"Sex Differences in the Treatment of Neonatal Opioid Withdrawal Syndrome with Buprenorphine"



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"Sex Differences in the Treatment of Neonatal Opioid Withdrawal Syndrome with Buprenorphine"

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Abstract

Neonatal opioid withdrawal syndrome (NOWS) is a growing crisis in the United States that affects the most vulnerable population; newborn babies. Due to the only "cure" being maternal drug use prevention, and because opioid use is on the rise, it is greatly important to find a drug that works to not only decrease the severity of the withdrawal symptoms but limit the length of treatment and hospital stay. The purpose of this study is to identify buprenorphine as a potential treatment option for NOWS due to its long half-life, antagonistic effects on the mu receptor, and its success in adults with opioid use disorder (OUD). The other goal is to study whether there are sex differences in the morphine withdrawal period and in the buprenorphine withdrawal period. In order to induce NOWS in a rodent model, 1 mg/kg of morphine was injected into ⅔ of the litter while the other ⅓ was injected with saline in p3 old pups for 7 days to develop a dependence. Their withdrawal behaviors are then observed with a modified Finnegan scale on p10 and p11 and then on p12 ½ of the morphine group is injected with buprenorphine and the rest with saline is injected for 8 days. On p20 and p21 there is another observation period to identify if the withdrawal behaviors have increased or decreased from the initial observation period and their sex is determined on p22. Statistical analysis supports that there is a significant difference between the morphine and saline group, and also significant between the morphine+buprenorphine group and the morphine+saline group and no significance in relation to sex. What this means is that the initial morphine injection induced NOWS behaviors, buprenorphine decreased withdrawal score, and treatment and withdrawal is not dependent on the sex of the pup. Based on these results it is important for future studies to take into consideration if buprenorphine impacts cognitive development in adolescents, an issue that other NOWS drugs

cause.

Introduction

The current opioid epidemic has impacted the world for decades and its negative effects have recently been brought to light. The opioid epidemic has impacted 11.8 million Americans through the misuse of prescription opioids, such as oxycodone and morphine, that can develop into an opioid use disorder (Lyden & Binswanger, 2019). This is characterized through active drug-seeking even if there are active consequences (Lyden & Binswanger, 2019). This current epidemic has caused a steady increase in drug dependence, which has led to an increase in physician diagnosed opioid use disorder. One particular group of people that are especially vulnerable to this epidemic are babies born to birthing parents with an opioid use disorder (OUD) who continue to use opioids during gestation, which clinically leads to neonatal opioid withdrawal syndrome (NOWS). This can cause severe physical and behavioral health problems such as respiratory depression, cardiovascular issues, and low birth weight; furthermore, these afflictions can continue throughout their adolescence. There are current treatment options for NOWS, although better therapies are needed as the hospital stay and behavioral symptoms still last from weeks to as long as months. The most important aspects that any pharmacological approach needs to treat are the overall withdrawal symptoms that could potentially impact the health of an opioid-exposed infant for years. It is also crucial that there is more research on sex differences as female infants born with NOWS are less likely to be studied even though beneficial treatments could potentially differ between sexes.

