficacy of antidepressant medication in changing this behavior is well-documented (Becker et al., 2023). Changes in stress reactivity during forced swim test have also been shown to occur concurrently with anhedonic behaviors in rodents undergoing stress exposure (Hong et al., 2012).

Deficits in social interaction/play behaviors can also be used as an indicator of anhedonic behavior (Trezza et al., 2010). Changes in social play behaviors are impacted by manipulation of dopaminergic and opioidergic neurotransmission (Trezza & Vanderschuren, 2009). More specifically, MOR agonism and antagonism inversely alter social play behaviors (Beatty & Costello, 1982; Niesink & Van Ree, 1989; Trezza & Vanderschuren, 2008). Disrupted sociability correlates with longer periods of addiction disorder and higher rates of relapse (Bakken et al., 2007). Reduced social interaction is observed in mice during prolonged abstinence from opioids (Goeldner et al., 2011; Lalanne et al., 2017; Lutz et al., 2014).

Changes in typical grooming behaviors can also represent depression-like behavior. Opioid administration has been shown to reduce grooming behaviors in depressed rodents (Kalamarides et al., 2024). Abstinence from opioids, however, inversely increases grooming (Goeldner et al., 2011; Lalanne et al., 2017; Lutz et al., 2014; Schulteis et al., 1994). It is suggested that increased grooming is a compulsive coping mechanism for combating stress and anxiety (Kalueff & Tuohimaa, 2005).

Social tests of familiarity and novelty increase Fos expression in brain regions governing social reward and aversion processing (Gómez-Gómez et al., 2019). This Fos expression is altered by early life stressors like maternal separation (Mejía-Chávez et al., 2021). Other forms of early life stress can also lead to anhedonic behaviors later in life. Low bedding and nesting (LBN), for example, leads to reduced sucrose preference and social play behavior (Bolton et al., 2018; Molet et al., 2016). LBN generates maltreatment by rat mothers, including increased rough handling, shorter bouts of maternal care, and increased unpredictability in patterns of maternal behaviors (Molet et al., 2016; Rincón-Cortés & Sullivan, 2016). The mother-infant relationship is integral to the early development of reward systems, likely due to the importance of pattern recognition to reward learning and the presentation of maternal care patterns. Postnatal stress, including that due to poor maternal care, increases sensitivity to aversive experiences later in life (Ventura et al., 2013).

1.3 Impact of perinatal opioids on reward and anhedonia

Chronic drug use and abuse is known to generate compulsive drug seeking, negative affect, and drug craving, outlined by neurochemical changes in the mesolimbic, amygdalar, and corticolimbic systems, respectfully (G. F. Koob & Volkow, 2010). George Koob and Norah Volkow posit that these aspects of drug use are cyclical and generate increasingly intense drug use, ultimately leading to addiction (Koob & Volkow, 2010; see **Figure 1.2**). Occasional drug use follows this proposed cycle with conscious and impulsive consumption. As drug use continues, individuals' tolerance and dependence increase. The allostatic, or opposingly negative, response to the rewarding properties of drug use also increases (G. F. Koob & Volkow, 2016). Neuroadaptations in critical reward brain circuits, namely dysregulation of mesolimbic and corticolimbic systems, accompany continued drug use and consequent negative affect. These adaptations underlie tolerance development and drive the need for escalating doses to reach similar levels of pleasure and relief from negative emotional states (G. F. Koob & Volkow, 2016). Though adaptations in neurocircuitry may be attributed to a specific drug of abuse, the systems undergoing change are responsible for regulating natural reward, stress reactivity, and executive function. (G. F. Koob & Volkow, 2016). Predisposition, early life/adolescent drug use, and other forms of vulnerability contribute to the risk of transitioning from occasional drug use to addiction (G. F. Koob & Volkow, 2010, 2016; Kreek & Koob, 1998). Early life stress is also well-characterized as a risk factor for altered reward neurocircuitry and behaviors (Hanson et al., 2021). Emerging evidence suggests that the drug and stress-induced impact of prenatal opioid exposure poses lifelong risks for behavioral disorders, including those that contribute to future drug use (Horn et al., 2018; see **Table 1.1**). Prenatal opioid exposure is also heavily associated with genetic and molecular changes that can alter reward and stress responses (see **Table 1.2**).

1.3.1 Impact of perinatal opioids on reward behavior and circuitry

Familial drug use positively correlates with use in future generations of both humans and rodents (Nielsen et al., 2012; Vermeulen-Smit et al., 2012; Yohn et al., 2015). This is concerning for drugs of abuse that are easily accessible, such as alcohol. By age 18, nearly 60% of teens will have had at least one drink, with many reporting binge drinking experience (Substance Abuse and Mental Health Services Administration (SAMHSA), 2016). Significant evidence has shown that genetic and environmental factors lead to multigenerational alcohol use (Cadoret et al., 1986).

Despite the opioid crisis spanning nearly 30 years, little clinical data is available on multigenerational opioid use. Clinical communities must rely on meta-analyses of survey data, which is limited due to difficulties in maintaining longitudinal studies in drug abuse households. The few studies that do exist show that opioid and other drug use amongst adolescents has been linked to a familial history of opioid and alcohol use (Jedrzejczak, 2005; Russell et al., 2015). In particular, children born to mothers with opioid misuse/dependency are more likely to be diagnosed with alcohol abuse or opioid misuse later in life, even if raised in a foster or adopted home (Elam et al., 2020; Griesler et al., 2019; Nygaard et al., 2020; Ornoy et al., 2010;

Vidal et al., 2012). These studies struggle to isolate opioid use as the main contributing factor to multigenerational drug use, due to highly common factors of multidrug use, low socioeconomic status/resources, and stress. Therefore, rodent models play a critical role in elucidating the impact of perinatal opioid use on future reward behavior and neurocircuitry. As previously discussed, variability in POE models has resulted in conflicting results, but common outcomes have also emerged over decades of work.

Preference for drugs of abuse, especially opioids, is altered as a result of pre-, peri-, and postnatal opioid exposure in multiple species (Y. Chen, Du, et al., 2022; Gagin et al., 1997; Haydari et al., 2014; He et al., 2010; Smith et al., 2022; Timár et al., 2010; Torabi et al., 2017; Wang et al., 2017; L.-Y. Wu et al., 2009). Changes in opioid preference can also be manipulated by methadone, a long-acting opioid classically used during opioid maintenance therapy to reduce the negative effects of heroin and morphine (Chiang et al., 2015; Hovious & Peters, 1985). Enhanced drug preference has also been reported for methamphetamines, demonstrating that the effects of POE extend to other drug types (Shen et al., 2016). Some evidence contradicts these findings and suggests there is no impact of POE on opioid preferences in adulthood (J. Jiang et al., 2011; Riley & Vathy, 2006; Yazdanfar et al., 2021). There is also conflicting evidence of POE's impact on preference for sweetened solutions (Gagin et al., 1996; Torabi et al., 2017). Nonetheless, POE's impact on hedonics, which is largely governed by opioid signaling, is well-characterized.

POE-induced changes in dopaminergic-dependent reward behavior can last into adulthood, as well. Adult POE rats increase operant self-administration of heroin, cocaine, or morphine (Glick et al., 1977; Ramsey et al., 1993). Other studies, however, have reported no changes in cocaine, morphine, or sucrose self-administration as a result of POE (Riley & Vathy, 2006; I. Vathy et al., 2007). There is also evidence that POE increases locomotor sensitization for morphine and methamphetamine in adulthood (Byrnes, 2005; Robinson et al., 2020; L.-Y. Wu et al., 2009).

POE's reported effects on social reward are inconsistent. Our lab has shown that POE rats decreased social play and time socially interacting (Harder et al., 2023). Other evidence found that exposure to morphine from E8-E21 increased adolescent and adult social play, grooming, and approach behaviors (Buisman-Pijlman et al., 2009; Hol et al., 1996). These studies differ in that our protocol administers morphine across the entirety of pregnancy and post-birth, possibly extending the impact of morphine and limiting aggressive spontaneous withdrawal at birth.

A well-documented outcome of POE is site-specific changes in MOR expression across drug-related brain regions. Increases in MOR have been found in the NAc and CeA of POE rats, but decreases are reported in the spinal cord, amygdala, and thalamus (Chiou et al., 2003; Handelmann & Quirion, 1983; Kirby, 1983; I. Vathy et al., 2003). Some reports show changes in MOR expression are transient and return to control levels in post-adolescence (Bhat et al., 2006; Tempel et al., 1988). POE-induced changes in MOR are also hormone-dependent in both adult male and female rats (Slamberová et al., 2003). Reductions in OPRM1, the gene encoding for MOR have also been found in conjunction with reductions in pain threshold (Chiang et al., 2015). Long-acting opioid agonists, such as methadone and buprenorphine, that are prescribed in clinical OMTs produce similar negative outcomes in rats (Belcheva et al., 1998; Chiang et al., 2015; Kongstorp, Bogen, Steinsland, et al., 2020). It is, therefore, important that the potential risks of using these opioid alternatives continue to be considered.

Some studies report changes in dopaminergic signaling components in POE rats. Gestational oxycodone or buprenorphine alters NAc D1 dopamine receptor expression in association with changes in adult social and drug responding, respectively (Chiang et al., 2014; Smith et al., 2022). Prenatal morphine increases the number of TH+ cells in the PVN of adult male rats, which may be related to changes in sexual reward (I. Vathy et al., 2000). POE-induced changes are also found in signaling that supports dopaminergic neurotransmission and reward learning processes. POE enhances local field potentials in NAc and BLA and increased HDAC5 in the VTA leading to increased reward processing during morphine CPP testing (Y. Chen, Wang, et al., 2022). POE rats also do not exhibit morphine-associated increases in coherence in VTA-NAc, mPFC-NAc, and mPFC-BLA circuits, suggesting that network plasticity during reward is impaired. Hippocampal LTP evoked by electrical stimulation is reduced by POE in both male and female rats, highlighting a possible mechanism by which POE reduces spatial or reward learning (Ahmadalipour et al., 2018; Sarkaki et al., 2008; Shen et al., 2016; Truchet et al., 2002). Additionally, perinatal methadone exposure reduces CB1R-mediated LTD and glutamatergic signaling in DLS of adolescent male mice that underlie cue-induced reward responding (Grecco, Muñoz, et al., 2022).

1.3.2 Impact of perinatal opioids on stress and anxiety

The psychobiological weight of opioid abuse during pregnancy on both mother and infant qualifies this experience as early life stress (Horn et al., 2018). Early life changes in opioid and HPA systems are linked to altered stress and drug-seeking behaviors later in life (Bruchas et al., 2010; G. F. Koob, 2010; Pautassi et al., 2012). Parental stress exposure also increases the risk of stress and anxiety disorders in future generations (Bowers & Yehuda, 2016). Clinically, pregnant opioid-dependent moms undergo significant psychobiological stress that can extend into problematic maternal care, including reduced attentiveness, mood, and quality of support (Konijnenberg et al., 2016; Wachman et al., 2018). In rodent and nonhuman primate models of early life stress, offspring exhibit negative affect and increased stress reactivity due to stress-induced maternal care deficits (Bolton et al., 2018; Coplan et al., 1996). Together, this suggests that the observed outcomes of POE parallel those reported in other preclinical early life stress models.

Indeed, clinical data shows that maternal opioid dependency increases the risk of mental disorders, including anxiety and depression, in their children (Elam et al., 2020; Nygaard et al., 2020; Vidal et al., 2012). Opioid exposure at birth is also associated with behavioral disorders in young children and increases the risk of ADHD (Azuine et al., 2019). In one study, half of all NOWS infants were diagnosed with a mental disorder before age 5, and overall, were considered twice as likely to develop a conduct disorder or intellectual disability. They were also significantly more likely than non-NOWS children to express symptoms of stress hyperreactivity or emotional disturbance (Sherman et al., 2019). Increased anxiety and aggression are also found amongst the children of mothers who used methadone (de Cubas & Field, 1993). Perinatal opioid exposure reduces scores on the Vineland Social Maturity scale measuring social competence at 18 months and 3 years of age (Hunt et al., 2008). NOWS also produces higher testing scores for ADHD and autism spectrum disorder (ASD), especially in subscales related to social difficulties (Sandtorv et al., 2018).

History of morphine use in both or one rat parent can also lead to increased anxiety-like behaviors in male and female offspring (Byrnes, 2005; C.-Q. Li et al., 2014). Adolescent morphine use in females has even been reported to alter anxiety-like behaviors in grand-offspring (F2) (Vassoler & Byrnes, 2021). Parental morphine use also leads to reduced IGF-2 and dendritic branch growth in the dentate gyrus. These changes were prevented by environmental enrichment (EE), reinforcing previous findings showing EE's reduction of ELS-induced anxiety-like behaviors in rats (Baldini et al., 2013; Francis et al., 2002). Some studies, however, report no effect of POE on basal or stress-induced corticosterone release in rats (Laborie et al., 2005; Rimanóczy et al., 2003; Slamberová et al., 2004).

1.3.3 Sex differences in preclinical NOWS outcomes

Sex-specific differences in drug abuse are well-characterized with females overall being more at risk for dependency and neurobiological changes that maintain drug use (Bobzean et al., 2014; Kokane & Perrotti, 2020). In humans, however, male infants displayed increased NOWS diagnosis risk and severity (Charles et al., 2017; Jansson et al., 2007; Jones et al., 2010). Sex-specificity of NOWS outcomes has become a larger focus of preclinical research in recent years, as past research has primarily focused on male subjects (Simmons et al., 2023).

In reward research, female rats exposed to perinatal opioids are more likely than males to increase sensitization to heroin, morphine, alcohol, and even water (Y. Chen, Du, et al., 2022; Grecco, Haggerty, et al., 2022; Kvello et al., 2019). In some cases, locomotor reward behavior was enhanced (Y. Chen, Du, et al., 2022; Grecco, Haggerty, et al., 2022). In the case of alcohol, POE male rats, but not females, displayed increased aversion-resistant drinking compared to controls (Grecco, Haggerty, et al., 2022).

Several sex-specific outcomes of POE have been identified during stress and anxiety testing in rats. Female POE rats spent more time in the center of an open field test (OFT) or open arms of an elevated plus maze (EPM), indicating reduced anxiety (Y. Chen, Du, et al., 2022; Kvello et al., 2019). Similarly, female POE mice reduced stress-induced grooming responses during a sucrose splash test (Alipio, Brockett, et al., 2021). In contrast, POE males exhibit hyperactivity during OFT and decreased EPM open arm time (Alipio, Brockett, et al., 2021; Sithisarn et al., 2017). One study reported that POE females decreased OFT center time if exposed to prenatal buprenorphine, but both sexes decreased center time if exposed to morphine or methadone (H.-H. Chen et al., 2015). Despite the possible differences in sex-specific sensitivity to various opioid agonists, POE appears to consistently alter anxiety-like behaviors in rats.

When testing for acute stress responses, sex and age-specific changes emerge. POE males, but not females, increase acoustic startle responses and associated corticosterone release (K. L. Hamilton et al., 2005). These results were specific to adulthood; juvenile POE rats exhibited hyporeactivity to the test. Further, POE decreased immobility during forced swim test in adolescent females but increased immobility in adults; POE males did not display altered FST immobility (Y. Chen, Du, et al., 2022).

1.4 Dissertation Aims

Previous research investigating the impact of perinatal opioid exposure on the reward system of the brain has been limited to long-term changes in consumption and preference of narcotics and stimulants, including heroin, cocaine, and methamphetamine. A major gap in the literature is focused on POE-induced changes in alcohol consumption, despite the commonality of its use in adolescence and adulthood. Additionally, previous studies have suggested that changes in reward-seeking for drugs of abuse do not extend to naturally occurring rewards. Early life stress research suggests that negative experiences during the postnatal period may globally alter reward behaviors and lead to stress or anxiety. Although altered anxiety-like behaviors have been reported in POE rodents, the presentation of symptoms of depression, or anhedonia, remains to be determined.

To build on a growing body of literature demonstrating the long-term consequences of POE, we have developed a model of morphine exposure that replaces the typically truncated, direct injection style used by the field. In challenging this status quo, our lab has attempted to generate a more biologically relevant model that mimics the use of opioids prior to and across pregnancy in addition to following postnatal breastfeeding recommendations at the clinical level. This model utilizes subcutaneous osmotic minipumps that administer morphine (or saline) at regular intervals across the day. Opioid concentrations can also be adjusted to accommodate tolerance and increase blood volume associated with pregnancy, which mitigates problems associated with implantable delivery.

My dissertation research tests the hypothesis that perigestational opioid exposure during a critical period for dopaminergic and opioidergic development leads to future disrupted reward processing. We suggest that this disrupted reward manifests as changes in alcohol and sucrose self-administration. We also suggest that altered reward behavior is reflected in social interactions and acute stress response. These changes are precipitated not only by long-term *in utero* morphine exposure but also by changes in maternal behavior that have been reported in other studies of early life stress. **Thus, we propose our overarching hypothesis that perinatal**

exposure to morphine alters alcohol and naturally occurring reward behaviors in Sprague Dawley rats. Specifically, we hypothesize that POE leads to altered alcohol and sucrose self-administration in males and females. We also hypothesize that POE generates anhedonic behaviors in both sexes. Lastly, we hypothesize that these changes are preceded by morphine-driven fragmented and unpredictable maternal behavior during critical periods of development for the offspring. We will test these hypotheses with the following specific aims:

Specific Aim 1: Investigate changes in alcohol and sucrose-driven reward behaviors in male and female rats perinatally exposed to morphine. In adults, chronic opioid use is linked to increased risk of drug overdose or dependence. Chronic opioid use in pregnant women leads to negative consequences for their offspring, including increased risk of developing a substance use disorder. This aim will utilize a clinically relevant model of perinatal opioid exposure (POE) in Sprague Dawley rats to 1) examine its impact on alcohol and sucrose self-administration in males and females. Furthermore, 2) we will characterize expression of μ-opioid receptors in critical reward brain circuits known to underlie the actions of morphine, alcohol, and sucrose administration.

Specific Aim 2: Investigate anhedonic behaviors in male and female rats perinatally exposed to morphine and characterize maternal behavior in morphine-exposed rat dams. It is well-characterized that early life trauma leads to dysregulation of reward in humans and animals. Often, this type of chronic stress predicts the onset of depression and stress or anxiety disorders, which may contribute to future drug use. Healthcare professionals are increasingly recognizing the stress incurred by pregnant opioid-using moms and the need for mother-child dyad stress management and pair-bonding postpartum. This aim will 1) examine depression-like symptoms and acute stress reactivity, and 2) analyze behavior and brain activity associated with social interaction in adulthood. We will also 3) determine if anhedonic outcomes are related to fragmented or unpredictable maternal care due to chronic morphine use.

These studies are the first to use a clinically relevant model of perinatal opioid exposure to study reward and anhedonia in rats. Our results demonstrate that perinatal morphine-induced changes in reward-related behaviors extend to both alcohol and naturally rewarding stimuli. This highlights the importance of continued longitudinal attention for NOWS children and early life screening for stress and depression.

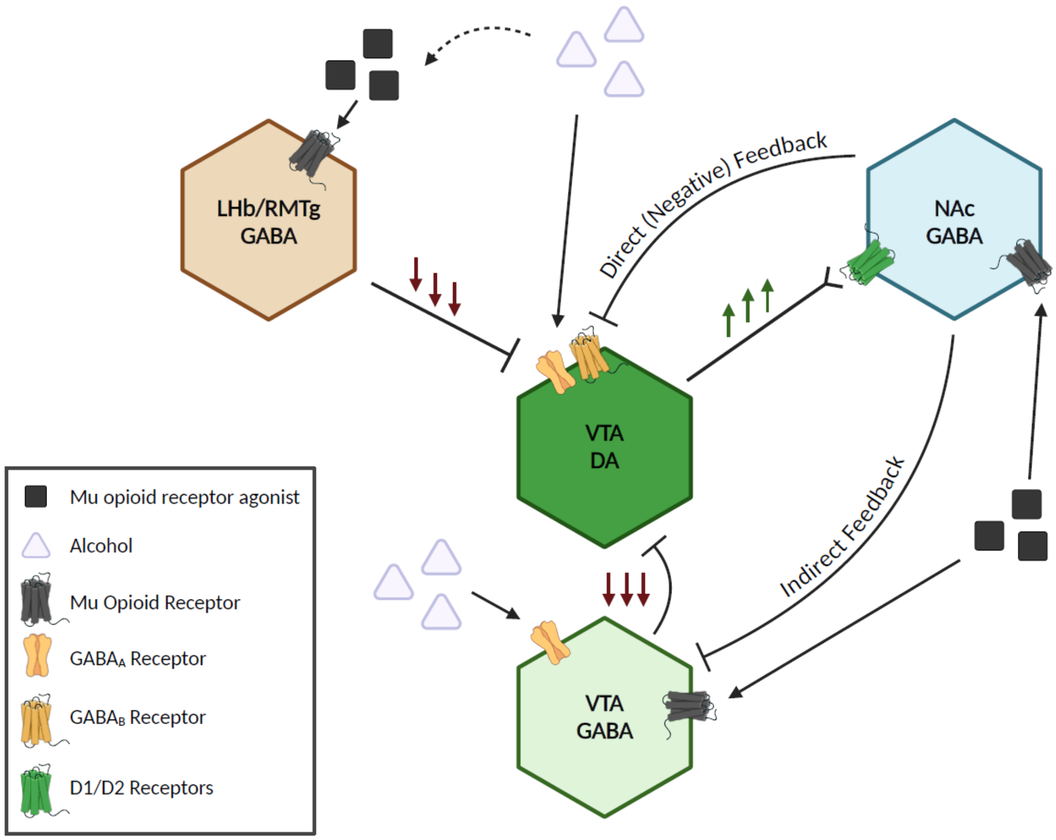

Figure 1.1 Mesolimbic actions of mu opioid agonists and alcohol. DA, dopamine; LHb, lateral habenula; NAc, nucleus accumbens; RMTg, rostromedial tegmental nucleus; VTA, ventral tegmental area

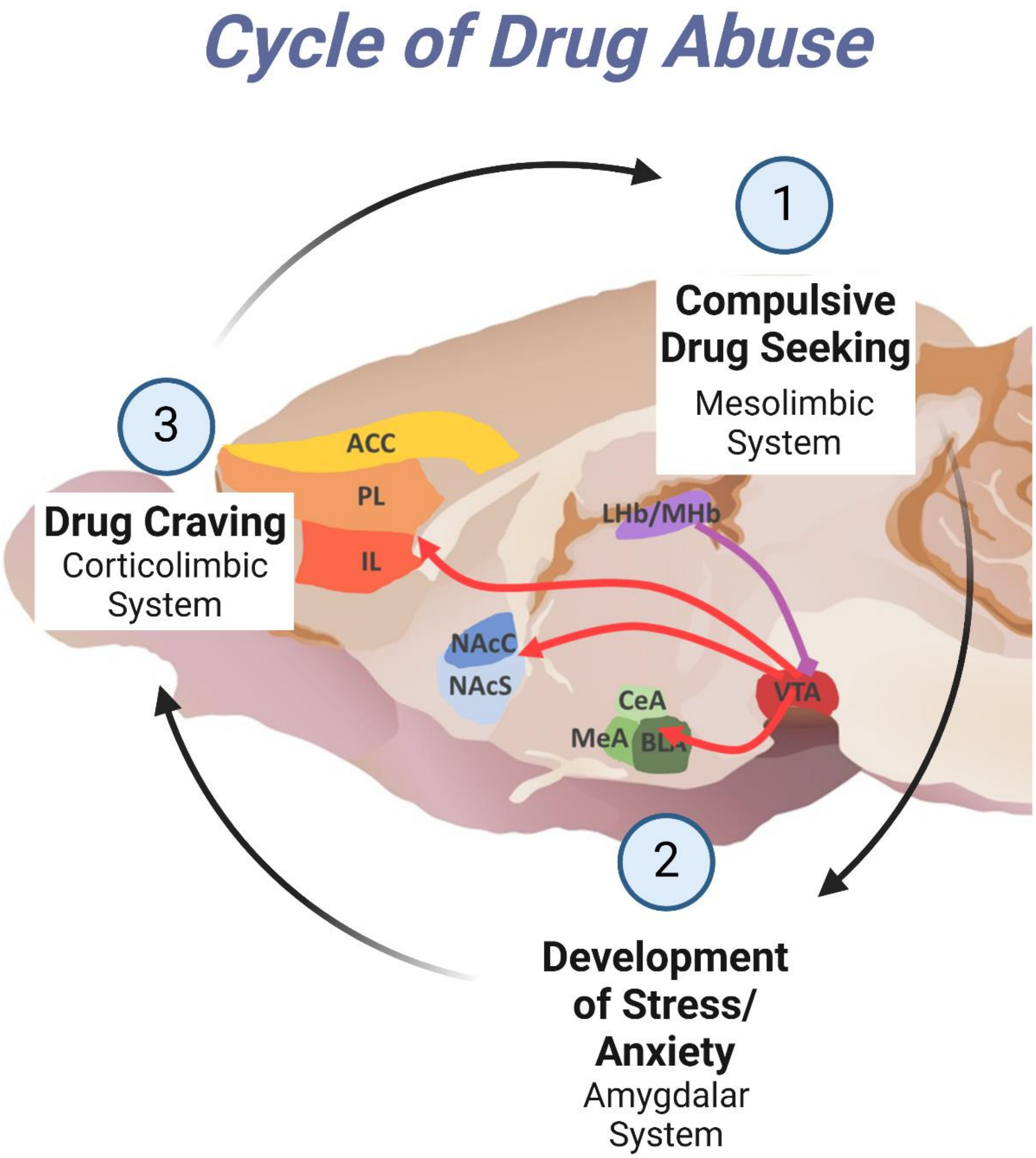

Figure 1.2 Cycle of drug abuse

Perinatal opioid-related behavioral changes in reward & stress

Subjects	Opioid Exposure	Outcomes	Reference
Clinical Evidence			
Humans	Familial history of use	↑ opioid misuse adolescent ♂ & ♀	(Russell et al., 2015)
Humans	Maternal prescription opioid misuse	↑ opioid misuse adolescent ♂ & ♀	(Elam et al., 2020)
Humans	Maternal/Paternal prescription opioid misuse	↑ opioid misuse age 12-17 ♂ & ♀	(Griesler et al., 2019)
Humans (mainly foster care/ adopted)	Prenatal opioid/ polysubstance exposure	↑ alcohol abuse disorder, ADHD, or depression age 17-22 ♂ & ♀	(Nygaard et al., 2020)
Humans (1 adopted group, 1 biological parent group)	Prenatal heroin exposure	↑ cognitive, social, or emotional disorders age 12-16 ♂ & ♀	(Ornoy et al., 2010)
Humans	Maternal/Paternal heroin dependence	↑ depression, ADHD, SUD age 6-18 ♂ & ♀	(Vidal et al., 2012)
Humans	Prenatal opioid exposure	↑ conduct disorder, emotional disturbance, ADHD children ♂ & ♀	(Azuine et al., 2019)
Humans	NOWS diagnosis	↑ language/coordination delay, conduct disorder, ADHD, adjustment reaction, acute stress reactivity, anxiety, emotional disturbance, ASD age 0-5 ♂ & ♀	(Sherman et al., 2019)
Humans	Prenatal methadone exposure	↑ anxiety, aggression, social rejection, depression, uncommunicative, ADHD children ♂ & ♀	(de Cubas & Field, 1993)
Humans	Prenatal opioid Exposure	↓ social maturity age 18mo-3 ♂ & ♀	(Hunt et al., 2008)
Humans	Prenatal opioid exposure	↑ADHD, ASD age 6-14 ♂ & ♀	(Sandtorv et al., 2018)

Humans	Prenatal opioid exposure	↑ diagnoses of behavioral, emotional, developmental, speech, and visual disorders Age 2+ infants ♂ & ♀	(Hall et al., 2019)
Reward Preference			
Sprague Dawley rats	Morphine (5-10mg/kg/day, s.c.) E11-E19	↓ morphine (10mg/kg, i.p.) P35 ♀ & n.c. ♂ ↑ morphine (10mg/kg, i.p.) P65 ♂ & n.c. ♀	(Y. Chen, Du, et al., 2022)
Fischer rats	Morphine (.75-12mg/day, s.c.) E12-E18	↑ morphine (2mg/kg, s.c.) P70-84 ♂ & ♀	(Gagin et al., 1997)
Wistar rats	Morphine (0.1-0.4 mg/ml in sucrose) E0-P8	↑ morphine (0.5 or 0.7mg/ml, oral) P37-49 ♂	(Haydari et al., 2014)
Chicks	Morphine (1mg/kg every other day) E5-E20)	↑ morphine (1mg/kg, i.p.), P1	(He et al., 2010)
Sprague Dawley rats	Oxycodone (0.1mg/kg/inf SA) Pregestation to P0	↓ extinction to oxycodone adult ♂, n.c. ♀	(Smith et al., 2022)
Wistar rats	Morphine (5-10mg/kg/day, s.c.) E0-P21 or E1-P0 or P0-P21	↑ morphine (1 or 3mg/kg, s.c.) P90-120 ♂ & ♀	(Timár et al., 2010)
Wistar rats	Morphine (5-10mg/kg twice daily, s.c.) E11-E18 then weaned off	↑ morphine (0.3, 0.5, or 0.7mg/ml, oral) P35-50 ♂ & ♀	(Torabi et al., 2017)
Chicks	Morphine (1mg/kg) E17 & E19	↑ morphine (1mg/kg, i.p.), P1	(Wang et al., 2017)
Sprague Dawley rats	Morphine (2-6mg/kg twice daily, s.c.) Pregestation to P0	↑ morphine (1mg/kg, i.p.), P60 ♂	(L.-Y. Wu et al., 2009)

Sprague Dawley rats	Methadone (5-7mg/kg twice daily, s.c.) E3-E20	↑ methadone (4mg/kg, s.c.), P64-71 ♂	(Chiang et al., 2015)
Sprague Dawley rats	Methadone (5 mg/kg/day, i.p.) Pregestation to P22	↑ morphine (0.5 mg/ml, oral) P85-90 ♂; n.c. methadone (0.5 mg/ml, oral)	(Hovious & Peters, 1985)
Sprague Dawley rats	Morphine (2-4mg/kg twice daily, s.c.) E3-E20	↓ extinction to methamphetamine adult ♂	(Shen et al., 2016)
Chicks	Morphine (1mg/kg every other day) E5-E8	↓ morphine P1 sex N/A	(J. Jiang et al., 2011)
Sprague Dawley rats	Morphine (5-10mg/kg twice daily, s.c.) E11-E18	n.c. morphine (0.1, 0.3, 1, 3 or 5mg/kg) P65 ♂	(Riley & Vathy, 2006)
Wistar rats	Morphine 2-7mg/kg/day, s.c.) Pregestation to P21	n.c. morphine >P70 ♂	(Yazdanfar et al., 2021)
Fischer rats	Morphine (.75-12mg/day, s.c.) E12-E18	↑ saccharin P70-84 ♂ & ♀	(Gagin et al., 1996)
C57BL/6J mice	Methadone (10mg/kg twice daily, s.c.) E0-P28	↓ alcohol (20%, oral) P34-61 ♂, but not ♀	(Grecco, Haggerty, et al., 2022)
Reward Self-Administration			
Sprague Dawley rats	Morphine (.5mg/ml in .4mg/ml saccharin) E0-P0	↑ morphine (0.04mg/kg) P90 ♀	(Glick et al., 1977)
Wistar rats	Morphine (10mg/kg/day, s.c.) E7-E9	↑ heroin (0.063mg/kg) & cocaine (0.125mg/kg) P70-84 ♂	(Ramsey et al., 1993)

Sprague Dawley rats	Morphine (5-10mg/kg twice daily, s.c.) E11-E18	n.c. sucrose (45mg pellets) P60-65 ♂ n.c. morphine (0.3, 1, 2 or 3 mg/kg) P65-100 ♂	(Riley & Vathy, 2006)
Sprague Dawley rats	Morphine (5-10mg/kg twice daily, s.c.) E11-E18	n.c. sucrose (45mg pellets) P60-65 ♂ & ♀ n.c. cocaine P65-95 ♂ & ♀	(I. Vathy et al., 2007)
C57BL/6J mice	Methadone (10mg/kg twice daily, s.c.) E0-P28	↑ alcohol (20%, oral) P34-61 ♂, but not ♀	(Grecco, Haggerty, et al., 2022)
Locomotor Sensitization			
C57BL/6 mice (Oprm1 AA SNP)	Morphine (10 mg/kg twice daily, s.c.) P1-P14	↑ morphine (20mg/kg, s.c.) P84 ♂ & ♀	(Robinson et al., 2020)
Sprague Dawley rats	Morphine (2-6mg/kg twice daily, s.c.) Pregestation to P0	↑ morphine (1mg/kg, i.p.), P60 ♂	(L.-Y. Wu et al., 2009)
Sprague Dawley rats	Buprenorphine (3mg/kg/day, s.c.) E3-E20	↑ methamphetamine (2mg/kg, i.p.) P56-84 ♂	(Chiang et al., 2014)
C57BL/6J mice	Methadone (10mg/kg twice daily, s.c.) E0-P28	↑ alcohol (20%, oral) P34-37 ♀, but not ♂	(Grecco, Haggerty, et al., 2022)
C57BL/6J mice	Heroin (1.05 mg/kg/day, s.c.) E12, E15, & E18	↑ heroin (0.7mg/kg, s.c.) P28 ♂ & ♀	(Kvello et al., 2019)
Sprague Dawley rats	Morphine (5-10mg/kg/day, s.c.) E11-E19	↑ morphine (10mg/kg, i.p.) P65 ♀ & n.c. ♂	(Y. Chen, Du, et al., 2022)
Social Reward			
Sprague Dawley rats	Morphine (8-16mg/kg/day, s.c. 3 inf/day via minipump) Pregestation to P7	↓ social play adolescent ♀ ↓ social contact adolescent ♂ & ♀	(Harder et al., 2023)

Wistar rats	Morphine (10mg/kg/day, sc.) E8-E21	↑ pinning social play P21 ♀ & ♂	(Buisman-Pijlman et al., 2009)
Sprague Dawley rats	Morphine (10mg/kg/day, s.c.) E8-E21	↑ pinning social play and social grooming P21 ↑ social approach P21, P28, P90	(Hol et al., 1996)
Stress & Anxiety			
Wistar rats	Morphine (10mg/kg twice daily, s.c.) E11-E18	n.c. stress induced plasma CORT adult ♂ & ♀	(Laborie et al., 2005)
Sprague Dawley rats	Morphine (5-10 mg/kg, twice daily, s.c.) E11-18	↓ restraint stress-induced ACTH, n.c. in CORT adult ♂	(Rimanóczy et al., 2003)
Sprague Dawley rats	Morphine (5-10 mg/kg, twice daily, s.c.) E11-18	↓ restraint stress-induced ACTH, n.c. in CORT adult ♀	(Slamberová et al., 2004)
Sprague Dawley rats	Morphine (5-10mg/kg/day, s.c.) E11-E19	↑ OFT center time P25 ♀, but not P52 ↑ EPM open arm time P53 ♀ ↓ FST immobility P32 ♀ ↑ FST immobility P54 ♀	(Y. Chen, Du, et al., 2022)
Wistar rats	Morphine (5-10mg/kg/day, s.c.) E11-E18	n.c. FST immobility P46/47	(Ahmadalipour & Rashidy-Pour, 2015)
C57BL/6J mice	Heroin (1.05mg/kg/day, s.c.) E12, E15, & E18	↑ OFT center time P28 ♀ n.c. OFT center time P28 ♂	(Kvello et al., 2019)

C57BL/6J mice	Fentanyl (10μg/ml in 2% saccharin, oral SA) E0-P21	↓ EPM open arm time adolescent ♂ n.c. EPM open arm time adolescent ♀ ↓ sucrose splash grooming adolescent ♀ n.c. sucrose splash grooming adolescent ♂	(Alipio, Haga, et al., 2021)
Sprague Dawley rats	Oxycodone (0.5 or 2mg/kg/day) E8-E21	↑ OFT distance traveled adult ♂ (low oxy group only)	(Sithisarn et al., 2017)
Sprague Dawley rats	Buprenorphine (3mg/kg/day, s.c.) E3-E20 Methadone (5-7mg/kg/day, s.c.) or morphine (2-4mg/kg/day, s.c.) E3-E20	↓ OFT center time adult ♀ ↓ OFT center time adult ♂ & ♀	(H.-H. Chen et al., 2015)
Sprague Dawley rats	*l*-alpha-acetylmethadol (LAAM) (0.2 or 1mg/kg/day) Pregestation-E10	↓ CORT response to social isolation or LPS injection (both LAAM groups) neonatal ♂ & ♀ ↑ CORT response to LPS (1mg/kg/day LAAM) adult ♂ & ♀	(K. L. Hamilton et al., 2005)

ADHD, attention deficit hyperactive disorder; ACTH, adrenocorticotropic hormone; ASD, autism spectrum disorder; CORT, corticosterone; E(#), embryonic day; EPM, elevated plus maze; inf, infusion; i.p., intraperitoneal; LAAM, l-alpha-acetylmethadol; LPS, lipopolysaccharide; NOWS, neonatal opioid withdrawal syndrome; n.c., no change; OFT, open field test; oxy, oxycodone; P(#), postnatal day; SA, self-administration; s.c., subcutaneous; SUD, substance use disorder

Table 1.1 Summary of perinatal opioid exposure-associated behavioral outcomes

Perinatal opioid-related molecular changes in brain reward systems

Subjects	**Opioid Exposure**	**Outcomes**	**Reference**
Clinical Evidence			
Humans	NOWS diagnosis	*PNOC* SNPs associated with ↑ risk of alcohol abuse full-term infants ♂ & ♀ *COMT* SNPs associated with ↑ risk of depression full-term infants ♂ & ♀	(Wachman et al., 2017)
Humans	NOWS diagnosis	↑ *OPRM1* methylation infants ♂ & ♀	(Wachman et al., 2014)
Humans	Prenatal opioid exposure	↑ *OPRM1* methylation newborns ♂ & ♀	(McLaughlin et al., 2017)
Humans	Prenatal opioid exposure	↑ plasma β-endorphin, β-lipotropin, & met-enkephalin newborns ♂ & ♀	(Panerai et al., 1983)
Mu Opioid Receptor Expression			
Sprague Dawley rats	Morphine (2-7mg/kg/day, sc., then via milk) Pregestation-P14	↓ MOR density in striatum, thalamus, & amygdala P14 sex N/A n.c. MOR density in midbrain, NAc, hpc, or cortex P14 sex N/A	(Chiou et al., 2003)
Albino rats	Morphine (1µg/pup in 5µl PBS) P1-P7	↑ MOR density in striatum & NAc ~P180 sex N/A	(Handelmann & Quirion, 1983)
Wistar rats	Morphine (20mg/kg/day, sc. Twice or four times daily) E12-E21	↓ MOR density in spinal cord P21 & P30 sex N/A ↑ MOR density in spinal cord P60 sex N/A	(Kirby, 1983)

Sprague Dawley rats	Morphine (5-10 mg/kg, twice daily) E11-E18	↑ MOR density in NAc, PMCoA, CeA young adult ♂ ↓ MOR density in BLA young adult ♂ n.c. MOR density in LA & BNST young adult ♂ n.c. MOR densities young adult ♀	(I. Vathy et al., 2003)
Sprague Dawley rats	Morphine (2mg/kg/day, i.p.) E13-E20	↑ MOR density P1 & P7 sex N/A ↑ MOR mRNA P1 & P7 sex N/A n.c. MOR density or mRNA P14 & P28 sex N/A (all whole brain measures)	(Bhat et al., 2006)
Sprague Dawley rats	Morphine (5-10 mg/kg, twice daily) E11-E18	↑ MOR density in CA1 OVX adult ♀ ↑ MOR density in CA3 hormone-treated adult OVX ♀ ↓ MOR density in CA3 adult OVX ♀ ↓ MOR density in DG hormone-treated adult GNX ♂, but not adult GNX ♂	(Slamberová et al., 2003)
Sprague Dawley rats	Morphine (75mg pellet) Implanted at E16	↓ DAMGO binding in whole brain P1-P8, but not P14 sex N/A	(Tempel et al., 1988)
Sprague Dawley rats	Morphine (75mg pellet) Implanted at E16 (5mg/kg/day, s.c.) Postnatal treatment for 4 or 8 days	↓ MOR density in striatum, NAc, & amygdala in 4-day postnatal group, but not 8-day P4, but not P8 ♂ & ♀	(Tempel, 1991b)
Sprague Dawley rats	Methadone (5-7mg/kg twice daily, s.c.) E3-E20	↓ MOR mRNA in spinal cord P30 ♂	(Chiang et al., 2015)

Sprague Dawley rats	Buprenorphine (3mg/kg/day, s.c.) E3-E20	n.c. MOR in NAc adult ♂	(Chiang et al., 2014)
Rats	Buprenorphine (0.3, 0.6, or 2.5mg/kg/day, s.c.) E6-E20 every other day	↓ MOR density P1 ♂ & ♀	(Belcheva et al., 1998)
Sprague Dawley rats	Methadone (10mg/kg/day) Pregestation-P0 Buprenorphine (1mg/kg/day) Pregestation-P0	↓ MOR binding in cerebrum P1 & P7 ♂ but not P14, P21, P77 ↓ MOR binding in cerebrum P1, P7, P14 ♂ but P21 or P77	(Kongstorp, Bogen, Stiris, et al., 2020)
Mesolimbic Signaling			
Sprague Dawley rats	Buprenorphine (3mg/kg/day, s.c.) E3-E20	↓ D1R in NAc adult ♂ n.c. D2 or D3R in NAc adult ♂ ↓ basal cAMP and D1R agonist-induced adenylyl cyclase activity in NAc adult ♂	(Chiang et al., 2014)
Sprague Dawley rats	Morphine (0.1-0.3 mg/ml in water, oral SA) E0-P21	↓ striatal dopamine P12 & P30 sex N/A ↑ striatal DOPAC P12 & P60 sex N/A ↓ striatal DOPAC P30 sex N/A ↑ met-enkephalin P0-P240 sex N/A ↑ substance P P12, P20, & P240 sex N/A ↓ TH mRNA in substantia nigra, but not VTA P7 sex N/A	(Gorio et al., 1996)

Sprague Dawley rats	Oxycodone (0.1mg/kg/inf SA) Pregestation to P0	↑ D1R density in NAc P55 ♂, but not P20 or P30 n.c. D2R density P20 or P30 ♂, or P20-55 ♀ ↓ microglial D1R volume P30 ♂	(Smith et al., 2022)
Sprague Dawley rats	Morphine (5-10 mg/kg, twice daily) E11-E18	↑ TH expression in PVN & LC, but not LH P65-75 ♂ ↓ TH expression in LC OVX P65-75 ♀	(I. Vathy et al., 2000)
Sprague Dawley rats	Morphine (5-10mg/kg/day, s.c.) E11-E19	↑ local field potential in NAc & BLA ↑ HDAC5 in VTA ↓ morphine reward-induced coherence of VTA-NAc, mPFC-NAc, and mPFC-BLA circuits (all in adult ♂)	(Y. Chen, Du, et al., 2022)
C57BL/6J mice	Methadone (10mg/kg twice daily, s.c.) E0-P28	↓ CB1-mediated LTD & glutamatergic signaling in DLS, but not DMS adolescent ♂, but not ♀	(Grecco, Muñoz, et al., 2022)
C57BL/6J mice	Fentanyl (10μg/ml in 2% saccharin, oral SA) E0-P21	↑ ACC function P35-P45 ♂ & ♀ ↓ primary somatosensory cortex (S1) function P35-P45 ♂ & ♀ ↓ glutamatergic and GABAergic receptor mRNAs in ACC and S1 P40 ♂ & ♀	(Alipio, Haga, et al., 2021)
C57BL/6J mice	Buprenorphine (5mg/kg/day) E7-P11	↑ excitatory synapses in ACC & NAc ↑ inhibitory synapses in PFC	(Boggess et al., 2021)

Hippocampal Signaling			
Wistar rats	Morphine (64mg/kg/day twice daily, s.c. during gestation, then reduced to 3.2mg/kg/day post-gestation) Pregestation-P10	↓ CA1 LTD adult ♂ & ♀	(Sarkaki et al., 2008)
Wistar rats	Morphine (5-10mg/kg/day, s.c.) E11-E18	↓ medial PP-DG LTP P50-57 ♂ & ♀ ↓ BDNF P50-57 ♀ n.c. BDNF P50-57 ♂	(Ahmadalipour et al., 2018)
Sprague Dawley rats	Methadone (5-7mg/kg/day, sc.) E3-E20	↓ DG excitability P70 ♀, but not ♂ ↑ DG sIPSC P70 ♀, but not ♂	(Gamble et al., 2022)

ACC, anterior cingulate cortex; BDNF, brain-derived neurotrophic factor; BLA, basolateral amygdala; BNST, bed nucleus of the stria terminalis; CA1, cornu ammonis 1; CA3, cornu ammonis 3; CB1, cannabinoid 1 receptor; CeA, central amygdala; *COMT*, catechol-O-methyltransferase; D(#)R, dopamine receptor #; DAMGO, [D-Ala2, N-MePhe4, Gly-ol]-enkephalin; DG, dentate gyrus; DLS, dorsolateral striatum; DMS, dorsomedial striatum; DOPAC, 3,4-Dihydroxyphenylacetic acid; E(#), embryonic day; GNX, gonadectomized; HDAC5, histone deacetylase 5; hpc, hippocampus; inf, infusion; i.p., intraperitoneal; LC, locus coeruleus; LH, lateral hypothalamus; LTD, long-term depression; LTP, long-term potentiation; MOR, µ-opioid receptor; mPFC, medial prefrontal cortex; NAc, nucleus accumbens; NAS, neonatal abstinence syndrome; n.c., no change; *OPRM1*, opioid receptor mu 1; OVX, ovariectomized; P(#), postnatal day; PBS, phosphate buffer solution; PMCoA, posteromedial cortex of the amygdala; *PNOC*, preproprotein; PP, perforant path; PVN, paraventricular nucleus; S1, primary somatosensory cortex; SA, self-administration; sIPSC, spontaneous inhibitory postsynaptic current; SNP, single nucleotide polymorphism; VTA, ventral tegmental area

Table 1.2 Summary of perinatal opioid exposure-associated molecular outcomes

2 PERINATAL OPIOID EXPOSURE ALTERS ALCOHOL-DRIVEN REWARD BEHAVIORS IN ADOLESCENT MALE AND FEMALE RATS

2.1 Introduction

Pregnant women are prescribed opioids at alarmingly high rates (approximately 9.5%-41.6% in the U.S. alone), and it is currently estimated that a baby is born every 15 minutes with symptoms of opioid withdrawal (Desai et al., 2014; Winkelman et al., 2018). Rates of opioid abuse have also risen among adolescents, and among those seeking treatment, a family history of opioid abuse has been identified as a primary contributing factor (Palamar et al., 2018; Russell et al., 2015). Indeed, familial drug use positively correlates with use in future generations in both humans and rodents (Cadoret et al., 1986; Vassoler et al., 2016). Gestational opioid exposure in humans is associated with several adverse developmental outcomes that may further contribute to drug use, including cognitive deficits, reduced socialization, and increased anxiety (Azuine et al., 2019; Larson et al., 2019). This raises concern for drugs of abuse that are easily accessible, such as alcohol. Indeed, by the end of high school, nearly 60% of teens will have had at least one drink, with many having participated in binge-level consumption (Johnston et al., 2020).

