Analysis of maternal behavior in oxycodone-exposed rats transitioned to methadone or abstinence

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1.0 Introduction

Opioid use disorder (OUD) during pregnancy is a growing public health concern due to its potential effects on maternal health, fetal development, and postpartum caregiving. Between 1999 and 2014, maternal opioid-related diagnoses at hospital delivery more than quadrupled. Prenatal opioid exposure is also associated with neonatal opioid withdrawal syndrome (NOWS), which can involve disturbances in neurological, autonomic, and gastrointestinal functioning following birth. Although the effects of prenatal opioid exposure on infants have been previously researched, its potential effects on the maternal brain and postpartum caregiving remain less understood, highlighting the need to investigate maternal behavior following opioid exposure and treatment during pregnancy (Wallin et al., 2021). Medications for opioid use disorder (MOUD), including methadone and buprenorphine, are commonly used during pregnancy to stabilize opioid exposure by reducing withdrawal symptoms and lowering the risk of relapse. Although these treatments provide important clinical benefits for infants born with NOWS, their potential effects on the maternal brain and postpartum caregiving remain incompletely understood (Suarez et al., 2022; Wallin et al., 2021). This is relevant as maternal behavior is a sensitive and complex system that depends on coordinated neurobiological changes during pregnancy and the postpartum period.

The transition to motherhood involves adaptations within the Maternal Brain Network (MBN), a collection of interconnected brain regions responsible for maternal motivation, caregiving, and responsiveness to offspring. This network is regulated by hormonal and neurotransmitter systems, including oxytocin, dopamine, and endogenous opioids. As exogenous opioids interact with systems involved in maternal behavior, exposure during pregnancy may interfere with the development of maternal caregiving (Numan & Young, 2016; Swain et al., 2019; Wallin et al., 2021). Despite increasing research on the effects of prenatal opioid exposure on infants, less attention has been directed toward postpartum maternal behavior. Understanding how methadone maintenance and opioid withdrawal influence caregiving is important because maternal interactions contribute to the early environment experienced by infants and subsequently influence behavioral and emotional development (Lonstein et al., 2015; Swain et al., 2019).

This study investigates the effects of gestational methadone maintenance versus withdrawal/abstinence on postpartum maternal behavior in rats. Maternal caregiving activities were assessed using behavioral video recordings collected on postnatal days (PND) 3, 6, 9, and 12. Behavioral transitions and entropy were also examined to characterize the patterns and variability of maternal behavior. The objective was to determine whether different approaches to managing opioid dependence during pregnancy are associated with changes in postpartum caregiving patterns.

2.0 Background and Literature Review

2.1 Neurobiology of the Maternal Brain Network

Maternal behavior is regulated by a coordinated network of brain regions that undergo functional changes during pregnancy and the postpartum period. The Maternal Brain Network (MBN) supports the transition from a nonmaternal state to one characterized by increased responsiveness to offspring, maternal motivation, and caregiving behaviors. Important components include the medial preoptic area (mPOA), ventral tegmental area (VTA), nucleus accumbens (NAc), ventral pallidum (VP), and periaqueductal gray (PAG) (Lonstein et al., 2015; Wallin et al., 2021). Such neural systems regulating maternal caregiving are largely conserved across mammals. Similar brain networks are involved in maternal motivation, reward, and responsiveness to offspring in both humans and rodents (Lonstein et al., 2015; Numan & Young, 2016). In rodent models, maternal caregiving is characterized by behaviors such as nursing, pup retrieval, licking and grooming, and nest building. These behaviors provide measurable indicators of maternal responsiveness and this study to investigate the neural mechanisms behind caregiving.

The mPOA is particularly important for the initiation and maintenance of maternal behavior. It integrates hormonal and sensory information associated with pregnancy, birth, and offspring, contributing to behaviors such as nursing, pup retrieval, and licking and grooming. The mPOA interacts with neural pathways involved in motivation and reward, allowing offspring-related stimuli to be rewarding as to promote maternal caregiving (Numan & Young, 2016). The mesolimbic dopamine system or “reward-circuit”, which includes the VTA to the NAc, plays an important role in maternal motivation. Dopamine signaling contributes to the rewarding properties of interactions with offspring, encouraging mothers to approach and care for their young. The VP also contributes to the expression of motivated maternal behavior through its connections with the mesolimbic reward pathway (Numan & Young, 2016; Swain et al., 2019). Other components of the MBN participate in maternal defense and responses to environmental stimuli (Figure 1). For example, the PAG is associated with maternal defensive responses, supporting protective behaviors toward offspring across mammalian species, including humans. Together, these regions support the coordination of caregiving, maternal motivation, and protective behavior (Wallin et al., 2021). Together, the MBN provides a neurobiological framework for understanding how changes in neurotransmitter signaling or exposure to exogenous opioids may influence maternal behavior.