Opioid drugs: definitions and mechanisms

Opioids are a class of drug that are either derived from or mimic the natural substance found in *Papaver somniferum,* also known as the opium poppy (National Institute of Diabetes and Digestive Kidney Diseases, 2020). Opiates that are naturally occurring and are derived from poppy include codeine and morphine (National Institute of Diabetes and Digestive Kidney Diseases, 2020). Opioids are divided further into subclasses by their chemical structure which include synthetic opioids, which are created to mimic natural alkaloids such as morphine or codeine, and include methadone and fentanyl. There is another subgroup called semisynthetic as they are derived from the natural alkaloids and include buprenorphine, hydrocodone, and oxycodone (Pathan & Williams, 2012). The way that opioids affect the nervous system is that they bind to opioid receptors in the brain which can either be mu, kappa, or delta (MERRER et al., 2009). Their ability to bind to the different receptors creates different behavioral effects: mu is responsible for euphoric and analgesia effects, kappa is responsible for analgesia and dysphoric effects, and delta is responsible for emotional stress (MERRER et al., 2009). Diving deeper, G-protein-coupled receptors have no direct link to an effector protein, which means that the message is sent through a G-protein (McDonald & Lambert, 2005). Any of the three receptors (mu, kappa, or delta) couple to the inhibitory G-proteins (McDonald & Lambert, 2005). Endogenous signaling peptides activate the opioid receptors; endorphins, enkephalins, and dynorphins (Reeves et al., 2022). Opioid receptors are Class A G-protein coupled receptors (GPCRs) that couple to inhibitory G proteins (Reeves et al., 2022). These receptors transduce extracellular messages using G protein (Gαi and Gβγ), mitogen-activated protein kinase (MAPK), and arrestin signaling pathways (Reeves et al., 2022). Opioid receptors work to

decrease neurotransmission through inhibiting voltage-gated calcium channels and activating inwardly rectifying potassium channels (Reeves et al., 2022). Opioid receptors can be located postsynaptically in neuronal soma and presynaptically in axon terminals in which postsynaptic opioid receptors inhibit neurotransmission by directly hyperpolarizing neurons, while presynaptic opioid receptors can indirectly reduce or enhance neural activity by reducing excitatory or inhibitory neurotransmission (Reeves et al., 2022). The opioid receptors and their endogenous ligands are differentially expressed and activated in many different parts of the brain (Reeves et al., 2022). Looking specifically at the mu receptor, they can be found throughout the central nervous system in areas that involve sensory, motor activity, and emotion (McDonald & Lambert, 2005). Examples of these areas include the amygdala and the cerebral cortex (McDonald & Lambert, 2005). Mu receptors are located presynaptically on primary afferent neurons within the dorsal horn of the spinal cord where they inhibit glutamate release (McDonald & Lambert, 2005). High densities of Mu receptors are found in the periaqueductal gray area (PAG) and the analgesia of some opioids is proposed to come about from removal of an inhibitory g-amino butyric acid (GABA)-ergic tone in this region of the brain (McDonald & Lambert, 2005). GABA is the main inhibitory transmitter in the brain and acts to reduce or prevent antinociceptive outflow from the PAG (McDonald & Lambert, 2005). The activation of the mu receptor is so common in pain relief due to its analgesic effects, which is due to mu receptor activation by an opioid agonist in the midbrain creates the formation of descending inhibitory impulses to the PAG which then stimulates the descending inhibitory neurons that trigger enkephalin-containing neurons that connects to the dorsal horn (Dhaliwal & Gupta, 2024). This ultimately leads to a decrease in nociceptive transmission from the periphery to the

thalamus (Dhaliwal & Gupta, 2024). The pharmacodynamic properties of the activation of the mu receptor is what creates the analgesic effects and is why medications that are agonists to the mu receptor are popular for both illicit and prescription use.

Because of these pharmacodynamic effects opioids are most commonly prescribed for pain management due to their behavioral effects such as euphoria, mood swings, drowsiness and mental clouding, and lack of consciousness (National Institute of Diabetes and Digestive Kidney Diseases, 2020). More specifically, they are prescribed by clinicians for pain management due to analgesia, but the effects stated in the previous sentence are common behavioral side effects of using opioids. More physiological effects include respiratory depression, decreased GI motility, sedation, nausea, vomiting, constipation, and intestinal bloating (National Institute of Diabetes and Digestive Kidney Diseases, 2020). Activation of the mu receptor can also lead to respiratory depression, which can ultimately lead to overdosing. How this occurs is through a reduction in the sensitivity of central and peripheral to hypercapnia, which is a buildup of carbon dioxide in the bloodstream (Almanza-Hurtado et al., 2022) (McDonald & Lambert, 2005).The reason why respiratory depression often leads to overdosing is due to high levels of sedation from an increased dosage of opioids (Palkovic et al., 2020). The higher the opioid dose the higher the depression is in the respiratory rate due to hypoxia, hypercapnia, and pain not having the ability to generate respiratory efforts (Palkovic et al., 2020). Opioids also have a direct link to cardiovascular health as well that can include a decrease in blood pressure that can cause vasodilation and a decrease in the cardiac efficiency and work (National Institute of Diabetes and Digestive Kidney Diseases, 2020). The route of administration for opioids depends on the type and the intended use; for example, methadone is given orally to OUD patients but fentanyl