Although chronic drug use has been shown to alter reward salience, limited research has explored the effects of gestational opioid exposure. Increased conditioned place preference for morphine was observed in adult male rats exposed to morphine during gestation, and adult male rats exposed to morphine for one week mid-gestation exhibited increased self-administration of morphine compared to controls (Torabi et al., 2017; L.-Y. Wu et al., 2009). Comparable changes have been reported for prenatal opioid exposure and future use of methamphetamines and cocaine, suggesting that changes in sensitivity to drug reward span multiple drug classes (Ramsey et al., 1993; Shen et al., 2016; I. Vathy et al., 2003). To date, however, it is unclear if this shift in reward sensitivity is limited to drugs of abuse or extends to natural rewards (Gagin et al., 1996; I. Vathy et al., 2007).

Although these studies suggest that gestational exposure to morphine alters the reward value for drugs of abuse, the majority of these studies employed a truncated opioid exposure period, typically using a daily or twice-daily opioid injection paradigm for seven to ten days in mid to late gestation. Importantly, this administration paradigm fails to recapitulate the clinical profile of a late adolescent opioid user who typically begins illicit drug use prior to pregnancy (Grecco & Atwood, 2020; Harder & Murphy, 2019). Our studies address this limitation by employing a clinically relevant model in which morphine is intermittently administered prior to and throughout gestation. Morphine exposure is continued through the first week post-partum to replicate further the clinical use of supplemental opioid therapy that protects newborns from withdrawal (Abdel-Latif et al., 2006; Bogen & Whalen, 2019; Welle-Strand et al., 2013).

Using our clinically relevant model, we investigated the impact of perinatal morphine exposure on alcohol consumption during adolescence and early adulthood. Our data reveal that perinatal morphine leads to effort-dependent changes in alcohol and sucrose consumption and suggest that opioid use during pregnancy alters the risk of drug abuse in the offspring.

2.2 Materials and Methods

2.2.1 Subjects

Female Sprague Dawley rats (age P60; Charles River Laboratories, Boston, MA) were used to generate male and female offspring perinatally exposed to morphine or sterile saline. Rats were co-housed in Optirat GenII individually ventilated cages (Animal Care Systems, Centennial, Colorado, USA) with corncob bedding in same-sex pairs or groups of three on a 12:12 hour light/dark cycle (lights on at 8:00 AM). Food (Lab Diet 5001 or Lab Diet 5015 for breeding pairs, St. Louis, MO, USA) and water were provided ad libitum throughout the experiment, except during operant procedures. All studies were approved by the Institutional Animal Care and Use Committee at Georgia State University and performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to reduce the number of rats used in these studies and minimize pain and suffering.

2.2.2 Perinatal opioid exposure design

Female Sprague Dawley rats were implanted with iPrecio SMP-200 microinfusion minipumps at postnatal day 60 (P60). Pumps were programmed to begin morphine or saline administration following one week of recovery from surgery. Initially, rats were administered 10 mg/kg across three doses per day, with doses increasing weekly by 2 mg/kg/day until 16 mg/kg/day was reached. One week after morphine initiation, females were paired with sexually-experienced males for two weeks to induce pregnancy. Morphine exposure to the dams continued throughout gestation. At approximately E18, pumps switched to twice-a-day dosing, as initial pilots using three-times-a-day dosing increased pup mortality early in life, possibly due to respiratory depression. Dams continued to receive morphine after parturition, such that pups received morphine through their mother's milk (Figure 1a). From approximately E18 to P4, daily dosage was 10.67 mg/kg/day. Morphine dosage was decreased to 9.33 mg/kg/day on P5 and again to 8 mg/kg/day on P6. On P7, morphine was removed. This protocol closely mirrors the clinical profile of a late adolescent female who develops an opioid use disorder. An essential part of this model is the administration of morphine prior to E15, the approximate date of μ-opioid receptor development in the fetal brain (Coyle & Pert, 1976). Pumps filled with sterile saline were used to generate control rats to account for the stress of surgery and pump refilling.

2.2.3 Alcohol self-administration

Male and female rats that received saline or morphine across gestation were weaned into reverse light conditions (12:12 hour dark/light; lights on at 8:00 PM) at P21 and aged to adolescence undisturbed. Beginning at P30, alcohol consumption was assessed using self-administration of ethanol gelatin (10% ethanol, 8% Polycose, and 2.5% gelatin in tap water; (Peris et al., 2006) using either a conditioned place preference (CPP) or operant conditioning paradigm (Figure 1b). Consumption of ethanol gelatin generates blood and brain ethanol levels in Sprague-Dawley rats, comparable to other paradigms involving liquid delivery (Nurmi et al., 1999). The protocol also avoids the necessity for alcohol-preferring rats that may exhibit different baseline expression of μ-opioid receptors (McBride et al., 1998).

Conditioned place preference. Eighteen male (11 saline, 7 morphine) and sixteen female (9 saline, 7 morphine) P30 rats were tested for CPP. Conditioned place preference (CPP) testing occured under red light in a two-compartment arena separated by an open door (Figure 2a).

Compartments were differentiated by bedding: one side contained white paper square bedding, whereas the other side had pelleted paper bedding (Teklad, Envigo, Indianapolis, IN); both types of bedding were distinct from the rats' typical home-cage bedding. One hour before being placed in the CPP arena for a 20-minute pretest, rats were habituated to the testing room. Side preference (defined as >50% time spent) was determined and used to generate pseudorandom unbiased and counterbalanced alcohol-bedding type assignments. Rats exhibiting >70% preference for one side during the pre-conditioning test were excluded. Before CPP, rats were conditioned to alcohol in a home cage-like setting. During alcohol-paired sessions, one of two ad libitum water bottles was replaced with a jar of approximately 20g of ethanol gelatin in a cage that contained one of the two bedding types. Sessions without alcohol contained two water bottles and the other bedding type. Following two 24-hour pairings, rats received twice daily exposures separated by three hours using the following schedule: Days 3-4, 6 hours; Days 5-6, 3 hours; Days 7-11, 1 hour. Order of alcohol/no alcohol sessions was randomly assigned and then alternated each day. Alcohol exposure time of day was balanced by alternating morning/afternoon:alcohol/no alcohol. Rats were single-housed during CPP conditioning but were otherwise group-housed. A post-conditioning test session was conducted on the 12th day. During the test, rats were placed in the center doorway, randomly facing an arena side/bedding type. Rats were recorded exploring the arena freely for 20 minutes. Preference scores were calculated by subtracting the time spent on the non-alcohol-paired side from the time spent on the alcohol-paired side.

<u>Noncontingent alcohol self-administration</u>. Twenty-eight male (15 saline, 13 morphine) and twenty-five female (11 saline, 14 morphine) P30 rats were used in the noncontingent operant

sessions. Like CPP, rats were isolated in a new home cage with a jar filled with ethanol gelatin for two consecutive 24-hour sessions. Free access to ethanol gelatin was gradually reduced across the next 4 days to 1-hour daily sessions for 10 total days (Figure 3a). Ethanol consumption was measured in g/kg/hr.

Operant procedures. During the last five days of noncontingent exposure, rats were trained to lever press in operant conditioning chambers (MED Associates, St. Albans, VT, USA) using light cues and negative reinforcement (80db white noise). Successful pressing of the "active" (versus inactive) lever was indicated by 5s termination of the chamber's house light and white noise and a light turned on above the lever. After five days of lever training, gelatin rewards were paired with the reinforcement cues. All rats underwent ten days of 30-minute sessions where an active lever press earned an approximately 0.2-gram ethanol gelatin reward (Fixed Ratio 1; FR1), followed by ten days of FR3 in which three presses were required for rewards. On the final day, motivational breakpoints were determined using a progressive ratio (PR) in which requirements increase as each reward is achieved. Throughout operant conditioning sessions, lever press data from active and inactive levers was collected using time signatures to monitor activity levels, total consumption, consumption across time, and lever differentiation. Within the chambers, infrared beams at the front and back monitored activity during sessions. Accuracy during the operant session was defined as (active presses)/(active press during reward receipt + inactive presses).

Relapse testing. Forced abstinence is a well-established assay used to measure the rewarding properties of alcohol after daily use is suspended (A. Lê & Shaham, 2002). Following breakpoint determination, rats were returned to their home cage for one day, one week, and two weeks of abstinence. Following abstinence, gelatin consumption was measured in one FR3 operant session. Relapse was measured by a rat's individual % change in alcohol consumed compared to their 10-day average during FR3.

2.2.4 Sucrose Administration

A separate cohort of rats was tested for noncontingent and subsequent operant natural reward behavior using 20% sucrose in tap water (Figure 4a). During home cage access, the sucrose mixture included 2.5% gelatin to mimic alcohol gel and prevent spillage. No one-day relapse test was administered due to five days-on/two days-off sucrose schedule.

2.2.5 Immunohistochemistry

Randomly selected littermates of rats used for reward behavior were sacrificed at postnatal ages 7, 14, and 30 to assess µ-opioid receptor (MOR) expression. Brains were collected via rapid decapitation and drop-fixed in 4% paraformaldehyde for 24 hours, followed by storage in 30% sucrose at 4°C until sectioning. Fixed tissue was sectioned coronally at 40-µm with a Leica SM2010R microtome and stored at -20°C in cryoprotectant-antifreeze solution (Watson et al., 1986). Forebrain and midbrain sections were processed for MOR immunoreactivity using standard techniques previously described (Fullerton et al., 2021; Harder et al., 2023). Free-floating sections were rinsed in potassium phosphate-buffered saline (KPBS), incubated in 3% hydrogen peroxide for 30 minutes, and rinsed again in KPBS at room temperature. Tissue was then incubated in rabbit anti-MOR primary antibody (Alomone Labs, Jerusalem, Israel; 1:7,500 for P7 and P14; 1:2,000 for P30) at room temperature for 1 hour, followed by 48 hours at 4°C. Primary antibody was rinsed using KPBS and incubated in biotinylated donkey anti-rabbit IgG (Jackson Immunoresearch, West Grove, PA, USA; 1:600) for 1 hour. After the tissue was rinsed with KPBS and sodium acetate (0.175M; pH 6.5), immunoreactivity was visualized using nickel sulfate 3,3'-diaminobenzidine solution (2 mg/10 ml) and 0.08% hydrogen peroxide in sodium acetate buffer. After 20 minutes, tissue was rinsed in sodium acetate and KPBS and then mounted on gelatin-subbed slides, air-dried, dehydrated with increasing concentrations of ethanol, cleared with xylene, and cover-slipped.

For immunohistochemistry data, 16-bit grayscale images were captured using a QImaging Retiga EXi CCD camera and IPLab Image Analysis Software (BD Biosciences). The nucleus accumbens (NAc), medial habenula (MHb), and ventral tegmental area (VTA) were bilaterally imaged at 10× magnification (2-3 sections per rat, average of 4-6 values). The average grayscale pixel value within each region was recorded for each rat.

2.2.6 Statistical analysis

All data met the requirements for parametric analyses (normality, homogeneity of variance). The effect of sex, treatment (morphine/saline), and day of schedule (where applicable) was assessed using two- or three-way mixed models ANOVA with an alpha level of $p<0.05$. Given that ANOVA testing cannot handle missing values, mixed models using Greenhouse-Geisser were implemented where outlier correction was necessary. Outliers were identified using GraphPad Prism's ROUTS method. Post hoc testing was performed using Tukey's or Sidak's. Experiments involving repeated measures were not tested for daily individual group differences. No significant litter effects were observed; therefore, group data comprised multiple litters. GraphPad Prism 9.1.0 was used for all statistical analyses.

2.3 Results

2.3.1 Conditioned place preference

To determine if gestational morphine exposure altered reward salience, morphine (POE) or saline (CON) exposed rats were first tested for alcohol preference using CPP (Figure 2a). Our analysis showed a significant interaction between sex and treatment ($F_{sex*tx}(1,29)=9.345$, $p=0.0048$; Figure 2b). POE males spent, on average, 135 ± 74 secs in EtOH-paired bedding vs CON males (-74 ± 56) ($p=0.064$). In contrast, neither POE nor CON females formed a CPP for EtOH-paired bedding ($p=0.328$). Indeed, most POE females spent less time in EtOH-paired bedding, suggesting conditioned place aversion. POE male and female rats also exhibited more room entries, or movement between the two arena areas, than CON rats ($F_{treatment}(1,27)=8.687$, $p=0.0065$; Figure 2c). POE males also significantly increased the number of room entries from pre- to post-conditioning ($p=0.0031$), suggesting increased searching for alcohol rewards. Alternatively, POE males may be displaying alcohol-associated locomotor hyperactivity. Post-conditioning room entry counts for POE males were also significantly higher than those of CON males ($p=0.0125$).

2.3.2 Alcohol Self-Administration

The results from our CPP studies suggested that POE males exhibited an increased preference for alcohol compared to CON males. We next tested if daily consumption of alcohol differed between morphine and saline-exposed adolescent rats. All rats were introduced to alcohol under “free access” (FA) conditions (see Figure 3a for schedule). During FA, rats were individually housed in standard cages and returned to their home cage at the end of the session.

All rats showed an increase in alcohol consumption over time ($F(7,632)=102.6$, $p<0.01$; Figure 3b). We also noted a significant interaction between sex and treatment ($F(1,97)=4.52$, $p=0.036$). This effect was driven by females ($F_{treatment}(1,46)=4.19$, $p=0.04$). From days 7-16, POE females consistently consumed, on average, 24% more EtOH than controls. Following free access, rats were trained to lever press for alcohol rewards. POE did not produce differences in lever press accuracy during training ($F_{treatment}(1,63)=2.072$, $p=0.155$), but males were less accurate than females ($F_{sex}(1,63)=5.050$, $p=0.028$; Figure 3c).

Across ten days of FR1, POE rats consumed significantly less alcohol than CON rats ($F_{treatment}(1,63)=6.424$, $p=0.01$; Figure 4a). This reduced alcohol consumption continued through ten subsequent days of FR3 ($F_{treatment}(1,63)=4.805$, $p=0.03$; Figure 4b). These differences were driven primarily by males (FR1: $F(1,31)=7.595$, $p=0.0097$; FR3: $F(1,31)=4.697$, $p=0.03$), with no differences in consumption observed in females (FR1: $p=0.243$; FR3: $p=0.218$). Following FR3, rats were tested using a progressive ratio (PR), an indicator of maximal effort for reward. Despite consuming significantly less alcohol under both FR1 & FR3 schedules, average PR values were similar across all groups with no effect of sex or treatment (Sex: $p=018$; Treatment: $p=0.32$; Figure 4c).

We next assessed relapse drinking, which is characterized as compulsive alcohol-seeking behavior. In rats, repeated cycles of drinking and deprivation, or forced abstinence, will drive increases in self-administration and alcohol seeking (A. Lê & Shaham, 2002). Here, relapse severity was determined by comparing FR3 alcohol consumption to that displayed after successive periods of forced abstinence (one day, one week, and two weeks). Our analyses showed no effect of sex or treatment in relapse potential, defined by consumption after abstinence compared to individual average FR3 consumption (Sex: p_{1day}=0.668, p_{1week}=0.99, p_{2weeks}=0.554; Treatment: p_{1day}=0.84, p_{1week}=0.148, p_{2weeks}=0.114; Figure 4d-f). However, interestingly, all POE females exhibited relapsed drinking behavior at both one week and two weeks, in contrast to the other groups.

Children exposed to opiates across gestation are at an increased risk for developing attention deficit disorders; thus, we also assessed operant responding rates as an index of task focus (Azuine et al., 2019; Nygaard et al., 2016; Sirnes et al., 2018). We first examined the latency to first operant response. As rats tend to press the lever when first learning the task inadvertently, FR1 sessions were not analyzed. Overall, in FR3, males were faster to earn their first alcohol reinforcement than females, averaging 56.4s compared to 88.4s ($F_{sex}(1,63)$=10.87, p=0016; Figure 5a). This effect was independent of treatment (p=0.48). We also assessed accuracy, defined as the percentage of all lever presses that elicited a reward. Despite males displaying reduced accuracy during lever press training, they were more accurate in their lever pressing than females during FR3 (F(1,63)=6.865, p=0.01, Figure 5b). After one day of forced abstinence, POE rats were faster to earn rewards than CON rats ($F_{day*treatment}(2,54)$=4.043, p=0.02; One Day: p=0.034; Figure 5c). On average, POE rats earned their first reward in 37.3s while control rats earned theirs in 89.6s. Overall, males earned their first reward faster than females ($F_{sex}(1,28)$=12.06, p=0.0017). No significant treatment differences in first reward latency or accuracy were noted at one week or two weeks relapse testing (One Week: p=0.92; Two Weeks: p=0.807; Figure 5c-d).

As discussed above, POE rats consumed less alcohol across the majority of FR1 & FR3 test days. We next examined if the reduced consumption compared to CON rats was consistent across the entire session or if there was a shift in response rates within each session. For this, reinforcements were binned into 5min periods. We found that the number of rewards earned was consistent between groups across 30min FR1 sessions (FR1: p_{sex}=0.69, $p_{treatment}$=0.306; Figure 5e). During FR3 sessions, there was an overall sex difference between males and females ($F_{sex}(1,63)=4.392, p=0.401$), but this was not the case within individual 5min bins (post hoc comparisons: all $p>0.05$; Figure 5f). No significant treatment difference in binned FR3 reinforcements was found ($p=0.16$), suggesting that POE and CON rats do not differ in their attention to the operant task at the beginning or end of a session.

2.3.3 Sucrose Self-Administration

We next assessed if the changes noted in alcohol-driven behaviors due to perinatal opioid exposure extend to natural rewards, specifically sucrose. Rats self-administered 20% sucrose solution under free access and operant conditions, similar to our alcohol testing paradigm (Figure 6a). No effect of treatment or sex was observed when rats were provided free access to sucrose ($p=0.84$; Figure 6b). Rats were next trained to lever press using white noise. CON rats were more accurate in lever pressing than POE rats ($F_{treatment}(1,32)=5.717, p=0.023$; Figure 6c). However, POE rats reached control rats' levels of accuracy by the end of training. Overall, female rats consumed significantly more sucrose than males across all FR1 sessions ($F_{sex}(1,32)=63.51, p<0.0001$, Figure 6d); CON female rats also consumed significantly more sucrose than POE females ($F_{treatment}(1,17)=4.617, p=0.046$). During FR3 sessions, there was both a treatment and sex difference in that female rats continued to consume more sucrose than males overall, while CON groups consistently consumed more than POE ($F_{sex}(1,32)=56.24$, $p<0.0001$, $F_{treatment}(1,32)=4.489$, $p=0.042$; Figure 6e). Sucrose breakpoint, as measured by progressive ratio testing, was higher in females compared to males but not altered by POE treatment ($Female_{average}=49.9$, $Male_{average}=35.1$; $F_{sex}(1,32)=16.37$, $p=0.0003$, $F_{treatment}(1,32)=2.872$,$p=0.099$; Figure 6f). After one week of forced abstinence, POE rats trended towards increased relapse potential compared to CON rats, demonstrating higher increases in sucrose consumption compared to their average FR3 consumption ($F_{treatment,}(1,32)=3.793$,$p=0.06$; Figure 6g). This was also observed following a subsequent two-week forced abstinence period ($F_{treatment}(1,32)=3.522$,$p=0.069$; Figure 6h). Overall, these results suggest that daily motivation for sucrose is decreased in POE rats, as indicated by reduced consumption under free access conditions. Forced abstinence, however, may trigger compulsive consumption of sucrose for POE rats, as they consistently demonstrated increased lever pressing during relapse testing. Together with our alcohol self-administration data, these results suggest that gestational morphine reduces motivation for operant rewards. Interestingly, although POE females consumed more alcohol than controls during free access conditions, this did not extend to sucrose intake.

As with alcohol, we examined POE-induced changes in sucrose reward latency, press accuracy, and reinforcement rate. No significant differences in first sucrose reward latency were found during FR3 ($p_{sex}=0.44$, $p_{treatment}=0.93$; Figure 7a). In these sessions, press accuracy was not affected by POE but was higher in males than in females ($p_{treatment}=0.087$, $p_{sex}=0.0019$; Figure 7b). Similarly, groups did not differ in their first sucrose reward latency during relapse sessions ($p_{sex}=0.16$, $p_{treatment}=0.31$; Figure 7c), but males were overall more accurate than females (p_{sex}=0.0276, $p_{treatment}$=0.16; Figure 7d). When analyzing sucrose reinforcement data in 5min intervals, POE rats earn fewer rewards overall compared to CON during FR1 and FR3 (FR1: $F_{treatment}(1,32)=5.353$, p=0.0273; FR3: $F_{treatment}(1, 32)=6.309$, p=0.0173; Figure 7e-f). Groups do not, however, differ within individual 5min periods (post hoc comparisons: all p>0.05). Therefore, all groups exhibit a similar reduction in sucrose rewards achieved across the average operant session.

2.3.4 μ-Opioid Receptor Expression

We next examined if the significant differences observed in alcohol self-administration were paralleled by changes in μ-opioid receptor protein (MOR). We used immunohistochemistry to identify MORs in key reward-associated brain regions at three developmental time points: P7, P14, and P30.

No significant effect of treatment or sex on MOR expression was noted for P7 in the nucleus accumbens (NAc), medial habenula (MHb), or ventral tegmental area (VTA) (NAc: $p_{treatment}$=0.10, p_{sex}=0.07; MHb: $p_{treatment}$=0.19, p_{sex}=0.18; VTA: $p_{treatment}$=0.42, p_{sex}=0.15; Figure 8a-c). At P14, increased NAc MOR was observed in POE females compared to CON females and POE males ($F_{sex*treatment}(1,19)=13.42$, p=0.0017; Figure 8d). There was no significant effect of treatment or sex in MHb or VTA MOR at P14 (MHb: $p_{treatment}$=0.67, p_{sex}=0.97; VTA: $p_{treatment}$=0.86, p_{sex}=0.06; Figure 8e-f). At P30, POE groups expressed significantly less MHb MOR than CON groups ($F_{treatment}(1,19)=4.848$, p=0.0402; Figure 8h). No group differences in NAc or VTA MOR expression were found at P30 (NAc: $p_{treatment}$=0.30, p_{sex}=0.56; VTA: $p_{treatment}$=0.53, p_{sex}=0.79; Figure 8g, i).

2.4 Discussion

The present studies were conducted to determine the impact of perinatal morphine exposure on alcohol and sucrose consumption in adolescent male and female Sprague Dawley rats. We report that maternal morphine exposure that begins before inception and continues after birth produces multiple sex-specific changes in alcohol-driven behaviors. Specifically, POE significantly altered CPP behaviors, including a preference for alcohol-paired cues and alcohol-seeking locomotion in males but not females. In contrast, POE female rats consumed more alcohol compared to controls under free access conditions. This increase did not extend to operant conditioning sessions where additional effort was required to earn alcohol. Thus, in contrast to our hypothesis that gestational morphine exposure would increase alcohol consumption regardless of required effort, POE rats significantly reduced their alcohol consumption across FR1 and FR3 compared to controls. This was also true for our natural reward choice, sucrose. POE female rats consumed less sucrose than controls during FR1, and both sexes consumed less than controls during FR3. Unlike alcohol, free access consumption of sucrose did not differ between groups. POE rats did, however, display elevated relapse behaviors towards sucrose.

Our data show that POE can induce both increases and decreases in alcohol consumption depending on the effort required to obtain the reward. These results are inconsistent with previous preclinical studies that report enhanced sensitivity and motivation for drugs of abuse after early life opioid exposure. For example, Timar et al. (2010) reported that pre-, peri, and postnatal morphine exposure (5-10mg/kg/day) increased preference for morphine in adulthood. Similar results were reported with shorter periods of prenatal exposure

(Gagin et al., 1997; Torabi et al., 2017; L.-Y. Wu et al., 2009). Other studies also report that POE's effects extend to other drugs of abuse. Morphine exposure for only two days of gestation reportedly increases heroin and cocaine I.V. self-administration in adult male rats (Ramsey et al., 1993). Locomotor sensitization to 2g/mg doses of methamphetamine is also increased in Sprague Dawley rats with prenatal exposure to 4-12mg/kg/day of buprenorphine, highlighting the potential impact of clinically recommended long-acting opioid agonists. Most of these studies failed to examine changes in reward behavior across varying scenarios of drug exposure, limiting the ability to draw conclusions about the long-term effects of POE on reward processing in more dynamic or real-world contexts. In fact, the only existing study on alcohol use post-POE found that twice daily 10mg/kg doses of methadone from E0 to P28 increased operant self-administration but decreased conditioned place preference in male mice (Grecco, Haggerty, et al., 2022). Studies on selective breeding for alcohol consumption in rats have reported similar results as well, in that rats with a low preference for alcohol consume more when required to lever press (Ritz et al., 1989). In contrast, rats displaying high alcohol consumption during the two-bottle choice were less willing to work for alcohol rewards (Ritz et al., 1989, 1994).

Our POE rats, especially females, exhibit similar behavior in that they consumed more alcohol than controls during noncontingent or "free access" sessions but less during operant conditioning sessions when effort was required. Importantly, decreased alcohol consumption during operant conditioning was not a result of impaired learning. Rather, our results suggest a disruption in motivation, which has been previously associated with both mesolimbic dopamine dysfunction and anhedonic phenotypes in rodents (Canonica & Zalachoras, 2022). For example, chronic social defeat or restraint stress decreased operant response for sucrose rewards (Bergamini et al., 2016; Xu et al., 2017). Conversely, isolation stress increases noncontingent alcohol consumption and CPP, in addition to inducing anxiety and depression-like behaviors (J.-S. Lee et al., 2021; Wukitsch et al., 2019).

For our POE males, increased CPP for alcohol may indicate increased sensitivity to its rewarding effects. This coincided with increased chamber shuttling during testing. Shuttling behavior may be related to drug-seeking locomotion or, alternatively, general hyperactivity, as seen in rat models of ADHD (Wultz et al., 1990). Interestingly, children with prenatal opioid exposure are at an increased risk for ADHD (Azuine et al., 2019; Sandtorv et al., 2018; Sherman et al., 2019).

POE females consistently elevated their alcohol consumption following one and two weeks without access. This relapse steadily increased as a function of time, suggesting that POE females may be more susceptible to losing control over compulsive drinking habits, a characteristic found in social drinkers with similar abstinent experiences (Ludwig et al., 1974). Drug abstinence can generate negative affect that drives stress-reducing relapse behavior (G. F. Koob, 2020). Negative affect is more prevalent in humans and rats exposed to perinatal opioids; therefore, their risk for relapse is greater (Bakhireva et al., 2019; N. C. F. Castro et al., 2021).

Several measures of alcohol behavior that were disrupted by POE were similarly disrupted for sucrose, a natural reward. Previous studies in rodents exposed to opioids have reported conflicting evidence. For example, sucrose consumption used for operant training was unaffected by POE in adult male and female rats (I. Vathy et al., 2007); however, others report an increased preference for saccharin in adult male and female rats exposed to morphine mid-gestation (Gagin et al., 1996). In contrast, Torabi et al. (2017) found that E11-P0 morphine exposure, followed by naloxone-precipitated withdrawal, decreased sucrose and increased morphine preference in adult male and female rats. In the present study, we observed no treatment differences for sucrose during free access; rather, it was not until effort was needed during operant conditioning that sucrose (and alcohol) consumption was decreased in POE rats, especially females. Compared to controls, POE rats demonstrated increased relapse for sucrose (and alcohol), suggesting that changes in reward-seeking behavior are not isolated to drugs of abuse.

Perinatal morphine-induced changes in MOR in reward-associated brain regions are well-characterized. Here, we report that POE increased MOR expression by 37% in the nucleus accumbens (NAc) of P14 females compared to controls. These results are consistent with previous studies reporting increased NAc MOR expression and binding in adult males and females exposed to gestational opioids (Handelmann & Quirion, 1983; Vassoler et al., 2016; I. Vathy et al., 2003). NAc MOR is critical to the rewarding properties of alcohol in rats, and its pharmacological manipulation leads to changes in consumption behaviors (Barson et al., 2009; Hyytiä & Kiianmaa, 2001). Studies involving direct manipulation of MOR will be necessary to determine if these changes in opioidergic signaling drive our observed changes in alcohol/sucrose reward.

Additionally, we found that medial habenula (MHb) MOR expression was decreased in POE rats at P30 compared to controls. The MHb has the highest region-specific expression of MOR in the brain and plays a significant role in the neural circuitry underlying opioid reward/aversion and social interaction (Allain et al., 2022; L.-J. Boulos et al., 2017; Gardon et al., 2014; Mechling et al., 2016). Reduced MHb MOR supports our previous study reporting reduced social interaction in periadolescent POE rats (Harder et al., 2023). Genetic ablation of dorsal MHb neurons, where MOR is primarily localized, also reduces sucrose preference in mice (Contestabile et al., 1987; Gardon et al., 2014; Hsu et al., 2014). Again, studies directly manipulating MOR within the MHb will be necessary to show causality.

Although changes in emotional states were not explicitly assessed, decreased motivation and preference for sucrose in rats are characteristics of anhedonia, or the inability to experience pleasure. Symptoms of depression are regularly reported in NOWS newborns and are thought to be produced by neuroendocrine and reward system changes (Hanson et al., 2021; Horn et al., 2018; Nygaard et al., 2020; Vidal et al., 2012; Wachman et al., 2018). Animal models of early life stress, such as low bedding and nesting (LBN), generate similarly dysfunctional phenotypes (Bolton et al., 2018; Williams et al., 2022). LBN also produces fragmented and unpredictable maternal care that is thought to train offspring to recognize rewarding patterns poorly (Molet et al., 2016).

Clinically, women undergoing opioid maintenance therapy (OMT) display suboptimal maternal behaviors that are associated with cognitive deficits in their children (Konijnenberg et al., 2015; Sarfi et al., 2011). During mother-child interactions, OMT moms exhibit significant reductions in several areas, such as sustained attention, mood, supportive presence, and responsiveness (Konijnenberg et al., 2016). These factors are critical for social and cognitive development that may alter future behavior in NOWS children (Larson et al., 2019). Fractured maternal behaviors may also be present in our morphine-exposed rat dams, as we have previously reported reduced nursing behavior during morphine infusions (Harder et al., 2023).

Our study is the first to report POE-induced changes in both alcohol and sucrose self-administration in rats and does so using a clinically relevant opioid exposure model. Admittedly, our results are descriptive. However, they lay the foundation for future studies examining the impact of gestational opioids on reward. Clinical studies report an altered risk for alcohol use disorders amongst those born with POE or in families with a history of parental opioid use disorder (Elam et al., 2020; Nygaard et al., 2020; Vidal et al., 2012). These findings highlight the potential long-term effects of perinatal opioid exposures on reward-related behaviors and underscore the need for further research to elucidate the underlying mechanisms. By bridging preclinical and clinical insights, our study provides a critical step toward understanding and addressing the intergenerational impact of opioid use disorders.

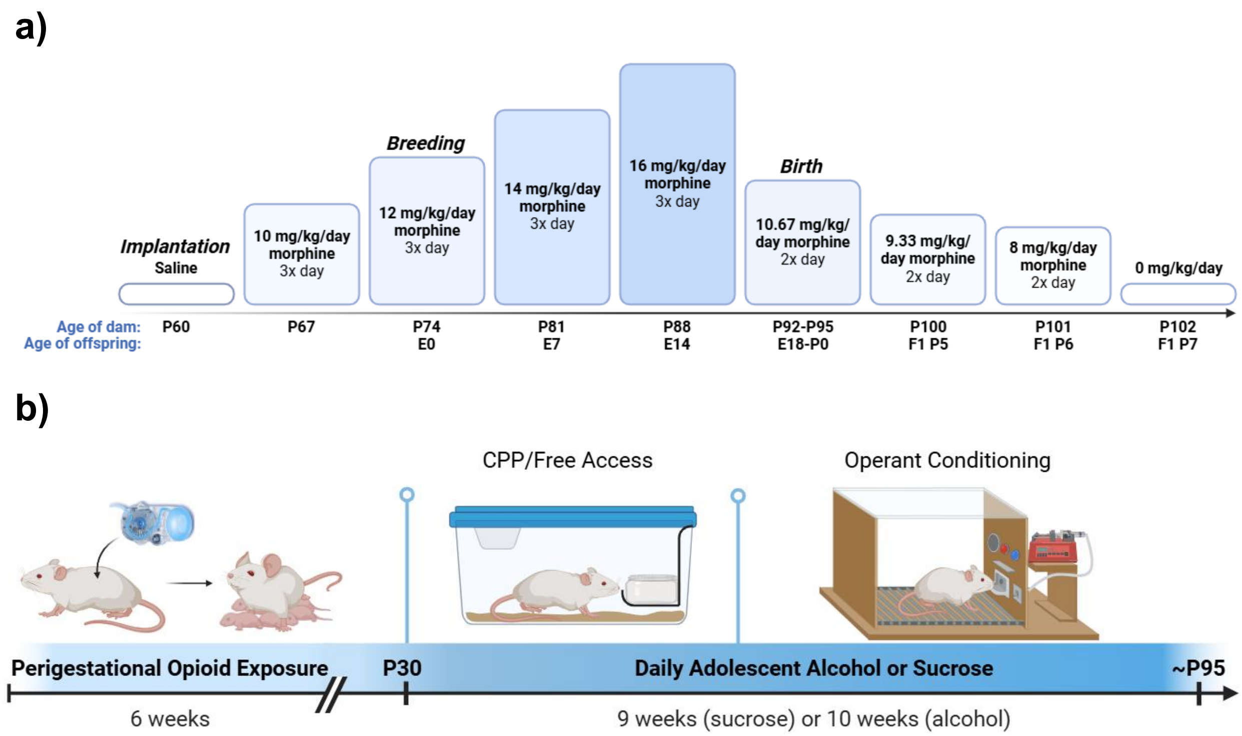

*Figure 2.1 Sprague Dawley rats are exposed to morphine or saline from conception to first postnatal week (**a**). Rats were tested for alcohol or sucrose preference using either CPP or free access followed by operant conditioning (**b**). Created with Biorender.*

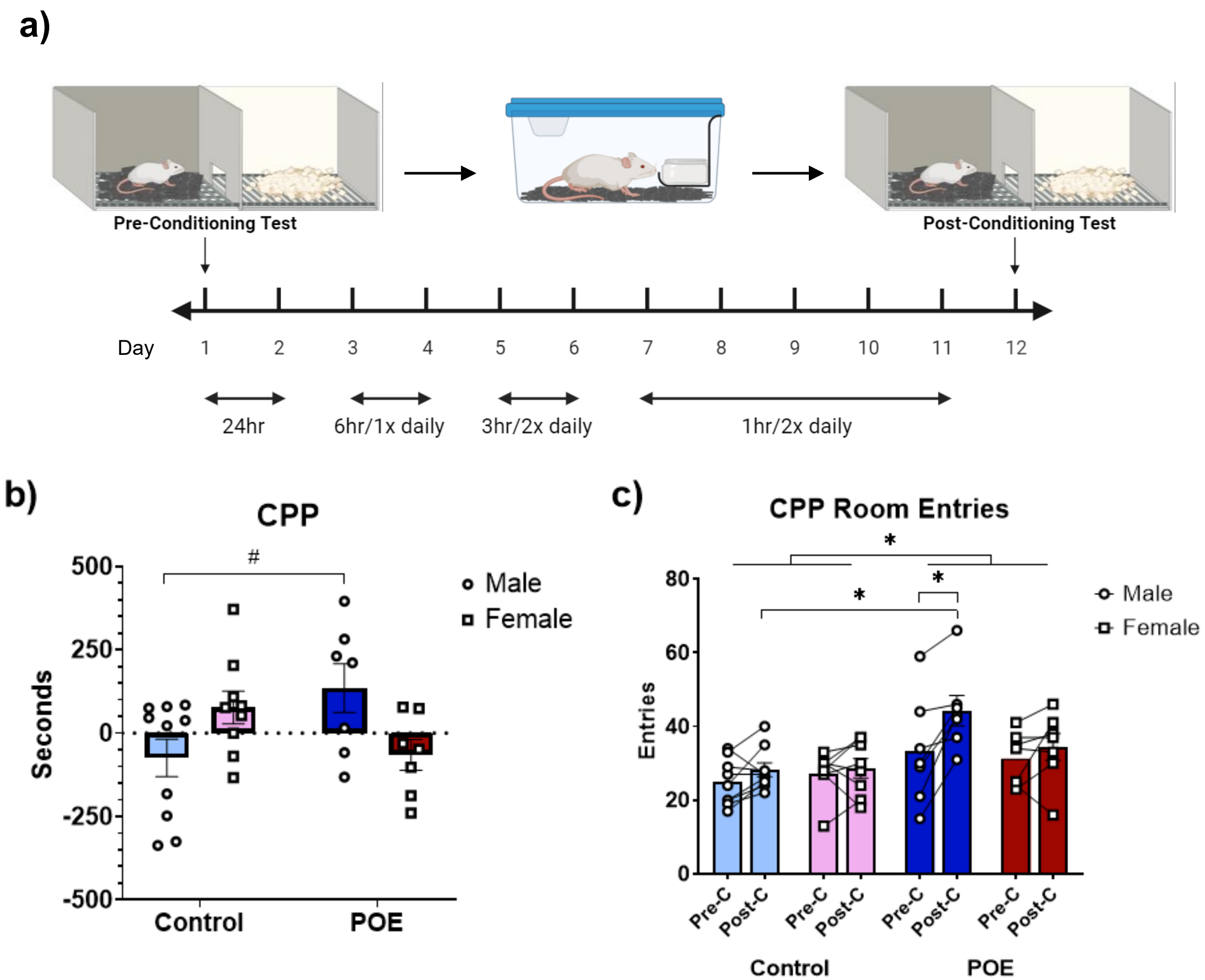

*Figure 2.2 Alcohol conditioned place preference was assessed in P30 rats across twelve days (**a**). POE male rats showed a trending increase in time spent on the alcohol-paired side of the arena compared to CON males, indicating an increased preference for alcohol (**b**). This effect was only evident in POE males. Overall POE rats showed increased exploratory behavior as demonstrated by an increase in room entries (**c**). #Trending difference between POE males vs CON males; p=0.06. *Significant difference between POE males vs CON males, POE male pre- vs post-conditioning, POE males vs CON males post-conditioning, or POE vs CON rats; p<0.05. Graphs indicate mean ± SEM. Created with Biorender.*