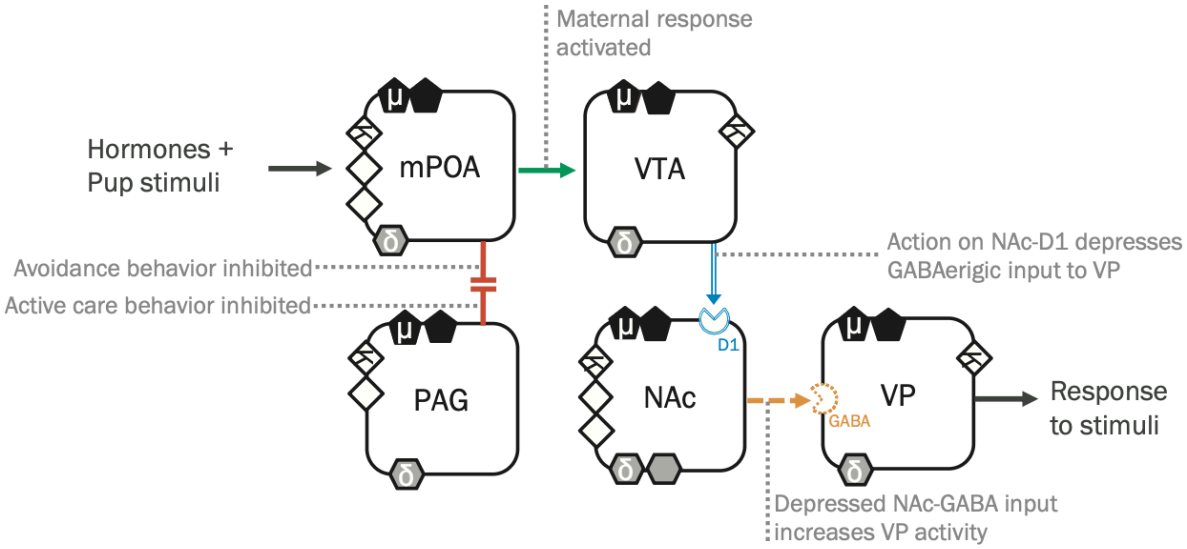


Figure 1. Simplified Maternal Brain Network Connections and Opioid Receptor Distribution. Hormonal and pup-related stimuli activate the medial preoptic area (mPOA), stimulating the ventral tegmental area (VTA). Dopamine signaling from the VTA to the nucleus accumbens (NAc) reduces inhibitory GABAergic signaling to the ventral pallidum (VP), allowing maternal caregiving behaviors to occur. The mPOA also inhibits the periaqueductal gray (PAG) during maternal care, while the PAG can temporarily inhibit the mPOA to facilitate transitions to other behaviors. The diagram also illustrates the distribution of mu (μ), kappa (κ), and delta (δ) opioid receptors throughout the network. Reproduced from Figure 2 in Wallin et al. (2021).

2.2 Hormonal Regulation of Maternal Sensitivity

The development of maternal behavior depends on hormonal and neurochemical adaptations occurring throughout pregnancy and after birth. Changes in reproductive hormones help prepare the maternal brain to respond to offspring, while neurotransmitter systems contribute to the motivation and reinforcement of caregiving behaviors (Lonstein et al., 2015).

Oxytocin is an important regulator of maternal responsiveness and mother-infant bonding. It contributes to the development of maternal behavior and supports the neural processes involved in recognizing and responding to offspring-related stimuli. Oxytocin also interacts with brain regions associated with maternal motivation and reward (Figure 1), helping facilitate caregiving behaviors during the postpartum period (Numan & Young, 2016; Swain et al., 2019). Dopamine drives maternal sensitivity by further contributing to the motivational and rewarding aspects of maternal care. Activity within the VTA-NAc pathway helps reinforce interactions with offspring, promoting behaviors such as pup approach and retrieval. The coordinated activity of oxytocin and dopamine systems supports the transition toward sustained maternal caregiving (Numan & Young, 2016). Endogenous opioids are another important component of maternal neurobiology. Opioid signaling modulates reward, emotional processing, and responses to offspring. Since endogenous opioid activity contributes to neurochemical changes during the transition to motherhood, alterations in opioid signaling may influence maternal motivation and responsiveness (Swain et al., 2019; Wallin et al., 2021). Together,

hormonal and neurotransmitter systems regulate maternal sensitivity by influencing how offspring-related stimuli are processed and how caregiving behaviors are initiated and maintained.

2.3 Dysregulation of the Maternal Brain Network

Dysregulation of the MBN are factors interfering with the initiation, organization, and/or maintenance of maternal caregiving behaviors. Since maternal behavior depends on coordinated activity among hormonal, motivational, and reward-related systems, disruptions within these pathways may alter maternal responsiveness to offspring (Lonstein et al., 2015; Wallin et al., 2021). These changes may be reflected in caregiving behaviors such as nursing, licking and grooming, pup retrieval, and attendance to nest, which are important indicators of maternal attention and responsiveness (Numan & Young, 2016; Wallin et al., 2021).

Stress is an important consideration in maternal behavior as it interferes with the coordination between neural systems involved in caregiving, emotional regulation, and responses to environmental challenges (Lambert & Byrnes, 2019). Maternal behavior is influenced by the balance between offspring-directed motivation and defensive responses to competing stimuli or threats (Lonstein et al., 2015). The MBN includes neural regions involved in both maternal caregiving (mPOA) and maternal defense (PAG). Hence, disruptions in stress-related and emotional processes may influence how mothers allocate their behavior between caring for offspring and responding to their environment (Wallin et al., 2021). In the context of opioid dependence, withdrawal represents an additional physiological and behavioral challenge with similar stress responses. Generally, OUD is known to disrupt maternal behavior by neural reward and motivation systems. The endogenous opioid system normally contributes to the neurochemical adaptations required for maternal care (Swain et al., 2019; Wallin et al., 2021). However, exogenous opioids interfere with these opioid receptors, altering signaling within the MBN, including regions involved in maternal motivation and responsiveness. (Wallin et al., 2021). OUD may also disrupt neural systems responsible for balancing maternal caregiving and defense.