is given intravenously in a hospital setting (Lotsch, 2005). One of the most common routes of administration is oral use, which is when the drug is primarily absorbed in the small intestine, and the bioavailability of the opioid is dependent on the amount of the drug absorbed by the intestinal epithelium (Kim & De Jesus, 2024). One of the problems with oral administration is that there is an increase in variable absorption rates, especially if there is an incorrect measurement of dose (Kim & De Jesus, 2024). The most common ROA for opioids is intravenously, which is because it can bypass the liver's first-pass metabolism due to direct injection into the veins with a syringe (Kim & De Jesus, 2024). Peripheral veins offer increased accessibility to the circulatory system, especially the upper extremities, due to the lower chances of thrombophlebitis (Kim & De Jesus, 2024). An advantage of IV is the rapid onset of action, avoids the gastrointestinal system, and has a predictable way of action and an increase in bioavailability (Kim & De Jesus, 2024). A different route of administration is intranasally, which means that the drug is absorbed by passive diffusion across the single-layered, well-vascularized respiratory epithelium directly into the systemic circulation (Kim & De Jesus, 2024). Advantages to this is increased permeability of the nasal mucosa compared to orally, quick absorption (usually within 30 minutes), and also avoids the gastrointestinal tract (Kim & De Jesus, 2024). Another common ROA of opioids is inhalation, also known as "free-basing." How the drug reaches the bloodstream is that the alveolar epithelium has a greater surface area for absorption of the inhaled opioid (Kim & De Jesus, 2024). Advantages to this ROA are large surface area, proximity to blood flow, and smaller doses are equivalent to larger doses of the same opioid when taken orally (Kim & De Jesus, 2024). The last ROA is transdermal/intramuscular. Transdermal is when the opioid is administered to the skin and absorbed, although the onset and

offset times are slow which makes it more short-term (Puntillo et al., 2021).

In a clinical setting the route of administration is dependent on the type of opioid and the potency. One of which is orally administered and is preferred in clinical settings due to its noninvasive administration and has shown efficacy (Puntillo et al., 2021). In opioids, oral administration is common to manage moderate to severe postoperative pain, although it should only be used for a short period of time, which is also consistent for use in acute pain (Puntillo et al., 2021). Common opioids prescribed orally are methadone and oxycodone (Puntillo et al., 2021). Intermittent IV administration is recommended for severe acute pain through a titration because of the rapid onset without the uncertainty of medicine absorption by other routes (Puntillo et al., 2021). The most common opioids administered through IV are morphine, hydromorphone, and ketamine (Puntillo et al., 2021). Another route of administration is transdermal, which is common in acute pain although other routes of administration are preferred (Puntillo et al., 2021). Fentanyl and buprenorphine patches are common for transdermal administration, although fentanyl patches are banned in many countries (Puntillo et al., 2021). It was found that the route of administration in illicit opioid use was dependent on the type of opioid and also the environment that the person is in (Young et al., 2010). What researchers described is that participants in the study from urban areas preferred to chew or swallow pills and the participants from rural areas preferred intranasal administration and intravenous injection (Young et al., 2010). Choosing between the two ROAs is dependent on the type of opioid, for example, participants preferred to use hydrocodone, methadone, oxycodone, and OxyContin intranasally and preferred to use morphine and hydromorphone intravenously (Young et al., 2010). A possible consideration as to why the participants choose one ROA over the other for