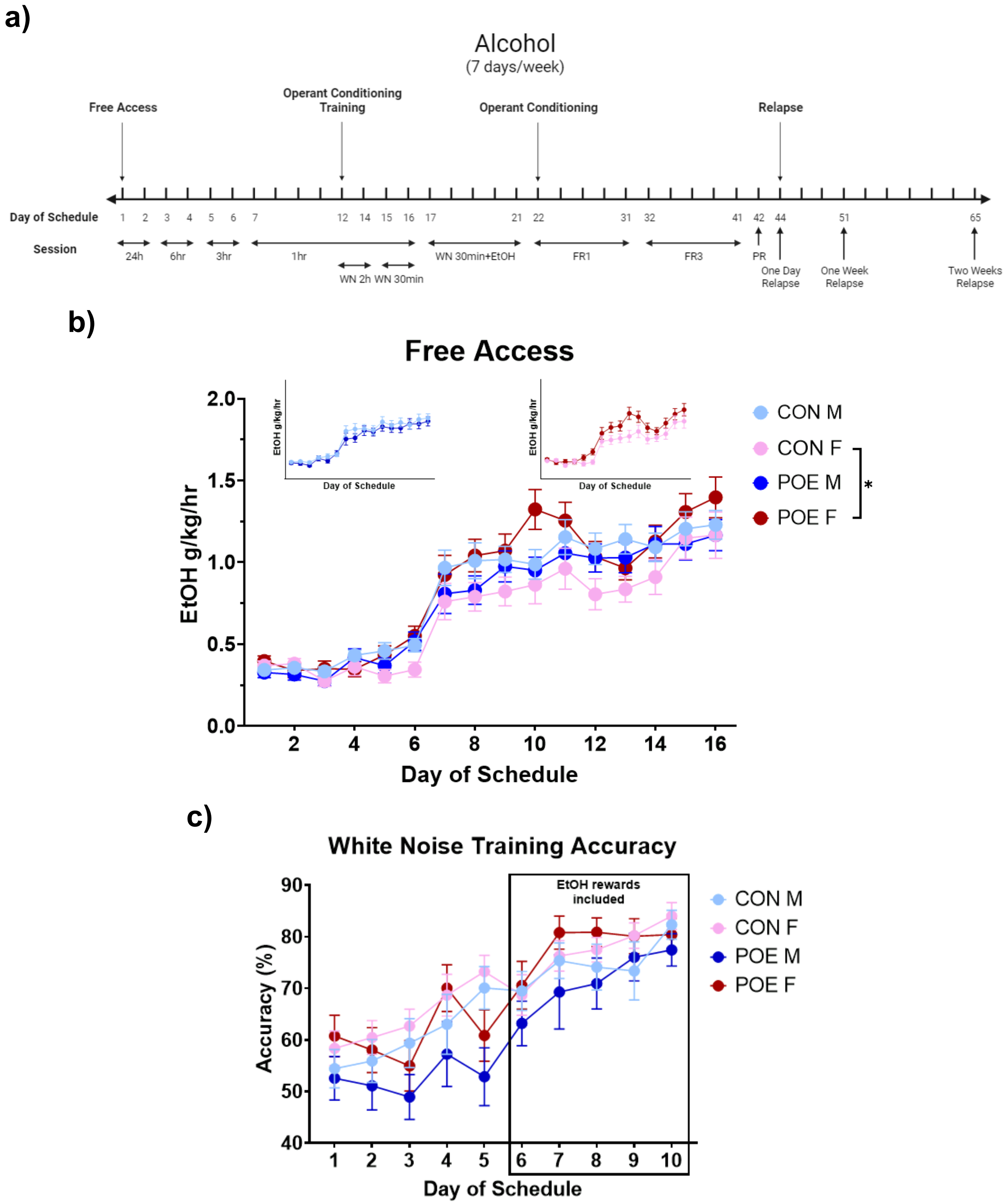

*Figure 2.3 POE & CON rats were tested for alcohol preference across 65 days (**a**). POE female rats consumed more g/kg/hr of alcohol than CON females during free access (**b**). POE did not alter press accuracy during training, but male rats were overall less accurate than females (**c**). *Significant difference between POE females vs CON females; p<0.05. Graphs indicate mean ± SEM.*

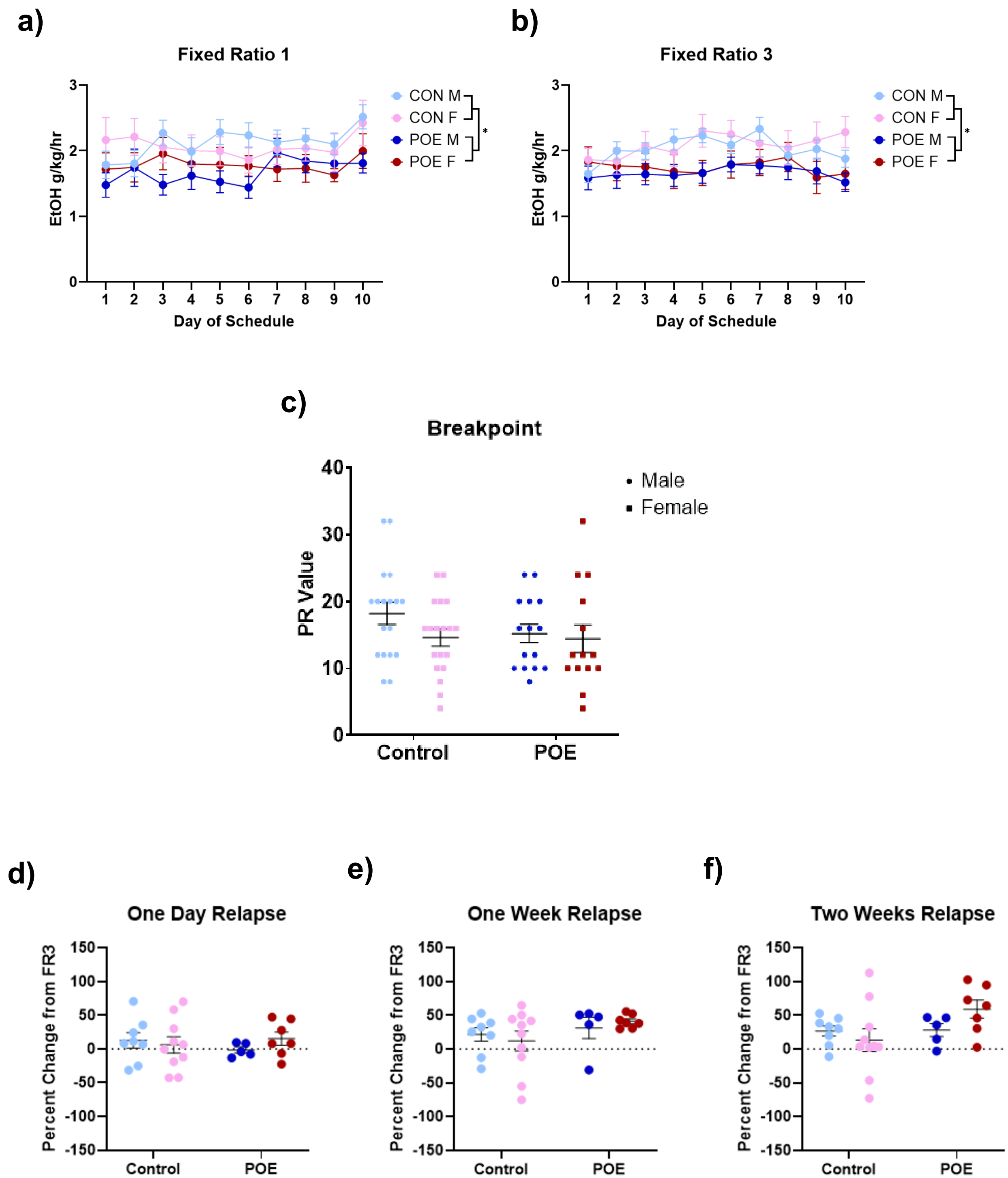

*Figure 2.4 POE rats consumed significantly less alcohol under both FR1 (**a**) and FR3 schedules (**b**). Groups did not differ in breakpoint for alcohol as determined by progressive ratio schedule (**c**). There was no significant effect of treatment for relapse potential (**d-f**). *Significant difference between POE vs CON rats; p<0.05. Graphs indicate mean ± SEM.*

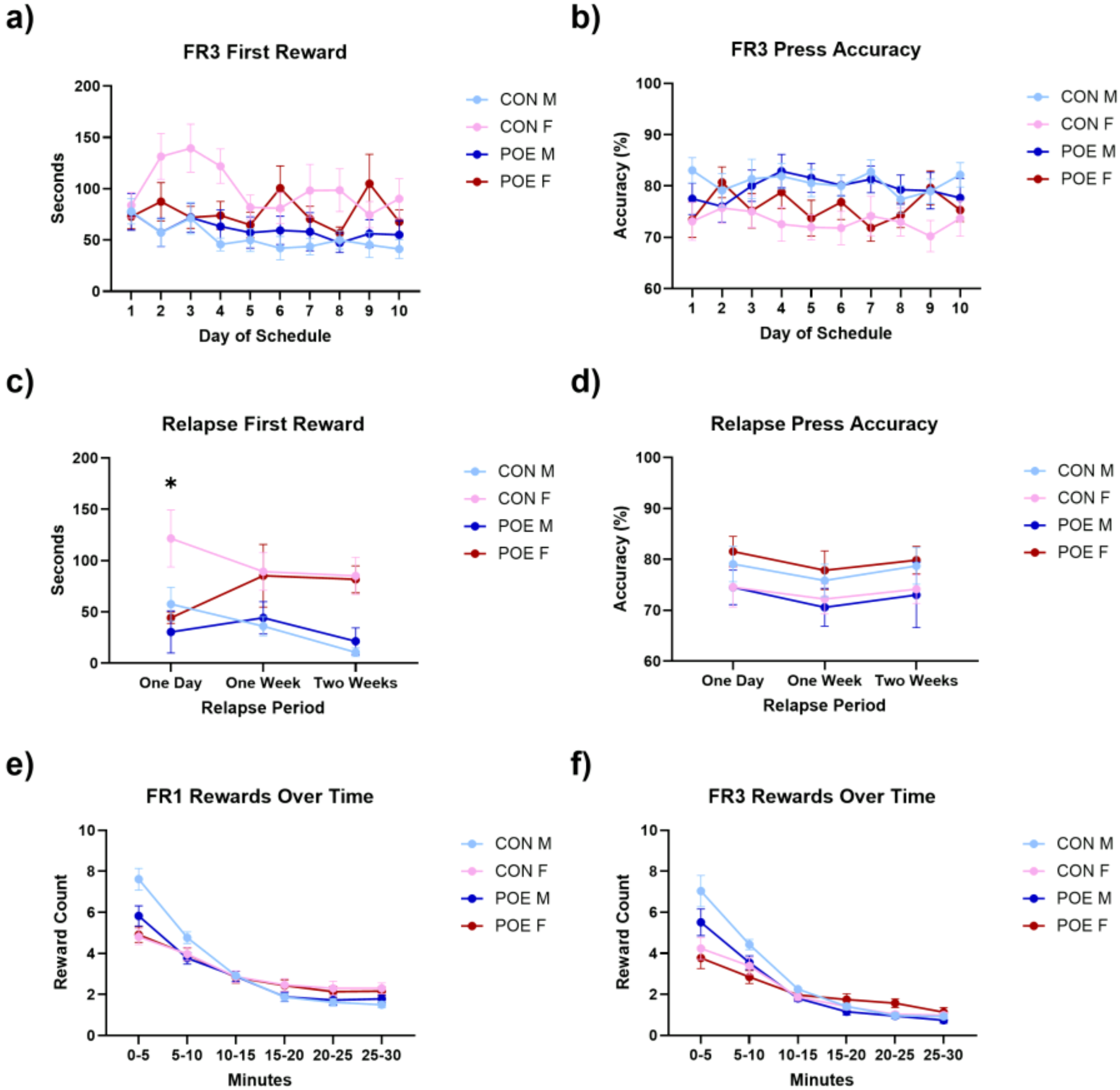

*Figure 2.5 During FR3, female rats took longer to earn their first reward than males (**a**) and were less accurate in their lever pressing (**b**). During relapse, POE rats were faster to earn their first reward than CON rats after one day forced abstinence, and males were faster than females across all three test days (**c**). There were no group differences in lever press accuracy during relapse testing (**d**). Average number of rewards earned over time, binned in 5min intervals, was generally similar between groups during FR1 (**g**) and FR3 (**h**). During FR3 sessions, males and females differed overall, but not within individual 5min periods. *Significant difference between POE vs CON rats. Graphs indicate mean ± SEM.*

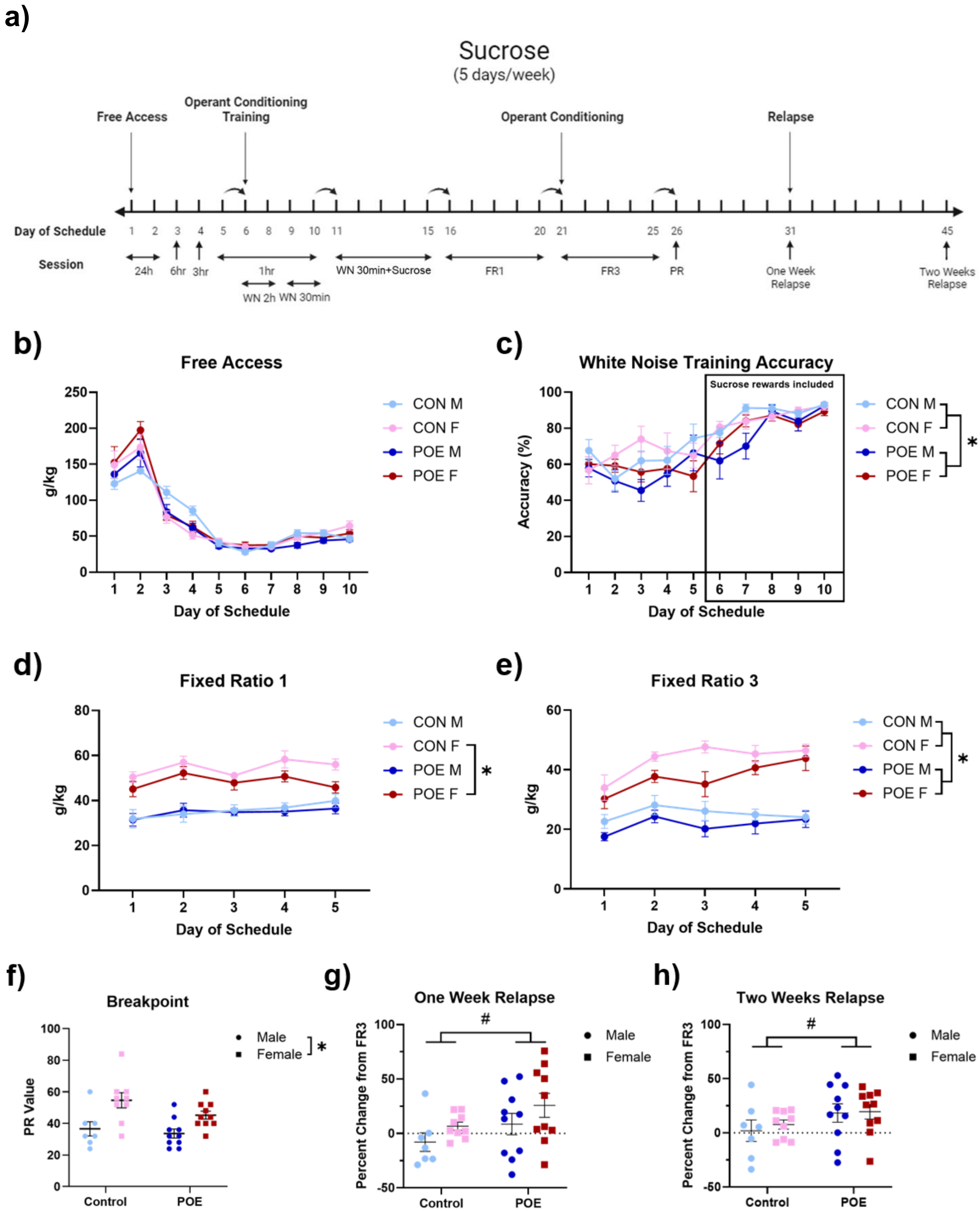

Figure 2.6 POE and CON rats were given daily intermittent access to sucrose and then transitioned to daily operant sessions to test consumption, breakpoint, and relapse. Our sucrose

*consumption schedule takes place across 45 days; arrows indicate 2-day breaks in behavioral testing (**a**). No treatment effects were observed for sucrose consumption under free access conditions (**b**). CON rats were more accurate than morphine rats in their active lever press (AP) accuracy during training (**c**). This is also true when considering only the last five days of training where sucrose rewards were included. POE female rats consumed significantly less sucrose than CON females during operant conditioning session at FR1 (**d**) and showed a trending decrease at FR3 (**e**). CON rats also consumed more sucrose than POE rats. Female rats exhibited higher breakpoints for sucrose than male rats (**f**). POE rats showed a trending increase in sucrose relapse compared to CON after one week and two weeks forced abstinence (**g-h**). *Significant difference between POE vs CON rats, males vs females, or POE females vs CON females; p<0.05. #Trending difference between POE vs CON rats; p<0.08. Graphs indicate mean ± SEM.*

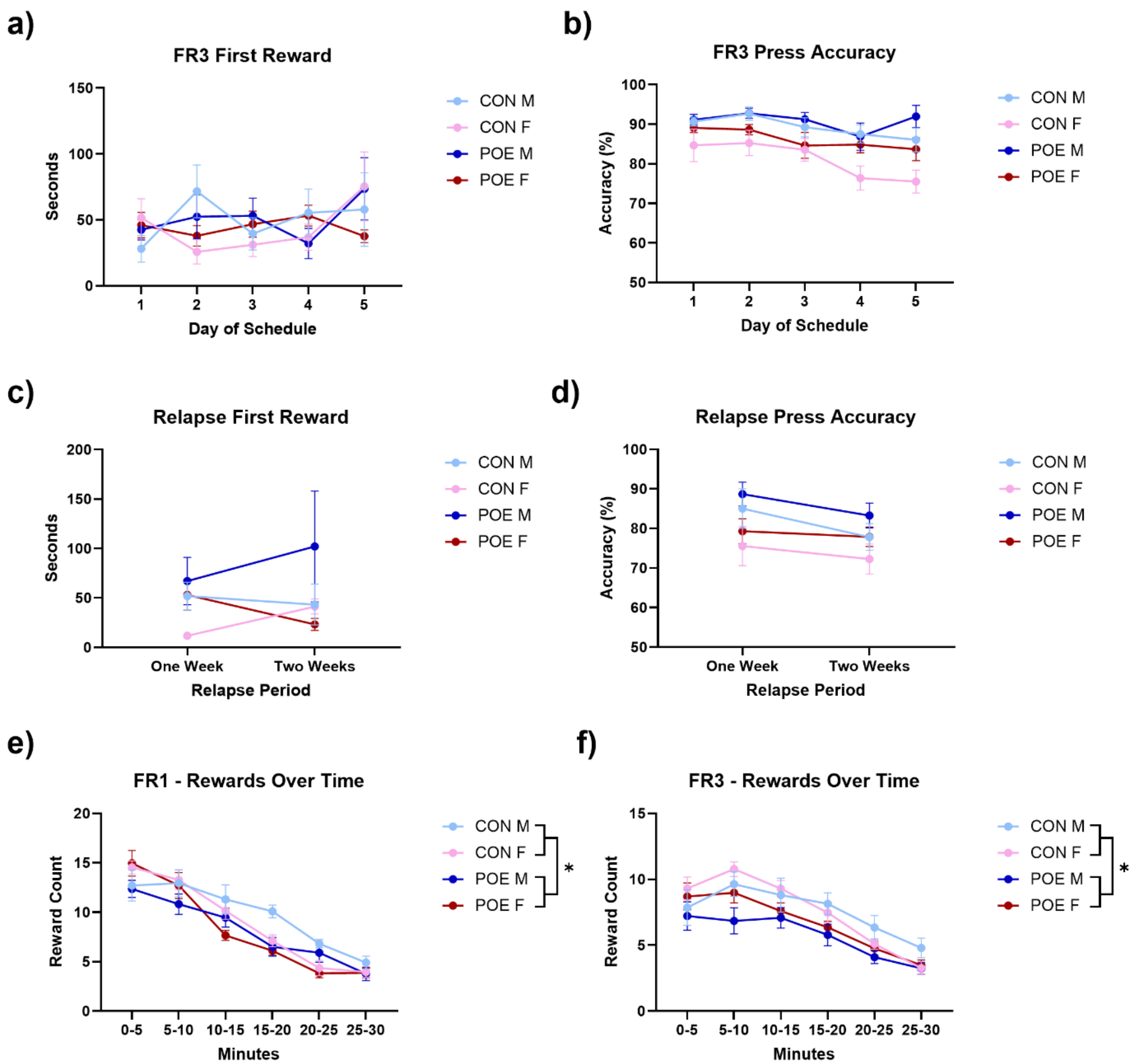

*Figure 2.7 During sucrose operant conditioning sessions, groups did not differ in their FR3 first reward latency (**a**). Male rats were more accurate in their lever pressing than females during FR3, but there were no significant differences between treatment groups (**b**). Latency to first reward after one and two weeks forced abstinence was not impacted by treatment or sex (**c**), but press accuracy was reduced in females compared to males (**d**). During FR1 and FR3, the average number of sucrose rewards earned, when binned into 5min intervals, was overall significantly different between POE and CON rats, but not within individual 5min periods (**e-f**). *Significant difference between POE vs CON rats, $p<0.05$. Graphs indicate mean ± SEM.*

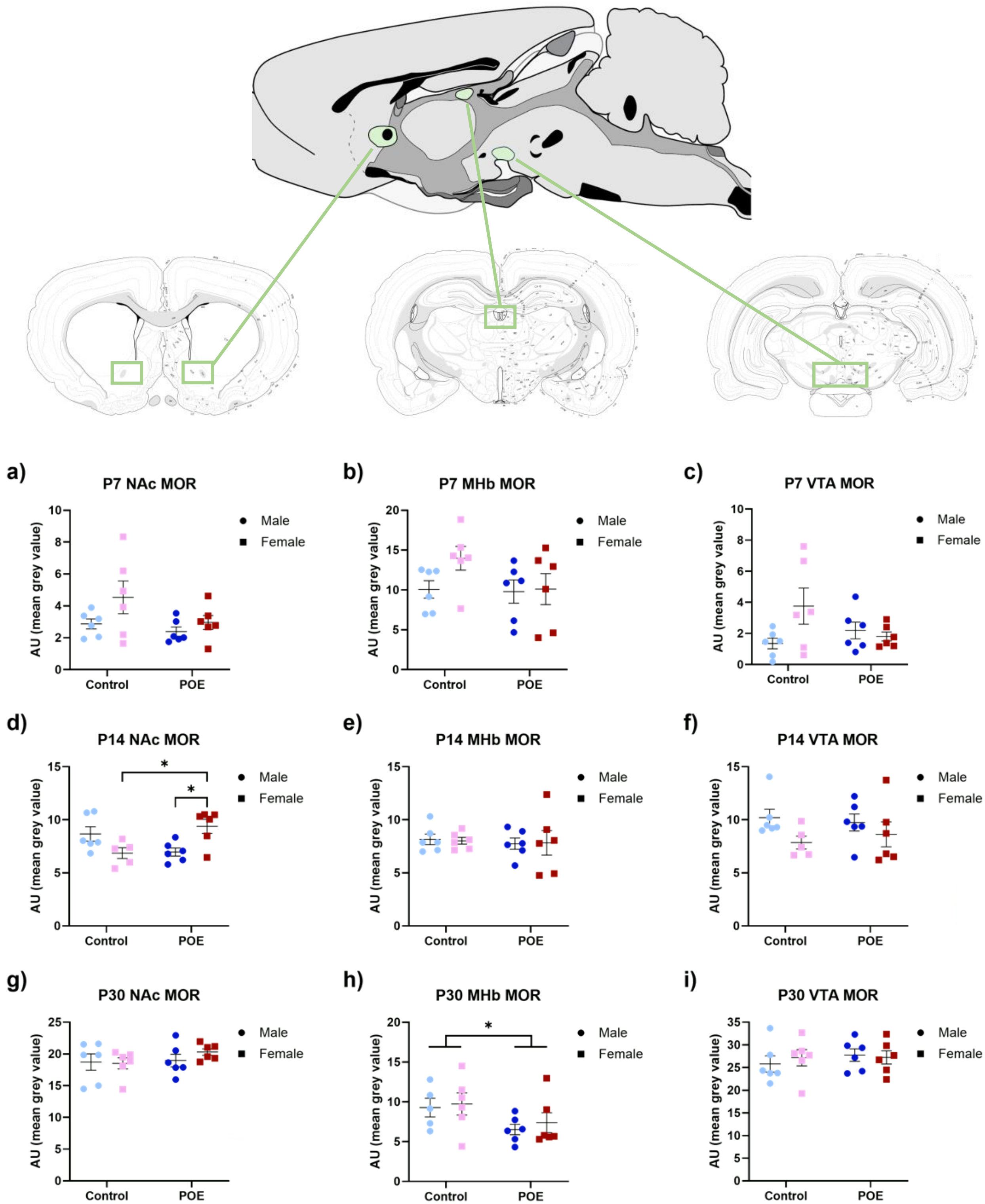

Figure 2.8 μ-Opioid receptor expression in nucleus accumbens (NAc; 1mm AP), medial habenula (MHb; -3mm AP), and ventral tegmental area (VTA; -5.2mm AP) at P7, P14, & P30. NAc MOR expression is elevated in POE female rats compared to CON female rats at P14, but no

*other groups differences were observed across P7, P14, or P30 (**a-c**). MHb MOR expression is significantly lower in POE rats compared to CON rats at P30, but no differences were noted at P7 or P14 (**d-f**). No group differences were found in VTA MOR across P7, P14, or P30 (**g-i**). *Significant difference between POE females vs CON females, or POE and CON rats; p<0.05. Graphs indicate mean± SEM.*

3 DISRUPTED MATERNAL BEHAVIOR IN MORPHINE-DEPENDENT PREGNANT RATS AND ANEDONIA IN THEIR OFFSPRING

3.1 Introduction

Across multiple decades, the United States has generally outpaced other nations in opioid use and related deaths, leading to a crisis that has only recently garnered the clinical attention it deserves (Pierce et al., 2021). One of the most significant at-risk groups for deleterious opioid consequences is pregnant women and their unborn children. Seven percent of women report using an opioid prescription during pregnancy, and of those women, one in five report misuse (Ko et al., 2020). As a consequence, between 2010 and 2017, the incidence rate of neonatal opioid withdrawal syndrome (NOWS) escalated across all races, income brackets, and insurance statuses from 4.0 to 7.3 in 1000 births (Hirai et al., 2021). While the immediate needs of these infants are well known and addressed in the hospital following delivery, the long-term behavioral and cognitive changes due to opioid exposure during pregnancy are understudied.

To address this gap in the literature, our lab has developed a novel perinatal opioid exposure (POE) model that administers daily pulsatile morphine to rat dams from before breeding to one week following parturition. Using this model, we have previously reported that gestational exposure to morphine reduces juvenile social play and alters both alcohol and sucrose intake during adolescence and early adulthood (Harder et al., 2023; Searles et al., 2023). Parallel changes in oxytocin and μ-opioid receptor expression in brain regions key for social behavior and reward signaling were also observed, suggesting an overall shift in reward processing. Similar results have been reported following early life manipulations in other models. For example, Molet et al. (2016) showed that housing rat dams and pups in impoverished environments for one week postnatally induced aberrant maternal behavior and anhedonia in male offspring. More specifically, male offspring that experienced early life stress had reduced sucrose preference and adolescent social play (Molet et al., 2016).

As opioid use during pregnancy is considered an "early life stressor" and our POE rats displayed aspects of anhedonia in their reduced social play and operant response for sucrose, we predicted our POE rats would show alterations in other hallmarks of anhedonia, namely, sucrose preference, adult social interaction, and forced swim. Additionally, due to the reported interaction between maternal care deficits in early life stress models and changes in reward-seeking behaviors and anhedonia in offspring, we also expanded upon our previously published analysis of maternal behavior to determine if POE induces suboptimal maternal care in more nuanced ways than simply gross time spent performing pup-directed actions (Bolton et al., 2018; Molet et al., 2016). Maternal care observations were used to calculate fragmentation, defined as a reduction in maternal care bout length, and entropy, or unpredictability in maternal care.

Based on the rationale above, the present study tested the hypothesis that perinatal morphine exposure leads to suboptimal maternal care, acting as an early life stressor and driving anhedonia in offspring.

3.2 Materials and Methods

3.2.1 Subjects

Female Sprague Dawley rats (age P60; Charles River Laboratories, Boston, MA) were used to generate male and female offspring. POE and control offspring were weaned at P21 into Optirat GenII individually ventilated cages (Animal Care Systems, Centennial, Colorado, USA) with corncob bedding in same-sex pairs or groups of three on a 12:12 hour light/dark cycle (lights on at 8:00 AM). Food (Lab Diet 5001 or Lab Diet 5015 for breeding pairs, St. Louis, MO, USA) and water were provided *ad libitum* throughout the experiment, except during maternal behavior recording and operant procedures. All studies were approved by the Institutional Animal Care and Use Committee at Georgia State University and performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to reduce the number of rats used in these studies and minimize pain and suffering.

3.2.2 Perinatal opioid exposure design

Female Sprague Dawley rats were implanted with iPrecio SMP-200 microinfusion minipumps at postnatal day 60 (P60). Pumps were programmed to begin morphine or saline administration following one week of recovery from surgery. One week after morphine initiation, females were paired with sexually-experienced males for two weeks to induce pregnancy. Morphine (or saline) exposure continued throughout gestation. Rats were initially administered 10 mg/kg across three doses per day, with doses increasing weekly by 2 mg/kg/day until 16 mg/kg/day was reached. At approximately E18, pumps switched to twice-a-day dosing, as initial pilots using three-times-a-day dosing increased pup mortality early in life, possibly due to respiratory depression. Dams continued to receive decreasing doses of morphine up to P7. This protocol closely mirrors the clinical profile of a late adolescent female who develops an opioid use disorder, becomes pregnant, and continues using opioids throughout pregnancy. An essential part of this model is the administration of morphine prior to E15, the approximate date of μ-opioid receptor development in the fetal brain (Coyle & Pert, 1976). Pumps filled with sterile saline were used to generate control (CON) rats to account for surgery and pump refilling stress. See Figure 1 for the overall dosing and testing schedule.

3.2.3 Maternal behavior analysis

Rat maternal behaviors were observed on P2, P4, and P6 twice daily for 1hr: 2hr after lights on (prior to morphine infusion) and 6hr after lights on (after morphine infusion). Following morphine cessation at P7, maternal behavior was observed once daily on P8, P10, P12, and P14, 4hr after lights on. All sessions were recorded for offline analysis. The following measures were scored: nursing, licking/grooming pups, nest building, self-grooming, returning pups to the nest, and time spent off nest. Time spent on individual behaviors was recorded and used to calculate behavior bout length to determine levels of fragmentation. A shorter average bout length for a type of behavior indicates higher fragmentation. Pattern analysis of behaviors was performed to calculate an entropy value, representing the predictability of behaviors. High predictability, where one behavior expectedly follows another, would be represented by a low entropy value. Calculation of entropy is described in depth by Molet et al. (2016).

3.2.4 Sucrose preference test

At P40, POE and control rats were tested for sucrose preference using a 1% sucrose solution during their dark phase. On day 1, rats were individually housed, and their water bottles were replaced with two 200mL bottles of 1% sucrose for 24hr. On day 2, their water was returned for 6hr and then replaced with one water bottle and one sucrose bottle for 18hr. On day 3, rats were water-deprived for 12hr and then provided one water bottle and one sucrose bottle for 12hr. The positions of the bottles were counterbalanced. After 12hr of two-bottle-choice, the experimental water/sucrose bottles were removed, and rats were returned to their original home cages. Each day, all bottles were weighed pre- and post-ingestion to determine volume (mL) consumed.

3.2.5 Forced swim test

Adult (P60) POE and control rats were tested for stress reactivity using the forced swim test (FST) (Victoria et al., 2014). An 80x200mm cylinder was filled with 25°C water deep enough that rats could touch the bottom while at the surface. All rats were given a 5min pre-swim one day prior to FST. On testing day, rats were placed in the water for a 5min FST; time immobile and latency to immobility were determined using ANY-Maze™ behavioral tracking software (Stoelting Co., Wood Dale, IL). Blood samples were collected from the saphenous vein into EDTA-hematology tubes (BD from Fisher Scientific) at four intervals relative to the FST to measure corticosterone levels: baseline (-60min), stress (5min), peak (30min), and recovery (75min). Blood samples were centrifuged at 3000 rcf at 4°C for 15 min, and plasma corticosterone was measured with ELISA (DetectX Corticosterone Multi-Format Kit, K014-H; Arbor Assays, Ann Arbor, MI). Correlation coefficients were measured against known standards ($r^2 > 0.98$). Inter- and intra-assay CVs were below 12%.

3.2.6 Social interaction test

At P73, POE and control rats were isolated for 24hr, then reunited with their cage mates in their original home cage. Behavior was recorded for 10min for offline analysis; time spent interacting, defined as sniffing, grooming, chasing, or playing, was measured.

3.2.7 Immunohistochemistry

Ninety minutes following the social interaction test, one cohort of rats was sacrificed to assess forebrain and midbrain Fos and tyrosine hydroxylase (TH) expression. Rats were transcardially perfused with 0.9% sodium chloride saline containing 2% sodium nitrate followed by 4% paraformaldehyde fixative. Brains were extracted and stored in 4% paraformaldehyde at 4°C overnight, then transferred into 30% sucrose until sectioning. Fixed tissue was sectioned coronally at 25-μm with a Leica SM2010R microtome and stored at -20°C in cryoprotectant-antifreeze solution (Watson et al., 1986). Free-floating sections were rinsed in potassium phosphate-buffered saline (KPBS), incubated in 3% hydrogen peroxide for 30 minutes, and rinsed again in KPBS at room temperature. Tissue was then incubated in mouse anti-c-Fos primary antibody (Abcam Inc., Cambridge, MA, USA.; 1:10,000) overnight at room temperature. Tissue was then rinsed and incubated in biotinylated donkey anti-mouse IgG (Jackson Immunoresearch, West Grove, PA, USA; 1:600) for 1 hour. Secondary antibody was amplified with avidin-biotin solution for 1 hour. After tissue was rinsed with KPBS and sodium acetate (0.175M; pH 6.5), immunoreactivity was visualized using nickel sulfate 3,3′-diaminobenzidine solution (2 mg/10 ml) and 0.08% hydrogen peroxide in sodium acetate buffer. After 20 minutes, tissue was rinsed in sodium acetate and KPBS. Tissue was then blocked with 5% normal donkey serum (VWR, Inc., Pittsburgh, Pennsylvania, USA) for 1 hour at room temperature and incubated in sheep anti-TH primary antibody (Fisher Scientific, Inc., Palatine, IL, USA; 1:1000) for 1 hour at room temperature followed by 24 hours at 4°C. Primary antibody was rinsed using KPBS and incubated in donkey anti-sheep AlexaFluor 555 secondary antibody (Fisher Scientific, Palatine, IL, USA; 1:200) for 1.5 hours at room temperature. Tissue was rinsed and mounted on gelatin-subbed slides, air-dried, and cover-slipped with ProLong Diamond Antifade Mountant (Fisher Scientific, Inc., Palatine, IL, USA).

16-bit images were captured using a Keyence BZ-X700 microscope and software for immunohistochemistry quantification. Subregions of the medial prefrontal cortex (mPFC), nucleus accumbens (NAc), habenula, amygdala, and ventral tegmental area (VTA) were bilaterally imaged at 10x magnification (2-3 sections per rat, average of 4-6 values). Fos+ cells were detected automatically using ImageJ software and then manually validated. Total number of Fos+ cells was averaged to produce a mean number per rat per region. In VTA sections, TH immunoreactivity was used to determine if Fos+ cells were dopaminergic.

3.2.8 Statistical analysis

All data met the requirements for parametric analyses (normality, homogeneity of variance). The effects of sex, treatment (morphine/saline), and session (where applicable) were assessed using two- or three-way mixed model ANOVA with an alpha level of 0.05. Given that repeated measures ANOVA cannot handle missing values, mixed models using Greenhouse-Geisser were implemented where outlier correction was necessary. Outliers were identified using GraphPad Prism's ROUTS method. Post hoc testing was performed using Tukey's or Sidak's. Experiments involving repeated measures were not tested for daily individual group differences. No significant litter effects were observed; therefore, group data comprised multiple litters (6 control/7 POE for maternal behavior; 2 control/2 POE for offspring behaviors). GraphPad Prism 9.5.1 was used for all statistical analyses.

3.3 Results

3.3.1 Maternal Care

To determine if opioid use during pregnancy produced suboptimal maternal care, we first determined whether maternal care fragmentation differed between POE and CON dams as reflected by mean bout length of each behavior. Our analysis identified significant interactions of treatment and session for nursing bout length ($F_{treatment*session}(9,83)=2.53$, $p=0.01$; Figure 2a). Post-hoc analysis showed that the mean bout length for nursing was significantly shorter in POE dams compared to CON at P2 PM and P4 PM assessments when circulating levels of morphine were high. To assess for patterns when morphine was present or absent, we also grouped data by category: low levels of morphine (P2-P6 AM), high levels of morphine (P2-P6 PM), and no morphine (P8-P14). Overall, the average nursing bout length was shorter for POE than CON during morphine highs (PM) ($F_{treatment*session}(2,21)=5.57$, $p=0.01$; Figure 2b). No differences were noted during low morphine (AM) or no morphine (P8-P14). Interestingly, CON rat mothers displayed significantly longer nursing bouts in the afternoon (when POE morphine levels were high) compared to P8-P14 (no morphine). Although mean licking/grooming bout length was longer in POE dams across the majority of timepoints examined, these differences were not significant (Figure 2c). Similarly, no significant differences were noted in bout length when data were grouped by morphine level (Figure 2d). Bouts of self-grooming behavior were significantly different in POE rats compared to CON ($F_{treatment*session}(9,88)=2.03$, $p=0.04$; Figure 2e). No significant differences in self-grooming behavior were noted when data were grouped by morphine level (Figure 2f), although bout length for POE dams was consistently longer than CON.

There was no significant treatment effect for off-nest mean bout length (Figure 2g-h). Following morphine cessation at P8, POE dams displayed a trending increase ($p=.07$) in their off-nest bout length compared to CON. However, no significant interaction of treatment and session was observed (Figure 2g). No treatment effects were observed in nest building mean bout length (Figure 2i-j).

We also assessed total time spent performing each behavior across the three morphine levels. There were significant treatment and session effects on total time spent nursing as well as a significant session x treatment interaction, with all dams nursing the most during morning sessions and POE dams nursing less than CON dams, especially in the morning when morphine levels were low ($F_{session\ x\ treatment}(2,22)=6.919$, $p=0.0047$; $F_{treatment}(1,11)=5.388$, $p=0.0405$; $F_{session}(2,22)=159.4$, $p<0.0001$; Figure 3a). There was a significant effect of session but no treatment effect on licking/grooming pups ($F_{session}(1.160,12.76)=36.62$, $p<0.0001$; Figure 3b). For self-grooming, there were significant main effects of session and treatment, with POE dams spending more time self-grooming than controls and both groups spending less time self-grooming during afternoon sessions (when morphine levels were high for the POE dams) ($F_{session}(1.511,16.62)=13.48$, $p=0.0007$; $F_{treatment}(1,11)=9.759$, $p=0.0097$; Figure 3c). For time off-nest and nest building, there were significant effects of session and no effect of treatment (Off Nest: $F_{session}(2,22)=19.57$, $p<0.0001$; $F_{treatment}(1,11)=1.355$, $p=0.2690$; Figure 3d; Nest Building: $F_{session}(1.038,11.42)=10.56$, $p=0.0070$; $F_{treatment}(1,11)=1.504$, $p=0.2456$; Figure 3e).

To determine if overall attention to pups is altered by chronic morphine, we categorized measures of total time spent nursing, licking/grooming, and carrying of pups as “pup-directed behavior”. We observed that POE moms spent significantly less time focused on their pups compared to CON ($F_{treatment}$ (1,11)=2.37, p<0.01; Figure 4a). This was particularly evident when morphine levels were high. Overall, across all sessions, the mean total percentage of time directed towards pups was 86.6% for CON versus 65.4% for POE, a 24.5% decrease.

We next used paired behavioral pattern analysis to calculate an entropy, or unpredictability, score; this score reflects the likelihood of one behavior mode following another. POE dams were less predictable (higher entropy) when morphine was low and more predictable when morphine was high ($F_{treatment*session}$(2,32)=3.96, p=0.03; Figure 4b-c). No significant differences were noted as a function of time for CON dams. Interestingly, POE dams were more unpredictable during morphine withdrawal (Low; AM) than they were following a morphine infusion (High; PM) or after morphine termination (None; P8-P14). This suggests that morphine abstinence/withdrawal in the morning leads to unpredictable behavior, while morphine “intoxication” in the afternoon mutes behavioral variability and increases predictability.

3.3.2 Sucrose Preference

Our maternal behavior analysis results suggest that POE dams display disrupted maternal care that varies in fragmentation and predictability. Disrupted maternal care has been previously associated with anhedonia or depressive-like behavior in the offspring. Thus, to assess anhedonia in our POE offspring, rats born to either CON or POE dams were tested for their preference for sucrose using two-bottle choice and stress reactivity using the forced swim test.

No effect of sex was observed in sucrose preference, so data were combined. Compared to CON, POE rats showed a decreased preference for sucrose ($F_{treatment}$(1,19)=6.24, p=0.02;

Figure 5a; 78.6% CON versus 71.4% POE, 9.2% decrease). POE and CON rats consumed similar amounts of sucrose during habituation, suggesting that preference was unrelated to caloric intake (Figure 5b).

3.3.3 Acute Stress Reactivity

Approximately two weeks following the sucrose preference test, adult POE and CON rats underwent the forced swim test (FST). We observed no significant effect of treatment on either time spent immobile or latency to immobility (Figure 6a-b). Blood corticosterone levels were determined at baseline, immediately after FST, and 30- and 75-min post-test. There was a significant interaction between treatment and sex ($F_{treatment*sex}(1,21)=4.73$, $p=0.04$; Figure 6c). POE males exhibited higher plasma corticosterone levels immediately after and 30-min post-FST compared to CON males ($F_{treatment}(1,28)=10.29$, $p<0.01$). No effect of treatment was observed in females. Overall, FST-induced corticosterone release was higher in females than in males ($F_{sex}(1,21)=65.44$, $p<0.01$).

3.3.4 Adult Social Interaction

Our lab has previously reported reduced juvenile play in POE female rats (Harder et al., 2023). Adult POE and CON rats were assessed for time spent socially interacting to determine if social aversion extends later into life. POE rats spent significantly less time interacting with cage mates compared to CON ($F_{treatment}(1,18)=9.27$, $p<0.01$; 382s CON versus 341s POE, 10.7% decrease; Figure 7a). Females overall spent less time interacting with their cage mates than males ($F_{sex}(1,18)=11.10$, $p<0.01$; 380s Male versus 335s Female, 11.8% decrease).

Next, we examined Fos expression in a cohort of rats 90 min after the social interaction test. Positive cell counts were conducted in seven brain regions associated with social reward and aversion (Figure 7b). The number of Fos^+ cells in an additional four regions averaged less than 10 and were excluded from our analysis: basolateral amygdala, central amygdala, lateral habenula, and medial habenula. Expression of Fos was significantly higher (86% increase) in the prelimbic cortex (PL) of POE rats compared to CON, but not in anterior cingulate (ACC) or infralimbic (IL) cortices ($F_{treatment}(1,11)=5.164$, $p=0.04$; Figure 7c-e). The number of Fos^+ cells was also 93% higher in the medial amygdala (MeA; $p=0.04$; Figure 7f).

Fos expression within the mesolimbic circuit was greater in POE rats than CON rats. Specifically, Fos expression was significantly higher in the NAc core (NAcC), NAc shell (NAcS), and VTA of POE rats compared to CON (NAcC: $F_{treatment}(1,12)=19.72$, $p<0.01$; NAcS: $F_{treatment}(1,12)=30.18$, $p<0.01$; VTA: $F_{treatment}(1,12)=13.57$, $p<0.01$; Figure 7g-i).

Dopaminergic signaling within the VTA is critical for encoding reward, while nondopaminergic signaling, from either GABAergic or glutamatergic interneurons, modulates dopamine activity to dampen reward encoding. Thus, we examined whether the observed Fos^+ cells in the VTA were co-localized with the dopaminergic marker tyrosine hydroxylase (TH). Treatment has no significant effect on dopaminergic Fos^+ cell counts in both POE and CON groups, with the majority of Fos^+ cells negative for TH (Figure 7j).

3.4 Discussion

The present study aimed to determine the effects of perinatal morphine on maternal care and anhedonia in the offspring. POE dams displayed longer bouts of pup- and self-grooming prior to morphine infusions, which may indicate morphine withdrawal. In the present study, the dosage of morphine was gradually decreased over roughly 10 days surrounding birth to minimize severe spontaneous withdrawal in both dams and pups. Consistent with this, POE dams did not display overt signs of withdrawal, such as wet dog shakes or teeth chattering. At P8, the first full day without morphine, longer bouts of self- and pup-grooming were observed in POE dams. Increased grooming is indicative of withdrawal and is suspected to be a compulsive coping mechanism for abstinence-induced stress or anxiety (Goeldner et al., 2011; Kalueff & Tuohimaa, 2005; Lalanne et al., 2017; Lutz & Kieffer, 2013; Schulteis et al., 1994). POE dams also had longer off-nest bouts at P8. As with increases in grooming at this timepoint, this measure could reflect social avoidance previously reported to accompany rodent opioid withdrawal (Ozdemir et al., 2024). Together, these data suggest that our POE dams did experience withdrawal, which would induce additional stress for both the dam and her offspring.