Preclinical studies demonstrate that opioid signaling influences interactions between the mPOA and PAG, suggesting that exogenous opioids may interfere with the regulation of caregiving and defensive responses (Wallin et al., 2021). Such studies are limited by type of opioid administered, timing of exposure, and behavioral measures examined (Byrnes & Vassoler, 2018). Opioid withdrawal may also introduce additional physiological stress, potentially affecting maternal motivation and responsiveness. Therefore, comparing methadone maintenance with forced withdrawal provides an opportunity to investigate whether differences in opioid exposure and withdrawal conditions are associated with changes in maternal caregiving.

2.4 Medications for Opioid Use Disorder During Pregnancy and Postpartum

MOUD are used to manage opioid dependence during pregnancy by reducing withdrawal symptoms, stabilizing opioid exposure, and decreasing the likelihood of relapse. Methadone and buprenorphine are commonly used medications that act on opioid receptor systems, although they differ in their pharmacological properties (Wallin et al., 2021) Methadone is a full μ-opioid receptor agonist used in maintenance treatment, while buprenorphine is a partial agonist at the μ-opioid and κ-opioid receptors. Both medications provide alternatives to repeated cycles of opioid use and withdrawal (Wallin et al., 2021). Their use during pregnancy is intended to support maternal stability and reduce risks associated with continued illicit opioid use, including exposure to infectious diseases. (Suarez et al., 2022).

Suarez et al. (2022) compared maternal and neonatal outcomes associated with buprenorphine and methadone treatment during pregnancy. Their findings demonstrated that compared to methadone treatment, buprenorphine exposure was associated with lower rates of NOWS (52.0% vs. 69.2%), preterm birth (14.4% vs. 24.9%), and low birth weight (8.3% vs. 14.9%). However, maternal outcomes, including cesarean delivery and severe maternal complications, were similar between treatment groups. Despite the neonatal advantages, methadone may offer benefits for treatment retention. Lin et al. (2025) examined treatment discontinuation during pregnancy and through one year postpartum, finding that individuals receiving buprenorphine were more likely to discontinue treatment than those receiving methadone, both during pregnancy (32.8% vs. 25.6%) and through one year postpartum (58.8% vs. 49.0%). Together, these findings highlight differences in neonatal outcomes and treatment retention between MOUD approaches, emphasizing the importance of evaluating both maternal and offspring outcomes throughout pregnancy and the postpartum period.

Although MOUD offer important benefits, exposure to opioid agonists during pregnancy may still influence the developing maternal brain. The effects of opioid maintenance therapies on the MBN and maternal behavior still remain unknown (Wallin et al., 2021). Specifically, the effect of continued methadone maintenance compared with opioid withdrawal on postpartum maternal caregiving requires further investigation. Evaluating these conditions in an animal model may help clarify how gestational opioid treatment is associated with maternal behavioral patterns and mother-offspring interactions.

3.0 Methods

The present study involved an analysis of maternal behavior recordings obtained from a previously conducted experiment in the Byrnes Lab examining the effects of gestational methadone maintenance versus withdrawal/abstinence in rats. Sections 3.1-3.4; animal preparation, catheterization, oxycodone self-administration, and gestational drug administration, were completed as part of the previous unpublished study conducted by Lia Kelly. The current

project focuses on scoring existing home-cage video recordings and examining postpartum maternal behavioral patterns.

3.1 Animals

Adult female Sprague Dawley rats (n = 14) were obtained from Charles River Laboratories and housed in a temperature- and humidity-controlled vivarium with unrestricted access to food and water. All animal procedures were approved by the Tufts University Institutional Animal Care and Use Committee (IACUC) and conducted in accordance with National Institutes of Health guidelines

3.2 Catheterization Surgery

Subjects were surgically implanted with indwelling jugular catheters under isoflurane anesthesia (2–3% in oxygen). Catheters were flushed daily with heparinized saline mixed with antibiotic solution (Gentamicin) to maintain patency. When not in use, catheters were sealed with plastic obdurators. Animals were allowed a minimum of five days to recover prior to the start of self-administration training.

3.3 Oxycodone Self-Administration (SA) Acquisition

Following recovery, subjects were trained to self-administer intravenous oxycodone in standard operant conditioning chambers equipped with two levers: an active lever delivering a drug infusion of oxycodone (0.1mg/kg/infusion) and an inactive lever with no programmed consequence. Sessions lasted six hours per day for 2.5 weeks. Active lever presses were paired with a cue light and 20-second timeout. Data were recorded and analyzed using Med-PC IV software. Following an acquisition period, females were mated with abstinent males.

3.4 Gestational Opioid Administration

On gestational day (GD) 9 or 10, confirmed pregnant subjects subcutaneously with Alzet osmotic minipumps to establish two treatment conditions. The methadone-maintained group (METH; $n = 7$) received methadone dissolved in distilled water at a dose of 10 mg/kg/day. The vehicle-treated group (VEH; $n = 7$) received sterile saline and served as a model of forced opioid withdrawal following oxycodone dependence. Contents of the minipumps were released into the local subcutaneous space at a flow rate of 2.5 µl per hour for up to 28 days. Minipumps were weighed pre- and post-filling to confirm uniform solution volume across treatment groups. Minipumps were weighed before and after filling to verify consistent solution volumes. Daily oxycodone self-administration sessions were discontinued following minipump implantation. The day of birth was designated postnatal day (PND) 0.