certain opioids is if it is in pill form or comes in a liquid. For example, liquid morphine is easier to inject intravenously compared to intranasally. A risk factor for transitioning to using opioids intravenously includes increased frequency of use and an increase in previous substance abuse (Young et al., 2010). A reason for the switch is that intravenously administering a drug allows it to be directly injected into the bloodstream, which is faster than taking it orally or intranasally.The different routes of administration can impact the blood plasma concentrations, where IV would be one of the fastest routes clinically as the medical professionals are directly injecting the drug into the bloodstream (Lotsch, 2005). The half-life of opioids depends on the type of opioid it is and how it is administered. The half life of methadone is about eight minutes and morphine half life is about two to three hours (Lotsch, 2005). The same is true for peak plasma concentrations as it depends on the route of administration and the type of opioid, for example, fentanyl has a faster peak plasma concentration as it is more potent compared to methadone and is also administered IV compared to methadone that is administered orally (Lotsch, 2005).

The Opioid Epidemic and NOWS

Three million Americans say that they either have or had opioid use disorder (OUD) diagnosed by a medical professional (Azadfard et al., 2023). According to the DSM-IV, the criteria for someone having an OUD is: use of an opioid (or multiple), usual dose of opioid has diminished and higher dose is required, experience opioid withdrawal syndrome, using opioids more often or longer than intended, unsuccessful attempts to reduce or quit opioids, time spent obtaining, using, or recovering from opioids, bothered/distressed by cravings for opioids, using

opioids in physically hazardous situations, opioid use despite known psychological or physical health problem caused or exacerbated, failure to meet major role obligations because of opioids, continued use of opioids despite social problems, and important activities reduced or given up because of opioids (Marsden et al., 2019). About 75% of overdoses in 2021 were caused by an opioid, and this number has seen to be on the rise as it has already increased by 15% since 2021 (CDC, 2023). OUDs are found to be more common in vulnerable communities and impact minority groups and women (Lippold, 2019). The WHO published a study that found that women and people from lower socioeconomic backgrounds were more likely to suffer from an opioid overdose compared to others (WHO, 2023). More specifically, a group of people that OUDs have a large impact on are pregnant women as there is a significant "parallel" between the current opioid epidemic in non-pregnant people and those that are (American Society of Addiction Medicine, 2021). What this entails is that pregnant women are directly affected by the current opioid epidemic and women that were dependent on opioids before pregnancy continue during pregnancy as well (American Society of Addiction Medicine, 2021). Another issue with OUDs in pregnant people is that not only does it affect their overall health, but it also has a significant negative impact on the fetus as well that continues into postnatal care (American Society of Addiction Medicine, 2021).

Many populations have been disproportionately affected by the opioid epidemic. More specifically, African American people in urban communities (Gondré-Lewis et al., 2022). There is a steep increase in opioid related deaths in the black community as heroin use has switched from rural communities and into more urban areas (Gondré-Lewis et al., 2022). In this study there is also an increase in fentanyl related deaths, specifically in the Maryland, Pennsylvania,

and District of Columbia areas (Gondré-Lewis et al., 2022). Compared to white people, black people were more likely to be impacted by opioid related death and this study raises awareness for national recognition of the shift in "profiling" of opioid related deaths (Gondré-Lewis et al., 2022). The researchers discuss how the government has been launching opioid epidemic "relief" yet there has been a 1040% increase in synthetic opioid use in America from 2013 to 2019 (Gondré-Lewis et al., 2022). With an increase in opioid use comes an increase in opioid overdose, which the researchers bring to light that black people are dying disproportionately from fentanyl "laced" drugs compared to white people (Gondré-Lewis et al., 2022).