Overall, our results indicate that gestational opioid exposure induces suboptimal maternal care as POE dams exhibited shorter average bouts of nursing, particularly following morphine administration. These results are consistent with our previous study reporting decreased time spent nursing and increased time licking/grooming pups in POE litters, particularly after the dam receives morphine (Harder et al., 2023). Interestingly, control dams exhibited longer bouts of nursing during P2-P6 afternoon “high morphine” observations than P8-P14 midday observations. This may be explained by decreases in nursing bout length that occurs in the five days after birth; however, hour-to-hour circadian variation in nursing bout length has not been studied (Lapp et al., 2023). Nursing behavior is necessary for offspring, providing both nutritive value and maintenance of body temperature, thus avoiding hypothermia associated with morphine withdrawal (Belknap, 1989; Eriksson & Rönnbäck, 1989). Clinically, both breastfeeding and skin-to-skin contact, even during ongoing opioid maintenance therapy, improves infant health and reduces the need for direct pharmacological treatment in newborns (Abdel-Latif et al., 2006; Atwood et al., 2016; Demirci et al., 2015; Holmes et al., 2016; Kocherlakota, 2014; MacMillan et al., 2018; Patrick et al., 2016; Pritham, 2013; Short et al., 2016; Welle-Strand et al., 2013). Thus, our observation of fragmented maternal care by POE dams, vital for pup survival, is of particular concern for the long-term outcomes of POE offspring.

Compared to CON dams, POE dams varied in the predictability of their maternal behavior, such that morning care (prior to morphine) was less predictable than afternoon care (following morphine). The unpredictability leading up to the morphine infusion may signal reward anticipation or withdrawal; in contrast, the increased predictability of maternal care can be explained by a significant reduction in the different types of behaviors displayed during high levels of morphine. Indeed, during sessions of high morphine levels, POE dams spent significantly less time performing actions that were directed towards pups and more time self-grooming or off-nest. Additionally, the notably broader range in unpredictability scores of the POE dams across all morphine levels (ranging from 0.2 to 1.6 compared to 0.9 to 1.3 for CON) highlights the variability of morphine-exposed dams. This high level of variability would disrupt the pups' ability to predict maternal care across the first postnatal week and likely hinder reward processing later in life (Novick et al., 2018).

Unpredictable and fragmented maternal care produces postpartum stress that is associated with increased depression, anxiety, and anhedonia in the offspring at both clinical and preclinical levels (Bolton et al., 2018, 2018; Coplan et al., 1996; Levis et al., 2022; Spadoni et al., 2022). In rodents, this is characterized by reduced engagement with rewarding stimuli. For example, suboptimal care induced by resource scarcity reduces socialization and sucrose preference in Sprague Dawley rats (Bolton et al., 2018; Molet et al., 2016). Here, we report adolescent POE rats have reduced preference for sucrose and spend less time socially interacting with cage mates in adulthood. These results and our previous findings of reduced juvenile play suggest POE may promote anhedonia in the offspring (Harder et al., 2023). These behavioral changes likely result from a combination of indirect drug exposure (through the placenta and postnatal milk) and receipt of suboptimal maternal care.

In the present study, POE males had higher levels of CORT following the FST, consistent with previous data reporting increased corticosterone release during stress response to lipopolysaccharide in males but not females following prenatal opiate exposure (K. L. Hamilton et al., 2005). Interestingly, despite this sex-specific physiological response to the acute stressor, we found no treatment differences in behavioral response to the FST. Previously in our lab, we have reported a male-specific reduction of corticosterone release following adult FST when a single dose of morphine was given on the day of birth, indicating that chronic versus acute morphine exposure in early life may have opposing effects on stress reactivity (Victoria et al., 2014). Furthermore, the literature regarding the impact of gestational opioid exposure on FST performance seems to highlight the importance of testing age in the consistency of results. For example, Ahmadalipour & Rashidy-Pour (2015) reported prenatal morphine exposure did not impact FST performance at P46, while Chen et al. (2022) reported prenatal morphine exposure in females decreased immobility in the FST at P32 yet increased immobility at P54. Thus, additional FST at multiple timepoints may better elucidate the impact of our POE model on acute stress reactivity across the lifespan.

Gestational morphine resulted in reduced social interaction in adults in the current study, consistent with our previous report of reduced social play behavior in adolescent POE females and reduced time spent together in POE males and females (Harder et al., 2023). In line with our findings, low sociability and social avoidance have been reported in rodents following chronic opioid use in adulthood (Goeldner et al., 2011; Lalanne et al., 2017; Lutz & Kieffer, 2013). However, contrary to our findings, Hol et al. (1996) showed that social play, grooming, and approach behaviors were increased in adolescent and adult rats with prenatal morphine exposure. Notably, the opposing directionality in sociability following early life morphine exposure is likely due to inconsistent dosing timelines, which has contributed to many other experimental outcome discrepancies in the field, particularly regarding reward behavior (Grecco & Atwood, 2020). More specifically, previous studies examining the long-term impact of gestational morphine exposure on social play typically administered daily injections of morphine within a truncated period, typically E8-21, or utilized extended dosing regimens that extended until weaning (Buisman-Pijlman et al., 2009; Hol et al., 1996).

In humans, exposure to opioids *in utero* reduces socialization, coinciding with cognitive deficits and increased anxiety that augment social disorders (Azuine et al., 2019; Larson et al., 2019). Gestational exposure to morphine also reduces social competency scores in infancy and early childhood, increasing the risk of developing ADHD and autism spectrum disorder (Hunt et al., 2008; Sandtorv et al., 2018). Social response in rodents has been altered via pharmacological manipulation of MOR or dopaminergic neurotransmission, highlighting the roles of these systems in social behavior (Beatty & Costello, 1982; Niesink & Van Ree, 1989; Trezza & Vanderschuren, 2008, 2009). Opioidergic and dopaminergic brain regions implicated in social reward show elevated Fos expression following social testing; aberrant functional connectivity in rats that received poor maternal care and early life stress has also been reported (Bolton et al., 2018; Gómez-Gómez et al., 2019; Mejía-Chávez et al., 2021). We found significantly elevated Fos expression in the VTA and NAc in POE rats compared to CON, especially females, the majority of which was nondopaminergic. While aversive stimuli can trigger dopaminergic signaling as an associative learning response, nondopaminergic VTA signaling promotes feelings of anxiety and underlies defensive behavior (Barbano et al., 2020; Cohen et al., 2012; Tan et al., 2012; Zhou et al., 2019). Together with our data, this suggests that POE decreases sociability in a familiar setting during adulthood and increases nondopaminergic activity in the mesolimbic pathway that typically contributes to aversion.

Reduced responses to natural rewards, such as social interaction and sucrose, are a hallmark of anhedonic behavior and are often accompanied by changes in opioidergic or dopaminergic signaling (Der-Avakian & Markou, 2012; Kringelbach & Berridge, 2016; Nestler & Carlezon, 2006). Previous studies have reported activation of the μ-opioid receptor (MOR) in the habenula suppresses depression-like behavior. Specifically, Park et al. (2024) found that DAMGO activation of MOR attenuated aversion and decreased immobility in the forced swim test following acute traumatic stress in rats. Similarly, Bailly et al. (2023) reported that optical stimulation of MOR+ habenular neurons increased both avoidance behavior and immobility in the tail suspension task. These findings, along with our previous report of reduced MOR in the MHb of POE rats, suggest that perinatal morphine reduces MOR-mediated inhibition of habenula neurons, thus facilitating depressive-like behavior (Searles et al., 2023). This lack of opioid inhibition and currently reported nondopaminergic VTA activation following social interaction identify two independent cellular pathways through which POE-induced alterations in neural signaling promote an anhedonic phenotype.

In summary, we demonstrate that perinatal morphine, which is known to alter preference and motivation for drugs of reward, produces a decreased preference for both sucrose solution and social stimulation. We also show that, in males, POE generates hypothalamic-pituitary-axis hyperactivity during acute stress. These results highlight underlying depression-like symptoms that are produced by the physical and psychological stress of drug exposure in both rodent models and clinical populations. Further, we show that morphine exposure across pregnancy generates fragmentation and inconsistency in maternal care that has been demonstrated in other models of early life adversity to contribute to anhedonia. While not directly measured, we believe the changes in maternal care induced by morphine also contribute to our previously reported changes in alcohol consumption. Future studies will aim to directly manipulate maternal care through cross-fostering and elucidate the extent to which the observed anhedonic profile of POE offspring is driven by suboptimal maternal care. Additionally, future analysis correlating offspring measures with the severity of maternal care deficits can further delineate the influence of drug use and rearing conditions on offspring outcomes.

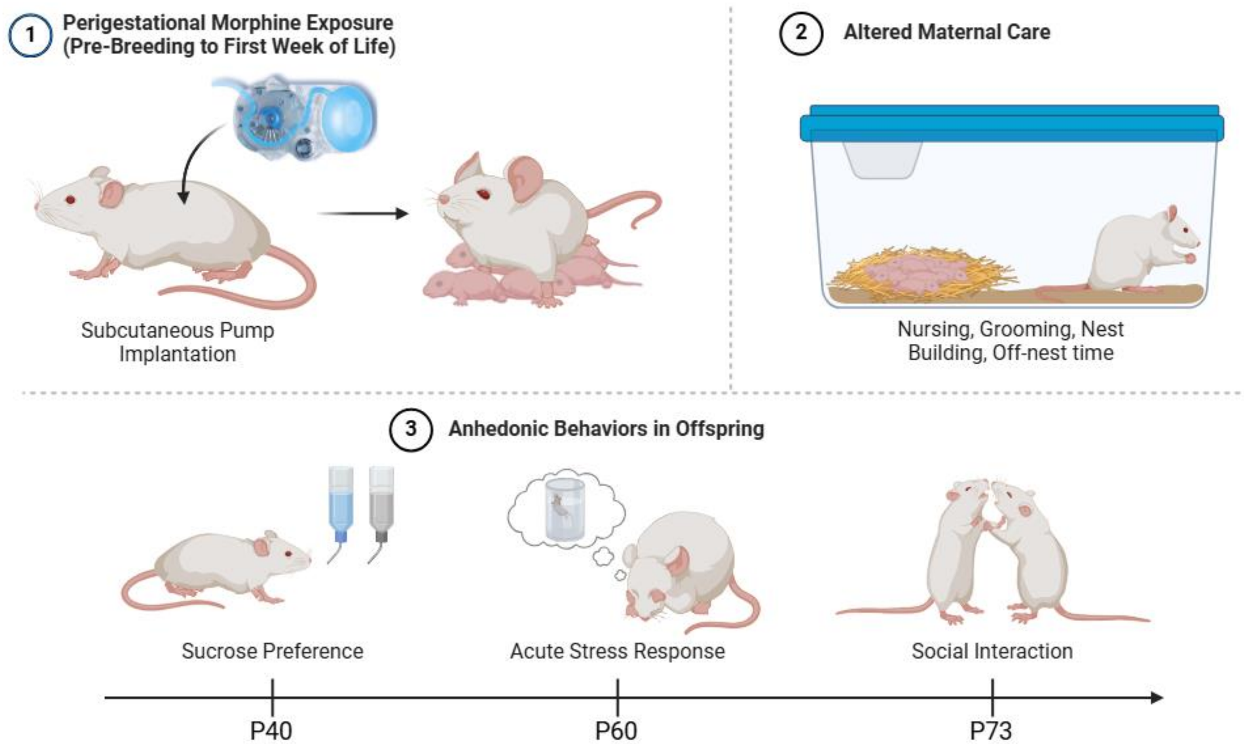

Figure 3.1 At P60, female Sprague Dawley rats are implanted with subcutaneous pumps to deliver morphine (or saline) prior to breeding through postnatal day 7 (P7). Maternal behavior was monitored at P2, P4, and P6 twice daily (before and after a scheduled infusion of morphine). Following morphine termination at P7, maternal behavior was observed once a day on P8, P10, P12, and P14. In adolescence, POE and CON rats were assessed for anhedonic behavior using sucrose preference test. Adult POE and CON rats were assessed for stress reactivity using FST and social behavior using social interaction test. Created with Biorender.

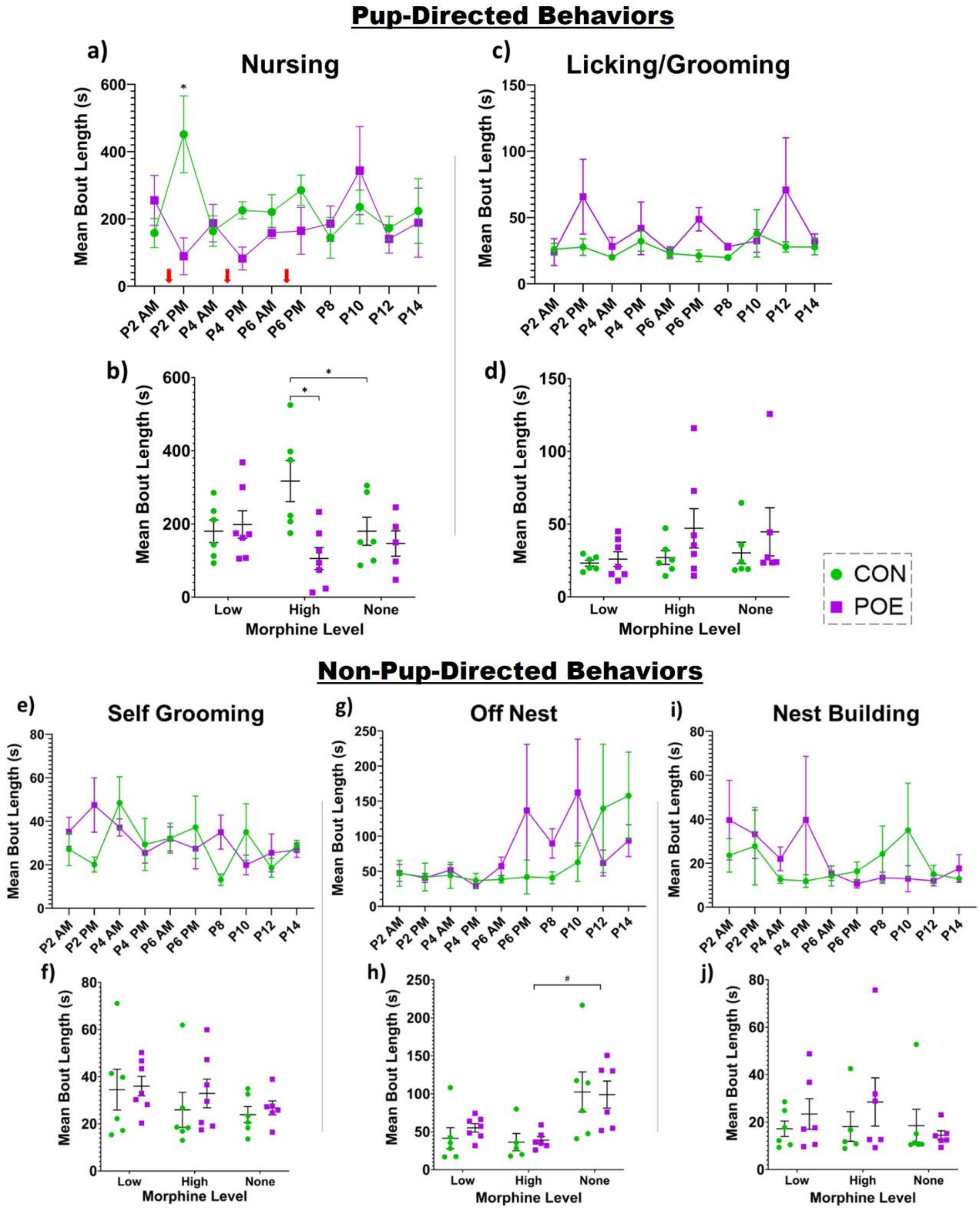

*Figure 3.2 Maternal behavior was assessed for differences in bout length and unpredictability. Specifically, we measured nursing (**a-b**), licking/grooming of pups (**c-d**), self-*

*grooming (**e-f**), off-nest time (**g-h**), and nest building (**i-j**). Red arrows indicate when dams received a bolus of morphine. *Significant difference: Nursing (CON vs POE P2 PM and P4 PM; CON vs POE high morphine level; CON high vs none), Licking/Grooming (CON vs POE P6 PM and P8), Self-Grooming (CON vs POE P8); **#**Statistical trend ($p<0.08$): Off-Nest (CON vs POE P8; POE high vs none). Graphs indicate mean ± SEM.*

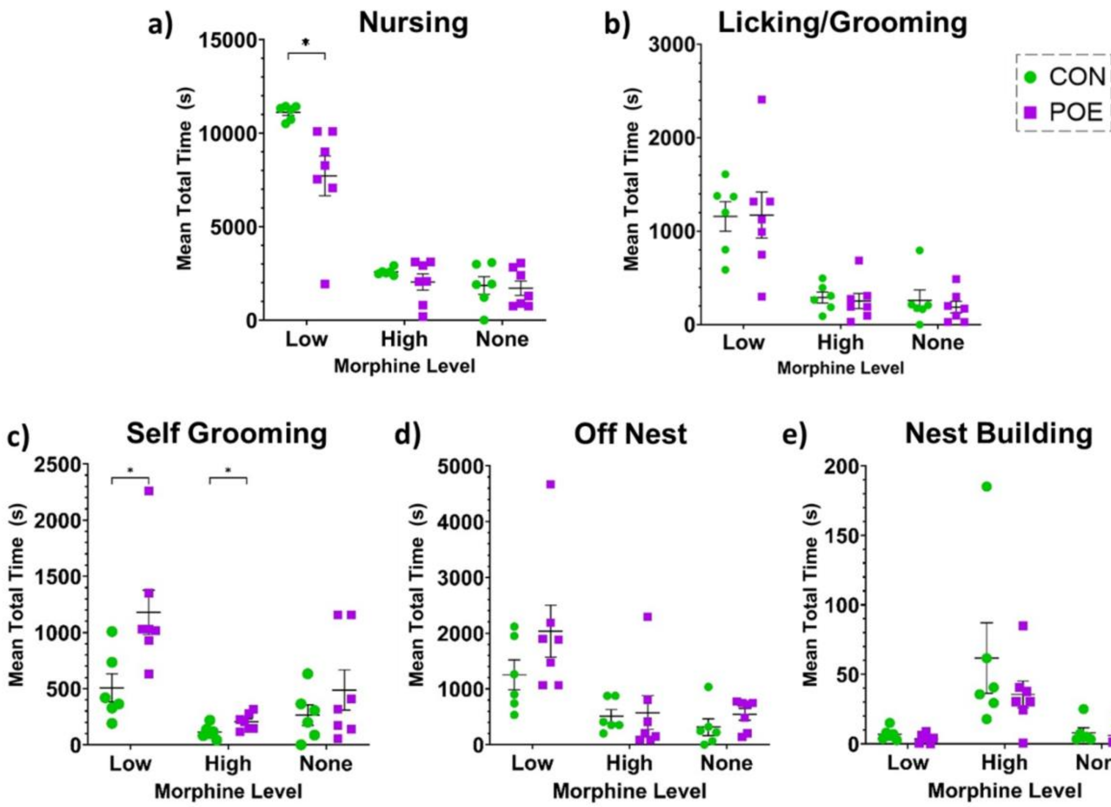

*Figure 3.3 Total time dams spent in each behavioral state was analyzed across morphine levels for nursing (**a**), licking/grooming of pups (**b**), self-grooming (**c**), off nest (**d**), and nest building (**e**). *Significant difference: Total Time Nursing (CON vs POE low morphine levels), Total Time Self-Grooming (CON vs POE low and high morphine levels). Graphs indicate mean ± SEM.*

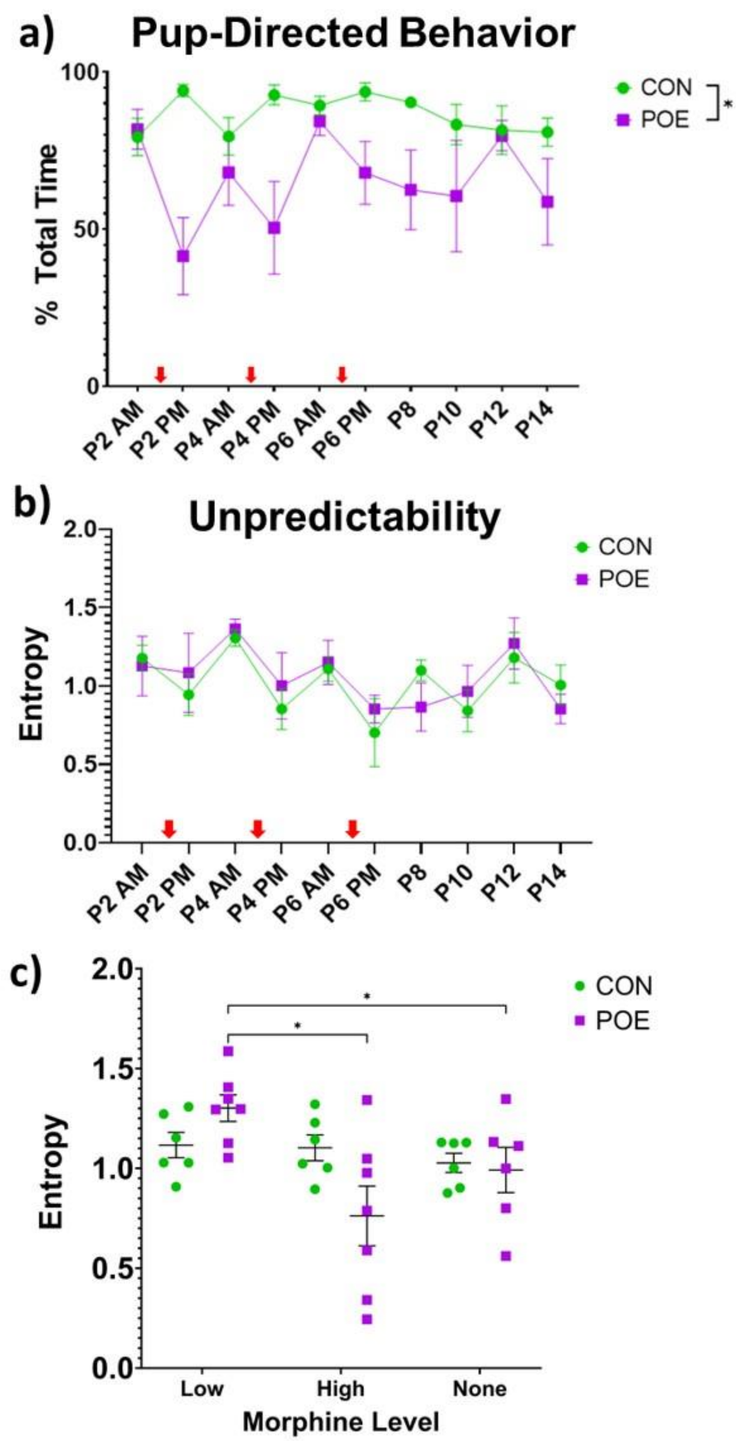

*Figure 3.4 POE dams spent less time performing pup-directed behaviors (nursing, licking/grooming, carrying) compared to CON (**a**). POE dams also exhibited increased entropy, or unpredictable behavioral patterns, when circulating morphine levels were low, and decreased entropy when morphine levels were high (**b-c**). Red arrows indicate when dams received a bolus of morphine. *Significant difference: %Pup-directed behavior (CON vs POE); Unpredictability (CON vs POE low and high morphine levels). Graphs indicate mean ± SEM.*

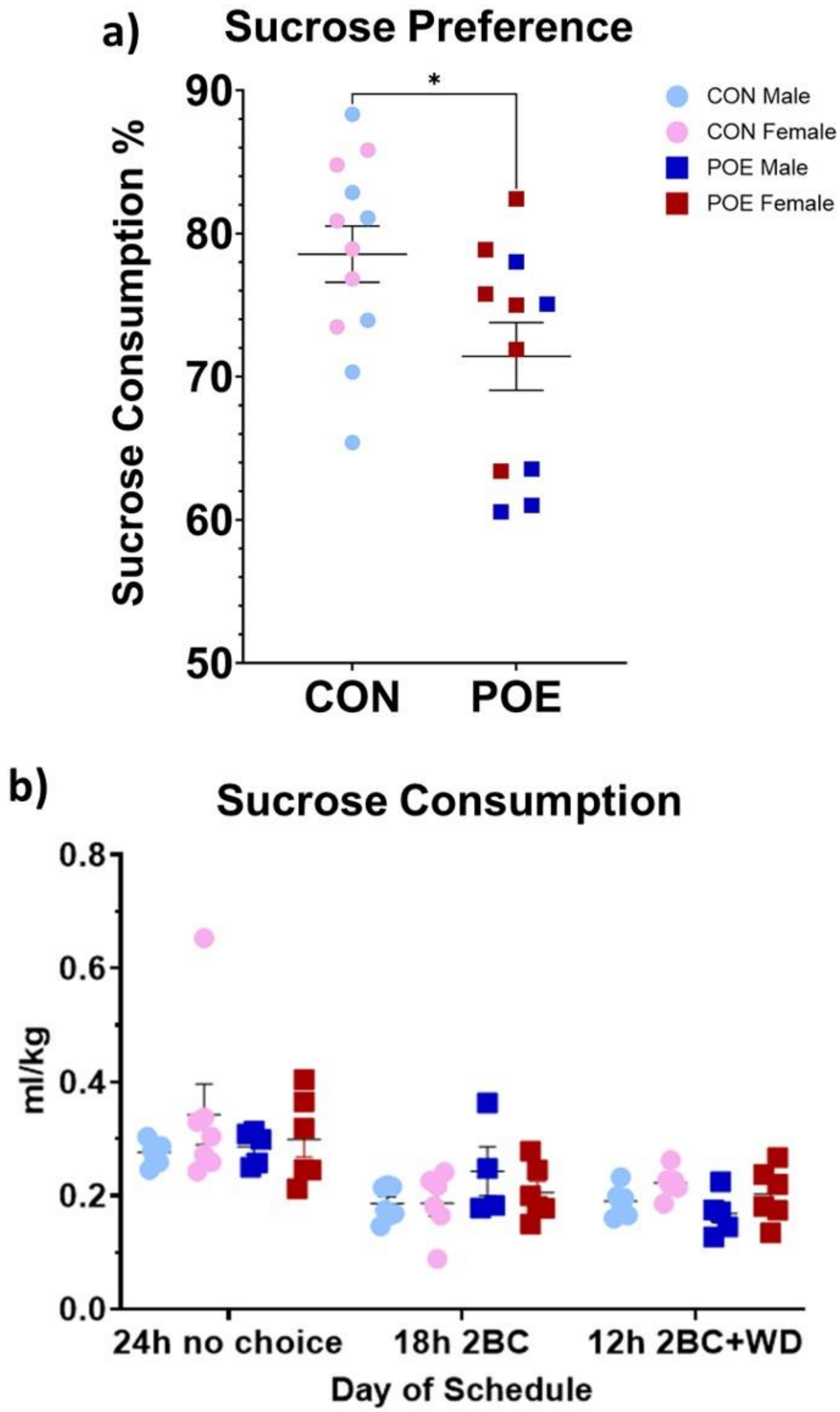

*Figure 3.5 Adolescent offspring were measured for sucrose preference using two-bottle choice for 1% sucrose solution vs water. POE rats exhibited a significant decrease in their sucrose preference compared to CON during testing (**a**). This result was not due to differences in sucrose consumption during reward habituation (**b**). *Significant difference between POE vs CON sucrose preference. Graphs indicate mean ± SEM.*

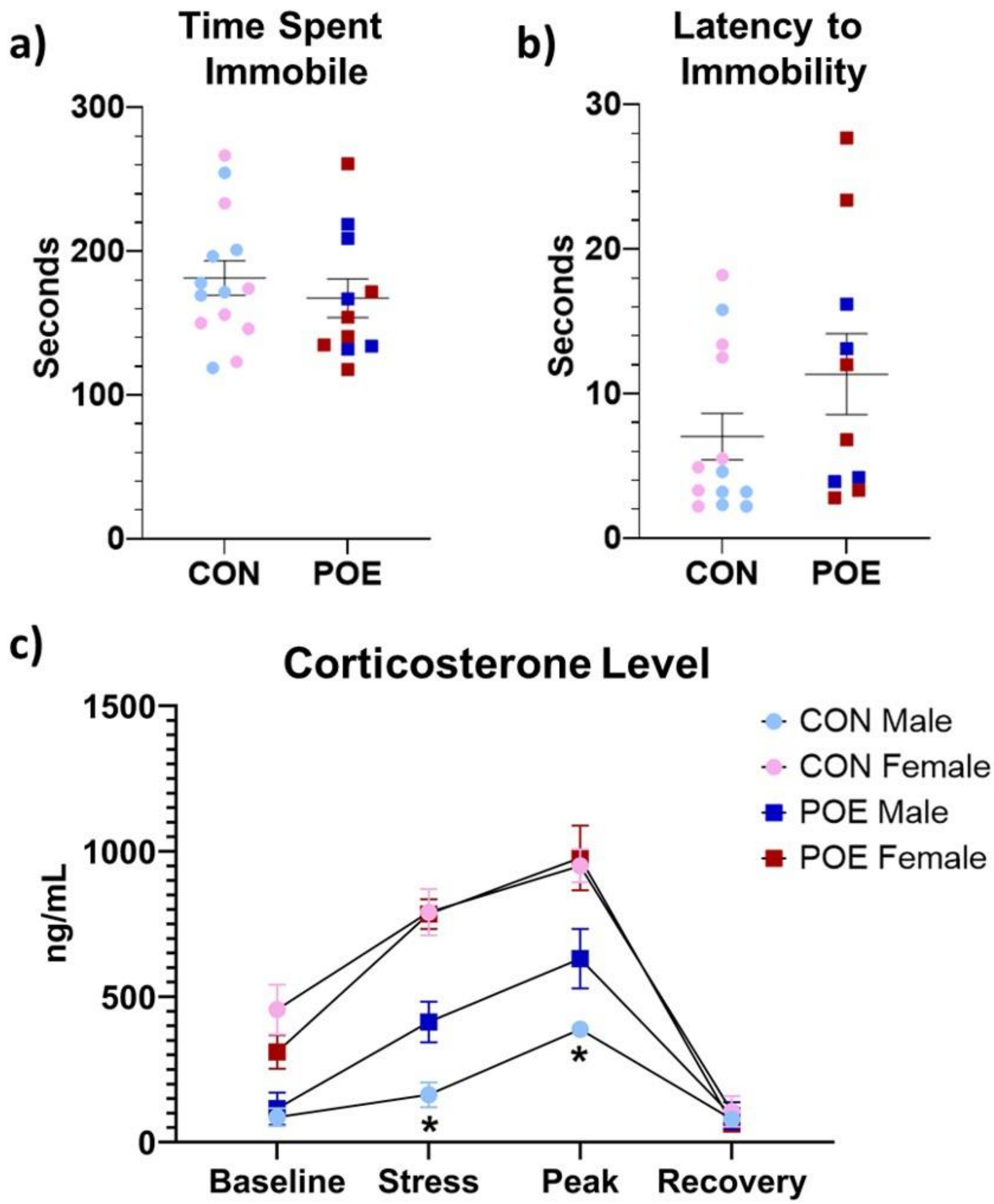

*Figure 3.6 Adult POE and CON performance in the forced swim test (FST). Depression-like behavior, as measured by time spent immobile and latency to immobility, was similar between treatment groups (**a-b**). HPA reactivity, as measured by plasma corticosterone levels across four key timepoints relative to the test, indicated that POE males had an increased stress response to FST, specifically immediately following and at peak (30-min post-test) measures. Female rats exhibited similar corticosterone levels across FST (**c**). *Significant differences between CON vs POE stress corticosterone and CON vs POE peak corticosterone. Graphs indicate mean ± SEM.*

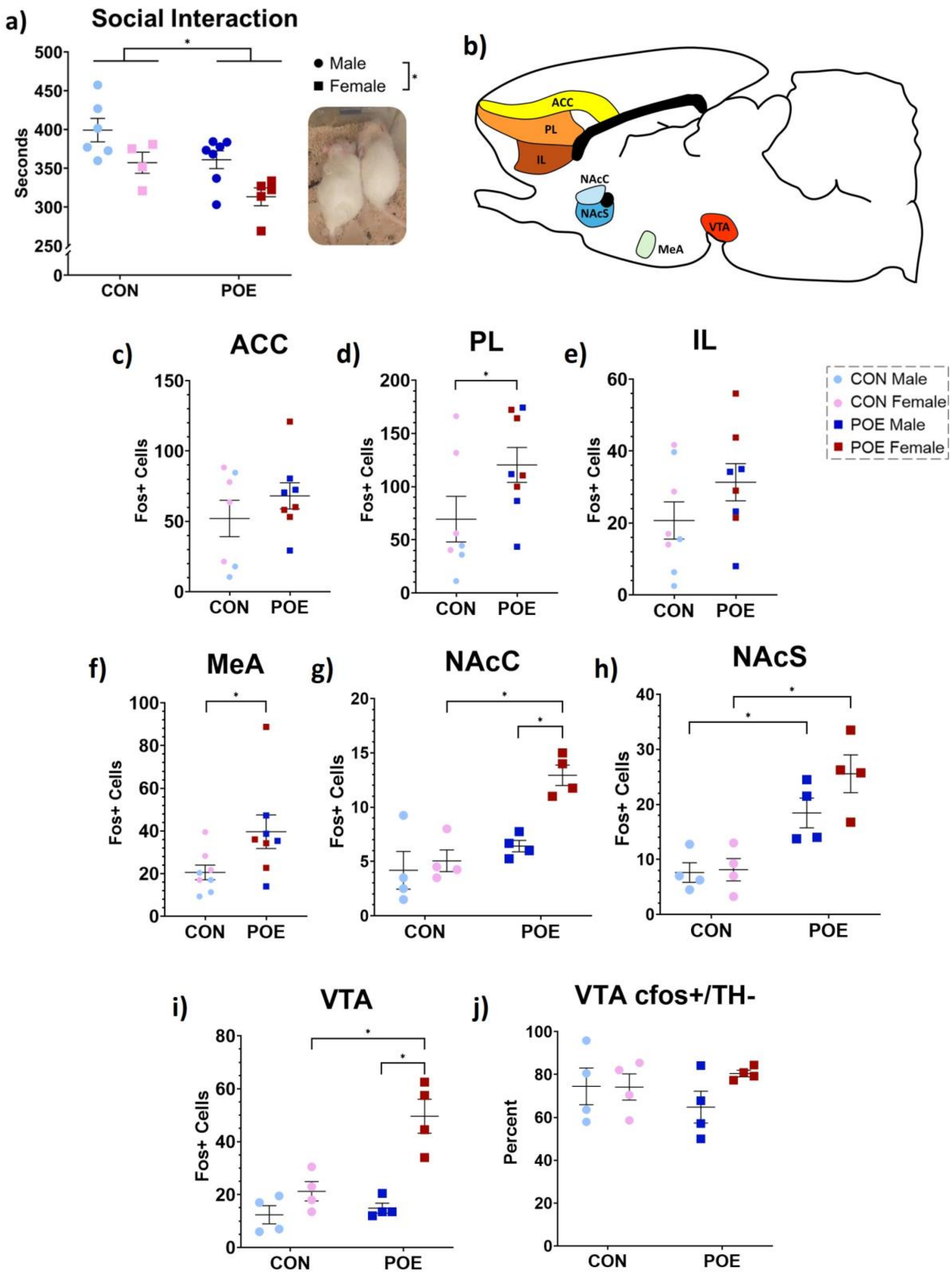

Figure 3.7 Adult POE and CON rats were tested for social interaction time as a measure of anhedonic behavior. Dual-housed rats were isolated for 24 hours and then reunited with cage mates. POE rats significantly reduce their social interaction time compared to CON rats. Female

*rats also spent less time with their cage mates overall (**a**). Rats were sacrificed 30-min post-test to determine cellular activity levels in brain regions governing social reward and aversion (**b**). Post-social interaction Fos expression was unchanged in the anterior cingulate cortex (ACC; **c**), increased in the prelimbic cortex (PL; **d**), unchanged in the infralimbic cortex (IL; **e**), and increased in the medial amygdala (MeA; **f**) in POE rats. POE sex-specifically increased Fos$^+$ cell counts in nucleus accumbens core and shell, as well as ventral tegmental area (NAcC, NAcS, VTA; **g-i**). Percentage of Fos$^+$ cells in the VTA that were nondopaminergic was comparable across groups (**j**). *Significant differences: Social Interaction Time (CON vs POE; males vs females); PL Fos (CON vs POE); MeA Fos (CON vs POE); NAcC Fos (POE females vs all other groups), NAcS Fos (CON males vs POE males; CON females vs POE females), VTA Fos (POE females vs all other groups). Graphs indicate mean ± SEM.*

4 DISCUSSION

4.1 Summary: Perinatal exposure to morphine leads to long-term changes in reward-directed behaviors

It is clear that despite widespread use of opioid therapy strategies, pregnant women with an opioid use disorder and their exposed offspring are subject to a spectrum of negative outcomes. For newborns, these outcomes pose risks well into adulthood that contribute to disordered social behavior and increased propensity for drug use. Multigenerational drug use is indeed present in both humans and controllable animal models, where factors of drug access, rearing behavior, at-home stress, and multidrug use can be independently examined. To appropriately address the neurodevelopmental impact of perinatal opioids on future reward behavior, preclinical investigations must recontextualize these seemingly confounding variables as unavoidable components of NOWS diagnoses. In doing so, we aim to examine their contributions to reward from a broader perspective. Clinical studies confirm that attempting to isolate perinatal drug exposure through adoption does not rescue the enduring impact of NOWS. Instead, the medical community encourages increasing the nurturing aspects of motherhood following childbirth to improve infant outcomes. It is believed that suboptimal maternal care produced by the stress of maternal opioid use directly contributes to the developmental trajectory of NOWS patients.

Data presented in Chapters 2 and 3 demonstrate that POE alters reward behaviors towards both drugs (alcohol) and naturally rewarding stimuli (sucrose, social interaction). POE increased preference for alcohol in males and noncontingent, or free, intake in females. Interestingly, POE rats later consistently displayed decreases in self-administration during operant conditioning, where learned behavior was required to earn rewards. This attenuation in alcohol reward was not due to a diminished ability to learn the task. Next, we found that both preference and intake of sucrose under similar exposure conditions (free access, fixed ratio schedules) were reduced by POE. This study is the first to uncover perinatal opioid-induced changes in alcohol or sucrose consumption in rats.

POE rats that displayed a reduced preference for sucrose during adolescence also spent less time socially interacting with cagemates as adults. This outlines a lifelong blunted response to naturally rewarding stimuli in POE rats. Our lab previously reported that POE rats reduce socially rewarding play behavior across adolescence (ages P25-45; Harder et al., 2023); therefore, reductions in social behavior are present at multiple life stages. During adult social interactions, nondopaminergic mesolimbic brain activity was increased in POE rats, as shown by Fos expression, pinpointing brain regions contribution to their social avoidance.

In humans, early life opioid exposure consistently leads to an increased risk of depression, stress, and/or anxiety disorders. This includes hyperreactivity to acute stress and increased emotional disturbance. POE-related changes in rodents have been thoroughly studied with mixed results. Differences in anxiolytic vs anxiogenic responses to stressful stimuli are often sex and age-dependent, and findings are inconsistent across multiple studies. We found that male POE rats displayed elevated HPA response to forced swim stress. Other studies have shown similar changes in corticosterone release in POE rats faced with an immunological challenge (lipopolysaccharide, LPS) in adulthood (K. L. Hamilton et al., 2005). Hyperreactivity of the HPA axis is a biomarker for depression; thus, the simultaneous presentation of reduced engagement with naturally rewarding stimuli and elevated acute stress response supports our overall characterization of an anhedonic phenotype in these rats wherein their ability to experience pleasure normally is diminished (Stetler & Miller, 2011).

It is well-established that responses to these reward stimuli are influenced by MOR signaling. Several methods of modeling perinatal opioid exposure in rodents show that chronic exposure to exogenous opioids during early development will permanently change MOR expression or function, leading to differences in sensitivity to rewarding and aversive stimuli. Indeed, our POE rats exhibited reduced MOR expression during adolescence in the medial habenula, demonstrating a lasting effect of POE in a region associated with reward/aversion processing.

Our POE paradigm improves on previous approaches to modeling human use of drugs across pregnancy, where exposure is typically not continuous throughout gestation. Clinical practices and post-birth diagnoses are designed around continuous opioid use throughout the natal period, which our model recapitulates. In addition, by not implementing cross-fostering, we were able to analyze maternal behaviors during and after a period of opioid use. We found that rat dams undergoing morphine exposure showed reduced care for their pups and increased grooming behaviors, potentially as a display of stress coping. Fragmentation in nursing bouts and altered predictability in care patterns disrupt offspring's earliest opportunities to hone prediction error. This outcome alone has been shown to generate anhedonic behaviors in rodents (Kangas et al., 2022; Molet et al., 2016; Spadoni et al., 2022). Potential changes in these aspects of maternal care have not been previously analyzed in POE rodent models.

4.2 Perinatal exposure to morphine alters alcohol reward

Manipulation of opioidergic systems can either promote or diminish alcohol consumption. In fact, one of the few clinically-approved medications for treating alcohol use is naltrexone, a μ-opioid receptor antagonist. POE, across several rodent-based paradigms, consistently alters MOR density in brain regions associated with drinking behavior. Despite this, only one study, published during the time of the present work, has addressed the potential impact of POE in rodents on alcohol consumption. In that study, female mice receiving daily injections of methadone from E0 to P28 increased their intake and locomotor sensitization to alcohol while males displayed decreased conditioned place preference (CPP) (Grecco, Haggerty, et al., 2022).

In the present study, male POE rats increased their CPP for alcohol, indicating an increased sensitivity to alcohol reward. Within the CPP arena, POE males significantly increased their chamber shuttling from pre- to post-conditioning. Self-administration of alcohol during the conditioning phase may have led to increases in this behavior, with POE rats actively seeking alcohol reward. This result is consistent with Grecco et al. (2022) who reported that perinatal methadone increases alcohol-induced locomotion during conditioning sessions in mice. Room switching was higher for our POE rats compared to controls, regardless of demonstrated alcohol preference. Similar two-chamber shuttling has been reported in ADHD-like spontaneous hypertensive rats (SHRs), a rodent model for attention-deficit hyperactive disorder (ADHD) (Watterson et al., 2015). This behavior is dose-dependently reduced with Ritalin, a clinical treatment for ADHD (Wultz et al., 1990). Our observed increases in room shuttling, suggestive of ADHD, is consistent with previous studies reporting that individuals with prenatal opioid exposure or NOWS diagnosis are more likely to develop ADHD in early childhood or adolescence (Azuine et al., 2019; de Cubas & Field, 1993; Nygaard et al., 2020; Sandtorv et al., 2018; Sherman et al., 2019; Vidal et al., 2012). The use of black and white bedding materials prevented accurate measurement of locomotor behavior with automated tracking software. In the present study, locomotor behavior could not be reliably recorded. Future studies will be necessary to determine if POE induced hyperlocomotion during alcohol-seeking and alcohol-drinking conditions.