3.5 Behavioral Video Scoring

The present study analyzed previously recorded home-cage videos of maternal behavior collected on PND3, PND6, PND9, and PND12. Maternal behavior was examined across observation periods at 8:00 AM, 1:00 PM, and 6:00 PM. Behavioral observations were scored using Behavioral Observation Research Interactive Software (BORIS) to characterize maternal caregiving and non-maternal activities across the early postpartum period. Behavioral scoring was conducted blinded to treatment group assignment to minimize potential observer bias. A behavioral ethogram consisting of 14 categories was used to classify observed activities (Table 1).

Key	Code	Behavior	Operational Definition
1	LG	Licking/grooming pups	Mother uses her tongue to lick or groom one or more pups.
2	AN	Active nursing	Mother nurses pups while maintaining an elevated, arched-back posture.
3	LN	Low nursing	Mother nurses pups while maintaining a low, relatively flat posture over them.
4	SN	Side nursing	Mother lies on her side while pups nurse.
5	O	Off pups	Mother is not in physical contact with pups.
6	E	Eating	Mother consumes food.
7	D	Drinking	Mother drinks water.
8	SG	Self-grooming	Mother licks, scratches, or cleans her own body.
9	C	Carrying pups	Mother picks up and transports a pup, typically using her mouth.
0	NB	Nest building	Mother gathers, moves, or rearranges nesting material.
Q	M	Moving on nest	Mother changes position or moves within the nest area.
W	L	Locomotor activity	Mother walks or moves around the cage.
E	V	Vertical activity	Mother stands or rears on her hind legs.
R	P	Pups off nest	One or more pups are located outside the designated nest area.

Table 1. Ethogram of Maternal Behaviors Recorded Using BORIS Software. Key: keyboard shortcut pressed to record a behavior. Code: abbreviation assigned to each behavior for identification and data analysis. Behavior name: specific maternal activity being observed. Operational definition: observable actions qualifying the behavior. All behaviors were classified as state events to measure behavioral durations and transitions.

3.6 Statistical Analysis

Behavioral durations were quantified from coded recordings to determine the amount of time dams spent performing individual maternal and nonmaternal activities. Durations were compared between methadone maintained (METH) and vehicle treated oxycodone withdrawn (i.e. withdrawal/abstinence) (VEH) groups across PND 3, 6, 9, 12 and observation timepoints 8:00 AM, 1:00 PM, and 6:00 PM, to evaluate differences in postpartum caregiving.

Behavioral transitions were analyzed using a finite Markov chain to calculate behavioral entropy as a measure of behavioral predictability. Lower entropy values indicated more

consistent and predictable behavioral sequences, whereas higher values indicated greater variability and less predictable transitions. An entropy value of zero indicated the absence of behavioral transitions, such as when a dam remained nursing throughout an entire recording.

For entropy calculations, the 10 recorded behaviors were consolidated. Active, low, and side nursing were combined into a single nursing category, while eating and drinking were grouped together. Individual recordings were excluded from entropy calculations if the dam was not visible for a portion of the recording or did not engage in maternal behavior. These exclusions minimized potential bias from incomplete observations or insufficient scorable maternal activity. Consequently, sample sizes varied across entropy analyses and differed from those used in behavioral duration comparisons.

Behavioral transition flowcharts were generated to visualize the organization and relative frequency of transitions between maternal behaviors. The flowcharts represented four behavioral categories, with selected behaviors combined or excluded from the 10-category entropy analysis.

Analysis of variance (ANOVA) was used to evaluate the effects of treatment group and observation time on behavioral measurements. The F-statistic was used to assess variation associated with the experimental factors relative to unexplained variation, while the corresponding p-values were used to determine statistical significance. Fractional degrees of freedom in the repeated-measures ANOVA results reflected adjustments to the degrees of freedom. Statistical significance was defined as $p < .05$.

4.0 Results

4.1 Oxycodone Self-Administration Liter Outcomes

In the previous study, of the 14 female rats initially assigned to treatment groups (METH, $n = 7$; VEH, $n = 7$), all seven methadone-maintained dams successfully gave birth, whereas three vehicle-treated dams failed to deliver litters, leaving four VEH dams with offspring. This study also reported that METH dams produced significantly larger litters than VEH dams, $F(1, 16) = 6.727$, $p = .0196$. These differences in reproductive outcomes reduced the number of animals available for subsequent maternal behavioral analyses.

4.2 Maternal Behavior Durations

Maternal behavioral durations were compared between METH and VEH dams on PND3 (Figure 2) and PND6 (Figure 3). Behaviors examined included nursing, licking and grooming pups, and nonmaternal behaviors (e.g. off nest or self grooming). No statistically significant differences in individual behavioral durations were observed between treatment groups on either PND3 or PND6 (all $p > .05$).