Neonatal Opioid Withdrawal Syndrome

Neonatal Opioid Withdrawal Syndrome (NOWS) is a growing crisis in the United States that has affected over 30,000 babies since 2014 (Jansson & Patrick, 2019). That number is on the rise with prenatal opioid exposure affecting 2% of all births (Bakhireva ~~N~~ et al., 2018). NOWS occurs when the birth parent actively uses opioids while pregnant, and the baby is born dependent and has observable withdrawal behaviors (Mangat, et. al, 2019). The opioids in question can be either prescription used illicitly, prescription used licitly, or bought and used illicitly (Tobon, Habecker, & Forray, 2019). It is found that between 14-22% of pregnant women are prescribed opioids with 6.5% reporting illicit use of their prescription (Tobon, Habecker, & Forray, 2019). The health of the newborn baby is also influenced by when the birth parent took/if they are currently taking opioids, extent of use, type of opioid, and route of administration (Tobon, Habecker, & Forray, 2019). The third trimester is when the fetus is most vulnerable due to increased maternal and placental blood flow and decreased thickness and

increased surface area of the placenta (Sachdeva et al., 2009). This also may occur even if the mother is actively involved in medication assisted therapies such as methadone (Bakhireva N et al., 2018). The symptoms the neonates may experience include; excessive or continuous high pitched crying, lack of sleep, hyperactive motor control, tremors and increased muscle tone, excoriation (scratching of the skin), and fevers (Mangat et al., 2019). The tool used to diagnose and observe the severity of NOWS seen in newborns is rated on a scale from one to three on the Finnegan scale, with three being the most severe (Jansson & Patrick, 2019). The rankings are determined on things such as heart rate, amount of tremors, hours of sleep, and amount of crying (Jansson & Patrick, 2019).

There are many long-term consequential effects that arise due to opioid exposure prenatally such as changes in neurodevelopment, delayed cognition, a decrease in school performance, negative behavior, vision, and mortality (Anbalagan & Mendez, 2023). Studies have shown that there are substantial differences in neurodevelopmental outcome in NOWS children compared to children not exposed to opioids and has been shown to affect them from 12 months of age through adolescence (Anbalagan & Mendez, 2023). The more physiological consequences of exposure include visual-motor problems including strabismus, nystagmus, and visual acuity impairment secondary to refractive errors (Anbalagan & Mendez, 2023). The cognitive effects include lower IQ scores, difficulty with verbal comprehension, impaired short-term memory, and executive function difficulties are all more common in school age children compared to toddlers and infants (Anbalagan & Mendez, 2023). This can carry over to middle school age children as there are consistent findings with low mean academic scores that most commonly impact seventh graders (Anbalagan & Mendez, 2023). It was also common to see that there was a decrease in brain volume, alteration in the maturation of connective neuronal

tracks, and relative microcephaly (Anbalagan & Mendez, 2023). Behaviorally it has been observed that NOWS babies are more likely to be diagnosed with ADHD, adjustment disorders, and conduct disorders (Anbalagan & Mendez, 2023). They can be more aggressive and have anxiety disorders, and researchers have found that NOWS babies are more likely to be rehospitalized during childhood due to trauma, maltreatment, and behavioral problems (Anbalagan & Mendez, 2023). There is also a higher mortality rate in children who were exposed to opioids prenatally which is why it is crucial that there be better treatment options found for NOWS (Grossman & Berkwitt, 2019).

There are many treatment options for newborns diagnosed with NOWS such as benzodiazepines, barbiturates, naloxone, chlorpromazine, and clonidine (Kushnir, Garretson, Mariappan, & Stahl, 2022). These drugs represent a number of different classes, including opioids receptor agonists, GABA receptor agonists, and drugs that affect monoamine systems. The drug found to be the most successful in decreasing the symptoms from NOWS is phenobarbital, a sedative and hypnotic barbiturate. Phenobarbital is a positive allosteric modulator on the GABA-A receptor (Quinlan et al., 2018). When this GABA-A is activated only in the developmental stages, depolarizing GABAergic potentials activate voltage dependent calcium channels and elevate the calcium ions (Ganguly et al., 2001). This explains why GABA-A is activated postnatally instead of prenatally. The reason it is so productive in ceasing the effects is because it is an anti-seizure medication that helps to treat the trembling, irritability, and pain often associated with opioid withdrawal (Stevens, 2021). In clinical studies, phenobarbital is used when no other treatment options show significant results and decrease in symptom severity (Bakhireva N et al., 2018). According to research conducted by a Cochrane systematic review, “the use of phenobarbital to ameliorate symptoms associated with NAS (neonatal