For POE males, alcohol intake during intermittent free access was not different from control males but was comparatively decreased during operant conditioning. Unlike other drugs of abuse, rats are likely to titrate their alcohol responses to avoid the aversive properties of intoxication, reaching a level of satiety within a short period of time. POE males experiencing enhanced rewarding effects of alcohol may reach satiety sooner, and therefore, reduce their consumption. Routine sampling of blood alcohol levels in future studies will determine if potential changes in alcohol metabolism influence intake. Alternatively, leptin signaling, which has a dampening effect on reward and promotes satiety, could be measured in POE rats. Leptin's relationship with alcohol, specifically, is unclear, but NOWS patients exhibit higher levels of the leptin receptor gene, LEPR, which may contribute to altered feeding behavior (Bach et al., 2021; Yen et al., 2019).

For females, POE did not affect alcohol CPP. Interestingly, their alcohol intake during free access was increased compared to controls; during operant conditioning, alcohol intake decreased, similar to POE males. While these results would appear contradictory, it has been previously reported that instrumental responding specifically can be impaired by chronic stress, which we hypothesize is a primary feature of our POE model. Similarly, rodent models of chronic social defeat and restraint stress also reduce operant responding (Bergamini et al., 2016; Xu et al., 2017). Conversely, chronic isolation or restraint stress increases alcohol CPP and noncontingent consumption in mice (J.-S. Lee et al., 2021); these increases coincide with exacerbated anxiety and depression-like behaviors.

It has also been reported that rats may alter their intake of alcohol depending on the effort requirements of an experimental design. In a study comparing selectively-bred male rat strains, only alcohol-preferring Wistar rats (P rats) outperformed other strains across multiple operant reward requirement ratios (1, 2, 4, 8, 16, 32) (Ritz et al., 1994). “High alcohol drinking”, or HAD, rats, genetically selected for their high alcohol consumption during noncontingent scenarios, did not differ from non-preferring (NP) rats during operant testing. NP rats also displayed higher breakpoints for alcohol than HAD rats. Similarly, ALKO Alcohol Accepting (AA) rats bred for their high alcohol consumption during two-bottle choice consumed less alcohol (8%) than control Sprague Dawley rats at multiple fixed operant ratios (Ritz et al., 1989). Together, these studies suggest that alcohol preference is not always a predictor of reinforcement in rats.

POE may provide tolerance to the inherent aversive properties of oral alcohol administration, specifically during the acquisition period (noncontingent free access or CPP conditioning). For POE females, aversion-resistance manifests as increased alcohol consumption. For POE males, CPP increases. Future studies should assess for aversion-resistant drinking in our rats, as it is reported that perinatal methadone exposure in male mice increases quinine-adulterated alcohol drinking (Grecco, Haggerty, et al., 2022). Generally, rats, including

Sprague Dawleys, require several weeks of intermittent or 24-hour alcohol access to reach chronic, stable “maintenance” intake (Cuitavi et al., 2021; Quintanilla et al., 2012, 2016). Our rats do not stabilize their consumption until reaching operant conditioning. Unlike other studies, we did not employ noncontingent intermittent access or two-bottle choice for several weeks to dramatically escalate daily intake. Therefore, we may have missed the opportunity to demonstrate potential for tolerance in our POE and control rats, where increasing amounts of alcohol would be necessary to reach the same level of satiety.

POE and control rats were tested for relapse potential using forced abstinence, a protocol designed to mimic the human experience of voluntary or mandated abstinence from alcohol (Martin-Fardon & Weiss, 2013; Reichel & Bevins, 2009). Following abstinence, we measured the consumption of alcohol after successively increasing periods of no access without using cue or context extinction. Expected increases in drinking are based on the alcohol deprivation effect (ADE) that can be produced with repeated periods of forced abstinence. Abstinence can span hours to weeks and reliably increase alcohol intake (A. Lê & Shaham, 2002; Sinclair & Li, 1989) It is also proposed that ADE-like effects will drive increased saccharin intake (Sinclair & Bender, 1978; Sinclair & Li, 1989).

Unlike controls, male and female POE rats consistently consumed more alcohol after one and two weeks of abstinence. This was relative to their stabilized FR3 intake. POE females also progressively increased this relapsed consumption across one-day, one-week, and two-week periods, suggesting that relapse susceptibility may be abstinence duration-dependent. Stress or anxiety produced by drug abstinence exacerbates relapse potential, driving individuals to alleviate negative emotional states given the opportunity (G. F. Koob & Volkow, 2010;

Solomon & Corbit, 1974). Protracted alcohol abstinence is in part driven by alterations in corticotropin-releasing factor (CRF) systems and accompanied by elevated anxiety-like behaviors (Valdez et al., 2002). Early life adversity and stressful life experiences are strongly associated with relapse and alcohol use in humans, especially women, but their effects in rats remain unclear (Simpson & Miller, 2002; Van Dam et al., 2014). Stress history in rats does not disproportionately affect female rat alcohol intake post-abstinence (Carvalho et al., 2022; Moench & Logrip, 2021). Timing of adverse experiences relative to glucocorticoid system development in males and females may underlie sex differences in relapse potential. For example, sex differences in vulnerability to stressful experiences are associated with testosterone levels during HPA axis development (Schwarz et al., 2012). However, stress in adulthood (following HPA axis development) can also sex-specifically alter glucocorticoid sensitivity and relapse for alcohol (Logrip & Gainey, 2020). The implications of early life stress will be discussed later in this chapter, but the direct impact of suboptimal maternal care in our POE rats should, in the future, be examined for its association with changes in relapse-related intake of alcohol.

Clinical treatment of alcohol relapse and dependence with naltrexone and nalmefene, both MOR antagonists, warrants exploration in the context of early life opioid exposure (Niciu & Arias, 2013). Acute naltrexone has been shown to be more effective in individuals who exhibit high-risk characteristics, such as family history of drug use (King et al., 1997). High-risk individuals also present diminished basal opioid activity and central opioid pathways that are more sensitive to ethanol (Gianoulakis, 1998).

4.3 Perinatal exposure to morphine alters sucrose reward

Previous studies examining the impact of gestational opioid exposure on consumption of sweetened solutions are contradictory. For example, daily injections of morphine during mid-gestation (E11-18) did not affect sucrose intake in adult male or female Sprague Dawley rats during operant conditioning (Riley & Vathy, 2006; I. Vathy et al., 2007). Decreased preference to 32% sucrose was reported in adolescent rats exposed to morphine during mid-to-late gestation (E11-P0) and naloxone-precipitated withdrawal (Torabi et al., 2017). However, this study is challenging to interpret, as precipitated withdrawal may exaggerate biological outcomes and high-dose sucrose is a poor tool for measuring hedonic value. In contrast, Gagin et al. (1996) reports that mid-gestation morphine increases saccharin preference in adult male and female Fischer rats. The dearth of studies investigating these naturally rewarding sweeteners is surprising considering that endogenous opioid systems are well-established contributors to both homeostatic and hedonic values of food stimuli in both humans and rodents (Berridge, 2000; Bertino et al., 1991, 1991; D. C. Castro & Berridge, 2014). Agonism and antagonism of MOR signaling produce increases and decreases, respectively, in feeding and positive affective reactions to sucrose (Berridge, 2000; Cleary et al., 1996; Eikemo et al., 2016; I. Morales et al., 2020; Papaleo et al., 2007).

To our knowledge, we are the first lab to extensively study the impact of POE on sucrose reward. In doing so, we report that changes in reward behaviors towards drugs of abuse (alcohol) are not distinct from naturally rewarding stimuli. Similar to our alcohol experiments, adolescent POE rats consumed less sucrose than controls during operant conditioning sessions. For alcohol, we suspect that decreased consumption may be related to differences in reward efficacy, wherein increased reward value would drive POE rats to consume less and reach satiety sooner. Sucrose intake is a strong predictor of alcohol intake in both rats and humans (Eiler et al., 2005; Kampov-Polevoy et al., 1999; A. D. Lê et al., 2006). Alternatively, POE rats may be less motivated to consume alcohol than controls, but this outcome is not supported by similar breakpoint measurements between treatment groups.

Reduced operant responding may also represent an anhedonic state, in which rodents are less motivated to perform or have difficulty learning instrumental tasks (Der-Avakian & Markou, 2012; Xu et al., 2017). Indeed, during sucrose lever press training, POE rats were statistically less accurate than controls. This difference was not observed in alcohol operant training but may still represent changes in reward learning. In humans, NOWS is strongly associated with depression, cognitive disorders, and even delays in motor coordination development (Nygaard et al., 2020; Ornoy et al., 2010; Sherman et al., 2019). These outcomes, together with increased risk for ADHD, may contribute to differences in school performance for NOWS children and, here, in learned task performance (Fill et al., 2018; Nygaard et al., 2016; Oei et al., 2017; Rees et al., 2020). In rats, POE can produce memory impairments in association with spatial learning, novel object recognition, and avoidance retention (Ahmadalipour et al., 2018; Gamble et al., 2022; Nasiraei-Moghadam et al., 2013). Sucrose lever press accuracy in our POE rats was different from controls after training, but future experiments testing for lever choice reversal or extinction may be useful in addressing the impact of POE on instrumental learning.

POE also induced an upward trend in relapsed sucrose intake in both males and females compared to controls. This was true after both one and two weeks of abstinence. Therefore, week-long breaks in regular reward access appear to elicit POE-induced differences in both alcohol and sucrose. We did not directly measure physiological withdrawal from alcohol or sucrose in our rats, but these negative affect phases of the relapse cycle are related to increased basal ACTH, plasma corticosterone, and ventral tegmental or amygdalar CRF (Grieder et al., 2014; G. Koob & Kreek, 2007). These measurements should be recorded in future studies during abstinence periods to determine the impact of stress on augmented relapse.

As mentioned earlier, disrupted operant responding (FR1/FR3) towards both alcohol and sucrose suggests an anhedonic phenotype in our POE rats. To characterize this, we broadened our studies to include depression-like behavior analysis. First, we observed reduced sucrose preference in both male and female adolescent POE rats. To our knowledge, this is the first report of POE-induced changes in two-bottle choice (2BC) preference test using noncaloric (1%) sucrose. Sucrose lacks inherent aversion; therefore, reduced preference during sucrose 2BC suggests an attenuated ability to feel pleasure. This is consistent with previous studies showing that low sucrose preference is a hallmark of anhedonic profiles in rodents.

Caloric or sweetening additives in our jello shots may have contributed to changes in alcohol intake in our POE rats. By design, jello shot mixtures are intended to increase the palatability of alcohol for rodents. In our initial CPP pilot studies using intraperitoneal injection of alcohol, we found consistent aversion to alcohol in all groups. This did not occur when rats orally self-administered the jello shot mix. Given changes in intake and preference of sucrose at caloric and hedonic concentrations, respectively, it remains possible that sweetening additives to alcohol jello shots affected our results. A considerably longer intermittent alcohol protocol would be needed to replace the advantages of jello shot vehicle and still overcome alcohol’s taste aversion. We believe this barrier to throughput has prevented other researchers from tackling POE's effects on alcohol in rats. However, the elimination of palatable additives may delineate differences in preference for alcohol and sucrose in POE rats. When primed with morphine (2.5mg/kg), Sprague Dawley rats are preferentially motivated to consume sweetened ethanol (6%) over water, but not water sweetened with sucrose (5%) (Stromberg et al., 1997). This suggests that opioid signaling may have a disproportionate impact on sucrose over alcohol. How morphine withdrawal or history of morphine use would impact this test is unclear.

4.4 Anhedonia phenotype characterized by increased acute stress response and reduced social engagement

NOWS patients are more likely to exhibit depression, anxiety, or emotional disorders, which may contribute to future drug misuse (Azuine et al., 2019; de Cubas & Field, 1993; Hall et al., 2019; G. Koob & Kreek, 2007; Ornoy et al., 2010; Sherman et al., 2019; Vidal et al., 2012). Comorbidity of depression-like behavior and increased drug intake has been reported in rats exposed to prenatal morphine (Y. Chen, Du, et al., 2022). Our results showing reduced sucrose preference in male and female POE rats suggest that our gestation-spanning protocol produces anhedonia. To further assess if our POE model induced an anhedonic phenotype, adolescent rats tested for sucrose preference were later tested for sociality using the social interaction test. They were also tested for depression-like behavior using the forced swim test (FST). In a subgroup of rats, we measured blood corticosterone (CORT) at four timepoints during the test to capture the inverted-U of its release.

We found differences in either latency to immobility or time spent immobile between treatment groups, suggesting that POE did not induce a depressive-like state. However, FST did significantly elevate CORT levels in POE males immediately following the test and again at 30min post-swim (peak) compared to controls. This suggests that morphine administration across gestation induces HPA hyperreactivity to acute stress. No treatment differences were observed in CORT levels at baseline or following recovery (60 min post-swim).

Evidence on the impact of perinatal morphine on CORT and stress reactivity is mixed. Prenatal morphine injections (E11-E18) did not impact CORT release in adult rats exposed to either ether or restraint stress (Laborie et al., 2005; Rimanóczy et al., 2003; Slamberová et al., 2004). In contrast, prenatal exposure to the long-acting opioid, *l*-alpha-acetylmethadol (LAAM), decreased CORT response to immunological challenge (LPS) at P18 but increased CORT levels in adults under the same conditions (K. L. Hamilton et al., 2005). Age-specific changes in FST-evoked immobility have also been reported in POE rat with decreases at P32 and increases at P54 in females with no change in males (Y. Chen, Du, et al., 2022). This study also reported anxiolytic effects of POE in both adolescence and adulthood during open field (OFT) and elevated plus maze (EPM) tests. Other studies report that prenatal buprenorphine/methadone (E3-E20) or perinatal fentanyl exposure (E0-P21) increase anxiety-like behaviors during OFT and EPM (Alipio, Haga, et al., 2021; H.-H. Chen et al., 2015). Perinatal fentanyl also reduced sucrose splash test grooming, a sign of negative affect, in adolescent females. These conflicting results are likely due to differences in the opioid administration paradigm used, including gestational age at onset, type of opioid administered, and age of behavioral testing. Clinical data report more consistent findings, with NOWS diagnosis leading to increased risk for stress-related disorders and increased acute stress reactivity in NOWS patients aged 1-5 (Sherman et al., 2019). Increased salivary cortisol has also been reported in NOWS infants (Rodriguez et al., 2020). The mechanism(s) whereby NOWS impacts HPA axis-regulated stress responses later in life remains unclear.

In our previous study, we found that POE reduces juvenile social play in females and increases time not in contact in both sexes (Harder et al., 2023). Adolescent POE males and females also exhibited fewer nape attacks and pins during play. As adults, Sprague Dawley rats rarely show nape attacks or pining behaviors with cagemates; therefore, we focused our analysis on social interaction. Following 24 hrs of isolation, rats were assessed for time spent interacting with their cagemate (sniffing, chasing, grooming, and playing); time not in contact served as an inverse measure. Here we report that our POE rats spent significantly less time socially interacting with cagemates, preferring to spend time alone (not in contact). In our earlier study, we found that changes in adolescent play time were associated with differences in oxytocin (OT) expression (Harder et al., 2023). Altered OT signaling is associated with social deficits in patients with autism spectrum disorder and ADHD, both of which are more common in individuals born with NOWS (Levi-Shachar et al., 2020; Sandtorv et al., 2018; Zhang et al., 2017). Social disorders are also more likely in children and teenagers born with NOWS, with deficits in social maturity detectable in NOWS patients as young as 18 months old (de Cubas & Field, 1993; Hunt et al., 2008; Ornoy et al., 2010). What is unclear is if reductions in sociability in NOWS infants are due to gestational opioids, or due to challenges associated with concurrent reductions in emotional maturity and cognitive development.

Based on previous studies demonstrating a critical role for oxytocin in social behavior (Burkett et al., 2016; Ferguson et al., 2001; Takayanagi et al., 2005), we hypothesize that POE reduces OT signaling in key brain regions in adulthood. These studies are currently underway. It is likely that OT also plays a role in other reward deficits observed in our POE rats. OT release in the central amygdala is associated with depression-like behavior in adult Wistar rats (Ebner et al., 2005), and circulating levels of OT are reduced in humans with major depressive disorder (Frasch et al., 1995). Reductions in cagemate interactions in our adult rats demonstrate a potentially lifelong impact of POE on social reward. Avoidance and antisocial behaviors are reported in young people with a history of NOWS, but no longitudinal studies have explored this outcome in adults.

4.5 Mesolimbocortical control of reward and aversion through opioid-sensitive signaling

Several studies have elucidated the impact of chronic opioid administration during development on the structure and function of opioid neural circuits (Hauser & Knapp, 2018). In the context of drug abuse, brain regions associated with reward express μ-opioid receptor (MOR), the primary target of morphine and its derivatives, and are understandably of considerable interest. Presently, we report that POE decreased medial habenula (MHb) MOR expression in adolescent rats. MHb contains one of the highest region-specific concentrations of MOR in the rodent brain and is commonly associated with aversion signaling (Bailly et al., 2023; L. J. Boulos et al., 2020). Downstream habenular signaling to GABAergic neurons of the rostromedial tegmental area (RMTg) and ventral tegmental area (VTA) maintains a hyperpolarizing gate preventing dopamine release (Matsui & Williams, 2011). Reducing habenular MOR lowers the inhibitory actions of endogenous opioids in this region. Therefore, disinhibition of dopamine release during social engagement is affected. In mice, knockout of habenular MOR significantly reduces social preference for both familiar and novel conspecifics (Allain et al., 2022). Genetic ablation of dorsal MHb neurons, where MOR is primarily localized, also reduces sucrose preference in mice (Contestabile et al., 1987; Gardon et al., 2014; Hsu et al., 2014). In our study, POE did not alter MOR in the VTA and only transiently (at P14, but not P30) in the nucleus accumbens (NAc). In perinatal opioid exposure research, site-specific changes in MOR density are commonly reported despite variability in POE model drug choice and length of exposure; however, the results are often contradictory, with reports that POE increases, decreases, or does not affect MOR in the nucleus accumbens and adjacent striatal subregions (Chiang et al., 2014; Chiou et al., 2003; Handelmann & Quirion, 1983; Tempel, 1991b; I. Vathy et al., 2003).

To begin elucidating brain regions implicated in social reward that may be impacted by perinatal morphine, we used Fose expression as a proxy for neural activity. Previous studies on early life adversity (LBN) have reported increased activation in amygdalar and prefrontal cortex subregions during social play (Bolton et al., 2018). Our POE studies generated similar results; during adult social interactions, medial amygdala (MeA) and prelimbic (PL) cortex were more active in POE rats. A trend toward increased activity was also observed in the basolateral amygdala (BLA). No significant changes in neuronal activity were detected in the anterior cingulate cortex (ACC), infralimbic (IL) cortex, or central amygdala (CeA). Medial and lateral habenula regions showed very low levels of Fos and were not analyzed. Mesolimbocortical dopamine signaling plays a prominent role in encoding reward behaviors, so we also probed Fos expression in VTA and nucleus accumbens core/shell (NAc/NAcS). All three regions showed higher Fos expression in POE females compared to controls. As female social behavior was not disproportionately affected by POE in the current study, the findings of increased Fos are unclear. However, social deficits in adolescent POE females were more severe (Harder et al., 2023).

In combination with cell-type identification, we also found that, in the VTA, the majority of Fos+ cells were nondopaminergic. This was true in both POE and control rats. VTA GABAergic and glutamatergic interneurons modulate dopaminergic activity in association with aversion, depression, and stress (Barbano et al., 2020; Cai & Tong, 2022; M. Morales & Margolis, 2017; Root et al., 2014; Tan et al., 2012; van Zessen et al., 2012). Nondopaminergic VTA information also contributes to aversion signaling in the habenula and nucleus accumbens (Qi et al., 2016; Root et al., 2014). Fast-scan cyclic voltammetry would be useful for determining if mesolimbic dopamine flux is altered by elevated nondopaminergic activity during social behavior.

The role of the mesolimbic system in rewarding behaviors is well-established. VTA production and output of dopamine to striatal, prefrontal, and amygdalar regions is critical to encoding reward events (G. F. Koob & Volkow, 2016; Terauchi et al., 2023). These connections are opioid sensitive and, therefore, vulnerable to change due to experiences with chronic morphine exposure (G. F. Koob & Volkow, 2010; Pitchers et al., 2014). Elevated nondopaminergic activity may be related to VTA interneuron reductions in dopamine release to NAc. This would be evident in recordings of hypothetically reduced nucleus accumbens activity. However, we observed POE-associated increases in both NAcC and NAcS Fos expression, especially in females. Thus, increased NAc Fos expression may relate to nondopaminergic inputs. Glutamatergic VTA projections to the NAc drive social avoidance, fear, and anxiety-like behavior (McGovern et al., 2024; Qi et al., 2016). Glutamatergic VTA interneurons are MOR-sensitive, but it is unclear if VTA-NAc populations are as well (McGovern et al., 2023). POMC neurons of the arcuate nucleus release β-endorphin directly to NAc subregions, meaning that during pleasurable events, reduced dopamine input from the VTA would not prevent opioid signaling (Mansour et al., 1988; Song & Choi, 2023). Indeed, intra-NAc infusion of opioid agonists and antagonists to either core or shell alters social interactions in rats (Trezza et al., 2011). Nucleus accumbens also receives glutamatergic and GABAergic input from mPFC regions that underlies reward and avoidance behaviors (Bossert et al., 2012; Britt et al., 2012; A. T. Lee et al., 2014).

The amygdala is traditionally associated with early life stress memory and fear conditioning. The MeA, in particular, receives dopaminergic input from the VTA that is associated with bonding in humans (Atzil et al., 2017). In rats, MeA is associated with social and sex rewards (Hodges et al., 2019; Holder et al., 2010). Social isolation stress reduces social interaction and MeA synaptophysin expression, a marker of synaptic plasticity, suggesting that this region is sensitive to chronic stress and influences future social behaviors.

In the present study, we observed elevated Fos expression in the PL and IL of POE rats. PL activity increases during fear experiences and excites the central amygdala via the BLA (Kapp et al., 1979; LeDoux, 2000). PL-NAc projections are associated with emotional aversion (Long et al., 2024). In contrast, IL projects to intercalated neurons of the amygdala to suppress fear and the NAcS to promote extinction of conditioned behaviors (J. Peters et al., 2009). Increased PL activity in our POE rats suggests that this behavior promotes aversion signaling. MOR in the PL is required for morphine sensitization and linked to suppression of physiological stress responses (Fassini et al., 2014; C. Jiang et al., 2021). POE-induced changes in mPFC MOR may contribute to changes in reward behaviors and are so far unexplored by the field. Moreover, subregions of the amygdala are sensitive to MOR signaling and have extensive connectivity with reward and aversion areas of the brain (Reeves et al., 2022). Amygdalar subregions included in this experiment and others, such as the bed nucleus of stria terminalis (BNST), should be included in future MOR expression analyses.

4.6 Maternal care deficits contribute to changes in reward-directed behaviors in rats

Opioid-using mothers experience stress from caring for their newborns while overcoming drug dependence (Velez & Jansson, 2008). Caring for NOWS infants is considerably more stressful and challenging due to the symptoms associated with opioid withdrawal. Specifically, these infants have a unique combination of excessive crying, irritability, gastrointestinal stress, and disorganized behavior. Societal stigmas associated with maternal opioid use further exacerbate maternal stress and negatively impact the quality of both self-care and offspring rearing. Clinicians believe that stress and suboptimal maternal care worsen negative outcomes in NOWS children (Wachman et al., 2018). Preclinical rodent models also suggest that poor maternal care is detrimental to pup health, with models of early life stress in rats reporting fragmented and unpredictable behavior that is linked to increased developmental disorders in offspring.

Although some studies report no impact of buprenorphine or fentanyl on maternal care, others have shown that morphine exposure during and after pregnancy reduces nursing and pup licking (Alipio, Haga, et al., 2021; Cruz et al., 2010; Kongstorp, Bogen, Stiris, et al., 2020). Our lab previously reported that POE dams spend less time nursing their pups compared to controls, especially during periods of high circulating morphine (Harder et al., 2023). Previous studies examining the impact of POE on maternal behavior have traditionally measured total time spent. However, more recent studies have applied a more detailed analysis approach, examining individual bout lengths of each behavior (fragmentation) and the patterns in behavior sequences (entropy). Here, we report that, compared to controls, POE dams reduced their time spent nursing during periods of low circulating morphine and “fragmented” their nursing into smaller bouts when morphine levels were high. This suggests that peaks and troughs of morphine exposure across the day can significantly alter nursing behaviors that are vital to pup survival. Entropy, a measure of behavioral unpredictability, was also altered in POE dams depending on the circulating levels of morphine in their system. Specifically, prior to a scheduled bolus of morphine, POE dams were unpredictable in their care patterns compared to controls. During this time, they engaged in significantly more self-grooming. We also observed non-significant increases in time off their nest. In contrast, after receiving morphine, POE dams became more predictable. During morphine highs, POE dams exhibited lower overall pup attention compared to controls, characterized by spending less total time nursing or licking/grooming their offspring. Therefore, when dams became more predictable, their maternal care remained deficient. Control rats did not vary in their behavioral predictability. Together, our data suggest that morphine introduced an unstable environment for POE pups.

Similar patterns of fragmentation and entropy have been reported in models of chronic early life adversity (ELA) and are associated with negative affect in offspring. Low bedding and nesting (LBN) stress is a model of simulated poverty that alters maternal care and reduces offspring engagement with sucrose and social rewards (Molet et al., 2016). LBN-induced unpredictability also reduces response bias during probabilistic reward task, which is predictive of anhedonia in rodents, nonhuman primates, and humans (Kangas et al., 2022). Stress-related

CeA activation increased in LBN offspring during social play, illustrating that fragmented and unpredictable care alters the encoding of naturally rewarding stimuli (Bolton et al., 2018; Fraser et al., 2024). Unstable bonding between rat pups and their mothers may lead to deficits in social bonding later in life.

Repeated cross-fostering, another model of ELA, destabilizes maternal care and increases sensitivity to aversive stimuli and FST immobility in offspring. These changes were associated with reduced dopamine signaling in the mPFC (Ventura et al., 2013). Unstable maternal care may also contribute to our observed changes in acute stress response. Although we did not observe changes in FST immobility, CORT levels induced by FST were elevated in POE males.

Importantly, patterns of maternal care are rewarding for offspring and provide opportunities to hone neural circuits implicated in prediction error during early development. We believe that our observations of fragmented and unpredictable maternal behavior by POE dams support our model's clinical relevance. NOWS severity and hospitalization length are associated with both higher stress in mothers and dysfunction in parent-child bonding (Bakhireva et al., 2019). This bonding at early ages is crucial for cognitive development, and humans and rats exposed to unpredictable maternal care exhibit poor cognitive performance (E. P. Davis et al., 2017). Unpredictable maternal sensory signaling (auditory, visual, tactile) is also associated with reduced effortful control, or capacity for emotional and behavioral regulation, in five-year-old children (Holmberg et al., 2022). Further, self-reported childhood experiences of unpredictable parenting consistently predicted symptoms of depression, anxiety, and anhedonia in adults (Spadoni et al., 2022). These studies have helped shift clinical recommendations for families experiencing opioid dependency and NOWS towards increasing mother-child bonding by rooming dyads together, increasing skin-to-skin contact, and swaddling (Atwood et al., 2016; Holmes et al., 2016; MacMillan et al., 2018; Patrick et al., 2016). Rescue of dam-pup bonding in our POE rats may serve to be protective against the changes in reward and aversion behaviors that we have so far reported. Measuring stress levels and reward response in the dams may also elucidate a therapeutic target for their behavior. Glucocorticoid or mineralocorticoid receptor-mediated suppression is a clinical treatment for stress that may relieve hypothetically high baseline HPA-axis activity in dams, although mixed results have thus far been reported (Barroca et al., 2021).

Alternatively, OT has been strongly implicated in maternal behavior and may also provide a therapeutic option (Lesse et al., 2017). Increases in maternal mesolimbic circuit activity produced by offspring suckling are blocked by OT antagonism (Febo et al., 2005). This is likely associated with dopamine release during these interactions (Byrnes et al., 2002; Hansen et al., 1993). This suggests that administration of OT in our POE rats during periods of reduced nursing may increase this behavior. Further, OT administration in POE offspring may alleviate symptoms of anhedonia produced by drug withdrawal and fragmented maternal care (Ebner et al., 2005).

4.7 Future directions to improve outcomes for children born to opioid using moms pre- and post-birth

Clinicians report success following the implementation of the “Eat, Sleep, Console” method that encourages consistent bonding experiences between mother and child (Blount et al., 2019). Breastfeeding is continued despite ongoing maternal opioid maintenance therapies which, in part, supplements pharmacological intervention for offspring (Pritham, 2013). These practices have been shown to shorten the length of hospitalization and promote an environment of positivity that reduces maternal stress and fights societal stigmas surrounding NOWS diagnosis (Abdel-Latif et al., 2006; Demirci et al., 2015; Kocherlakota, 2014; Short et al., 2016; Welle-Strand et al., 2013). Ultimately, improving maternal care is a successful therapeutic route that should receive more attention. Clinical and preclinical data, including our own, demonstrates that failure to provide adequate rearing increases the likelihood of depression, anxiety, and stress later in life.

Specific targeting of stress and depression with OT is a promising avenue for improving parent-child bonding and post-infancy anhedonia. Low peripartum levels of OT have been reported in new mothers that show dampened reward circuit activation in response to infant cues (Strathearn et al., 2009). Intranasal OT improves offspring protective behaviors in mothers with postpartum depression, but its effectiveness in treating negative mood remains unclear (Mah et al., 2015; Thul et al., 2020). Plasma OT is also reduced in drug-addicted mothers who exhibit low attachment scores with their newborns, further supporting OT therapy as particularly beneficial for opioid-using mothers (Light et al., 2004). Last, OT treatment reduces symptoms of opioid withdrawal, as well as withdrawal-associated depression and anxiety, when used in combination with group therapy (Naderi et al., 2020). Therefore, OT may also be rehabilitative for mothers undergoing maintenance therapy by attenuating drug craving during and after pregnancy and improving maternal-infant bond. Supplemental OT therapy may also be useful to NOWS patients experiencing depression, as plasma levels are negatively correlated with anxiety and depression severity in patients with major depressive disorder (MDD) (Scantamburlo et al., 2007).

The effectiveness of OT in treating drug- and stress-induced negative affect is supported by preclinical data. Both acute and chronic OT administration effectively reduce stress responses in rats (Manbeck et al., 2014; Windle et al., 1997, 2004), but its potential use in reversing behaviors modified by gestational opioid exposure is unknown. There is evidence that OT limits drug use in rats by engaging mesolimbic dopamine circuitry. For example, intra-NAc injection of OT reduces the rewarding effects of morphine, while systemic OT antagonist administration augments morphine tolerance (Kovács et al., 1984; Sarnyai et al., 1988). ICV OT also reduces alcohol self-administration by blocking VTA-NAc dopamine signaling (S. T. Peters et al., 2017). Given this evidence, exploring whether OT can mitigate the long-term behavioral and neurochemical alterations induced by perinatal opioid exposure represents a promising avenue for future research. Understanding these mechanisms could pave the way for developing novel OT-based interventions to address substance use disorders and their intergenerational consequences.

The need for more clinical studies focused on the long-term outcomes associated with neonatal opioid withdrawal syndrome is abundantly clear. To date, analysis of drug and alcohol use in offspring of opioid-using mothers is virtually nonexistent. The difficulties in maintaining longitudinal studies with NOWS patients pose an obvious challenge; however, growing attention brought on by rising opioid-related deaths in the United States has generated momentum in building larger cohorts of NOWS patients. Improvements in ICU outcomes for newborns in recent years are tied to both standardization of nonpharmacological protocols and increased follow-up care for families (Jilani & Giroir, 2020). Prioritizing long-term research efforts and leveraging these advances will provide critical insights into the developmental trajectories of NOWS patients. Such studies are essential for informing evidence-based interventions aimed at mitigating the intergenerational impact of opioid use disorders.

REFERENCES

Abdel-Latif, M. E., Pinner, J., Clews, S., Cooke, F., Lui, K., & Oei, J. (2006). Effects of breast milk on the severity and outcome of neonatal abstinence syndrome among infants of drug-dependent mothers. *Pediatrics*, *117*(6), e1163-1169. https://doi.org/10.1542/peds.2005-1561

ACOG Committee on Health Care for Underserved Women & American Society of Addiction Medicine. (2012). ACOG Committee Opinion No. 524: Opioid abuse, dependence, and addiction in pregnancy. *Obstetrics and Gynecology*, *119*(5), 1070–1076. https://doi.org/10.1097/AOG.0b013e318256496e

Adhikary, S., & Williams, J. T. (2022). Cellular Tolerance Induced by Chronic Opioids in the Central Nervous System. *Frontiers in Systems Neuroscience*, *16*. https://doi.org/10.3389/fnsys.2022.937126

Agirregoitia, E., Peralta, L., Mendoza, R., Expósito, A., Ereño, E. D., Matorras, R., & Agirregoitia, N. (2012). Expression and localization of opioid receptors during the maturation of human oocytes. *Reproductive BioMedicine Online*, *24*(5), 550–557. https://doi.org/10.1016/j.rbmo.2012.02.007

Ahmadalipour, A., Ghodrati-Jaldbakhan, S., Samaei, S. A., & Rashidy-Pour, A. (2018). Deleterious effects of prenatal exposure to morphine on the spatial learning and hippocampal BDNF and long-term potentiation in juvenile rats: Beneficial influences of postnatal treadmill exercise and enriched environment. *Neurobiology of Learning and Memory*, *147*, 54–64. https://doi.org/10.1016/j.nlm.2017.11.013

Ahmadalipour, A., & Rashidy-Pour, A. (2015). Effects of treadmill running exercise during the adolescent period of life on behavioral deficits in juvenile rats induced by prenatal morphine exposure. *Physiology & Behavior*, *139*, 26–33. https://doi.org/10.1016/j.physbeh.2014.10.038

Alcaro, A., Huber, R., & Panksepp, J. (2007). Behavioral Functions of the Mesolimbic Dopaminergic System: An Affective Neuroethological Perspective. *Brain Research Reviews*, *56*(2), 283–321. https://doi.org/10.1016/j.brainresrev.2007.07.014

Alipio, J. B., Brockett, A. T., Fox, M. E., Tennyson, S. S., deBettencourt, C. A., El-Metwally, D., Francis, N. A., Kanold, P. O., Lobo, M. K., Roesch, M. R., & Keller, A. (2021). Enduring consequences of perinatal fentanyl exposure in mice. *Addiction Biology*, *26*(2), e12895. https://doi.org/10.1111/adb.12895

Alipio, J. B., Haga, C., Fox, M. E., Arakawa, K., Balaji, R., Cramer, N., Lobo, M. K., & Keller, A. (2021). Perinatal Fentanyl Exposure Leads to Long-Lasting Impairments in Somatosensory Circuit Function and Behavior. *The Journal of Neuroscience*, *41*(15), 3400–3417. https://doi.org/10.1523/JNEUROSCI.2470-20.2020

Allain, F., Carter, M., Dumas, S., Darcq, E., & Kieffer, B. L. (2022). The mu opioid receptor and the orphan receptor GPR151 contribute to social reward in the habenula. *Scientific Reports*, *12*(1), 20234. https://doi.org/10.1038/s41598-022-24395-z

Allouche, S., Noble, F., & Marie, N. (2014). Opioid receptor desensitization: Mechanisms and its link to tolerance. *Frontiers in Pharmacology*, *5*, 280. https://doi.org/10.3389/fphar.2014.00280

Anand, K. J. S., Anderson, B. J., Holford, N. H. G., Hall, R. W., Young, T., Shephard, B., Desai, N. S., & Barton, B. A. (2008). Morphine pharmacokinetics and pharmacodynamics in preterm and term neonates: Secondary results from the NEOPAIN trial. *BJA: British Journal of Anaesthesia*, *101*(5), 680. https://doi.org/10.1093/bja/aen248

Arttamangkul, S., Heinz, D. A., Bunzow, J. R., Song, X., & Williams, J. T. (2018). Cellular tolerance at the μ-opioid receptor is phosphorylation dependent. *eLife*, *7*, e34989. https://doi.org/10.7554/eLife.34989

Atwood, E. C., Sollender, G., Hsu, E., Arsnow, C., Flanagan, V., Celenza, J., Whalen, B., & Holmes, A. V. (2016). A Qualitative Study of Family Experience With Hospitalization for Neonatal Abstinence Syndrome. *Hospital Pediatrics*, *6*(10), 626–632. https://doi.org/10.1542/hpeds.2016-0024

Atzil, S., Touroutoglou, A., Rudy, T., Salcedo, S., Feldman, R., Hooker, J. M., Dickerson, B. C., Catana, C., & Barrett, L. F. (2017). Dopamine in the medial amygdala network mediates human bonding. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(9), 2361–2366. https://doi.org/10.1073/pnas.1612233114

Auger, N., Rhéaume, M.-A., Low, N., Lee, G. E., Ayoub, A., & Luu, T. M. (2020). Impact of Prenatal Exposure to Opioids, Cocaine, and Cannabis on Eye Disorders in Children. *Journal of Addiction Medicine*, *14*(6), 459–466. https://doi.org/10.1097/ADM.0000000000000621

Avena, N. M., Rada, P., Moise, N., & Hoebel, B. G. (2006). Sucrose sham feeding on a binge schedule releases accumbens dopamine repeatedly and eliminates the acetylcholine satiety response. *Neuroscience*, *139*(3), 813–820. https://doi.org/10.1016/j.neuroscience.2005.12.037

Azuine, R. E., Ji, Y., Chang, H.-Y., Kim, Y., Ji, H., DiBari, J., Hong, X., Wang, G., Singh, G. K., Pearson, C., Zuckerman, B., Surkan, P. J., & Wang, X. (2019). Prenatal Risk Factors and Perinatal and Postnatal Outcomes Associated With Maternal Opioid Exposure in an Urban, Low-Income, Multiethnic US Population. *JAMA Network Open*, *2*(6), e196405. https://doi.org/10.1001/jamanetworkopen.2019.6405

Bach, P., Koopmann, A., & Kiefer, F. (2021). The Impact of Appetite-Regulating Neuropeptide Leptin on Alcohol Use, Alcohol Craving and Addictive Behavior: A Systematic Review of Preclinical and Clinical Data. *Alcohol and Alcoholism*, *56*(2), 149–165. https://doi.org/10.1093/alcalc/agaa044

Baik, J.-H. (2013). Dopamine Signaling in reward-related behaviors. *Frontiers in Neural Circuits*, *7*, 152. https://doi.org/10.3389/fncir.2013.00152

Bailly, J., Allain, F., Schwartz, E., Tirel, C., Dupuy, C., Petit, F., Diana, M. A., Darcq, E., & Kieffer, B. L. (2023). Habenular neurons expressing mu opioid receptors promote negative affect in a projection-specific manner. *Biological Psychiatry*, *93*(12), 1108–1117. https://doi.org/10.1016/j.biopsych.2022.09.013

Bakhireva, L. N., Holbrook, B. D., Shrestha, S., Leyva, Y., Ashley, M., Cano, S., Lowe, J., Stephen, J. M., & Leeman, L. (2019). Association between prenatal opioid exposure, neonatal opioid withdrawal syndrome, and neurodevelopmental and behavioral outcomes at 5-8 months of age. *Early Human Development*, *128*, 69–76. https://doi.org/10.1016/j.earlhumdev.2018.10.010

Bakken, K., Landheim, A. S., & Vaglum, P. (2007). Axis I and II disorders as long-term predictors of mental distress: A six-year prospective follow-up of substance-dependent patients. *BMC Psychiatry*, *7*, 29. https://doi.org/10.1186/1471-244X-7-29

Baldini, S., Restani, L., Baroncelli, L., Coltelli, M., Franco, R., Cenni, M. C., Maffei, L., & Berardi, N. (2013). Enriched Early Life Experiences Reduce Adult Anxiety-Like Behavior in Rats: A Role for Insulin-Like Growth Factor 1. *The Journal of Neuroscience*, *33*(28), 11715–11723. https://doi.org/10.1523/JNEUROSCI.3541-12.2013

Barbano, M. F., Wang, H.-L., Zhang, S., Miranda-Barrientos, J., Estrin, D. J., Figueroa-González, A., Liu, B., Barker, D. J., & Morales, M. (2020). VTA glutamatergic neurons mediate innate defensive behaviors. *Neuron*, *107*(2), 368-382.e8. https://doi.org/10.1016/j.neuron.2020.04.024

Barroca, N. C. B., Baes, C. V. W., Martins-Monteverde, C. M. S., Bosaipo, N. B., Umeoka, M. S. da S., Tejada, J., Antunes-Rodrigues, J., Castro, M. de, Juruena, M. F., Garcia-Cairasco, N., & Umeoka, E. H. de L. (2021). Evaluation of the HPA Axis' Response to Pharmacological Challenges in Experimental and Clinical Early-Life Stress-Associated Depression. *eNeuro*, *8*(1). https://doi.org/10.1523/ENEURO.0222-20.2020

Barson, J. R., Carr, A. J., Soun, J. E., Sobhani, N. C., Leibowitz, S. F., & Hoebel, B. G. (2009). Opioids in the nucleus accumbens stimulate ethanol intake. *Physiology & Behavior*, *98*(4), 453–459. https://doi.org/10.1016/j.physbeh.2009.07.012

Bartus, R. T., Emerich, D. F., Hotz, J., Blaustein, M., Dean, R. L., Perdomo, B., & Basile, A. S. (2003). Vivitrex®, an Injectable, Extended-Release Formulation of Naltrexone, Provides Pharmacokinetic and Pharmacodynamic Evidence of Efficacy for 1 Month in Rats.