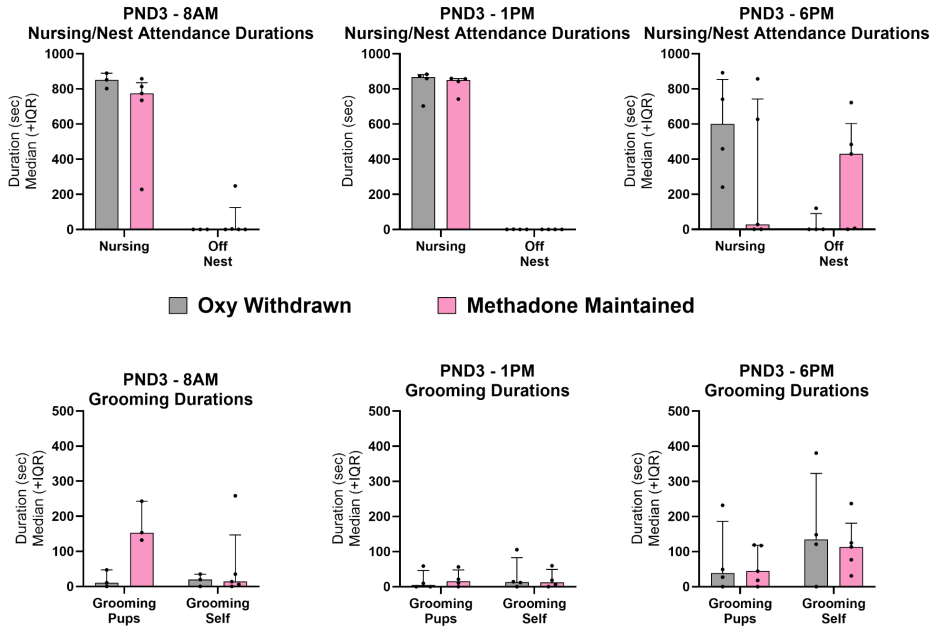


Figure 2. Maternal Behavioral Durations in Methadone Maintained and Withdrawn Dams on PND3. Comparison of time spent performing recorded maternal and nonmaternal behaviors in the METH and VEH groups on postnatal day 3. No statistically significant differences were detected between treatment groups (all $p > .05$). Pink = METH, methadone maintained; Gray = VEH oxycodone withdrawn.

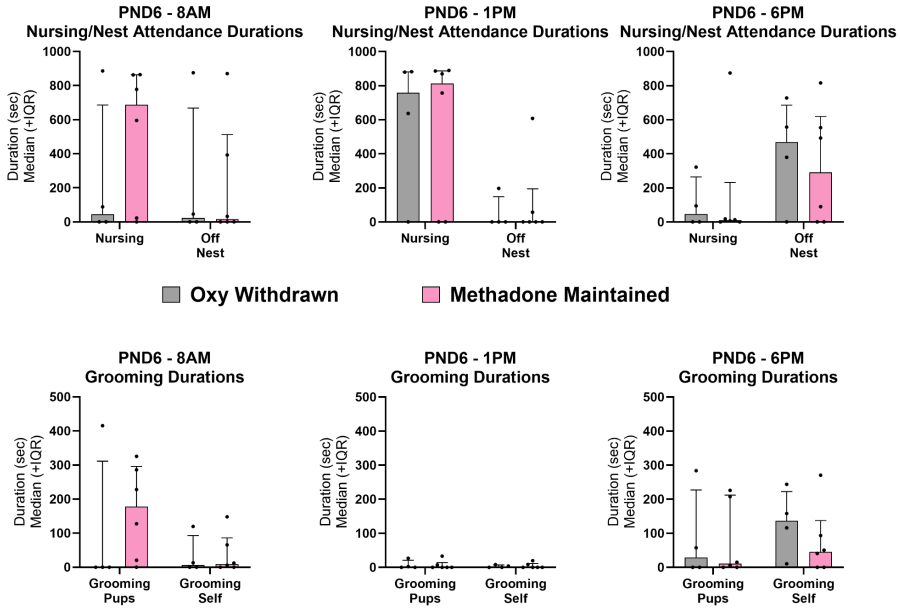


Figure 3. Maternal Behavioral Durations in Methadone Maintained and Withdrawn Dams on PND3. Comparison of time spent performing recorded maternal and nonmaternal behaviors in the METH and VEH groups on postnatal day 6. No statistically significant differences were detected between treatment groups (all $p > .05$). Pink = METH, methadone maintained; Gray = VEH oxycodone withdrawn.

4.3 Maternal Behavior Transitions

Behavioral transition flowcharts were used to visualize the organization of maternal behaviors in METH and VEH dams. These flowcharts represented four behavioral categories and illustrated the relative likelihood of transitions between successive activities.

On PND3 (Figure 4), the METH group exhibited more frequent transitions between behaviors, including increased transitions to nonmaternal activities and time spent off the nest. These patterns suggest a more disrupted maternal behavioral profile, consistent with the higher behavioral entropy observed in METH dams. In contrast, VEH dams exhibited fewer behavioral transitions and more sustained engagement in maternal caregiving activities, consistent with the expectation that dams remain focused on their pups during the early postpartum period. By PND6 (Figure 5), behavioral transition patterns appeared more similar between treatment groups, suggesting that the differences observed on PND3 became less pronounced.