abstinence syndrome) resulted in a significant reduction in the duration of daily supportive care needed versus supportive care alone" (Stevens, 2021, p. 15). This research supports phenobarbital as an active drug with properties associated with it that can be used to treat the severe symptoms of NOWS. The second medication, methadone, that is oftentimes used and has been researched and proven that it has effects that can decrease symptoms, is an opioid receptor agonist (Mangat et al., 2019). Methadone is a synthetic opioid and a full agonist on the mu receptor which activates the modulation of neurochemicals involved in analgesia, euphoria, and sedation (Durrani & Bansal, 2023). It is also the most commonly used treatment in American hospitals with about 80% of medical centers employing it in their treatment options (Mangat et al., 2019). A common pharmaceutical used to treat opioid withdrawal symptoms in adults is buprenorphine (Kraft et al., 2017). It is currently used as a treatment for OUD in adults and its prescription for OUDs in adults has doubled since 2009 (Chambers et al., 2023). An advantage to buprenorphine is its long half-life; if someone were to miss a dose there would be a minimization of the chance of the withdrawal effects occurring (Coe, Lofwall, & Walsh, 2019). Buprenorphine is also used consistently in adults due to the ceiling effect that results in a limited euphoric effect, therefore removing one of the biggest reasons that it is abused as an opioid (Coe, Lofwall, & Walsh, 2019). Another advantage to the ceiling effect is the lack of respiratory depression, which is one of the main aspects of overdosing, so it is a safer alternative to fentanyl and methadone (Dalal et al., 2021).

Although methadone, phenobarbital, and buprenorphine are all used to treat OUDs it is important to keep into consideration that each of these drugs have an abuse liability. Between 2000 and 2008 the number of methadone tablets sold increased from 110,670, 209 to 728,344,627 and the calls to poison control increased from 1,387 to 4,765 (Maxwell &

McCance-Katz, 2010). The distribution of the tablets used for pain management has now surpassed the liquid methadone distributed to treat OUDs in adults and 24% of methadone deaths were directly linked to the pain management tablets (Maxwell & McCance-Katz, 2010). The proportion increase of buprenorphine was much larger compared to methadone due to a non-injectable formula that was not available on the market until 2002 (Maxwell & McCance-Katz, 2010). Between 2000 and 2008 the number of poison control calls increased from 13 to 2,607 (Maxwell & McCance-Katz, 2010). Something interesting to note is that illicit buprenorphine use is oftentimes used by people who have OUDs to treat it themselves (Chilcoat et al., 2019). A percentage of these people are "self medicating" due to the belief that their current prescription is not enough (Chilcoat et al., 2019). A smaller percentage of people from this study used buprenorphine for the euphoric effects that increase abuse liability (Chilcoat et al., 2019). Phenobarbital also has a high abuse liability due to it being a barbiturate (Orbán, 1976). Using barbiturates has resulted in "intoxication" and "hypnotic effects" even though phenobarbital is used in epilepsy as an anticonvulsant (Orbán, 1976). Because phenobarbital is used strictly in medical settings people may turn to other drugs that give the same "high," an example being benzodiazepines because they produce similar hypnotic effects (Orbán, 1976). Oftentimes people become dependent during medical treatment with phenobarbital (Orbán, 1976).