Neuropsychopharmacology, *28*(11), 1973–1982. https://doi.org/10.1038/sj.npp.1300274

Beatty, W. W., & Costello, K. B. (1982). Naloxone and play fighting in juvenile rats. *Pharmacology, Biochemistry, and Behavior*, *17*(5), 905–907. https://doi.org/10.1016/0091-3057(82)90470-1

Becker, L., Mallien, A. S., Pfeiffer, N., Brandwein, C., Talbot, S. R., Bleich, A., Palme, R., Potschka, H., & Gass, P. (2023). Evidence-based severity assessment of the forced swim test in the rat. *PLOS ONE*, *18*(10). https://doi.org/10.1371/journal.pone.0292816

Beckwith, A. M., & Burke, S. A. (2015). Identification of Early Developmental Deficits in Infants With Prenatal Heroin, Methadone, and Other Opioid Exposure. *Clinical Pediatrics*, *54*(4), 328–335. https://doi.org/10.1177/0009922814549545

Belcheva, M. M., Bohn, L. M., Ho, M. T., Johnson, F. E., Yanai, J., Barron, S., & Coscia, C. J. (1998). Brain opioid receptor adaptation and expression after prenatal exposure to buprenorphine. *Brain Research. Developmental Brain Research*, *111*(1), 35–42. https://doi.org/10.1016/s0165-3806(98)00117-5

Belknap, J. K. (1989). Components of the opioid withdrawal syndrome in mice are thermoregulatory responses. *Pharmacology Biochemistry and Behavior*, *34*(2), 241–245. https://doi.org/10.1016/0091-3057(89)90306-7

Bergamini, G., Cathomas, F., Auer, S., Sigrist, H., Seifritz, E., Patterson, M., Gabriel, C., & Pryce, C. R. (2016). Mouse psychosocial stress reduces motivation and cognitive function in operant reward tests: A model for reward pathology with effects of agomelatine. *European Neuropsychopharmacology: The Journal of the European College of*

Neuropsychopharmacology, *26*(9), 1448–1464. https://doi.org/10.1016/j.euroneuro.2016.06.009

Bergamini, G., Mechtersheimer, J., Azzinnari, D., Sigrist, H., Buerge, M., Dallmann, R., Freije, R., Kouraki, A., Opacka-Juffry, J., Seifritz, E., Ferger, B., Suter, T., & Pryce, C. R. (2018). Chronic social stress induces peripheral and central immune activation, blunted mesolimbic dopamine function, and reduced reward-directed behaviour in mice. *Neurobiology of Stress*, *8*, 42–56. https://doi.org/10.1016/j.ynstr.2018.01.004

Berretta, S., Pantazopoulos, H., Caldera, M., Pantazopoulos, P., & Paré, D. (2005). Infralimbic cortex activation increases c-Fos expression in intercalated neurons of the amygdala. *Neuroscience*, *132*(4), 943–953. https://doi.org/10.1016/j.neuroscience.2005.01.020

Berridge, K. C. (1996). Food reward: Brain substrates of wanting and liking. *Neuroscience and Biobehavioral Reviews*, *20*(1), 1–25. https://doi.org/10.1016/0149-7634(95)00033-b

Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: Microstructure of affective taste reactivity patterns. *Neuroscience and Biobehavioral Reviews*, *24*(2), 173–198. https://doi.org/10.1016/s0149-7634(99)00072-x

Bertino, M., Beauchamp, G. K., & Engelman, K. (1991). Naltrexone, an opioid blocker, alters taste perception and nutrient intake in humans. *The American Journal of Physiology*, *261*(1 Pt 2), R59-63. https://doi.org/10.1152/ajpregu.1991.261.1.R59

Bhat, R., Chari, G., Gulati, A., Aldana, O., Velamati, R., & Bhargava, H. (1990). Pharmacokinetics of a single dose of morphine in preterm infants during the first week of life. *The Journal of Pediatrics*, *117*(3), 477–481. https://doi.org/10.1016/s0022-3476(05)81102-3

Bhat, R., Chari, G., & Rao, R. (2006). Effects of prenatal cocaine, morphine, or both on postnatal opioid (mu) receptor development. *Life Sciences*, *78*(13), 1478–1482. https://doi.org/10.1016/j.lfs.2005.07.023

Bielajew, C., & Shizgal, P. (1980). Dissociation of the substrates for medial forebrain bundle self-stimulation and stimulation-escape using a two-electrode stimulation technique. *Physiology & Behavior*, *25*(5), 707–711. https://doi.org/10.1016/0031-9384(80)90373-x

Bienkowski, P., Kostowski, W., & Koros, E. (1999). Ethanol-reinforced behaviour in the rat: Effects of naltrexone. *European Journal of Pharmacology*, *374*(3), 321–327. https://doi.org/10.1016/S0014-2999(99)00245-9

Bischoff, A. R., Dalle Molle, R., Mucellini, A. B., Pokhvisneva, I., Levitan, R. D., Meaney, M. J., & Silveira, P. P. (2024). Accumbal μ-opioid receptors and salt taste-elicited hedonic responses in a rodent model of prenatal adversity, and their correlates using human functional genomics. *Stress*, *27*(1), 2294954. https://doi.org/10.1080/10253890.2023.2294954

Blatchford, K. E., Diamond, K., Westbrook, R. F., & McNally, G. P. (2005). Increased vulnerability to stress following opiate exposures: Behavioral and autonomic correlates. *Behavioral Neuroscience*, *119*(4), 1034–1041. https://doi.org/10.1037/0735-7044.119.4.1034

Blount, T., Painter, A., Freeman, E., Grossman, M., & Sutton, A. G. (2019). Reduction in Length of Stay and Morphine Use for NAS With the "Eat, Sleep, Console" Method. *Hospital Pediatrics*, *9*(8), 615–623. https://doi.org/10.1542/hpeds.2018-0238

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

Bogen, D. L., & Whalen, B. L. (2019). Breastmilk feeding for mothers and infants with opioid exposure: What is best? *Seminars in Fetal & Neonatal Medicine*, *24*(2), 95–104. https://doi.org/10.1016/j.siny.2019.01.001

Bogen, D. L., Whalen, B. L., Kair, L. R., Vining, M., & King, B. A. (2017). Wide Variation Found in Care of Opioid-Exposed Newborns. *Academic Pediatrics*, *17*(4), 374–380. https://doi.org/10.1016/j.acap.2016.10.003

Boggess, T., Williamson, J. C., Niebergall, E. B., Sexton, H., Mazur, A., Egleton, R. D., Grover, L. M., & Risher, W. C. (2021). Alterations in Excitatory and Inhibitory Synaptic Development Within the Mesolimbic Dopamine Pathway in a Mouse Model of Prenatal Drug Exposure. *Frontiers in Pediatrics*, *9*, 794544. https://doi.org/10.3389/fped.2021.794544

Bohn, L. M., Gainetdinov, R. R., Lin, F. T., Lefkowitz, R. J., & Caron, M. G. (2000). Mu-opioid receptor desensitization by beta-arrestin-2 determines morphine tolerance but not dependence. *Nature*, *408*(6813), 720–723. https://doi.org/10.1038/35047086

Bolton, J. L., Molet, J., Regev, L., Chen, Y., Rismanchi, N., Haddad, E., Yang, D. Z., Obenaus, A., & Baram, T. Z. (2018). Anhedonia following early-life adversity involves aberrant interaction of reward and anxiety circuits and is reversed by partial silencing of amygdala corticotropin-releasing hormone gene. *Biological Psychiatry*, *83*(2), 137–147. https://doi.org/10.1016/j.biopsych.2017.08.023

Bonson, K. R., Grant, S. J., Contoreggi, C. S., Links, J. M., Metcalfe, J., Weyl, H. L., Kurian, V., Ernst, M., & London, E. D. (2002). Neural systems and cue-induced cocaine craving. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *26*(3), 376–386. https://doi.org/10.1016/S0893-133X(01)00371-2

Borgland, S. L., Connor, M., Osborne, P. B., Furness, J. B., & Christie, M. J. (2003). Opioid agonists have different efficacy profiles for G protein activation, rapid desensitization, and endocytosis of mu-opioid receptors. *The Journal of Biological Chemistry*, *278*(21), 18776–18784. https://doi.org/10.1074/jbc.M300525200

Bossert, J. M., Stern, A. L., Theberge, F. R. M., Marchant, N. J., Wang, H.-L., Morales, M., & Shaham, Y. (2012). Role of Projections from Ventral Medial Prefrontal Cortex to Nucleus Accumbens Shell in Context-Induced Reinstatement of Heroin Seeking. *Journal of Neuroscience*, *32*(14), 4982–4991. https://doi.org/10.1523/JNEUROSCI.0005-12.2012

Boulos, L. J., Ben Hamida, S., Bailly, J., Maitra, M., Ehrlich, A. T., Gavériaux-Ruff, C., Darcq, E., & Kieffer, B. L. (2020). Mu opioid receptors in the medial habenula contribute to naloxone aversion. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *45*(2), 247–255. https://doi.org/10.1038/s41386-019-0395-7

Boulos, L.-J., Darcq, E., & Kieffer, B. L. (2017). Translating the Habenula-From Rodents to Humans. *Biological Psychiatry*, *81*(4), 296–305. https://doi.org/10.1016/j.biopsych.2016.06.003

Bowers, M. E., & Yehuda, R. (2016). Intergenerational Transmission of Stress in Humans. *Neuropsychopharmacology*, *41*(1), 232–244. https://doi.org/10.1038/npp.2015.247

Bozarth, M. A. (1987). Neuroanatomical boundaries of the reward-relevant opiate-receptor field in the ventral tegmental area as mapped by the conditioned place preference method in rats. *Brain Research*, *414*(1), 77–84. https://doi.org/10.1016/0006-8993(87)91327-8

Britt, J. P., Benaliouad, F., McDevitt, R. A., Stuber, G. D., Wise, R. A., & Bonci, A. (2012). Synaptic and Behavioral Profile of Multiple Glutamatergic Inputs to the Nucleus Accumbens. *Neuron*, *76*(4), 790–803. https://doi.org/10.1016/j.neuron.2012.09.040

Bruchas, M. R., Land, B. B., & Chavkin, C. (2010). The Dynorphin-Kappa Opioid System as a Modulator of Stress-induced and Pro-addictive Behaviors. *Brain Research*, *1314C*, 44. https://doi.org/10.1016/j.brainres.2009.08.062

Buisman-Pijlman, F. T. A., Gerrits, M. a. F. M., & Van Ree, J. M. (2009). Increased opioid release in specific brain areas in animals exposed to prenatal morphine and emotional stress later in life. *Neuroscience*, *159*(1), 405–413. https://doi.org/10.1016/j.neuroscience.2008.11.010

Bulgin, D., Patrick, S. W., McElroy, T., McNeer, E., Dupont, W. D., & Murry, V. M. (2023). Patient and Community Factors Affecting Treatment Access for Opioid Use Disorder. *Obstetrics and Gynecology*, *142*(2), 339–349. https://doi.org/10.1097/AOG.0000000000005227

Burkett, J. P., Andari, E., Johnson, Z. V., Curry, D. C., de Waal, F. B. M., & Young, L. J. (2016). Oxytocin-dependent consolation behavior in rodents. *Science*, *351*(6271), 375–378. https://doi.org/10.1126/science.aac4785

Byrnes, E. M. (2005). Transgenerational consequences of adolescent morphine exposure in female rats: Effects on anxiety-like behaviors and morphine sensitization in adult offspring. *Psychopharmacology*, *182*(4), 537–544. https://doi.org/10.1007/s00213-005-0122-4

Byrnes, E. M., Rigero, B. A., & Bridges, R. S. (2002). Dopamine antagonists during parturition disrupt maternal care and the retention of maternal behavior in rats. *Pharmacology, Biochemistry, and Behavior*, *73*(4), 869–875. https://doi.org/10.1016/s0091-3057(02)00941-3

Byrnes, E. M., & Vassoler, F. M. (2018). Modeling prenatal opioid exposure in animals: Current findings and future directions. *Frontiers in Neuroendocrinology*, *51*, 1–13. https://doi.org/10.1016/j.yfrne.2017.09.001

Cadoret, R. J., Troughton, E., O'Gorman, T. W., & Heywood, E. (1986). An Adoption Study of Genetic and Environmental Factors in Drug Abuse. *Archives of General Psychiatry*, *43*(12), 1131–1136. https://doi.org/10.1001/archpsyc.1986.01800120017004

Cai, J., & Tong, Q. (2022). Anatomy and Function of Ventral Tegmental Area Glutamate Neurons. *Frontiers in Neural Circuits*, *16*, 867053. https://doi.org/10.3389/fncir.2022.867053

Canonica, T., & Zalachoras, I. (2022). Motivational disturbances in rodent models of neuropsychiatric disorders. *Frontiers in Behavioral Neuroscience*, *16*, 940672. https://doi.org/10.3389/fnbeh.2022.940672

Carlsson, A., Falck, B., & Hillarp, N. A. (1962). Cellular localization of brain monoamines. *Acta Physiologica Scandinavica. Supplementum*, *56*(196), 1–28.

Carvalho, M., Morais-Silva, G., Caixeta, G. A. B., Marin, M. T., & Amaral, V. C. S. (2022). Alcohol Deprivation Differentially Changes Alcohol Intake in Female and Male Rats Depending on Early-Life Stressful Experience. *NeuroSci*, *3*(2), 214–225. https://doi.org/10.3390/neurosci3020016

Castro, D. C., & Berridge, K. C. (2014). Opioid Hedonic Hotspot in Nucleus Accumbens Shell: Mu, Delta, and Kappa Maps for Enhancement of Sweetness “Liking” and “Wanting.” *The Journal of Neuroscience*, *34*(12), 4239–4250. https://doi.org/10.1523/JNEUROSCI.4458-13.2014

Castro, N. C. F., Silva, I. S., Cartágenes, S. C., Fernandes, L. M. P., Ribera, P. C., Barros, M. A., Prediger, R. D., Fontes-Júnior, E. A., & Maia, C. S. F. (2021). Morphine Perinatal Exposure Induces Long-Lasting Negative Emotional States in Adult Offspring Rodents. *Pharmaceutics*, *14*(1), 29. https://doi.org/10.3390/pharmaceutics14010029

Chaffin, M., Kelleher, K., & Hollenberg, J. (1996). Onset of physical abuse and neglect: Psychiatric, substance abuse, and social risk factors from prospective community data. *Child Abuse & Neglect*, *20*(3), 191–203. https://doi.org/10.1016/S0145-2134(95)00144-1

Charles, M. K., Cooper, W. O., Jansson, L. M., Dudley, J., Slaughter, J. C., & Patrick, S. W. (2017). Male Sex Associated With Increased Risk of Neonatal Abstinence Syndrome. *Hospital Pediatrics*, *7*(6), 328–334. https://doi.org/10.1542/hpeds.2016-0218

Chen, H.-H., Chiang, Y.-C., Yuan, Z. F., Kuo, C.-C., Lai, M.-D., Hung, T.-W., Ho, I., & Chen, S.-T. (2015). Buprenorphine, methadone, and morphine treatment during pregnancy: Behavioral effects on the offspring in rats. *Neuropsychiatric Disease and Treatment*, *11*, 609–618. https://doi.org/10.2147/NDT.S70585

Chen, Y., Du, M., Kang, N., Guan, X., Liang, B., Chen, Z., & Zhang, J. (2022). Prenatal Morphine Exposure Differentially Alters Addictive and Emotional Behavior in Adolescent and Adult Rats in a Sex-Specific Manner. *Neurochemical Research*, *47*(8), 2317–2332. https://doi.org/10.1007/s11064-022-03619-8

Chen, Y. I., Choi, J.-K., Xu, H., Ren, J., Andersen, S. L., & Jenkins, B. G. (2010). Pharmacologic Neuroimaging of the Ontogeny of Dopamine Receptor Function. *Developmental Neuroscience*, *32*(2), 125–138. https://doi.org/10.1159/000286215

Chen, Y., Wang, X., Xiao, M., Kang, N., Zeng, W., & Zhang, J. (2022). Prenatal morphine exposure increases gamma oscillation and theta coherence in the rat reward system. *Neurotoxicology*, *90*, 246–255. https://doi.org/10.1016/j.neuro.2022.04.007

Chiang, Y.-C., Hung, T.-W., & Ho, I.-K. (2014). Development of sensitization to methamphetamine in offspring prenatally exposed to morphine, methadone and buprenorphine. *Addiction Biology*, *19*(4), 676–686. https://doi.org/vathy

Chiang, Y.-C., Ye, L.-C., Hsu, K.-Y., Liao, C.-W., Hung, T.-W., Lo, W.-J., Ho, I.-K., & Tao, P.-L. (2015). Beneficial effects of co-treatment with dextromethorphan on prenatally methadone-exposed offspring. *Journal of Biomedical Science*, *22*(1), 19. https://doi.org/10.1186/s12929-015-0126-2

Chiou, L.-C., Yeh, G.-C., Fan, S.-H., How, C.-H., Chuang, K.-C., & Tao, P.-L. (2003). Prenatal morphine exposure decreases analgesia but not K+ channel activation. *Neuroreport*, *14*(2), 239–242. https://doi.org/10.1097/00001756-200302100-00016

Christie, M. J. (2008). Cellular neuroadaptations to chronic opioids: Tolerance, withdrawal and addiction. *British Journal of Pharmacology*, *154*(2), 384–396. https://doi.org/10.1038/bjp.2008.100

Cleary, J., Weldon, D. T., O'Hare, E., Billington, C., & Levine, A. S. (1996). Naloxone effects on sucrose-motivated behavior. *Psychopharmacology*, *126*(2), 110–114. https://doi.org/10.1007/BF02246345

Cohen, J. Y., Haesler, S., Vong, L., Lowell, B. B., & Uchida, N. (2012). Neuron-type specific signals for reward and punishment in the ventral tegmental area. *Nature*, *482*(7383), 85–88. https://doi.org/10.1038/nature10754

Colantuoni, C., Rada, P., McCarthy, J., Patten, C., Avena, N. M., Chadeayne, A., & Hoebel, B. G. (2002). Evidence that intermittent, excessive sugar intake causes endogenous opioid dependence. *Obesity Research*, *10*(6), 478–488. https://doi.org/10.1038/oby.2002.66

Colantuoni, C., Schwenker, J., McCarthy, J., Rada, P., Ladenheim, B., Cadet, J. L., Schwartz, G. J., Moran, T. H., & Hoebel, B. G. (2001). Excessive sugar intake alters binding to dopamine and mu-opioid receptors in the brain. *Neuroreport*, *12*(16), 3549–3552. https://doi.org/10.1097/00001756-200111160-00035

Contestabile, A., Villani, L., Fasolo, A., Franzoni, M. F., Gribaudo, L., Oktedalen, O., & Fonnum, F. (1987). Topography of cholinergic and substance P pathways in the habenulo-interpeduncular system of the rat. An immunocytochemical and microchemical approach. *Neuroscience*, *21*(1), 253–270. https://doi.org/10.1016/0306-4522(87)90337-x

Coplan, J. D., Andrews, M. W., Rosenblum, L. A., Owens, M. J., Friedman, S., Gorman, J. M., & Nemeroff, C. B. (1996). Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: Implications for the pathophysiology of mood and anxiety disorders. *Proceedings of the National Academy of Sciences of the United States of America*, *93*(4), 1619–1623.

Coyle, J. T., & Pert, C. B. (1976). Ontogenetic development of [3H]naloxone binding in rat brain. *Neuropharmacology*, *15*(9), 555–560. https://doi.org/10.1016/0028-3908(76)90107-6

Cruz, A. de M., Maiorka, P. C., Canteras, N. S., Sukikara, M. H., & Felicio, L. F. (2010). Morphine treatment during pregnancy modulates behavioral selection in lactating rats. *Physiology & Behavior*, *101*(1), 40–44. https://doi.org/10.1016/j.physbeh.2010.04.013

Cuitavi, J., Lorente, J. D., Campos-Jurado, Y., Polache, A., & Hipólito, L. (2021). Neuroimmune and Mu-Opioid Receptor Alterations in the Mesocorticolimbic System in a Sex-Dependent Inflammatory Pain-Induced Alcohol Relapse-Like Rat Model. *Frontiers in Immunology*, *12*, 689453. https://doi.org/10.3389/fimmu.2021.689453

Dahlstroem, A., & Fuxe, K. (1964). EVIDENCE FOR THE EXISTENCE OF MONOAMINE-CONTAINING NEURONS IN THE CENTRAL NERVOUS SYSTEM. I. DEMONSTRATION OF MONOAMINES IN THE CELL BODIES OF BRAIN STEM NEURONS. *Acta Physiologica Scandinavica. Supplementum*, SUPPL 232:1-55.

Davies, M. (2003). The role of GABAA receptors in mediating the effects of alcohol in the central nervous system. *Journal of Psychiatry and Neuroscience*, *28*(4), 263–274.

Davis, E. P., Stout, S. A., Molet, J., Vegetabile, B., Glynn, L. M., Sandman, C. A., Heins, K., Stern, H., & Baram, T. Z. (2017). Exposure to unpredictable maternal sensory signals influences cognitive development across species. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(39), 10390–10395. https://doi.org/10.1073/pnas.1703444114

Davis, J. M., Shenberger, J., Terrin, N., Breeze, J. L., Hudak, M., Wachman, E. M., Marro, P., Oliveira, E. L., Harvey-Wilkes, K., Czynski, A., Engelhardt, B., D'Apolito, K., Bogen, D., & Lester, B. (2018). Comparison of Safety and Efficacy of Methadone vs Morphine for Treatment of Neonatal Abstinence Syndrome. *JAMA Pediatrics*, *172*(8), 741–748. https://doi.org/10.1001/jamapediatrics.2018.1307

de Cubas, M. M., & Field, T. (1993). Children of methadone-dependent women: Developmental outcomes. *The American Journal of Orthopsychiatry*, *63*(2), 266–276. https://doi.org/10.1037/h0079429

Demirci, J. R., Bogen, D. L., & Klionsky, Y. (2015). Breastfeeding and Methadone Therapy: The Maternal Experience. *Substance Abuse*, *36*(2), 203–208. https://doi.org/10.1080/08897077.2014.902417

Der-Avakian, A., & Markou, A. (2012). The Neurobiology of Anhedonia and Other Reward-Related Deficits. *Trends in Neurosciences*, *35*(1), 68–77. https://doi.org/10.1016/j.tins.2011.11.005

Desai, R. J., Hernandez-Diaz, S., Bateman, B. T., & Huybrechts, K. F. (2014). Increase in prescription opioid use during pregnancy among Medicaid-enrolled women. *Obstetrics and Gynecology*, *123*(5), 997–1002. https://doi.org/10.1097/AOG.0000000000000208

Di Chiara, G., & Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America*, *85*(14), 5274–5278. https://doi.org/10.1073/pnas.85.14.5274

Doberczak, T. M., Kandal, S. R., & Wilets, I. (1991). Neonatal opiate abstinence syndrome in term and preterm infants. *The Journal of Pediatrics*, *118*(6), 933–937. https://doi.org/10.1016/S0022-3476(05)82214-0

Ebner, K., Bosch, O. J., Krömer, S. A., Singewald, N., & Neumann, I. D. (2005). Release of Oxytocin in the Rat Central Amygdala Modulates Stress-Coping Behavior and the Release of Excitatory Amino Acids. *Neuropsychopharmacology*, *30*(2), 223–230. https://doi.org/10.1038/sj.npp.1300607

Eikemo, M., Løseth, G. E., Johnstone, T., Gjerstad, J., Willoch, F., & Leknes, S. (2016). Sweet taste pleasantness is modulated by morphine and naltrexone. *Psychopharmacology*, *233*(21), 3711–3723. https://doi.org/10.1007/s00213-016-4403-x

Eiler, W. J. A., Woods, J. E., Masters, J., McKay, P. F., Hardy, L., Goergen, J. J., Mensah-Zoe, B., Cook, J. B., Johnson, N. J., & June, H. L. (2005). Brain stimulation reward performance and sucrose maintained behaviors in alcohol-preferring and -nonpreferring rats. *Alcoholism, Clinical and Experimental Research*, *29*(4), 571–583. https://doi.org/10.1097/01.alc.0000158934.50534.b7

Elam, K. K., Sternberg, A., Waddell, J. T., Blake, A. J., & Chassin, L. (2020). Mother and Father Prescription Opioid Misuse, Alcohol Use Disorder, and Parent Knowledge in Pathways to

Adolescent Alcohol Use. *Journal of Youth and Adolescence*, *49*(8), 1663–1673. https://doi.org/10.1007/s10964-020-01266-2

El-Ghundi, M., O'Dowd, B. F., & George, S. R. (2007). Insights into the Role of Dopamine Receptor Systems in Learning and Memory. *Reviews in the Neurosciences*, *18*(1). https://doi.org/10.1515/REVNEURO.2007.18.1.37

Eriksson, P. S., & Rönnbäck, L. (1989). Effects of prenatal morphine treatment of rats on mortality, bodyweight and analgesic response in the offspring. *Drug and Alcohol Dependence*, *24*(3), 187–194. https://doi.org/10.1016/0376-8716(89)90055-0

Farrington, E. A., McGuinness, G. A., Johnson, G. F., Erenberg, A., & Leff, R. D. (1993). Continuous intravenous morphine infusion in postoperative newborn infants. *American Journal of Perinatology*, *10*(1), 84–87. https://doi.org/10.1055/s-2007-994711

Fassini, A., Scopinho, A. A., Resstel, L. B. M., & Correa, F. M. A. (2014). Opioid receptors in the prelimbic cortex modulate restraint stress-induced cardiovascular responses in the rat. *Neuropharmacology*, *85*, 367–374. https://doi.org/10.1016/j.neuropharm.2014.04.019

Fattore, L., Viganò, D., Fadda, P., Rubino, T., Fratta, W., & Parolaro, D. (2007). Bidirectional regulation of mu-opioid and CB1-cannabinoid receptor in rats self-administering heroin or WIN 55,212-2. *The European Journal of Neuroscience*, *25*(7), 2191–2200. https://doi.org/10.1111/j.1460-9568.2007.05470.x

Febo, M., Numan, M., & Ferris, C. F. (2005). Functional Magnetic Resonance Imaging Shows Oxytocin Activates Brain Regions Associated with Mother–Pup Bonding during Suckling. *Journal of Neuroscience*, *25*(50), 11637–11644. https://doi.org/10.1523/JNEUROSCI.3604-05.2005

Ferguson, J. N., Aldag, J. M., Insel, T. R., & Young, L. J. (2001). Oxytocin in the Medial Amygdala is Essential for Social Recognition in the Mouse. *Journal of Neuroscience*, *21*(20), 8278–8285. https://doi.org/10.1523/JNEUROSCI.21-20-08278.2001

Ferré, S. (2015). The GPCR heterotetramer: Challenging classical pharmacology. *Trends in Pharmacological Sciences*, *36*(3), 145–152. https://doi.org/10.1016/j.tips.2015.01.002

Fill, M.-M. A., Miller, A. M., Wilkinson, R. H., Warren, M. D., Dunn, J. R., Schaffner, W., & Jones, T. F. (2018). Educational Disabilities Among Children Born With Neonatal Abstinence Syndrome. *Pediatrics*, *142*(3), e20180562. https://doi.org/10.1542/peds.2018-0562

Font, L., Luján, M. Á., & Pastor, R. (2013). Involvement of the endogenous opioid system in the psychopharmacological actions of ethanol: The role of acetaldehyde. *Frontiers in Behavioral Neuroscience*, *7*. https://doi.org/10.3389/fnbeh.2013.00093

Fouriezos, G., Hansson, P., & Wise, R. A. (1978). Neuroleptic-induced attenuation of brain stimulation reward in rats. *Journal of Comparative and Physiological Psychology*, *92*(4), 661–671. https://doi.org/10.1037/h0077500

Francis, D. D., Diorio, J., Plotsky, P. M., & Meaney, M. J. (2002). Environmental Enrichment Reverses the Effects of Maternal Separation on Stress Reactivity. *The Journal of Neuroscience*, *22*(18), 7840–7843. https://doi.org/10.1523/JNEUROSCI.22-18-07840.2002

Frasch, A., Zetzsche, T., Steiger, A., & Jirikowski, G. F. (1995). Reduction of plasma oxytocin levels in patients suffering from major depression. *Advances in Experimental Medicine and Biology*, *395*, 257–258.

Fraser, K. M., Kim, T. H., Castro, M., Drieu, C., Padovan-Hernandez, Y., Chen, B., Pat, F., Ottenheimer, D. J., & Janak, P. H. (2024). Encoding and context-dependent control of reward consumption within the central nucleus of the amygdala. *iScience*, *27*(5), 109652. https://doi.org/10.1016/j.isci.2024.109652

Fu, R., Chen, X., Zuo, W., Li, J., Kang, S., Zhou, L.-H., Siegel, A., Bekker, A., & Ye, J.-H. (2016). Ablation of μ opioid receptor-expressing GABA neurons in rostromedial tegmental nucleus increases ethanol consumption and regulates ethanol-related behaviors. *Neuropharmacology*, *107*, 58–67. https://doi.org/10.1016/j.neuropharm.2016.02.027

Fullerton, E. F., Rubaharan, M., Karom, M. C., Hanberry, R. I., & Murphy, A. Z. (2021). Advanced age attenuates the antihyperalgesic effect of morphine and decreases μ-opioid receptor expression and binding in the rat midbrain periaqueductal gray in male and female rats. *Neurobiology of Aging*, *98*, 78–87. https://doi.org/10.1016/j.neurobiolaging.2020.10.020

Gagin, R., Cohen, E., & Shavit, Y. (1996). Prenatal exposure to morphine alters analgesic responses and preference for sweet solutions in adult rats. *Pharmacology, Biochemistry, and Behavior*, *55*(4), 629–634. https://doi.org/10.1016/s0091-3057(96)00278-x

Gagin, R., Kook, N., Cohen, E., & Shavit, Y. (1997). Prenatal morphine enhances morphine-conditioned place preference in adult rats. *Pharmacology, Biochemistry, and Behavior*, *58*(2), 525–528. https://doi.org/10.1016/s0091-3057(97)00281-5

Gamble, M. E., Marfatia, R., & Diaz, M. R. (2022). Prenatal methadone exposure leads to long-term memory impairments and disruptions of dentate granule cell function in a sex-dependent manner. *Addiction Biology*, *27*(5), e13215. https://doi.org/10.1111/adb.13215

Gardon, O., Faget, L., Chu Sin Chung, P., Matifas, A., Massotte, D., & Kieffer, B. L. (2014). Expression of mu opioid receptor in dorsal diencephalic conduction system: New insights for the medial habenula. *Neuroscience*, *277*, 595–609. https://doi.org/10.1016/j.neuroscience.2014.07.053

Gates, M. A., Coupe, V. M., Torres, E. M., Fricker-Gates, R. A., & Dunnett, S. B. (2004). Spatially and temporally restricted chemoattractive and chemorepulsive cues direct the formation of the nigro-striatal circuit. *The European Journal of Neuroscience*, *19*(4), 831–844. https://doi.org/10.1111/j.1460-9568.2004.03213.x

Georges, F., Normand, E., Bloch, B., & Le Moine, C. (1998). Opioid receptor gene expression in the rat brain during ontogeny, with special reference to the mesostriatal system: An in situ hybridization study. *Developmental Brain Research*, *109*(2), 187–199. https://doi.org/10.1016/S0165-3806(98)00082-0

Gerdin, E., Salmonson, T., Lindberg, B., & Rane, A. (1990). Maternal kinetics of morphine during labour. *Journal of Perinatal Medicine*, *18*(6), 479–487. https://doi.org/10.1515/jpme.1990.18.6.479

Gianoulakis, C. (1998). Alcohol-Seeking Behavior. *Alcohol Health and Research World*, *22*(3), 202–210.

Glick, S. D., Strumpf, A. J., & Zimmerberg, B. (1977). Effect of in utero administration of morphine on the subsequent development of self-administration behavior. *Brain Research*, *132*(1), 194–196. https://doi.org/10.1016/0006-8993(77)90720-x

Goeldner, C., Lutz, P.-E., Darcq, E., Halter, T., Clesse, D., Ouagazzal, A.-M., & Kieffer, B. L. (2011). Impaired emotional-like behavior and serotonergic function during protracted abstinence from chronic morphine. *Biological Psychiatry*, *69*(3), 236–244. https://doi.org/10.1016/j.biopsych.2010.08.021

Gómez-Gómez, Y. M., Sánchez-Aparicio, P., Mejía-Chávez, S., García-García, F., Pascual-Mathey, L. I., Aguilera-Reyes, U., Galicia, O., & Venebra-Muñoz, A. (2019). C-Fos immunoreactivity in the hypothalamus and reward system of young rats after social novelty exposure. *Neuroreport*, *30*(7), 510–515. https://doi.org/10.1097/WNR.0000000000001236

Gorio, A., Malosio, M. L., Vergani, L., & Di Giulio, A. M. (1996). Exposure to perinatal morphine promotes developmental changes in rat striatum. *International Journal of Developmental Neuroscience*, *14*(4), 471–479. https://doi.org/10.1016/0736-5748(95)00106-9

Grace, A. A., Floresco, S. B., Goto, Y., & Lodge, D. J. (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends in Neurosciences*, *30*(5), 220–227. https://doi.org/10.1016/j.tins.2007.03.003

Grecco, G. G., & Atwood, B. K. (2020). Prenatal Opioid Exposure Enhances Responsiveness to Future Drug Reward and Alters Sensitivity to Pain: A Review of Preclinical Models and Contributing Mechanisms. *eNeuro*, *7*(6), ENEURO.0393-20.2020. https://doi.org/10.1523/ENEURO.0393-20.2020

Grecco, G. G., Haggerty, D. L., Reeves, K. C., Gao, Y., Maulucci, D., & Atwood, B. K. (2022). Prenatal opioid exposure reprograms the behavioural response to future alcohol reward. *Addiction Biology*, *27*(2), e13136. https://doi.org/10.1111/adb.13136

Grecco, G. G., Muñoz, B., Di Prisco, G. V., Doud, E. H., Fritz, B. M., Maulucci, D., Gao, Y., Mosley, A. L., Baucum, A. J., & Atwood, B. K. (2022). Prenatal Opioid Exposure Impairs Endocannabinoid and Glutamate Transmission in the Dorsal Striatum. *eNeuro*, *9*(2), ENEURO.0119-22.2022. https://doi.org/10.1523/ENEURO.0119-22.2022

Grieder, T. E., Herman, M. A., Contet, C., Tan, L. A., Vargas-Perez, H., Cohen, A., Chwalek, M., Maal-Bared, G., Freiling, J., Schlosburg, J. E., Clarke, L., Crawford, E., Koebel, P., Repunte-Canonigo, V., Sanna, P. P., Tapper, A. R., Roberto, M., Kieffer, B. L., Sawchenko, P. E., … George, O. (2014). VTA CRF neurons mediate the aversive effects of nicotine withdrawal and promote intake escalation. *Nature Neuroscience*, *17*(12), 1751–1758. https://doi.org/10.1038/nn.3872

Griesler, P. C., Hu, M.-C., Wall, M. M., & Kandel, D. B. (2019). Nonmedical Prescription Opioid Use by Parents and Adolescents in the US. *Pediatrics*, *143*(3), e20182354. https://doi.org/10.1542/peds.2018-2354

Grossarth, S., Osmundson, S. S., Wiese, A. D., Phillips, S. E., Pham, A., Leech, A. A., Patrick, S. W., Spieker, A. J., Grijalva, C. G., & Adgent, M. A. (2023). Maternal Opioid Use Disorder and the Risk of Postneonatal Infant Mortality. *JAMA Pediatrics*, *177*(7), 675–683. https://doi.org/10.1001/jamapediatrics.2023.1047

Haber, S. N., Groenewegen, H. J., Grove, E. A., & Nauta, W. J. H. (1985). Efferent connections of the ventral pallidum: Evidence of a dual striato pallidofugal pathway. *Journal of Comparative Neurology*, *235*(3), 322–335. https://doi.org/10.1002/cne.902350304

Hall, E. S., McAllister, J. M., & Wexelblatt, S. L. (2019). Developmental Disorders and Medical Complications Among Infants with Subclinical Intrauterine Opioid Exposures. *Population Health Management*, *22*(1), 19–24. https://doi.org/10.1089/pop.2018.0016

Hamilton, K. L., Harris, A. C., Gewirtz, J. C., Sparber, S. B., & Schrott, L. M. (2005). HPA axis dysregulation following prenatal opiate exposure and postnatal withdrawal. *Neurotoxicology and Teratology*, *27*(1), 95–103. https://doi.org/10.1016/j.ntt.2004.09.004

Hamilton, R., Mulvihill, A., Butler, L., Chow, A., Irving, E., McCulloch, D. L., McNeil, A., Michael, K., Spowart, K. M., Waterson-Wilson, J., & Mactier, H. (2024). Impaired vision in children prenatally exposed to methadone: An observational cohort study. *Eye*, *38*(1), 118–126. https://doi.org/10.1038/s41433-023-02644-3

Handelmann, G. E., & Quirion, R. (1983). Neonatal exposure to morphine increases mu opiate binding in the adult forebrain. *European Journal of Pharmacology*, *94*(3–4), 357–358. https://doi.org/10.1016/0014-2999(83)90429-6

Hanlon, E. C., Baldo, B. A., Sadeghian, K., & Kelley, A. E. (2004). Increases in food intake or food-seeking behavior induced by GABAergic, opioid, or dopaminergic stimulation of the nucleus accumbens: Is it hunger? *Psychopharmacology*, *172*(3), 241–247. https://doi.org/10.1007/s00213-003-1654-0

Hansen, S., Bergvall, A. H., & Nyiredi, S. (1993). Interaction with pups enhances dopamine release in the ventral striatum of maternal rats: A microdialysis study. *Pharmacology, Biochemistry, and Behavior*, *45*(3), 673–676. https://doi.org/10.1016/0091-3057(93)90523-v

Hanson, J. L., Williams, A. V., Bangasser, D. A., & Peña, C. J. (2021). Impact of Early Life Stress on Reward Circuit Function and Regulation. *Frontiers in Psychiatry*, *12*, 744690. https://doi.org/10.3389/fpsyt.2021.744690

Harder, H. J., & Murphy, A. Z. (2019). Early life opioid exposure and potential long-term effects. *Neurobiology of Stress*, *10*, 100156. https://doi.org/10.1016/j.ynstr.2019.100156

Harder, H. J., Searles, C. T., Vogt, M. E., & Murphy, A. Z. (2023). Perinatal opioid exposure leads to decreased social play in adolescent male and female rats: Potential role of oxytocin signaling in brain regions associated with social reward. *Hormones and Behavior*, *153*, 105384. https://doi.org/10.1016/j.yhbeh.2023.105384

Hauser, K. F., & Knapp, P. E. (2018). Opiate Drugs with Abuse Liability Hijack the Endogenous Opioid System to Disrupt Neuronal and Glial Maturation in the Central Nervous System. *Frontiers in Pediatrics*, *5*, 294. https://doi.org/10.3389/fped.2017.00294

Haydari, S., Miladi-Gorji, H., Mokhtari, A., & Safari, M. (2014). Effects of voluntary exercise on anxiety-like behavior and voluntary morphine consumption in rat pups borne from morphine-dependent mothers during pregnancy. *Neuroscience Letters*, *578*, 50–54. https://doi.org/10.1016/j.neulet.2014.06.026

He, X., Bao, Y., Li, Y., & Sui, N. (2010). The effects of morphine at different embryonic ages on memory consolidation and rewarding properties of morphine in day-old chicks. *Neuroscience Letters*, *482*(1), 12–16. https://doi.org/10.1016/j.neulet.2010.06.074

Hernández-Montiel, H. L., Tamariz, E., Sandoval-Minero, M. T., & Varela-Echavarría, A. (2008). Semaphorins 3A, 3C, and 3F in mesencephalic dopaminergic axon pathfinding. *The Journal of Comparative Neurology*, *506*(3), 387–397. https://doi.org/10.1002/cne.21503

Herranz, G. S., Vílchez, M. angeles L., Ledo, J. D., & Sierra, A. M. (2014). Children Born to Heroin-Addicted Mothers: What's the Outcome 25 Years Later? *Journal of Addiction Research & Therapy*, *5*(2), Article 2.

Herz, A. (1997). Endogenous opioid systems and alcohol addiction. *Psychopharmacology*, *129*(2), 99–111. https://doi.org/10.1007/s002130050169

Heyser, C. J., Roberts, A. J., Schulteis, G., & Koob, G. F. (1999). Central administration of an opiate antagonist decreases oral ethanol self-administration in rats. *Alcoholism, Clinical and Experimental Research*, *23*(9), 1468–1476.