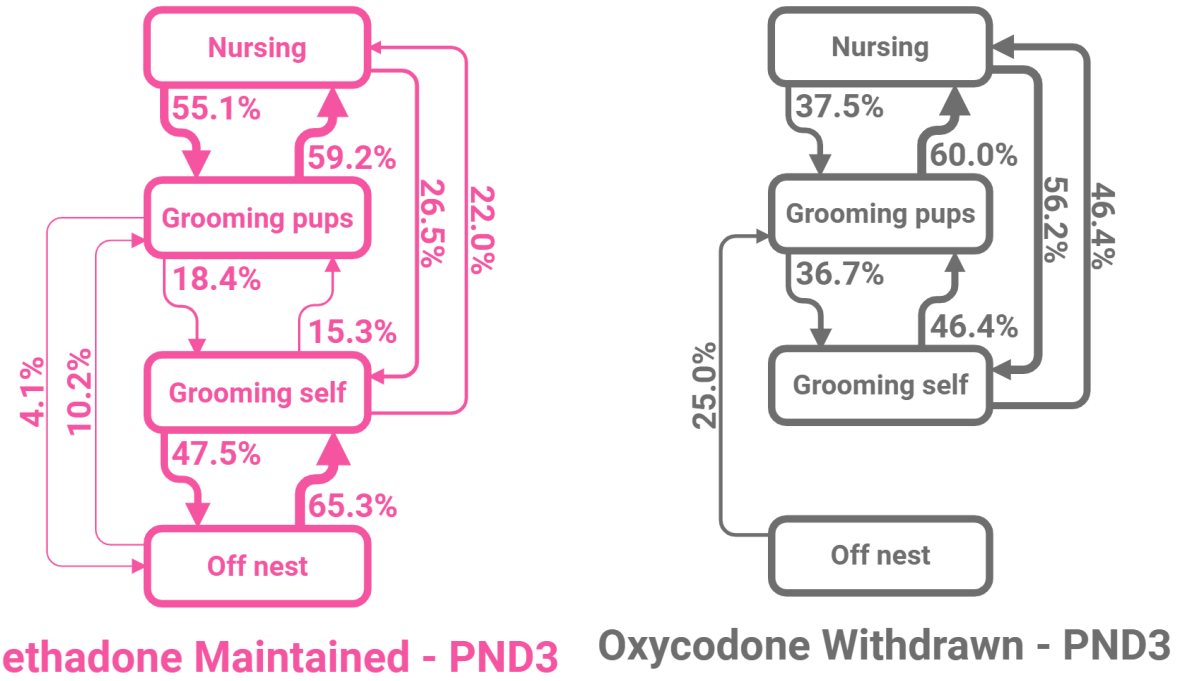


Figure 4. Maternal Behavioral Transition Patterns on PND3. Flowcharts illustrate the sequence and relative frequency of transitions between four behavioral categories in METH and VEH dams on postnatal day 3. Arrows represent transitions between behaviors and statistical values indicate transition probabilities. Pink = METH, methadone maintained; Gray = VEH oxycodone withdrawn.

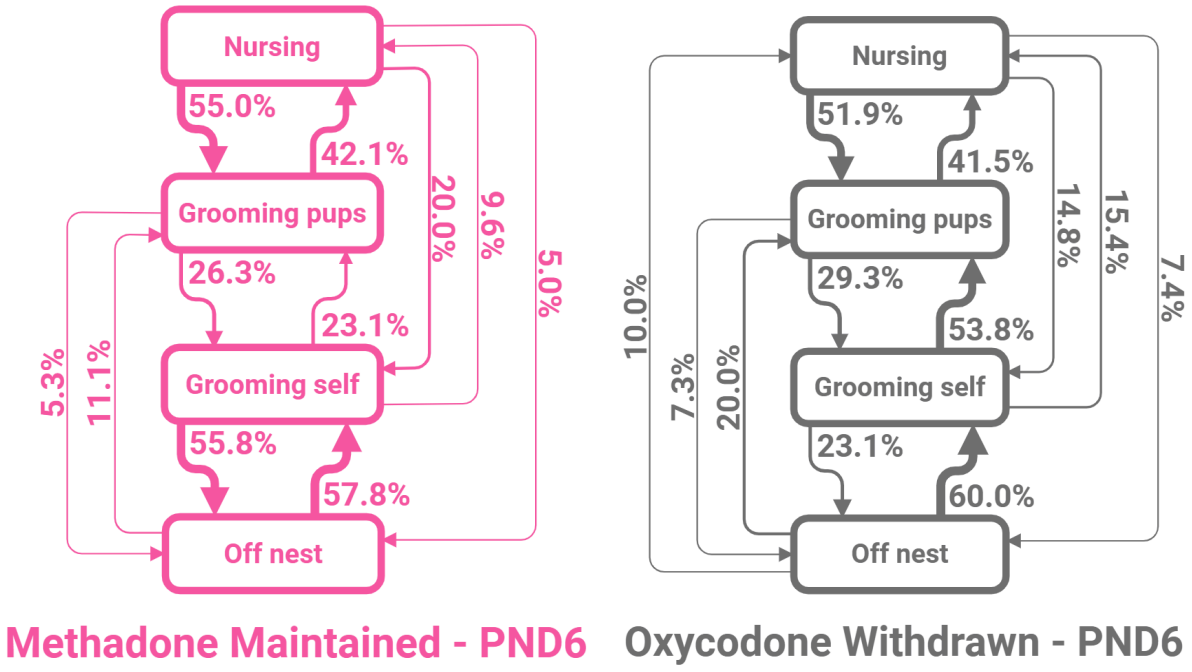


Figure 5. Maternal Behavioral Transition Patterns on PND6. Flowcharts illustrate the sequence and relative frequency of transitions between four behavioral categories in METH and VEH dams on postnatal day 6. Arrows represent transitions between behaviors and statistical values indicate transition probabilities. Pink = METH, methadone maintained; Gray = VEH oxycodone withdrawn.

4.4 Behavioral Entropy Analysis

Behavioral entropy was used to quantify the predictability of maternal behavioral sequences. Entropy values were compared between METH and VEH dams at 8:00 AM, 1:00 PM, and 6:00 PM. Lower entropy values represented more predictable behavioral sequences, while higher values represented less predictable sequences (Figure 6).

On PND3, the METH group exhibited higher behavioral entropy than the VEH group. However, the difference between treatment groups was not statistically significant, $F(1, 22) = 0.02255$, $p = .0603$, although a trend toward significance was observed.

On PND6, the METH and the VEH group exhibited similar behavioral entropy. However, the behavioral entropy varied significantly across observation timepoints; $F(1.899, 7.595) = 5.929$, $p = .0291$. The F-statistic indicates that variation in behavioral entropy associated with observation time was significant. The fractional degrees of freedom are consistent with a correction for repeated measurements. As the p-value was below the significance threshold of 0.05, these results indicate that maternal behavioral predictability varied significantly across the daily observation timepoints, 8:00 AM, 1:00 PM, and 6:00 PM.

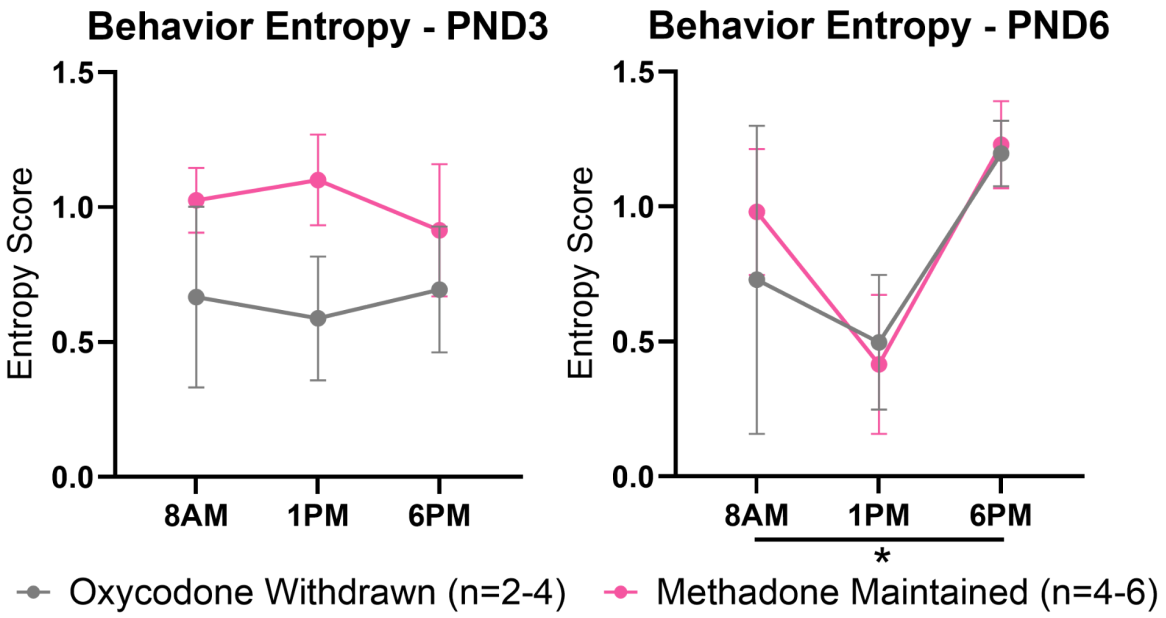


Figure 6. Comparison of Behavioral Entropy in Methadone Maintained and Withdrawn Dams on PND3 vs. PND6. Comparison of behavioral entropy between METH and VEH dams at 8:00 AM, 1:00 PM, and 6:00 PM. Higher entropy values indicate less predictable behavioral sequences. PND3 treatment group did not reach statistical significance, p = .0603. Statistical difference was observed on PND6, p = .0291. Sample sizes varied according to the availability of complete, scorable recordings. Pink = METH, methadone maintained; Gray = VEH oxycodone withdrawn.

5.0 Discussion

This study investigates the effects of gestational methadone maintenance versus withdrawal/abstinence on postpartum maternal behavior in rats. Maternal behavioral durations, behavioral transitions, and entropy were analyzed to evaluate differences in caregiving activity and behavioral organization. Overall, no statistically significant differences in individual behavioral durations were observed between methadone maintained (METH) and withdrawal/abstinence (VEH) dams on PND 3 and PND6. However, behavioral transition patterns suggested greater behavioral disruption in METH dams on PND3, consistent with their higher entropy scores. By PND6, behavioral organization appeared more similar between treatment groups, although a significant effect of observation time on entropy was identified.

A defining finding from the previous study was the difference in pregnancy outcomes between treatment groups. All seven METH dams successfully delivered litters, whereas three of the seven VEH dams failed to give birth. These findings suggest that methadone maintenance may provide greater reproductive stability than withdrawal/abstinence during pregnancy. The absence of litters in three VEH dams raises the possibility of pregnancy loss associated with withdrawal-related physiological stress. The small sample size prevents a definitive conclusion

regarding the cause of unsuccessful deliveries. However, these findings may have implications for understanding the risks of opioid withdrawal during pregnancy.