Hirai, A. H., Ko, J. Y., Owens, P. L., Stocks, C., & Patrick, S. W. (2021). Neonatal Abstinence Syndrome and Maternal Opioid-Related Diagnoses in the US, 2010-2017. *JAMA*, *325*(2), 146–155. https://doi.org/10.1001/jama.2020.24991

Hodges, T. E., Louth, E. L., Bailey, C. D. C., & McCormick, C. M. (2019). Adolescent social instability stress alters markers of synaptic plasticity and dendritic structure in the medial amygdala and lateral septum in male rats. *Brain Structure & Function*, *224*(2), 643–659. https://doi.org/10.1007/s00429-018-1789-8

Hol, T., Niesink, M., van Ree, J. M., & Spruijt, B. M. (1996). Prenatal exposure to morphine affects juvenile play behavior and adult social behavior in rats. *Pharmacology, Biochemistry, and Behavior*, *55*(4), 615–618. https://doi.org/10.1016/s0091-3057(96)00274-2

Holder, M. K., Hadjimarkou, M. M., Zup, S. L., Blutstein, T., Benham, R. S., McCarthy, M. M., & Mong, J. A. (2010). Methamphetamine facilitates female sexual behavior and enhances neuronal activation in the medial amygdala and ventromedial nucleus of the hypothalamus. *Psychoneuroendocrinology*, *35*(2), 197–208. https://doi.org/10.1016/j.psyneuen.2009.06.005

Holmberg, E., Kataja, E.-L., Davis, E. P., Pajulo, M., Nolvi, S., Lahtela, H., Nordenswan, E., Karlsson, L., Karlsson, H., & Korja, R. (2022). Unpredictable maternal sensory signals in caregiving behavior are associated with child effortful control. *PloS One*, *17*(12), e0279384. https://doi.org/10.1371/journal.pone.0279384

Holmes, A. V., Atwood, E. C., Whalen, B., Beliveau, J., Jarvis, J. D., Matulis, J. C., & Ralston, S. L. (2016). Rooming-In to Treat Neonatal Abstinence Syndrome: Improved Family-Centered Care at Lower Cost. *Pediatrics*, *137*(6), e20152929. https://doi.org/10.1542/peds.2015-2929

Hong, S., Flashner, B., Chiu, M., Hoeve, E. ver, Luz, S., & Bhatnagar, S. (2012). Social isolation in adolescence alters behaviors in the forced swim and sucrose preference tests in female but not in male rats. *Physiology & Behavior*, *105*(2), 269–275. https://doi.org/10.1016/j.physbeh.2011.08.036

Honkanen, A., Vilamo, L., Wegelius, K., Sarviharju, M., Hyytiä, P., & Korpi, E. R. (1996). Alcohol drinking is reduced by a μ1- but not by a δ-opioid receptor antagonist in alcohol-preferring rats. *European Journal of Pharmacology*, *304*(1), 7–13. https://doi.org/10.1016/0014-2999(96)00118-5

Horn, S. R., Roos, L. E., Berkman, E. T., & Fisher, P. A. (2018). Neuroendocrine and immune pathways from pre- and perinatal stress to substance abuse. *Neurobiology of Stress*, *9*, 140–150. https://doi.org/10.1016/j.ynstr.2018.09.004

Hovious, J. R., & Peters, M. A. (1985). Opiate self-administration in adult offspring of methadone-treated female rats. *Pharmacology, Biochemistry, and Behavior*, *22*(6), 949–953. https://doi.org/10.1016/0091-3057(85)90301-6

Hsu, Y.-W. A., Wang, S. D., Wang, S., Morton, G., Zariwala, H. A., Iglesia, H. O. de la, & Turner, E. E. (2014). Role of the Dorsal Medial Habenula in the Regulation of Voluntary Activity, Motor Function, Hedonic State, and Primary Reinforcement. *The Journal of Neuroscience*, *34*(34), 11366. https://doi.org/10.1523/JNEUROSCI.1861-14.2014

Hudak, M. L., Tan, R. C., THE COMMITTEE ON DRUGS, THE COMMITTEE ON FETUS AND NEWBORN, Frattarelli, D. A. C., Galinkin, J. L., Green, T. P., Neville, K. A., Paul, I. M., Van Den Anker, J. N., Papile, L.-A., Baley, J. E., Bhutani, V. K., Carlo, W. A., Cummings, J., Kumar, P., Polin, R. A., Wang, K. S., & Watterberg, K. L. (2012). Neonatal Drug Withdrawal. *Pediatrics*, *129*(2), e540–e560. https://doi.org/10.1542/peds.2011-3212

Hunt, R. W., Tzioumi, D., Collins, E., & Jeffery, H. E. (2008). Adverse neurodevelopmental outcome of infants exposed to opiate in-utero. *Early Human Development*, *84*(1), 29–35. https://doi.org/10.1016/j.earlhumdev.2007.01.013

Hyytiä, P., & Kiianmaa, K. (2001). Suppression of ethanol responding by centrally administered CTOP and naltrindole in AA and Wistar rats. *Alcoholism, Clinical and Experimental Research*, *25*(1), 25–33. https://doi.org/10.1111/j.1530-0277.2001.tb02123.x

Jansson, L. M., Dipietro, J. A., Elko, A., & Velez, M. (2007). Maternal vagal tone change in response to methadone is associated with neonatal abstinence syndrome severity in exposed neonates. *The Journal of Maternal-Fetal & Neonatal Medicine: The Official Journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians*, *20*(9), 677–685. https://doi.org/10.1080/14767050701490327

Jansson, L. M., & Patrick, S. W. (2019). Neonatal Abstinence Syndrome. *Pediatric Clinics of North America*, *66*(2), 353–367. https://doi.org/10.1016/j.pcl.2018.12.006

Jarjour, S., Bai, L., & Gianoulakis, C. (2009). Effect of Acute Ethanol Administration on the Release of Opioid Peptides From the Midbrain Including the Ventral Tegmental Area. *Alcoholism: Clinical and Experimental Research*, *33*(6), 1033–1043. https://doi.org/10.1111/j.1530-0277.2009.00924.x

Jedrzejczak, M. (2005). Family and environmental factors of drug addiction among young recruits. *Military Medicine*, *170*(8), 688–690. https://doi.org/10.7205/milmed.170.8.688

Jennings, J. H., Sparta, D. R., Stamatakis, A. M., Ung, R. L., Pleil, K. E., Kash, T. L., & Stuber, G. D. (2013). Distinct extended amygdala circuits for divergent motivational states. *Nature*, *496*(7444), 224–228. https://doi.org/10.1038/nature12041

Jhou, T. C., Fields, H. L., Baxter, M. G., Saper, C. B., & Holland, P. C. (2009). The rostromedial tegmental nucleus (RMTg), a GABAergic afferent to midbrain dopamine neurons, encodes aversive stimuli and inhibits motor responses. *Neuron*, *61*(5), 786–800. https://doi.org/10.1016/j.neuron.2009.02.001

Ji, H., & Shepard, P. D. (2007). Lateral habenula stimulation inhibits rat midbrain dopamine neurons through a GABA(A) receptor-mediated mechanism. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *27*(26), 6923–6930. https://doi.org/10.1523/JNEUROSCI.0958-07.2007

Jiang, C., Wang, X., Le, Q., Liu, P., Liu, C., Wang, Z., He, G., Zheng, P., Wang, F., & Ma, L. (2021). Morphine coordinates SST and PV interneurons in the prelimbic cortex to disinhibit pyramidal neurons and enhance reward. *Molecular Psychiatry*, *26*(4), 1178–1193. https://doi.org/10.1038/s41380-019-0480-7

Jiang, J., He, X., Wang, M.-Y., & Sui, N. (2011). Early prenatal morphine exposure impairs performance of learning tasks and attenuates in vitro heterosynaptic long-term potentiation of intermediate medial mesopallium in day-old chicks. *Behavioural Brain Research*, *219*(2), 363–366. https://doi.org/10.1016/j.bbr.2010.12.034

Jilani, S. M., & Giroir, B. P. (2020). Neonatal Abstinence Syndrome: Leveraging Health Information Technology to Develop a Data-Driven National Policy Approach. *Public Health Reports*, *135*(2), 173–176. https://doi.org/10.1177/0033354920902476

Johnson, B. A., & Ait-Daoud, N. (2000). Neuropharmacological treatments for alcoholism: Scientific basis and clinical findings. *Psychopharmacology*, *149*(4), 327–344. https://doi.org/10.1007/s002130000371

Johnson, S. W., & North, R. A. (1992). Opioids excite dopamine neurons by hyperpolarization of local interneurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *12*(2), 483–488. https://doi.org/10.1523/JNEUROSCI.12-02-00483.1992

Johnston, L. D., Miech, R. A., O'Malley, P. M., Bachman, J. G., Schulenberg, J. E., & Patrick, M. E. (2020). *Monitoring the Future National Survey Results on Drug Use, 1975-2019: Overview, Key Findings on Adolescent Drug Use*. Institute for Social Research. https://eric.ed.gov/?id=ED604018

Jones, H. E., O'Grady, K. E., Johnson, R. E., Velez, M., & Jansson, L. M. (2010). Infant neurobehavior following prenatal exposure to methadone or buprenorphine: Results from the neonatal intensive care unit network neurobehavioral scale. *Substance Use & Misuse*, *45*(13), 2244–2257. https://doi.org/10.3109/10826084.2010.484474

K. Rehni, A., S. Jaggi, A., & Singh, N. (2013). Opioid Withdrawal Syndrome: Emerging Concepts and Novel Therapeutic Targets. *CNS & Neurological Disorders - Drug Targets*, *12*(1), 112–125. https://doi.org/10.2174/1871527311312010017

Kalamarides, D. J., Singh, A., & Dani, J. A. (2024). Protracted opioid withdrawal behaviors are reduced by nitric oxide inhibition in mice. *Addiction Neuroscience*, *12*, 100167. https://doi.org/10.1016/j.addicn.2024.100167

Kalueff, A. V., & Tuohimaa, P. (2005). The grooming analysis algorithm discriminates between different levels of anxiety in rats: Potential utility for neurobehavioural stress research. *Journal of Neuroscience Methods*, *143*(2), 169–177. https://doi.org/10.1016/j.jneumeth.2004.10.001

Kampov-Polevoy, A. B., Garbutt, J. C., & Janowsky, D. S. (1999). Association between preference for sweets and excessive alcohol intake: A review of animal and human studies. *Alcohol and Alcoholism*, *34*(3), 386–395. https://doi.org/10.1093/alcalc/34.3.386

Kang, J., Kim, H. J., Kim, T., Lee, H., Kim, M., Lee, S. W., Kim, M. S., Koyanagi, A., Smith, L., Fond, G., Boyer, L., Rahmati, M., Sánchez, G. F. L., Dragioti, E., Cortese, S., Shin, J. I., Yon, D. K., & Solmi, M. (2024). Prenatal opioid exposure and subsequent risk of neuropsychiatric disorders in children: Nationwide birth cohort study in South Korea. *BMJ*, *385*, e077664. https://doi.org/10.1136/bmj-2023-077664

Kangas, B. D., Short, A. K., Luc, O. T., Stern, H. S., Baram, T. Z., & Pizzagalli, D. A. (2022). A cross-species assay demonstrates that reward responsiveness is enduringly impacted by adverse, unpredictable early-life experiences. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *47*(3), 767–775. https://doi.org/10.1038/s41386-021-01250-9

Kapp, B. S., Frysinger, R. C., Gallagher, M., & Haselton, J. R. (1979). Amygdala central nucleus lesions: Effect on heart rate conditioning in the rabbit. *Physiology and Behavior*, *23*(6), 1109–1117. https://doi.org/10.1016/0031-9384(79)90304-4

Katz, R. J. (1982). Animal model of depression: Pharmacological sensitivity of a hedonic deficit. *Pharmacology Biochemistry and Behavior*, *16*(6), 965–968. https://doi.org/10.1016/0091-3057(82)90053-3

Kawakami, S. E., Quadros, I. M. H., & Suchecki, D. (2016). Naltrexone Prevents in Males and Attenuates in Females the Expression of Behavioral Sensitization to Ethanol Regardless of Maternal Separation. *Frontiers in Endocrinology*, *7*. https://doi.org/10.3389/fendo.2016.00135

Khachaturian, H., Lewis, M. E., Haber, S. N., Akil, H., & Watson, S. J. (1984). Proopiomelanocortin peptide immunocytochemistry in rhesus monkey brain. *Brain Research Bulletin*, *13*(6), 785–800. https://doi.org/10.1016/0361-9230(84)90237-5

King, A. C., Volpicelli, J. R., Frazer, A., & O'Brien, C. P. (1997). Effect of naltrexone on subjective alcohol response in subjects at high and low risk for future alcohol dependence. *Psychopharmacology*, *129*(1), 15–22. https://doi.org/10.1007/s002130050156

Kirby, M. L. (1983). Changes in [3H]naloxone binding in spinal cord of rats treated prenatally with morphine. *Neuropharmacology*, *22*(3, Part 1), 303–307. https://doi.org/10.1016/0028-3908(83)90244-7

Kirkland, J. M., Edgar, E. L., Patel, I., Feustel, P., Belin, S., & Kopec, A. M. (2024). Synaptic pruning during adolescence shapes adult social behavior in both males and females. *Developmental Psychobiology*, *66*(3), e22473. https://doi.org/10.1002/dev.22473

Ko, J. Y., D'Angelo, D. V., Haight, S. C., Morrow, B., Cox, S., Salvesen von Essen, B., Strahan, A. E., Harrison, L., Tevendale, H. D., Warner, L., Kroelinger, C. D., & Barfield, W. D. (2020). Vital Signs: Prescription Opioid Pain Reliever Use During Pregnancy - 34 U.S. Jurisdictions, 2019. *MMWR. Morbidity and Mortality Weekly Report*, *69*(28), 897–903. https://doi.org/10.15585/mmwr.mm6928a1

Kocherlakota, P. (2014). Neonatal abstinence syndrome. *Pediatrics*, *134*(2), e547-561. https://doi.org/10.1542/peds.2013-3524

Kokane, S. S., & Perrotti, L. I. (2020). Sex Differences and the Role of Estradiol in Mesolimbic Reward Circuits and Vulnerability to Cocaine and Opiate Addiction. *Frontiers in Behavioral Neuroscience*, *14*. https://doi.org/10.3389/fnbeh.2020.00074

Kongstorp, M., Bogen, I. L., Steinsland, S., Nerem, E., Salih, T. W., Stiris, T., & Andersen, J. M. (2020). Prenatal exposure to methadone or buprenorphine alters μ-opioid receptor binding and downstream signaling in the rat brain. *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience*, *80*(5), 443–453. https://doi.org/10.1002/jdn.10043

Kongstorp, M., Bogen, I. L., Stiris, T., & Andersen, J. M. (2020). Prenatal exposure to methadone or buprenorphine impairs cognitive performance in young adult rats. *Drug and Alcohol Dependence*, *212*, 108008. https://doi.org/10.1016/j.drugalcdep.2020.108008

Konijnenberg, C., Lund, I. O., & Melinder, A. (2015). Behavioural outcomes of four-year-old children prenatally exposed to methadone or buprenorphine: A test of three risk models. *Early Child Development and Care*, *185*(10), 1641–1657. https://doi.org/10.1080/03004430.2015.1016506

Konijnenberg, C., Sarfi, M., & Melinder, A. (2016). Mother-child interaction and cognitive development in children prenatally exposed to methadone or buprenorphine. *Early Human Development*, *101*, 91–97. https://doi.org/10.1016/j.earlhumdev.2016.08.013

Koob, G. F. (1992). Drugs of abuse: Anatomy, pharmacology and function of reward pathways. *Trends in Pharmacological Sciences*, *13*(5), 177–184. https://doi.org/10.1016/0165-6147(92)90060-j

Koob, G. F. (2010). The Role of CRF and CRF-related Peptides in the Dark Side of Addiction. *Brain Research*, *1314C*, 3. https://doi.org/10.1016/j.brainres.2009.11.008

Koob, G. F. (2020). Neurobiology of Opioid Addiction: Opponent Process, Hyperkatifeia, and Negative Reinforcement. *Biological Psychiatry*, *87*(1), 44–53. https://doi.org/10.1016/j.biopsych.2019.05.023

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of Addiction. *Neuropsychopharmacology*, *35*(1), 217–238. https://doi.org/10.1038/npp.2009.110

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

Koob, G., & Kreek, M. J. (2007). Stress, Dysregulation of Drug Reward Pathways, and the Transition to Drug Dependence. *The American Journal of Psychiatry*, *164*(8), 1149–1159. https://doi.org/10.1176/appi.ajp.2007.05030503

Kopec, A. M., Smith, C. J., Ayre, N. R., Sweat, S. C., & Bilbo, S. D. (2018). Microglial dopamine receptor elimination defines sex-specific nucleus accumbens development and social behavior in adolescent rats. *Nature Communications*, *9*(1), 3769. https://doi.org/10.1038/s41467-018-06118-z

Kovács, G. L., Izbéki, F., Horváth, Z., & Telegdy, G. (1984). Effects of oxytocin and a derivative (Z-prolyl-d-leucine) on morphine tolerance/withdrawal are mediated by the limbic system. *Behavioural Brain Research*, *14*(1), 1–8. https://doi.org/10.1016/0166-4328(84)90014-7

Kraft, W. K., Adeniyi-Jones, S. C., Chervoneva, I., Greenspan, J. S., Abatemarco, D., Kaltenbach, K., & Ehrlich, M. E. (2017). Buprenorphine for the Treatment of the Neonatal Abstinence

Syndrome. *The New England Journal of Medicine*, *376*(24), 2341–2348. https://doi.org/10.1056/NEJMoa1614835

Krans, E. E., Kim, J. Y., James, A. E., Kelley, D., & Jarlenski, M. P. (2019). Medication-Assisted Treatment Utilization Among Pregnant Women With Opioid Use Disorder. *Obstetrics and Gynecology*, *133*(5), 943–951. https://doi.org/10.1097/AOG.0000000000003231

Kreek, M. J., Bart, G., Lilly, C., LaForge, K. S., & Nielsen, D. A. (2005). Pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments. *Pharmacological Reviews*, *57*(1), 1–26. https://doi.org/10.1124/pr.57.1.1

Kreek, M. J., & Koob, G. F. (1998). Drug dependence: Stress and dysregulation of brain reward pathways. *Drug and Alcohol Dependence*, *51*(1–2), 23–47. https://doi.org/10.1016/s0376-8716(98)00064-7

Kringelbach, M. L., & Berridge, K. C. (2016). Neuroscience of Reward, Motivation, and Drive. In *Recent Developments in Neuroscience Research on Human Motivation* (Vol. 19, pp. 23–35). Emerald Group Publishing Limited. https://doi.org/10.1108/S0749-742320160000019020

Krishnan, V., Han, M.-H., Graham, D. L., Berton, O., Renthal, W., Russo, S. J., Laplant, Q., Graham, A., Lutter, M., Lagace, D. C., Ghose, S., Reister, R., Tannous, P., Green, T. A., Neve, R. L., Chakravarty, S., Kumar, A., Eisch, A. J., Self, D. W., … Nestler, E. J. (2007). Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*, *131*(2), 391–404. https://doi.org/10.1016/j.cell.2007.09.018

Kvello, A. M. S., Andersen, J. M., Øiestad, E. L., Steinsland, S., Aase, A., Mørland, J., & Bogen, I. L. (2019). A Monoclonal Antibody against 6-Acetylmorphine Protects Female Mice

Offspring from Adverse Behavioral Effects Induced by Prenatal Heroin Exposure. *The Journal of Pharmacology and Experimental Therapeutics*, *368*(1), 106–115. https://doi.org/10.1124/jpet.118.251504

Laborie, C., Dutriez-Casteloot, I., Montel, V., Dickès-Coopman, A., Lesage, J., & Vieau, D. (2005). Prenatal morphine exposure affects sympathoadrenal axis activity and serotonin metabolism in adult male rats both under basal conditions and after an ether inhalation stress. *Neuroscience Letters*, *381*(3), 211–216. https://doi.org/10.1016/j.neulet.2005.01.083

Lalanne, L., Ayranci, G., Filliol, D., Gavériaux-Ruff, C., Befort, K., Kieffer, B. L., & Lutz, P.-E. (2017). Kappa opioid receptor antagonism and chronic antidepressant treatment have beneficial activities on social interactions and grooming deficits during heroin abstinence. *Addiction Biology*, *22*(4), 1010–1021. https://doi.org/10.1111/adb.12392

Lapp, H. E., Salazar, M. G., & Champagne, F. A. (2023). Automated maternal behavior during early life in rodents (AMBER) pipeline. *Scientific Reports*, *13*(1), 18277. https://doi.org/10.1038/s41598-023-45495-4

Larson, J. J., Graham, D. L., Singer, L. T., Beckwith, A. M., Terplan, M., Davis, J. M., Martinez, J., & Bada, H. S. (2019). Cognitive and Behavioral Impact on Children Exposed to Opioids During Pregnancy. *Pediatrics*, *144*(2), e20190514. https://doi.org/10.1542/peds.2019-0514

Lê, A. D., Li, Z., Funk, D., Shram, M., Li, T. K., & Shaham, Y. (2006). Increased vulnerability to nicotine self-administration and relapse in alcohol-naive offspring of rats selectively bred for high alcohol intake. *The Journal of Neuroscience: The Official Journal of the*

Society for Neuroscience, *26*(6), 1872–1879. https://doi.org/10.1523/JNEUROSCI.4895-05.2006

Lê, A., & Shaham, Y. (2002). Neurobiology of relapse to alcohol in rats. *Pharmacology & Therapeutics*, *94*(1–2), 137–156. https://doi.org/10.1016/s0163-7258(02)00200-0

Lecca, S., Melis, M., Luchicchi, A., Muntoni, A. L., & Pistis, M. (2012). Inhibitory Inputs from Rostromedial Tegmental Neurons Regulate Spontaneous Activity of Midbrain Dopamine Cells and Their Responses to Drugs of Abuse. *Neuropsychopharmacology*, *37*(5), 1164–1176. https://doi.org/10.1038/npp.2011.302

LeDoux, J. E. (2000). Emotion circuits in the brain. *Annual Review of Neuroscience*, *23*, 155–184. https://doi.org/10.1146/annurev.neuro.23.1.155

Lee, A. T., Vogt, D., Rubenstein, J. L., & Sohal, V. S. (2014). A Class of GABAergic Neurons in the Prefrontal Cortex Sends Long-Range Projections to the Nucleus Accumbens and Elicits Acute Avoidance Behavior. *Journal of Neuroscience*, *34*(35), 11519–11525. https://doi.org/10.1523/JNEUROSCI.1157-14.2014

Lee, J.-S., Lee, S.-B., Kim, D.-W., Shin, N., Jeong, S.-J., Yang, C.-H., & Son, C.-G. (2021). Social isolation-related depression accelerates ethanol intake via microglia-derived neuroinflammation. *Science Advances*, *7*(45), eabj3400. https://doi.org/10.1126/sciadv.abj3400

Lesse, A., Rether, K., Gröger, N., Braun, K., & Bock, J. (2017). Chronic Postnatal Stress Induces Depressive-like Behavior in Male Mice and Programs second-Hit Stress-Induced Gene Expression Patterns of OxtR and AvpR1a in Adulthood. *Molecular Neurobiology*, *54*(6), 4813–4819. https://doi.org/10.1007/s12035-016-0043-8

Levis, S. C., Birnie, M. T., Bolton, J. L., Perrone, C. R., Montesinos, J. S., Baram, T. Z., & Mahler, S. V. (2022). Enduring disruption of reward and stress circuit activities by early-life adversity in male rats. *Translational Psychiatry*, *12*(1), 251. https://doi.org/10.1038/s41398-022-01988-w

Levi-Shachar, O., Gvirts, H. Z., Goldwin, Y., Bloch, Y., Shamay-Tsoory, S., Zagoory-Sharon, O., Feldman, R., & Maoz, H. (2020). The effect of methylphenidate on social cognition and oxytocin in children with attention deficit hyperactivity disorder. *Neuropsychopharmacology*, *45*(2), 367–373. https://doi.org/10.1038/s41386-019-0522-5

Lewis, R. G., Florio, E., Punzo, D., & Borrelli, E. (2021). The Brain's Reward System in Health and Disease. *Advances in Experimental Medicine and Biology*, *1344*, 57–69. https://doi.org/10.1007/978-3-030-81147-1_4

Li, C.-Q., Luo, Y.-W., Bi, F.-F., Cui, T.-T., Song, L., Cao, W.-Y., Zhang, J.-Y., Li, F., Xu, J.-M., Hao, W., Xing, X.-W., Zhou, F. H., Zhou, X.-F., & Dai, R.-P. (2014). Development of Anxiety-Like Behavior via Hippocampal IGF-2 Signaling in the Offspring of Parental Morphine Exposure: Effect of Enriched Environment. *Neuropsychopharmacology*, *39*(12), 2777–2787. https://doi.org/10.1038/npp.2014.128

Li, L., Sun, H., Ding, J., Niu, C., Su, M., Zhang, L., Li, Y., Wang, C., Gamper, N., Du, X., & Zhang, H. (2017). Selective targeting of M-type potassium Kv7.4 channels demonstrates their key role in the regulation of dopaminergic neuronal excitability and depression-like behaviour. *British Journal of Pharmacology*, *174*(23), 4277–4294. https://doi.org/10.1111/bph.14026

Light, K. C., Grewen, K. M., Amico, J. A., Boccia, M., Brownley, K. A., & Johns, J. M. (2004). Deficits in plasma oxytocin responses and increased negative affect, stress, and blood pressure in mothers with cocaine exposure during pregnancy. *Addictive Behaviors*, *29*(8), 1541–1564. https://doi.org/10.1016/j.addbeh.2004.02.062

Logrip, M. L., & Gainey, S. C. (2020). Sex differences in the long-term effects of past stress on alcohol self-administration, glucocorticoid sensitivity and phosphodiesterase 10A expression. *Neuropharmacology*, *164*, 107857. https://doi.org/10.1016/j.neuropharm.2019.107857

Long, J.-H., Wang, P.-J., Xuan, L., Juan, Y., Wu, G.-Y., Teng, J.-F., Sui, J.-F., Li, Y.-M., Yang, L., Li, H.-L., & Liu, S.-L. (2024). Prelimbic cortex-nucleus accumbens core projection positively regulates itch and itch-related aversion. *Behavioural Brain Research*, *468*, 114999. https://doi.org/10.1016/j.bbr.2024.114999

Lu, H., & Rosenbaum, S. (2014). Developmental Pharmacokinetics in Pediatric Populations. *The Journal of Pediatric Pharmacology and Therapeutics : JPPT*, *19*(4), 262–276. https://doi.org/10.5863/1551-6776-19.4.262

Ludwig, A. M., Wikler, A., & Stark, L. H. (1974). The first drink: Psychobiological aspects of craving. *Archives of General Psychiatry*, *30*(4), 539–547. https://doi.org/10.1001/archpsyc.1974.01760100093015

Lutz, P.-E., Ayranci, G., Chu-Sin-Chung, P., Matifas, A., Koebel, P., Filliol, D., Befort, K., Ouagazzal, A.-M., & Kieffer, B. L. (2014). Distinct Mu, Delta, and Kappa Opioid Receptor Mechanisms Underlie Low Sociability and Depressive-Like Behaviors During Heroin

Abstinence. *Neuropsychopharmacology*, *39*(11), 2694–2705. https://doi.org/10.1038/npp.2014.126

Lutz, P.-E., & Kieffer, B. L. (2013). The multiple facets of opioid receptor function: Implications for addiction. *Current Opinion in Neurobiology*, *23*(4), 473–479. https://doi.org/10.1016/j.conb.2013.02.005

MacMillan, K. D. L., Rendon, C. P., Verma, K., Riblet, N., Washer, D. B., & Volpe Holmes, A. (2018). Association of Rooming-in With Outcomes for Neonatal Abstinence Syndrome: A Systematic Review and Meta-analysis. *JAMA Pediatrics*, *172*(4), 345–351. https://doi.org/10.1001/jamapediatrics.2017.5195

Mague, S. D., & Blendy, J. A. (2010). OPRM1 SNP (A118G): Involvement in disease development, treatment response, and animal models. *Drug and Alcohol Dependence*, *108*(3), 172–182. https://doi.org/10.1016/j.drugalcdep.2009.12.016

Mah, B. L., Bakermans-Kranenburg, M. J., Van IJzendoorn, M. H., & Smith, R. (2015). Oxytocin promotes protective behavior in depressed mothers: A pilot study with the enthusiastic stranger paradigm. *Depression and Anxiety*, *32*(2), 76–81. https://doi.org/10.1002/da.22245

Manbeck, K. E., Shelley, D., Schmidt, C. E., & Harris, A. C. (2014). Effects of oxytocin on nicotine withdrawal in rats. *Pharmacology Biochemistry and Behavior*, *116*, 84–89. https://doi.org/10.1016/j.pbb.2013.11.002

Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H., & Watson, S. J. (1988). Anatomy of CNS opioid receptors. *Trends in Neurosciences*, *11*(7), 308–314. https://doi.org/10.1016/0166-2236(88)90093-8

Martin-Fardon, R., & Weiss, F. (2013). Modeling Relapse in Animals. *Current Topics in Behavioral Neurosciences*, *13*, 403–432. https://doi.org/10.1007/7854_2012_202

Matsui, A., & Williams, J. T. (2011). Opioid-Sensitive GABA Inputs from Rostromedial Tegmental Nucleus Synapse onto Midbrain Dopamine Neurons. *Journal of Neuroscience*, *31*(48), 17729–17735. https://doi.org/10.1523/JNEUROSCI.4570-11.2011

Matsumoto, M., & Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature*, *447*(7148), 1111–1115. https://doi.org/10.1038/nature05860

McBride, W. J., Chernet, E., McKinzie, D. L., Lumeng, L., & Li, T. K. (1998). Quantitative autoradiography of mu-opioid receptors in the CNS of alcohol-naive alcohol-preferring P and -nonpreferring NP rats. *Alcohol (Fayetteville, N.Y.)*, *16*(4), 317–323. https://doi.org/10.1016/s0741-8329(98)00021-4

McGovern, D. J., Ly, A., Ecton, K. L., Huynh, D. T., Prévost, E. D., Gonzalez, S. C., McNulty, C. J., Rau, A. R., Hentges, S. T., Daigle, T. L., Tasic, B., Baratta, M. V., & Root, D. H. (2024). Ventral tegmental area glutamate neurons mediate nonassociative consequences of stress. *Molecular Psychiatry*, *29*(6), 1671–1682. https://doi.org/10.1038/s41380-022-01858-3

McGovern, D. J., Polter, A. M., Prévost, E. D., Ly, A., McNulty, C. J., Rubinstein, B., & Root, D. H. (2023). Ventral tegmental area glutamate neurons establish a mu-opioid receptor gated circuit to mesolimbic dopamine neurons and regulate opioid-seeking behavior. *Neuropsychopharmacology*, *48*(13), 1889–1900. https://doi.org/10.1038/s41386-023-01637-w

McLaughlin, P., Mactier, H., Gillis, C., Hickish, T., Parker, A., Liang, W.-J., & Osselton, M. D. (2017). Increased DNA Methylation of ABCB1, CYP2D6, and OPRM1 Genes in Newborn Infants of Methadone-Maintained Opioid-Dependent Mothers. *The Journal of Pediatrics*, *190*, 180-184.e1. https://doi.org/10.1016/j.jpeds.2017.07.026

Mechling, A. E., Arefin, T., Lee, H.-L., Bienert, T., Reisert, M., Ben Hamida, S., Darcq, E., Ehrlich, A., Gaveriaux-Ruff, C., Parent, M. J., Rosa-Neto, P., Hennig, J., von Elverfeldt, D., Kieffer, B. L., & Harsan, L.-A. (2016). Deletion of the mu opioid receptor gene in mice reshapes the reward-aversion connectome. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(41), 11603–11608. https://doi.org/10.1073/pnas.1601640113

Mejía-Chávez, S., Venebra-Muñoz, A., García-García, F., Corona-Morales, A. A., & Orozco-Vargas, A. E. (2021). Maternal Separation Modifies the Activity of Social Processing Brain Nuclei Upon Social Novelty Exposure. *Frontiers in Behavioral Neuroscience*, *15*, 651263. https://doi.org/10.3389/fnbeh.2021.651263

Meye, F. J., Valentinova, K., Lecca, S., Marion-Poll, L., Maroteaux, M. J., Musardo, S., Moutkine, I., Gardoni, F., Huganir, R. L., Georges, F., & Mameli, M. (2015). Cocaine-evoked negative symptoms require AMPA receptor trafficking in the lateral habenula. *Nature Neuroscience*, *18*(3), 376–378. https://doi.org/10.1038/nn.3923

Michaels, T. I., Stone, E., Singal, S., Novakovic, V., Barkin, R. L., & Barkin, S. (2021). Brain reward circuitry: The overlapping neurobiology of trauma and substance use disorders. *World Journal of Psychiatry*, *11*(6), 222–231. https://doi.org/10.5498/wjp.v11.i6.222

Milliren, C. E., Gupta, M., Graham, D. A., Melvin, P., Jorina, M., & Ozonoff, A. (2018). Hospital Variation in Neonatal Abstinence Syndrome Incidence, Treatment Modalities, Resource Use, and Costs Across Pediatric Hospitals in the United States, 2013 to 2016. *Hospital Pediatrics*, *8*(1), 15–20. https://doi.org/10.1542/hpeds.2017-0077

Miranda-Morales, R. S., Molina, J. C., Spear, N. E., & Abate, P. (2012). Naloxone attenuation of ethanol-reinforced operant responding in infant rats in a re-exposure paradigm. *Psychopharmacology*, *219*(1), 235–246. https://doi.org/10.1007/s00213-011-2402-5

Moench, K. M., & Logrip, M. L. (2021). Housing Condition Differentially Impacts Escalation of Alcohol Intake, Relapse-Like Drinking, Anxiety-Like Behavior, and Stress History Effects by Sex. *Alcoholism, Clinical and Experimental Research*, *45*(2), 480–489. https://doi.org/10.1111/acer.14540

Molet, J., Heins, K., Zhuo, X., Mei, Y. T., Regev, L., Baram, T. Z., & Stern, H. (2016). Fragmentation and high entropy of neonatal experience predict adolescent emotional outcome. *Translational Psychiatry*, *6*(1), e702. https://doi.org/10.1038/tp.2015.200

Morales, I., Rodríguez-Borillo, O., Font, L., & Pastor, R. (2020). Effects of naltrexone on alcohol, sucrose, and saccharin binge-like drinking in C57BL/6J mice: A study with a multiple bottle choice procedure. *Behavioural Pharmacology*, *31*(2 & 3), 256–271. https://doi.org/10.1097/FBP.0000000000000553

Morales, M., & Margolis, E. B. (2017). Ventral tegmental area: Cellular heterogeneity, connectivity and behaviour. *Nature Reviews Neuroscience*, *18*(2), 73–85. https://doi.org/10.1038/nrn.2016.165

Naderi, S., Vousooghi, N., Sadighii, N., Batouli, S. A. H., Mirzaii, F., & Razaghi, E. M. (2020). Oxytocin Blocks Opioid Withdrawal Symptoms Only When Combined with Group Therapy: A Double-Blind, Randomized Controlled Clinical Trial. *Iranian Journal of Psychiatry and Behavioral Sciences*, *14*(3), Article 3. https://doi.org/10.5812/ijpbs.104627

Nair-Roberts, R. G., Chatelain-Badie, S. D., Benson, E., White-Cooper, H., Bolam, J. P., & Ungless, M. A. (2008). Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience*, *152*(4), 1024–1031. https://doi.org/10.1016/j.neuroscience.2008.01.046

Nakamura, S., Ito, Y., Shirasaki, R., & Murakami, F. (2000). Local Directional Cues Control Growth Polarity of Dopaminergic Axons Along the Rostrocaudal Axis. *The Journal of Neuroscience*, *20*(11), 4112–4119. https://doi.org/10.1523/JNEUROSCI.20-11-04112.2000

Nanovskaya, T. N., Nekhayeva, I. A., Hankins, G. D. V., & Ahmed, M. S. (2008). Transfer of methadone across the dually perfused preterm human placental lobule. *American Journal of Obstetrics and Gynecology*, *198*(1), 126.e1-4. https://doi.org/10.1016/j.ajog.2007.06.073

Nasiraei-Moghadam, S., Sherafat, M. A., Safari, M.-S., Moradi, F., Ahmadiani, A., & Dargahi, L. (2013). Reversal of prenatal morphine exposure-induced memory deficit in male but not female rats. *Journal of Molecular Neuroscience: MN*, *50*(1), 58–69. https://doi.org/10.1007/s12031-012-9860-z

Nestler, E. J., & Carlezon, W. A. (2006). The mesolimbic dopamine reward circuit in depression. *Biological Psychiatry*, *59*(12), 1151–1159. https://doi.org/10.1016/j.biopsych.2005.09.018

Niciu, M. J., & Arias, A. J. (2013). Targeted Opioid Receptor Antagonists in the Treatment of Alcohol Use Disorders. *CNS Drugs*, *27*(10), 777–787. https://doi.org/10.1007/s40263-013-0096-4

Nielsen, D. A., Utrankar, A., Reyes, J. A., Simons, D. D., & Kosten, T. R. (2012). Epigenetics of drug abuse: Predisposition or response. *Pharmacogenomics*, *13*(10), 1149. https://doi.org/10.2217/pgs.12.94

Niesink, R. J., & Van Ree, J. M. (1989). Involvement of opioid and dopaminergic systems in isolation-induced pinning and social grooming of young rats. *Neuropharmacology*, *28*(4), 411–418. https://doi.org/10.1016/0028-3908(89)90038-5

Nieto, S. J., & Kosten, T. A. (2017). Female Sprague-Dawley rats display greater appetitive and consummatory responses to alcohol. *Behavioural Brain Research*, *327*, 155–161. https://doi.org/10.1016/j.bbr.2017.03.037

Novick, A. M., Levandowski, M. L., Laumann, L. E., Philip, N. S., Price, L. H., & Tyrka, A. R. (2018). The effects of early life stress on reward processing. *Journal of Psychiatric Research*, *101*, 80–103. https://doi.org/10.1016/j.jpsychires.2018.02.002

Nurmi, M., Kiianmaa, K., & Sinclair, J. D. (1999). Brain ethanol levels after voluntary ethanol drinking in AA and Wistar rats. *Alcohol (Fayetteville, N.Y.)*, *19*(2), 113–118. https://doi.org/10.1016/s0741-8329(99)00022-1

Nygaard, E., Moe, V., Slinning, K., & Walhovd, K. B. (2015). Longitudinal cognitive development of children born to mothers with opioid and polysubstance use. *Pediatric Research*, *78*(3), 330–335. https://doi.org/10.1038/pr.2015.95

Nygaard, E., Slinning, K., Moe, V., Due-Tønnessen, P., Fjell, A., & Walhovd, K. B. (2018). Neuroanatomical characteristics of youths with prenatal opioid and poly-drug exposure. *Neurotoxicology and Teratology*, *68*, 13–26. https://doi.org/10.1016/j.ntt.2018.04.004

Nygaard, E., Slinning, K., Moe, V., Fjell, A., & Walhovd, K. B. (2020). Mental health in youth prenatally exposed to opioids and poly-drugs and raised in permanent foster/adoptive homes: A prospective longitudinal study. *Early Human Development*, *140*, 104910. https://doi.org/10.1016/j.earlhumdev.2019.104910

Nygaard, E., Slinning, K., Moe, V., & Walhovd, K. B. (2016). Behavior and Attention Problems in Eight-Year-Old Children with Prenatal Opiate and Poly-Substance Exposure: A Longitudinal Study. *PLOS ONE*, *11*(6), e0158054. https://doi.org/10.1371/journal.pone.0158054

Oei, J. L., Melhuish, E., Uebel, H., Azzam, N., Breen, C., Burns, L., Hilder, L., Bajuk, B., Abdel-Latif, M. E., Ward, M., Feller, J. M., Falconer, J., Clews, S., Eastwood, J., Li, A., & Wright, I. M. (2017). Neonatal Abstinence Syndrome and High School Performance. *Pediatrics*, *139*(2), e20162651. https://doi.org/10.1542/peds.2016-2651

Olabarrieta, E., Totorikaguena, L., Agirregoitia, N., & Agirregoitia, E. (2019). Implication of mu opioid receptor in the in vitro maturation of oocytes and its effects on subsequent fertilization and embryo development in mice. *Molecular Reproduction and Development*, *86*(9), 1236–1244. https://doi.org/10.1002/mrd.23248

Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative and Physiological Psychology*, *47*(6), 419–427. https://doi.org/10.1037/h0058775

Olds, M. E., & Olds, J. (1963). Approach-avoidance analysis of rat diencephalon. *The Journal of Comparative Neurology*, *120*, 259–295. https://doi.org/10.1002/cne.901200206

Ornoy, A., Daka, L., Goldzweig, G., Gil, Y., Mjen, L., Levit, S., Shufman, E., Bar-Hamburger, R., & Greenbaum, C. W. (2010). Neurodevelopmental and psychological assessment of adolescents born to drug-addicted parents: Effects of SES and adoption. *Child Abuse & Neglect*, *34*(5), 354–368. https://doi.org/10.1016/j.chiabu.2009.09.012

Oswald, L. M., & Wand, G. S. (2004). Opioids and alcoholism. *Physiology & Behavior*, *81*(2), 339–358. https://doi.org/10.1016/j.physbeh.2004.02.008

Ozdemir, D., Meyer, J., Kieffer, B. L., & Darcq, E. (2024). Model of negative affect induced by withdrawal from acute and chronic morphine administration in male mice. *Scientific Reports*, *14*(1), 9767. https://doi.org/10.1038/s41598-024-60759-3

Pacifici, G. M. (2016). Metabolism and pharmacokinetics of morphine in neonates: A review. *Clinics*, *71*(8), 474–480. https://doi.org/10.6061/clinics/2016(08)11

Palamar, J. J., Le, A., & Mateu-Gelabert, P. (2018). Not just heroin: Extensive polysubstance use among US high school seniors who currently use heroin. *Drug and Alcohol Dependence*, *188*, 377–384. https://doi.org/10.1016/j.drugalcdep.2018.05.001

Panerai, A. E., Martini, A., Di Giulio, A. M., Fraioli, F., Vegni, C., Pardi, G., Marini, A., & Mantegazza, P. (1983). Plasma beta-endorphin, beta-lipotropin, and met-enkephalin concentrations during pregnancy in normal and drug-addicted women and their newborn. *The Journal of Clinical Endocrinology and Metabolism*, *57*(3), 537–543. https://doi.org/10.1210/jcem-57-3-537

Pap, D., Gonda, X., Molnar, E., Lazary, J., Benko, A., Downey, D., Thomas, E., Chase, D., Toth, Z. G., Mekli, K., Platt, H., Payton, A., Elliott, R., Anderson, I. M., Deakin, J. F. W., Bagdy, G., & Juhasz, G. (2012). Genetic variants in the catechol-o-methyltransferase gene are associated with impulsivity and executive function: Relevance for major depression. *American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics*, *159B*(8), 928–940. https://doi.org/10.1002/ajmg.b.32098

Papaleo, F., Kieffer, B. L., Tabarin, A., & Contarino, A. (2007). Decreased motivation to eat in mu-opioid receptor-deficient mice. *The European Journal of Neuroscience*, *25*(11), 3398–3405. https://doi.org/10.1111/j.1460-9568.2007.05595.x

Papp, M., Wilner, P., & Muscat, R. (1991). An animal model of anhedonia: Attenuation of sucrose consumption and place preference conditioning by chronic unpredictable mild stress. *Psychopharmacology*, *104*(2). https://doi.org/10.1007/BF02244188

Park, H., Ryu, H., Zhang, S., Rhee, J., & Chung, C. (2024). Mu-opioid receptor activation in the habenula modulates synaptic transmission and depression-like behaviors. *Neurobiology of Disease*, *198*, 106543. https://doi.org/10.1016/j.nbd.2024.106543

Pastor, R., & Aragon, C. M. G. (2006). The Role of Opioid Receptor Subtypes in the Development of Behavioral Sensitization to Ethanol. *Neuropsychopharmacology*, *31*(7), 1489–1499. https://doi.org/10.1038/sj.npp.1300928

Patrick, S. W., Barfield, W. D., Poindexter, B. B., COMMITTEE ON FETUS AND NEWBORN, C. O. S. U. A. P., Cummings, J., Hand, I., Adams-Chapman, I., Aucott, S. W., Puopolo, K. M., Goldsmith, J. P., Kaufman, D., Martin, C., Mowitz, M., Gonzalez, L., Camenga, D. R., Quigley, J., Ryan, S. A., & Walker-Harding, L. (2020). Neonatal Opioid Withdrawal Syndrome. *Pediatrics*, *146*(5), e2020029074. https://doi.org/10.1542/peds.2020-029074

Patrick, S. W., Davis, M. M., Lehmann, C. U., & Cooper, W. O. (2015). Increasing incidence and geographic distribution of neonatal abstinence syndrome: United States 2009 to 2012. *Journal of Perinatology: Official Journal of the California Perinatal Association*, *35*(8), 650–655. https://doi.org/10.1038/jp.2015.36

Patrick, S. W., Faherty, L. J., Dick, A. W., Scott, T. A., Dudley, J., & Stein, B. D. (2019). Association Among County-Level Economic Factors, Clinician Supply, Metropolitan or Rural Location, and Neonatal Abstinence Syndrome. *JAMA*, *321*(4), 385–393. https://doi.org/10.1001/jama.2018.20851

Patrick, S. W., Kaplan, H. C., Passarella, M., Davis, M. M., & Lorch, S. A. (2014). Variation in treatment of neonatal abstinence syndrome in US Children's Hospitals, 2004–2011. *Journal of Perinatology*, *34*(11), 867–872. https://doi.org/10.1038/jp.2014.114

Patrick, S. W., Schumacher, R. E., Benneyworth, B. D., Krans, E. E., McAllister, J. M., & Davis, M. M. (2012). Neonatal Abstinence Syndrome and Associated Health Care Expenditures: United States, 2000-2009. *JAMA*, *307*(18), 1934–1940. https://doi.org/10.1001/jama.2012.3951

Patrick, S. W., Schumacher, R. E., Horbar, J. D., Buus-Frank, M. E., Edwards, E. M., Morrow, K. A., Ferrelli, K. R., Picarillo, A. P., Gupta, M., & Soll, R. F. (2016). Improving Care for Neonatal

Abstinence Syndrome. *Pediatrics*, *137*(5), e20153835. https://doi.org/10.1542/peds.2015-3835

Pautassi, R. M., Nizhnikov, M. E., Fabio, Ma. C., & Spear, N. E. (2012). EARLY MATERNAL SEPARATION AFFECTS ETHANOL-INDUCED CONDITIONING IN A nor-BNI INSENSITIVE MANNER, BUT DOES NOT ALTER ETHANOL-INDUCED LOCOMOTOR ACTIVITY. *Pharmacology, Biochemistry, and Behavior*, *100*(3), 630–638. https://doi.org/10.1016/j.pbb.2011.11.005

Peris, J., Zharikova, A., Li, Z., Lingis, M., MacNeill, M., Wu, M. T., & Rowland, N. E. (2006). Brain ethanol levels in rats after voluntary ethanol consumption using a sweetened gelatin vehicle. *Pharmacology, Biochemistry, and Behavior*, *85*(3), 562–568. https://doi.org/10.1016/j.pbb.2006.10.010

Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learning & Memory*, *16*(5), 279–288. https://doi.org/10.1101/lm.1041309

Peters, S. T., Bowen, M. T., Bohrer, K., McGregor, I. S., & Neumann, I. D. (2017). Oxytocin inhibits ethanol consumption and ethanol-induced dopamine release in the nucleus accumbens. *Addiction Biology*, *22*(3), 702–711. https://doi.org/10.1111/adb.12362

Pierce, M., Amsterdam, J. van, Kalkman, G. A., Schellekens, A., & Brink, W. van den. (2021). Is Europe facing an opioid crisis like the United States? An analysis of opioid use and related adverse effects in 19 European countries between 2010 and 2018. *European Psychiatry*, *64*(1), e47. https://doi.org/10.1192/j.eurpsy.2021.2219

Pitchers, K. K., Coppens, C. M., Beloate, L. N., Fuller, J., Van, S., Frohmader, K. S., Laviolette, S. R., Lehman, M. N., & Coolen, L. M. (2014). Endogenous Opioid-Induced Neuroplasticity of Dopaminergic Neurons in the Ventral Tegmental Area Influences Natural and Opiate Reward. *Journal of Neuroscience*, *34*(26), 8825–8836. https://doi.org/10.1523/JNEUROSCI.0133-14.2014

Pizzagalli, D. A. (2014). Depression, stress, and anhedonia: Toward a synthesis and integrated model. *Annual Review of Clinical Psychology*, *10*, 393–423. https://doi.org/10.1146/annurev-clinpsy-050212-185606

Pothos, E., Rada, P., Mark, G. P., & Hoebel, B. G. (1991). Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment. *Brain Research*, *566*(1–2), 348–350. https://doi.org/10.1016/0006-8993(91)91724-f

Pritham, U. A. (2013). Breastfeeding promotion for management of neonatal abstinence syndrome. *Journal of Obstetric, Gynecologic, and Neonatal Nursing: JOGNN*, *42*(5), 517–526. https://doi.org/10.1111/1552-6909.12242

Qi, J., Zhang, S., Wang, H.-L., Barker, D. J., Miranda-Barrientos, J., & Morales, M. (2016). VTA glutamatergic inputs to nucleus accumbens drive aversion by acting on GABAergic interneurons. *Nature Neuroscience*, *19*(5), 725–733. https://doi.org/10.1038/nn.4281

Quintanilla, M. E., Rivera-Meza, M., Berríos-Cárcamo, P., Salinas-Luypaert, C., Herrera-Marschitz, M., & Israel, Y. (2016). Beyond the “First Hit”: Marked Inhibition by N-Acetyl Cysteine of Chronic Ethanol Intake But Not of Early Ethanol Intake. Parallel Effects on

Ethanol-Induced Saccharin Motivation. *Alcoholism: Clinical and Experimental Research*, *40*(5), 1044–1051. https://doi.org/10.1111/acer.13031

Quintanilla, M. E., Tampier, L., Karahanian, E., Rivera-Meza, M., Herrera-Marschitz, M., & Israel, Y. (2012). Reward and Relapse: Complete Gene-Induced Dissociation in an Animal Model of Alcohol Dependence. *Alcoholism: Clinical and Experimental Research*, *36*(3), 517–522. https://doi.org/10.1111/j.1530-0277.2011.01606.x

Ramsey, N. F., Niesink, R. J., & Van Ree, J. M. (1993). Prenatal exposure to morphine enhances cocaine and heroin self-administration in drug-naive rats. *Drug and Alcohol Dependence*, *33*(1), 41–51. https://doi.org/10.1016/0376-8716(93)90032-l

Raynor, K., Kong, H., Chen, Y., Yasuda, K., Yu, L., Bell, G. I., & Reisine, T. (1994). Pharmacological characterization of the cloned kappa-, delta-, and mu-opioid receptors. *Molecular Pharmacology*, *45*(2), 330–334.