Behavioral duration data used to assess differences in quality of maternal care between methadone maintenance and withdrawal/abstinence groups. Differences in the amount of time dams spent performing individual versus maternal behaviors during the observation periods, were used as a parameter for quality. However, similar behavioral durations do not necessarily indicate identical patterns of maternal care. For example, two dams may spend comparable amounts of time nursing or grooming their pups but differ in the frequency and sequence of transitions between these activities. Therefore, analyzing behavioral transitions and entropy provides additional information about maternal behavioral organization that cannot be obtained from duration measurements alone.

Behavioral transition flowcharts suggested differences in the organization of maternal activities between treatment groups on PND3, with patterns stabilizing by PND6. On PND3, METH dams exhibited more frequent transitions between behaviors, including transitions to self-directed activities and time spent off the nest. These patterns suggest a more disrupted maternal behavioral profile during the early postpartum period, when dams would generally be expected to remain focused on caring for their offspring. In contrast, VEH dams exhibited fewer behavioral transitions and more sustained engagement in maternal caregiving activities. These observations were consistent with the higher behavioral entropy scores observed in METH dams, indicating less predictable behavioral sequences. Although METH dams exhibited higher entropy than VEH dams, the treatment-group difference did not reach statistical significance ($p = .0603$). Therefore, the observed differences suggest a possible association between methadone maintenance and disrupted maternal behavioral organization, but they do not establish a statistically significant treatment effect.

By PND6, behavioral transition patterns and entropy scores appeared more similar between METH and VEH dams. However, a statistically significant effect of observation time on behavioral entropy was identified, $F(1.899, 7.595) = 5.929$, $p = .0291$. This finding indicates that maternal behavioral predictability varied across the 8:00 AM, 1:00 PM, and 6:00 PM observation periods. The lower entropy observed at 1:00 PM may reflect more sustained maternal caregiving during the middle of the light phase, whereas higher entropy during the early and later observation periods may reflect increased transitions between maternal and nonmaternal activities. Previous research has described daily rhythms in maternal behavior, including greater time spent with offspring during the middle of the light phase (Canal-Corretger et al., 2000). In contrast, higher entropy during the early and later observation periods may reflect increased transitions between maternal caregiving and non-maternal activities. These patterns may also be influenced by regulation of the hypothalamic-pituitary-adrenal (HPA) axis, as cortisol levels in rats typically increase toward the onset of the dark phase (Mohawk et al., 2007). Furthermore, opioid exposure may alter HPA-axis regulation during pregnancy and postpartum (Wallin et al., 2021).

These findings are relevant to the MBN, which regulates maternal motivation, responsiveness to offspring, and caregiving behaviors through interconnected neural pathways involving dopamine, oxytocin, and endogenous opioid signaling even in humans (Numan & Young, 2016; Wallin et al., 2021). Since methadone acts on opioid receptors involved in maternal behavioral regulation, continued methadone exposure may influence the organization of postpartum caregiving. Previous literature has reported that opioid exposure can disrupt maternal behavior and MBN function (Wallin et al., 2021). Consequently, the increased behavioral transitions observed in METH dams may reflect altered regulation of maternal motivation and behavioral selection. However, the present findings cannot independently establish that methadone impairs or betters maternal care.

Limitations should be considered when interpreting these findings. First, the study included only two treatment conditions: methadone maintenance and forced withdrawal following oxycodone self-administration. The absence of an opioid-naive control group or a group maintained on oxycodone limits the ability to determine whether the observed behavioral patterns resulted specifically from methadone exposure, withdrawal, or prior oxycodone exposure. Additionally, the relatively small sample size limited statistical power, particularly because three VEH dams did not deliver litters. Sample sizes were further reduced in some entropy analyses when recordings were excluded due to incomplete visibility or insufficient scorable maternal behavior. Although recordings were collected on PND3, 6, 9, and 12, the present analysis focused on PND3 and PND6, limiting conclusions about behavioral changes throughout the postpartum period.

Future studies should incorporate larger sample sizes and additional control groups to distinguish the effects of methadone maintenance, opioid withdrawal, and prior oxycodone exposure. Larger sample sizes and analyses across later postnatal days (PND9 and PND12) would also help determine whether differences in behavioral organization persist or change over time. Additional research could provide further insight into whether changes in behavioral predictability are associated with meaningful differences in maternal caregiving.

Overall, the present study found no statistically significant differences in individual maternal behavioral durations between methadone-maintained and oxycodone-withdrawn dams on PND3 or PND6. However, METH dams exhibited more frequent behavioral transitions and higher entropy on PND3, suggesting a potentially more disrupted pattern of early postpartum caregiving. By PND6, behavioral organization appeared more similar between treatment groups, although entropy varied significantly across observation timepoints. Additionally, the greater number of successful deliveries in the METH group suggests a possible reproductive benefit of methadone maintenance relative to forced withdrawal. Together, these findings suggest that gestational methadone maintenance and withdrawal/abstinence may be associated with differences in the organization and predictability of maternal behavior that are not captured by behavioral duration measurements alone. Although the current data are insufficient to establish this hypothesis, the observed patterns provide a basis for further investigation.

6.0 Acknowledgments

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