Rees, P., Stilwell, P. A., Bolton, C., Akillioglu, M., Carter, B., Gale, C., & Sutcliffe, A. (2020). Childhood Health and Educational Outcomes After Neonatal Abstinence Syndrome: A Systematic Review and Meta-analysis. *The Journal of Pediatrics*, *226*, 149-156.e16. https://doi.org/10.1016/j.jpeds.2020.07.013

Reeves, K. C., Shah, N., Muñoz, B., & Atwood, B. K. (2022). Opioid Receptor-Mediated Regulation of Neurotransmission in the Brain. *Frontiers in Molecular Neuroscience*, *15*. https://doi.org/10.3389/fnmol.2022.919773

Reichel, C. M., & Bevins, R. A. (2009). Forced Abstinence Model of Relapse to Study Pharmacological Treatments of Substance Use Disorder. *Current Drug Abuse Reviews*, *2*(2), 184–194.

Reynolds, L. M., & Flores, C. (2021). Mesocorticolimbic Dopamine Pathways Across Adolescence: Diversity in Development. *Frontiers in Neural Circuits*, *15*, 735625. https://doi.org/10.3389/fncir.2021.735625

Richard, J. M., & Fields, H. L. (2016). Mu-opioid receptor activation in the medial shell of nucleus accumbens promotes alcohol consumption, self-administration and cue-induced reinstatement. *Neuropharmacology*, *108*, 14–23. https://doi.org/10.1016/j.neuropharm.2016.04.010

Riley, M. A., & Vathy, I. (2006). Mid- to late gestational morphine exposure does not alter the rewarding properties of morphine in adult male rats. *Neuropharmacology*, *51*(2), 295–304. https://doi.org/10.1016/j.neuropharm.2006.03.022

Rimanóczy, Á., Šlamberová, R., Riley, M. A., & Vathy, I. (2003). Adrenocorticotropin Stress Response but Not Glucocorticoid-Negative Feedback Is Altered by Prenatal Morphine Exposure in Adult Male Rats. *Neuroendocrinology*, *78*(6), 312–320. https://doi.org/10.1159/000074884

Rincón-Cortés, M., & Sullivan, R. M. (2016). Emergence of social behavior deficit, blunted corticolimbic activity and adult depression-like behavior in a rodent model of maternal maltreatment. *Translational Psychiatry*, *6*(10), e930. https://doi.org/10.1038/tp.2016.205

Ritz, M. C., Garcia, J. M., Protz, D., Rael, A.-M., & George, F. R. (1994). Ethanol-reinforced behavior in P, NP, HAD and LAD rats: Differential genetic regulation of reinforcement and motivation. *Behavioural Pharmacology*, *5*(4 And 5), 521–531. https://doi.org/10.1097/00008877-199408000-00013

Ritz, M. C., George, F. R., & Meisch, R. A. (1989). Ethanol self-administration in ALKO rats: II. Effects of selection and fixed-ratio size. *Alcohol*, *6*(3), 235–239. https://doi.org/10.1016/0741-8329(89)90024-4

Robinson, S. A., Jones, A. D., Brynildsen, J. K., Ehrlich, M. E., & Blendy, J. A. (2020). Neurobehavioral Effects of Neonatal Opioid Exposure in Mice: Influence of the OPRM1 SNP. *Addiction Biology*, *25*(5), e12806. https://doi.org/10.1111/adb.12806

Rodriguez, N., Vining, M., & Bloch-Salisbury, E. (2020). Salivary cortisol levels as a biomarker for severity of withdrawal in opioid-exposed newborns. *Pediatric Research*, *87*(6), 1033–1038. https://doi.org/10.1038/s41390-019-0601-7

Root, D. H., Mejias-Aponte, C. A., Qi, J., & Morales, M. (2014). Role of glutamatergic projections from ventral tegmental area to lateral habenula in aversive conditioning. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *34*(42), 13906–13910. https://doi.org/10.1523/JNEUROSCI.2029-14.2014

Rossen, L., Hamilton E., B., Abma, J., C.W., E., Beresovsky, V., Resendez, A., Chandra, A., & Martin, J. (2023). *Updated Methodology to Estimate Overall and Unintended Pregnancy Rates in the United States*. National Center for Health Statistics (U.S.). https://doi.org/10.15620/cdc:124395

Russell, B. S., Trudeau, J. J., & Leland, A. J. (2015). Social Influence on Adolescent Polysubstance Use: The Escalation to Opioid Use. *Substance Use & Misuse*, *50*(10), 1325–1331. https://doi.org/10.3109/10826084.2015.1013128

Russo, S. J., & Nestler, E. J. (2013). The Brain Reward Circuitry in Mood Disorders. *Nature Reviews. Neuroscience*, *14*(9), 10.1038/nrn3381. https://doi.org/10.1038/nrn3381

SAMHSA. (2014). *Results from the 2013 NSDUH: Summary of National Findings* (HHS Publication No. (SMA), pp. 14–4863). SAMHSA. https://www.samhsa.gov/data/sites/default/files/NSDUHresultsPDFWHTML2013/Web/NSDUHresults2013.htm

Sandtorv, L. B., Fevang, S. K. E., Nilsen, S. A., Bøe, T., Gjestad, R., Haugland, S., & Elgen, I. B. (2018). Symptoms Associated With Attention Deficit/Hyperactivity Disorder and Autism Spectrum Disorders in School-Aged Children Prenatally Exposed to Substances. *Substance Abuse: Research and Treatment*, *12*, 1178221818765773. https://doi.org/10.1177/1178221818765773

Sanlorenzo, L. A., Stark, A. R., & Patrick, S. W. (2018). Neonatal abstinence syndrome: An update. *Current Opinion in Pediatrics*, *30*(2), 182. https://doi.org/10.1097/MOP.0000000000000589

Sarfi, M., Smith, L., Waal, H., & Sundet, J. M. (2011). Risks and realities: Dyadic interaction between 6-month-old infants and their mothers in opioid maintenance treatment. *Infant Behavior & Development*, *34*(4), 578–589. https://doi.org/10.1016/j.infbeh.2011.06.006

Sarkaki, A., Assaei, R., Motamedi, F., Badavi, M., & Pajouhi, N. (2008). Effect of parental morphine addiction on hippocampal long-term potentiation in rats offspring. *Behavioural Brain Research*, *186*(1), 72–77. https://doi.org/10.1016/j.bbr.2007.07.041

Sarnyai, Z., Viski, S., Kriva´n, M., Szabo´, G., Kova´cs, G. L., & Telegdy, G. (1988). Endogenous oxytocin inhibits morphine tolerance through limbic forebrain oxytocin receptors. *Brain Research*, *463*(2), 284–288. https://doi.org/10.1016/0006-8993(88)90401-5

Scantamburlo, G., Hansenne, M., Fuchs, S., Pitchot, W., Maréchal, P., Pequeux, C., Ansseau, M., & Legros, J. J. (2007). Plasma oxytocin levels and anxiety in patients with major depression. *Psychoneuroendocrinology*, *32*(4), 407–410. https://doi.org/10.1016/j.psyneuen.2007.01.009

Schulteis, G., Markou, A., Gold, L. H., Stinus, L., & Koob, G. F. (1994). Relative sensitivity to naloxone of multiple indices of opiate withdrawal: A quantitative dose-response analysis. *The Journal of Pharmacology and Experimental Therapeutics*, *271*(3), 1391–1398.

Schwarz, J. M., Sholar, P. W., & Bilbo, S. D. (2012). Sex differences in microglial colonization of the developing rat brain. *Journal of Neurochemistry*, *120*(6), 948–963. https://doi.org/10.1111/j.1471-4159.2011.07630.x

Scott, C. S., Riggs, K. W., Ling, E. W., Fitzgerald, C. E., Hill, M. L., Grunau, R. V., Solimano, A., & Craig, K. D. (1999). Morphine pharmacokinetics and pain assessment in premature newborns. *The Journal of Pediatrics*, *135*(4), 423–429. https://doi.org/10.1016/s0022-3476(99)70163-0

Searles, C. T., Harder, H. J., Vogt, M. E., & Murphy, A. Z. (2023). Perigestational Opioid Exposure Alters Alcohol-Driven Reward Behaviors in Adolescent Rats. *bioRxiv: The Preprint Server for Biology*, 2023.11.14.567041. https://doi.org/10.1101/2023.11.14.567041

Seney, M. L., Kim, S.-M., Glausier, J. R., Hildebrand, M. A., Xue, X., Zong, W., Wang, J., Shelton, M. A., Phan, B. N., Srinivasan, C., Pfenning, A. R., Tseng, G. C., Lewis, D. A., Freyberg, Z., & Logan, R. W. (2021). Transcriptional Alterations in Dorsolateral Prefrontal Cortex and Nucleus Accumbens Implicate Neuroinflammation and Synaptic Remodeling in Opioid

Use Disorder. *Biological Psychiatry*, *90*(8), 550–562. https://doi.org/10.1016/j.biopsych.2021.06.007

Shen, Y.-L., Chen, S.-T., Chan, T.-Y., Hung, T.-W., Tao, P.-L., Liao, R.-M., Chan, M.-H., & Chen, H.-H. (2016). Delayed extinction and stronger drug-primed reinstatement of methamphetamine seeking in rats prenatally exposed to morphine. *Neurobiology of Learning and Memory*, *128*, 56–64. https://doi.org/10.1016/j.nlm.2015.12.002

Sherman, L. J., Ali, M. M., Mutter, R., & Larson, J. (2019). Mental Disorders Among Children Born With Neonatal Abstinence Syndrome. *Psychiatric Services*, *70*(2), 151–151. https://doi.org/10.1176/appi.ps.201800341

Short, V. L., Gannon, M., & Abatemarco, D. J. (2016). The Association Between Breastfeeding and Length of Hospital Stay Among Infants Diagnosed with Neonatal Abstinence Syndrome: A Population-Based Study of In-Hospital Births. *Breastfeeding Medicine*, *11*(7), 343–349. https://doi.org/10.1089/bfm.2016.0084

Simmons, S. C., Grecco, G. G., Atwood, B. K., & Nugent, F. S. (2023). Effects of prenatal opioid exposure on synaptic adaptations and behaviors across development. *Neuropharmacology*, *222*, 109312. https://doi.org/10.1016/j.neuropharm.2022.109312

Simon, H., Le Moal, M., & Cardo, B. (1975). Self-stimulation in the dorsal pontine tegmentum in the rat. *Behavioral Biology*, *13*(3), 339–347. https://doi.org/10.1016/s0091-6773(75)91388-7

Simpson, T. L., & Miller, W. R. (2002). Concomitance between childhood sexual and physical abuse and substance use problems. A review. *Clinical Psychology Review*, *22*(1), 27–77. https://doi.org/10.1016/s0272-7358(00)00088-x

Sinclair, J. D., & Bender, D. O. (1978). Compensatory behaviors: Suggestion for a common basis from deficits in hamsters. *Life Sciences*, *22*(16), 1407–1412. https://doi.org/10.1016/0024-3205(78)90634-3

Sinclair, J. D., & Li, T.-K. (1989). Long and short alcohol deprivation: Effects on AA and P alcohol-preferring rats. *Alcohol*, *6*(6), 505–509. https://doi.org/10.1016/0741-8329(89)90059-1

Sirnes, E., Griffiths, S. T., Aukland, S. M., Eide, G. E., Elgen, I. B., & Gundersen, H. (2018). Functional MRI in prenatally opioid-exposed children during a working memory-selective attention task. *Neurotoxicology and Teratology*, *66*, 46–54. https://doi.org/10.1016/j.ntt.2018.01.010

Sirnes, E., Oltedal, L., Bartsch, H., Eide, G. E., Elgen, I. B., & Aukland, S. M. (2017). Brain morphology in school-aged children with prenatal opioid exposure: A structural MRI study. *Early Human Development*, *106–107*, 33–39. https://doi.org/10.1016/j.earlhumdev.2017.01.009

Sithisarn, T., Legan, S. J., Westgate, P. M., Wilson, M., Wellmann, K., Bada, H. S., & Barron, S. (2017). The Effects of Perinatal Oxycodone Exposure on Behavioral Outcome in a Rodent Model. *Frontiers in Pediatrics*, *5*, 180. https://doi.org/10.3389/fped.2017.00180

Slamberová, R., Rimanóczy, A., Bar, N., Schindler, C. J., & Vathy, I. (2003). Density of mu-opioid receptors in the hippocampus of adult male and female rats is altered by prenatal morphine exposure and gonadal hormone treatment. *Hippocampus*, *13*(4), 461–471. https://doi.org/10.1002/hipo.10076

Slamberová, R., Rimanóczy, A., Riley, M. A., & Vathy, I. (2004). Hypothalamo-pituitary-adrenal axis-regulated stress response and negative feedback sensitivity is altered by prenatal morphine exposure in adult female rats. *Neuroendocrinology*, *80*(3), 192–200. https://doi.org/10.1159/000082359

Smidt, M. P., & Burbach, J. P. H. (2007). How to make a mesodiencephalic dopaminergic neuron. *Nature Reviews. Neuroscience*, *8*(1), 21–32. https://doi.org/10.1038/nrn2039

Smith, C. J., Lintz, T., Clark, M. J., Malacon, K. E., Abiad, A., Constantino, N. J., Kim, V. J., Jo, Y. C., Alonso-Caraballo, Y., Bilbo, S. D., & Chartoff, E. H. (2022). Prenatal opioid exposure inhibits microglial sculpting of the dopamine system selectively in adolescent male offspring. *Neuropsychopharmacology*, *47*(10), 1755–1763. https://doi.org/10.1038/s41386-022-01376-4

Solomon, R. L., & Corbit, J. D. (1974). An opponent-process theory of motivation: I. Temporal dynamics of affect. *Psychological Review*, *81*(2), 119–145. https://doi.org/10.1037/h0036128

Song, J., & Choi, S.-Y. (2023). Arcuate Nucleus of the Hypothalamus: Anatomy, Physiology, and Diseases. *Experimental Neurobiology*, *32*(6), 371–386. https://doi.org/10.5607/en23040

Spadoni, A. D., Vinograd, M., Cuccurazzu, B., Torres, K., Glynn, L. M., Davis, E. P., Baram, T. Z., Baker, D. G., Nievergelt, C. M., & Risbrough, V. B. (2022). Contribution of early-life unpredictability to neuropsychiatric symptom patterns in adulthood. *Depression and Anxiety*, *39*(10–11), 706–717. https://doi.org/10.1002/da.23277

Spanagel, R., Herz, A., & Shippenberg, T. S. (1992). Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. *Proceedings of the National Academy of Sciences*, *89*(6), 2046–2050. https://doi.org/10.1073/pnas.89.6.2046

Spanagel, R., & Zieglgänsberger, W. (1997). Anti-craving compounds for ethanol: New pharmacological tools to study addictive processes. *Trends in Pharmacological Sciences*, *18*(2), 54–59.

Squarzoni, P., Oller, G., Hoeffel, G., Pont-Lezica, L., Rostaing, P., Low, D., Bessis, A., Ginhoux, F., & Garel, S. (2014). Microglia Modulate Wiring of the Embryonic Forebrain. *Cell Reports*, *8*(5), 1271–1279. https://doi.org/10.1016/j.celrep.2014.07.042

Steiner, J. E. (1973). The gustofacial response: Observation on normal and anencephalic newborn infants. *Symposium on Oral Sensation and Perception*, *4*, 254–278.

Stetler, C., & Miller, G. E. (2011). Depression and hypothalamic-pituitary-adrenal activation: A quantitative summary of four decades of research. *Psychosomatic Medicine*, *73*(2), 114–126. https://doi.org/10.1097/PSY.0b013e31820ad12b

Strathearn, L., Fonagy, P., Amico, J., & Montague, P. R. (2009). Adult attachment predicts maternal brain and oxytocin response to infant cues. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *34*(13), 2655–2666. https://doi.org/10.1038/npp.2009.103

Stromberg, M. F., Meister, S., Volpicelli, J. R., & Ulm, R. R. (1997). Morphine enhances selection of both sucrose and ethanol in a two-bottle test. *Alcohol (Fayetteville, N.Y.)*, *14*(1), 55–62. https://doi.org/10.1016/s0741-8329(96)00107-3

Substance Abuse and Mental Health Services Administration (SAMHSA). (2016). *RESULTS FROM THE 2015 NATIONAL SURVEY ON DRUG USE AND HEALTH: DETAILED TABLES*. SAMHSA. http://www.samhsa.gov/data/sites/default/files/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015/NSDUH-DetTabs-2015.htm#tab2-19b

Sudakin, D. (2016). Naltrexone: Not Just for Opioids Anymore. *Journal of Medical Toxicology*, *12*(1), 71–75. https://doi.org/10.1007/s13181-015-0512-x

Tabakoff, B., & Hoffman, P. L. (2000). Animal Models in Alcohol Research. *Alcohol Research & Health*, *24*(2), 77–84.

Takayanagi, Y., Yoshida, M., Bielsky, I. F., Ross, H. E., Kawamata, M., Onaka, T., Yanagisawa, T., Kimura, T., Matzuk, M. M., Young, L. J., & Nishimori, K. (2005). Pervasive social deficits, but normal parturition, in oxytocin receptor-deficient mice. *Proceedings of the National Academy of Sciences*, *102*(44), 16096–16101. https://doi.org/10.1073/pnas.0505312102

Tan, K. R., Yvon, C., Turiault, M., Mirzabekov, J. J., Doehner, J., Labouèbe, G., Deisseroth, K., Tye, K. M., & Lüscher, C. (2012). GABA neurons of the VTA drive conditioned place aversion. *Neuron*, *73*(6), 1173–1183. https://doi.org/10.1016/j.neuron.2012.02.015

Tempel, A. (1991a). Visualization of mu opiate receptor downregulation following morphine treatment in neonatal rat brain. *Brain Research. Developmental Brain Research*, *64*(1–2), 19–26. https://doi.org/10.1016/0165-3806(91)90204-v

Tempel, A. (1991b). Visualization of μ opiate receptor downregulation following morphine treatment in neonatal rat brain. *Developmental Brain Research*, *64*(1), 19–26. https://doi.org/10.1016/0165-3806(91)90204-V

Tempel, A., & Espinoza, K. (1992). Morphine-induced downregulation of mu-opioid receptors and peptide synthesis in neonatal rat brain. *Annals of the New York Academy of Sciences*, *654*, 529–530. https://doi.org/10.1111/j.1749-6632.1992.tb26021.x

Tempel, A., Habas, J., Paredes, W., & Barr, G. A. (1988). Morphine-induced downregulation of mu-opioid receptors in neonatal rat brain. *Brain Research*, *469*(1–2), 129–133. https://doi.org/10.1016/0165-3806(88)90176-9

Terauchi, A., Yee, P., Johnson-Venkatesh, E. M., Seiglie, M. P., Kim, L., Pitino, J. C., Kritzer, E., Zhang, Q., Zhou, J., Li, Y., Ginty, D. D., Lee, W.-C. A., & Umemori, H. (2023). The projection-specific signals that establish functionally segregated dopaminergic synapses. *Cell*, *186*(18), 3845-3861.e24. https://doi.org/10.1016/j.cell.2023.07.023

Thoman, E. B., Denenberg, V. H., Sievel, J., Zeidner, L. P., & Becker, P. (1981). State organization in neonates: Developmental inconsistency indicates risk for developmental dysfunction. *Neuropediatrics*, *12*(1), 45–54. https://doi.org/10.1055/s-2008-1059638

Thul, T. A., Corwin, E. J., Carlson, N. S., Brennan, P. A., & Young, L. J. (2020). Oxytocin and postpartum depression: A systematic review. *Psychoneuroendocrinology*, *120*, 104793. https://doi.org/10.1016/j.psyneuen.2020.104793

Timár, J., Sobor, M., Király, K. P., Gyarmati, S., Riba, P., Al-Khrasani, M., & Fürst, S. (2010). Peri, pre and postnatal morphine exposure: Exposure-induced effects and sex differences in the behavioural consequences in rat offspring. *Behavioural Pharmacology*, *21*(1), 58–68. https://doi.org/10.1097/FBP.0b013e3283359f39

Torabi, M., Pooriamehr, A., Bigdeli, I., & Miladi-Gorji, H. (2017). Maternal swimming exercise during pregnancy attenuates anxiety/depressive-like behaviors and voluntary morphine consumption in the pubertal male and female rat offspring born from morphine dependent mothers. *Neuroscience Letters*, *659*, 110–114. https://doi.org/10.1016/j.neulet.2017.08.074

Trezza, V., Baarendse, P. J. J., & Vanderschuren, L. J. M. J. (2010). The Pleasures of Play: Pharmacological Insights into Social Reward Mechanisms. *Trends in Pharmacological Sciences*, *31*(10), 463–469. https://doi.org/10.1016/j.tips.2010.06.008

Trezza, V., Damsteegt, R., Achterberg, E. J. M., & Vanderschuren, L. J. M. J. (2011). Nucleus Accumbens μ-Opioid Receptors Mediate Social Reward. *The Journal of Neuroscience*, *31*(17), 6362–6370. https://doi.org/10.1523/JNEUROSCI.5492-10.2011

Trezza, V., & Vanderschuren, L. J. M. J. (2008). CANNABINOID AND OPIOID MODULATION OF SOCIAL PLAY BEHAVIOR IN ADOLESCENT RATS: DIFFERENTIAL BEHAVIORAL MECHANISMS. *European Neuropsychopharmacology : The Journal of the European College of Neuropsychopharmacology*, *18*(7), 519–530. https://doi.org/10.1016/j.euroneuro.2008.03.001

Trezza, V., & Vanderschuren, L. J. M. J. (2009). Divergent Effects of Anandamide Transporter Inhibitors with Different Target Selectivity on Social Play Behavior in Adolescent Rats. *The Journal of Pharmacology and Experimental Therapeutics*, *328*(1), 343–350. https://doi.org/10.1124/jpet.108.141069

Truchet, B., Chaillan, F. A., Soumireu-Mourat, B., & Roman, F. S. (2002). Learning and memory of cue-reward association meaning by modifications of synaptic efficacy in dentate gyrus and piriform cortex. *Hippocampus*, *12*(5), 600–608. https://doi.org/10.1002/hipo.10097

Vaccarino, F. J., Bloom, F. E., & Koob, G. F. (1985). Blockade of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat. *Psychopharmacology*, *86*(1), 37–42. https://doi.org/10.1007/BF00431681

Valdez, G. R., Roberts, A. J., Chan, K., Davis, H., Brennan, M., Zorrilla, E. P., & Koob, G. F. (2002). Increased ethanol self-administration and anxiety-like behavior during acute ethanol withdrawal and protracted abstinence: Regulation by corticotropin-releasing factor. *Alcoholism, Clinical and Experimental Research*, *26*(10), 1494–1501. https://doi.org/10.1097/01.ALC.0000033120.51856.F0

Valenti, O., Lodge, D. J., & Grace, A. A. (2011). Aversive Stimuli Alter Ventral Tegmental Area Dopamine Neuron Activity via a Common Action in the Ventral Hippocampus. *The Journal of Neuroscience*, *31*(11), 4280–4289. https://doi.org/10.1523/JNEUROSCI.5310-10.2011

Van Dam, N. T., Rando, K., Potenza, M. N., Tuit, K., & Sinha, R. (2014). Childhood Maltreatment, Altered Limbic Neurobiology, and Substance Use Relapse Severity via Trauma-Specific Reductions in Limbic Gray Matter Volume. *JAMA Psychiatry*, *71*(8), 917–925. https://doi.org/10.1001/jamapsychiatry.2014.680

van der Kooy, D., Mucha, R. F., O'Shaughnessy, M., & Bucenieks, P. (1982). Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. *Brain Research*, *243*(1), 107–117. https://doi.org/10.1016/0006-8993(82)91124-6

van Zessen, R., Phillips, J. L., Budygin, E. A., & Stuber, G. D. (2012). Activation of VTA GABA neurons disrupts reward consumption. *Neuron*, *73*(6), 1184–1194. https://doi.org/10.1016/j.neuron.2012.02.016

Vandenberg, K. A. (2007). State systems development in high-risk newborns in the neonatal intensive care unit: Identification and management of sleep, alertness, and crying. *The*

Journal of Perinatal & Neonatal Nursing, *21*(2), 130–139. https://doi.org/10.1097/01.JPN.0000270630.96864.9a

Vassoler, F. M., & Byrnes, E. M. (2021). Transgenerational effects on anxiety-like behavior following adolescent morphine exposure in female rats. *Behavioural Brain Research*, *406*, 113239. https://doi.org/10.1016/j.bbr.2021.113239

Vassoler, F. M., Wright, S. J., & Byrnes, E. M. (2016). Exposure to opiates in female adolescents alters mu opiate receptor expression and increases the rewarding effects of morphine in future offspring. *Neuropharmacology*, *103*, 112–121. https://doi.org/10.1016/j.neuropharm.2015.11.026

Vathy, I., He, H. J., Iodice, M., Hnatczuk, O. C., & Rimanóczy, A. (2000). Prenatal morphine exposure differentially alters TH-immunoreactivity in the stress-sensitive brain circuitry of adult male and female rats. *Brain Research Bulletin*, *51*(3), 267–273. https://doi.org/10.1016/s0361-9230(99)00227-0

Vathy, I., Slamberová, R., & Liu, X. (2007). Foster mother care but not prenatal morphine exposure enhances cocaine self-administration in young adult male and female rats. *Developmental Psychobiology*, *49*(5), 463–473. https://doi.org/10.1002/dev.20240

Vathy, I., Slamberová, R., Rimanóczy, A., Riley, M. A., & Bar, N. (2003). Autoradiographic evidence that prenatal morphine exposure sex-dependently alters mu-opioid receptor densities in brain regions that are involved in the control of drug abuse and other motivated behaviors. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *27*(3), 381–393. https://doi.org/10.1016/S0278-5846(02)00355-X

Vathy, I. U., Etgen, A. M., & Barfield, R. J. (1985). Effects of prenatal exposure to morphine on the development of sexual behavior in rats. *Pharmacology, Biochemistry, and Behavior*, *22*(2), 227–232. https://doi.org/10.1016/0091-3057(85)90382-x

Velez, M., & Jansson, L. M. (2008). The Opioid dependent mother and newborn dyad: Non-pharmacologic care. *Journal of Addiction Medicine*, *2*(3), 113–120. https://doi.org/10.1097/ADM.0b013e31817e6105

Ventura, R., Coccurello, R., Andolina, D., Latagliata, E. C., Zanettini, C., Lampis, V., Battaglia, M., D'Amato, F. R., & Moles, A. (2013). Postnatal aversive experience impairs sensitivity to natural rewards and increases susceptibility to negative events in adult life. *Cerebral Cortex (New York, N.Y.: 1991)*, *23*(7), 1606–1617. https://doi.org/10.1093/cercor/bhs145

Vermeulen-Smit, E., Koning, I. M., Verdurmen, J. E. E., Van der Vorst, H., Engels, R. C. M. E., & Vollebergh, W. A. M. (2012). The influence of paternal and maternal drinking patterns within two-partner families on the initiation and development of adolescent drinking. *Addictive Behaviors*, *37*(11), 1248–1256. https://doi.org/10.1016/j.addbeh.2012.06.005

Victoria, N. C., Karom, M. C., & Murphy, A. Z. (2014). Analgesia for Early-Life Pain Prevents Deficits in Adult Anxiety and Stress in Rats. *Developmental Neuroscience*, *37*(1), 1–13. https://doi.org/10.1159/000366273

Vidal, S. I., Vandeleur, C., Rothen, S., Gholam-Rezaee, M., Castelao, E., Halfon, O., Aubry, J.-M., Ferrero, F., & Preisig, M. (2012). Risk of Mental Disorders in Children of Parents with Alcohol or Heroin Dependence: A Controlled High-Risk Study. *European Addiction Research*, *18*(5), 253–264. https://doi.org/10.1159/000337328

Volkow, N. D., Wise, R. A., & Baler, R. (2017). The dopamine motive system: Implications for drug and food addiction. *Nature Reviews Neuroscience*, *18*(12), 741–752. https://doi.org/10.1038/nrn.2017.130

Volpicelli, J. R., Davis, M. A., & Olgin, J. E. (1986). Naltrexone blocks the post-shock increase of ethanol consumption. *Life Sciences*, *38*(9), 841–847. https://doi.org/10.1016/0024-3205(86)90601-6

Vosberg, D. E., Leyton, M., & Flores, C. (2020). The Netrin-1/DCC guidance system: Dopamine pathway maturation and psychiatric disorders emerging in adolescence. *Molecular Psychiatry*, *25*(2), 297–307. https://doi.org/10.1038/s41380-019-0561-7

Wachman, E. M., Hayes, M. J., Brown, M. S., Paul, J., Harvey-Wilkes, K., Terrin, N., Huggins, G. S., Aranda, J. V., & Davis, J. M. (2013). Association of OPRM1 and COMT Single-Nucleotide Polymorphisms With Hospital Length of Stay and Treatment of Neonatal Abstinence Syndrome. *JAMA*, *309*(17), 1821–1827. https://doi.org/10.1001/jama.2013.3411

Wachman, E. M., Hayes, M. J., Lester, B. M., Terrin, N., Brown, M. S., Nielsen, D. A., & Davis, J. M. (2014). Epigenetic Variation in the Mu-opioid Receptor Gene in Infants with Neonatal Abstinence Syndrome. *The Journal of Pediatrics*, *165*(3), 472–478. https://doi.org/10.1016/j.jpeds.2014.05.040

Wachman, E. M., Hayes, M. J., Sherva, R., Brown, M. S., Davis, J. M., Farrer, L. A., & Nielsen, D. A. (2015). Variations in Opioid Receptor Genes in Neonatal Abstinence Syndrome. *Drug and Alcohol Dependence*, *155*, 253–259. https://doi.org/10.1016/j.drugalcdep.2015.07.001

Wachman, E. M., Hayes, M. J., Sherva, R., Brown, M. S., Shrestha, H., Logan, B. A., Heller, N. A., Nielsen, D. A., & Farrer, L. A. (2017). Association of maternal and infant variants in PNOC and COMT genes with Neonatal Abstinence Syndrome severity. *The American Journal on Addictions*, *26*(1), 42–49. https://doi.org/10.1111/ajad.12483

Wachman, E. M., Schiff, D. M., & Silverstein, M. (2018). Neonatal Abstinence Syndrome: Advances in Diagnosis and Treatment. *JAMA*, *319*(13), 1362–1374. https://doi.org/10.1001/jama.2018.2640

Walhovd, K. B., Moe, V., Slinning, K., Due-Tønnessen, P., Bjørnerud, A., Dale, A. M., van der Kouwe, A., Quinn, B. T., Kosofsky, B., Greve, D., & Fischl, B. (2007). Volumetric cerebral characteristics of children exposed to opiates and other substances in utero. *NeuroImage*, *36*(4), 1331–1344. https://doi.org/10.1016/j.neuroimage.2007.03.070

Walhovd, K. B., Watts, R., Amlien, I., & Woodward, L. J. (2012). Neural Tract Development of Infants Born to Methadone-Maintained Mothers. *Pediatric Neurology*, *47*(1), 1–6. https://doi.org/10.1016/j.pediatrneurol.2012.04.008

Wang, Y., Berger, L., Durrance, C., Kirby, R. S., Kuo, D., Pac, J., & Ehrenthal, D. B. (2023). Duration and Timing of In Utero Opioid Exposure and Incidence of Neonatal Withdrawal Syndrome. *Obstetrics and Gynecology*, *142*(3), 603–611. https://doi.org/10.1097/AOG.0000000000005289

Wang, Y., Yao, Y., Li, Y., Nie, H., & He, X. (2017). Prenatal morphine exposure during late embryonic stage enhances the rewarding effects of morphine and induces the loss of membrane-bound protein kinase C-α in intermediate medial mesopallium in the chick. *Neuroscience Letters*, *639*, 25–30. https://doi.org/10.1016/j.neulet.2016.12.030

Watson, R. E., Wiegand, S. J., Clough, R. W., & Hoffman, G. E. (1986). Use of cryoprotectant to maintain long-term peptide immunoreactivity and tissue morphology. *Peptides*, *7*(1), 155–159. https://doi.org/10.1016/0196-9781(86)90076-8

Watterson, E., Daniels, C. W., Watterson, L. R., Mazur, G. J., Brackney, R. J., Olive, M. F., & Sanabria, F. (2015). Nicotine-induced place conditioning and locomotor activity in an adolescent animal model of attention deficit/hyperactivity disorder (ADHD). *Behavioural Brain Research*, *291*, 184–188. https://doi.org/10.1016/j.bbr.2015.05.031

Weller, A. E., Crist, R. C., Reiner, B. C., Doyle, G. A., & Berrettini, W. H. (2021). Neonatal Opioid Withdrawal Syndrome (NOWS): A Transgenerational Echo of the Opioid Crisis. *Cold Spring Harbor Perspectives in Medicine*, *11*(3), a039669. https://doi.org/10.1101/cshperspect.a039669

Welle-Strand, G. K., Skurtveit, S., Jansson, L. M., Bakstad, B., Bjarkø, L., & Ravndal, E. (2013). Breastfeeding reduces the need for withdrawal treatment in opioid-exposed infants. *Acta Paediatrica (Oslo, Norway: 1992)*, *102*(11), 1060–1066. https://doi.org/10.1111/apa.12378

Williams, A. V., Flowers, J., Coates, K. S., Ingram, A., Hehn, A. T., Dupuis, M., Wimmer, M. E., Venniro, M., & Bangasser, D. A. (2022). Early resource scarcity alters motivation for natural rewards in a sex- and reinforcer-dependent manner. *Psychopharmacology*, *239*(12), 3929–3937. https://doi.org/10.1007/s00213-022-06264-9

Windle, R. J., Kershaw, Y. M., Shanks, N., Wood, S. A., Lightman, S. L., & Ingram, C. D. (2004). Oxytocin Attenuates Stress-Induced c-fos mRNA Expression in Specific Forebrain Regions

Associated with Modulation of Hypothalamo–Pituitary–Adrenal Activity. *Journal of Neuroscience*, *24*(12), 2974–2982. https://doi.org/10.1523/JNEUROSCI.3432-03.2004

Windle, R. J., Shanks, N., Lightman, S. L., & Ingram, C. D. (1997). Central Oxytocin Administration Reduces Stress-Induced Corticosterone Release and Anxiety Behavior in Rats*. *Endocrinology*, *138*(7), 2829–2834. https://doi.org/10.1210/endo.138.7.5255

Winkelman, T. N. A., Villapiano, N., Kozhimannil, K. B., Davis, M. M., & Patrick, S. W. (2018). Incidence and Costs of Neonatal Abstinence Syndrome Among Infants With Medicaid: 2004-2014. *Pediatrics*, *141*(4), e20173520. https://doi.org/10.1542/peds.2017-3520

Wise, R. A. (1996). Addictive Drugs and Brain Stimulation Reward. *Annual Review of Neuroscience*, *19*(1), 319–340. https://doi.org/10.1146/annurev.ne.19.030196.001535

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience*, *5*(6), 483–494. https://doi.org/10.1038/nrn1406

Wise, R. A., & Rompre, P. P. (1989). Brain dopamine and reward. *Annual Review of Psychology*, *40*, 191–225. https://doi.org/10.1146/annurev.ps.40.020189.001203

Wu, C.-Y., Chen, H.-H., Tao, P.-L., & Yuan, Z. F. (2023). Comparisons of stress-related neuronal activation induced by restraint in adult male rat offspring with prenatal exposure to buprenorphine, methadone, or morphine. *The Chinese Journal of Physiology*, *66*(2), 65–72. https://doi.org/10.4103/cjop.CJOP-D-23-00015

Wu, L.-Y., Chen, J.-F., Tao, P.-L., & Huang, E. Y.-K. (2009). Attenuation by dextromethorphan on the higher liability to morphine-induced reward, caused by prenatal exposure of morphine in rat offspring. *Journal of Biomedical Science*, *16*(1), 106. https://doi.org/10.1186/1423-0127-16-106

Wukitsch, T. J., Reinhardt, E. K., Kiefer, S. W., & Cain, M. E. (2019). Voluntary ethanol consumption during early social isolation and responding for ethanol in adulthood. *Alcohol*, *77*, 1–10. https://doi.org/10.1016/j.alcohol.2018.09.004

Wultz, B., Sagvolden, T., Moser, E. I., & Moser, M. B. (1990). The spontaneously hypertensive rat as an animal model of attention-deficit hyperactivity disorder: Effects of methylphenidate on exploratory behavior. *Behavioral and Neural Biology*, *53*(1), 88–102. https://doi.org/10.1016/0163-1047(90)90848-z

Xia, Y., Driscoll, J. R., Wilbrecht, L., Margolis, E. B., Fields, H. L., & Hjelmstad, G. O. (2011). Nucleus Accumbens Medium Spiny Neurons Target Non-Dopaminergic Neurons in the Ventral Tegmental Area. *Journal of Neuroscience*, *31*(21), 7811–7816. https://doi.org/10.1523/JNEUROSCI.1504-11.2011

Xu, P., Wang, K., Lu, C., Dong, L., Chen, Y., Wang, Q., Shi, Z., Yang, Y., Chen, S., & Liu, X. (2017). Effects of the chronic restraint stress induced depression on reward-related learning in rats. *Behavioural Brain Research*, *321*, 185–192. https://doi.org/10.1016/j.bbr.2016.12.045

Xuei, X., Flury-Wetherill, L., Almasy, L., Bierut, L., Tischfield, J., Schuckit, M., Nurnberger, J. I., Foroud, T., & Edenberg, H. J. (2008). Association analysis of genes encoding the nociceptin receptor (OPRL1) and its endogenous ligand (PNOC) with alcohol or illicit drug dependence. *Addiction Biology*, *13*(1), 80–87. https://doi.org/10.1111/j.1369-1600.2007.00082.x

Yang, H., de Jong, J. W., Tak, Y., Peck, J., Bateup, H., & Lammel, S. (2018). Nucleus accumbens subnuclei regulate motivated behavior via direct inhibition and disinhibition of VTA dopamine subpopulations. *Neuron*, *97*(2), 434-449.e4. https://doi.org/10.1016/j.neuron.2017.12.022

Yazdanfar, N., Farnam, A., Sadigh-Eteghad, S., Mahmoudi, J., & Sarkaki, A. (2021). Enriched environment and social isolation differentially modulate addiction-related behaviors in male offspring of morphine-addicted dams: The possible role of μ-opioid receptors and ΔFosB in the brain reward pathway. *Brain Research Bulletin*, *170*, 98–105. https://doi.org/10.1016/j.brainresbull.2021.02.005

Yen, E., Kaneko-Tarui, T., Ruthazer, R., Harvey-Wilkes, K., Hassaneen, M., & Maron, J. L. (2019). Sex-Dependent Gene Expression in Infants with Neonatal Opioid Withdrawal Syndrome. *The Journal of Pediatrics*, *214*, 60-65.e2. https://doi.org/10.1016/j.jpeds.2019.07.032

Yeoh, S. L., Eastwood, J., Wright, I. M., Morton, R., Melhuish, E., Ward, M., & Oei, J. L. (2019). Cognitive and Motor Outcomes of Children With Prenatal Opioid Exposure. *JAMA Network Open*, *2*(7), e197025. https://doi.org/10.1001/jamanetworkopen.2019.7025

Yohn, N. L., Bartolomei, M. S., & Blendy, J. A. (2015). Multigenerational and Transgenerational Inheritance of Drug Exposure: The effects of alcohol, opiates, cocaine, marijuana, and nicotine. *Progress in Biophysics and Molecular Biology*, *118*(0), 21–33. https://doi.org/10.1016/j.pbiomolbio.2015.03.002

Yoo, S. H., Jansson, L. M., & Park, H.-J. (2017). Sensorimotor outcomes in children with prenatal exposure to methadone. *Journal of AAPOS: The Official Publication of the American Association for Pediatric Ophthalmology and Strabismus*, *21*(4), 316–321. https://doi.org/10.1016/j.jaapos.2017.05.025

Zalewska-Kaszubska, J., Gorska, D., Dyr, W., & Czarnecka, E. (2008). Voluntary alcohol consumption and plasma beta-endorphin levels in alcohol-preferring rats chronically treated with naltrexone. *Physiology & Behavior*, *93*(4), 1005–1010. https://doi.org/10.1016/j.physbeh.2008.01.007

Zhang, R., Zhang, H.-F., Han, J.-S., & Han, S.-P. (2017). Genes Related to Oxytocin and Arginine-Vasopressin Pathways: Associations with Autism Spectrum Disorders. *Neuroscience Bulletin*, *33*(2), 238–246. https://doi.org/10.1007/s12264-017-0120-7

Zhou, Z., Liu, X., Chen, S., Zhang, Z., Liu, Y., Montardy, Q., Tang, Y., Wei, P., Liu, N., Li, L., Song, R., Lai, J., He, X., Chen, C., Bi, G., Feng, G., Xu, F., & Wang, L. (2019). A VTA GABAergic Neural Circuit Mediates Visually Evoked Innate Defensive Responses. *Neuron*, *103*(3), 473-488.e6. https://doi.org/10.1016/j.neuron.2019.05.027

Zhu, Y., Wienecke, C. F. R., Nachtrab, G., & Chen, X. (2016). A thalamic input to the nucleus accumbens mediates opiate dependence. *Nature*, *530*(7589), 219–222. https://doi.org/10.1038/nature16954

Zito, K. A., Vickers, G., & Roberts, D. C. S. (1985). Disruption of cocaine and heroin self-administration following kainic acid lesions of the nucleus accumbens. *Pharmacology Biochemistry and Behavior*, *23*(6), 1029–1036. https://doi.org/10.1016/0091-3057(85)90110-8

Zukin, R. S., Sugarman, J. R., Fitz-Syage, M. L., Gardner, E. L., Zukin, S. R., & Gintzler, A. R. (1982). Naltrexone-induced opiate receptor supersensitivity. *Brain Research*, *245*(2), 285–292. https://doi.org/10.1016/0006-8993(82)90811-3