Buprenorphine as a Therapy for NOWS

Buprenorphine is a synthetic opioid analgesic that also binds to the mu opioid receptor (Coe, Lofwall, & Walsh, 2019). As a low efficacy agonist, buprenorphine is a partial agonist on the mu receptor which means that the complete effect of it will not have as strong of an impact

compared to other opioids (Coe, Lofwall, & Walsh, 2019). This partial agonist causes a ceiling effect on the mu receptor response that creates the euphoric effects and respiratory depression (Coe, Lofwall, & Walsh, 2019). Because it has a ceiling effect that shows that it has a lower abuse liability compared to full agonist on the mu receptor. This also shows that there is a limit to the euphoric and respiratory depression while taking buprenorphine, which gives it another benefit compared to methadone and phenobarbital . There is also a lower chance of overdosing because it has a low efficacy rate and only a partial agonist. Buprenorphine has a significantly higher affinity, the attractive force between a compound and a receptor, compared to fentanyl and morphine (Coe, Lofwall, & Walsh, 2019). This high affinity causes buprenorphine to be very hard to remove from a mu receptor which causes it to block other opioids from binding to it, which makes it a competitive agonist (Coe, Lofwall, & Walsh, 2019). Because of this it also blocks the effects of other opioids on both a physiological and behavioral level. The high affinity also provides it with the ability to alleviate withdrawal symptoms in OUD patients in clinical settings (Coe, Lofwall, & Walsh, 2019). Buprenorphine also has a slow dissociation from the mu receptor which would account for it having a longer half-life (Coe, Lofwall, & Walsh, 2019). This would also lead to less of a necessity to dose more than once a day because of how long its mechanism of action is. Because of buprenorphine acting as a partial agonist on the mu receptor and its ability to alleviate withdrawal symptoms on both a behavioral and physiological level it has the potential to be a successful drug in treating the current epidemic that is neonatal opioid withdrawal syndrome. Buprenorphine is also a partial antagonist on the delta and kappa receptors, which is understudied but has a possibility to have partial responsibility for the ceiling effect (Infantino et al., 2021).

Currently there is no cure for NOWS, other than maternal prevention, and only drugs that

can treat symptoms or try to limit the hospital stay. Treating to limit hospital stay and symptoms are the two main goals for treatment of NOWS when maternal prevention is either too late or difficult to manage. The current drugs used for treatment (phenobarbital and methadone) work to an extent but could potentially create negative behavioral effects in the future, more specifically phenobarbital (Quinlan et al., 2018). Phenobarbital has been seen to cause lower cognitive performance compared to babies that were not treated with phenobarbital (Quinlan et al., 2018). It also creates long lasting structural and functional changes of the brain that can cause apoptosis in the limbic system and motor function (Quinlan et al., 2018). The behavioral changes seen include anxiety-like behavior and schizophrenia-like behavior that also includes emotional changes (Quinlan et al., 2018). Buprenorphine is an up and coming drug that has only been tested on adults who have been clinically diagnosed with an OUD. Although it has not been approved by the Food and Drug Administration (FDA) for use in NOWS, it has promising effects in adults that could potentially carry over into infants. It has shown to have significant effects on their withdrawal symptoms almost entirely and it did not need to be administered as often as other drugs such as phenobarbital (Coe, Lofwall, & Walsh, 2019). Because of its long half-life it would not need to be administered daily which would be perfect for NOWS babies as it limits the amount of drugs being administered. This would also mean that its effects would last longer compared to other drugs which could limit how long they are hospitalized for. It was also seen in adults that their withdrawal symptoms did not come back when they missed doses, showing that there is a possibility this could be studied further as a possible top treatment for NOWS (Coe, Lofwall, & Walsh, 2019). What makes buprenorphine stand out against methadone and phenobarbital is its long half-life which signifies that it would be administered less frequently compared to phenobarbital and methadone. This is important for babies due to intravenous

injections having a more invasive aspect. Buprenorphine also has no connection to future developmental delays with cognition or emotion that methadone and phenobarbital have. This is an important aspect because future quality of life and success should be taken into account when deciding which drug that the NOWS baby should receive. Buprenorphine also has les drug-drug interaction which means that it is safe for patients with mild to moderate liver impairment, which is a birth effect of NOWS, and renal failure (Infantino et al., 2021). One other important reason why buprenorphine stands out is it's ceiling effect that methadone and phenobarbital do not have, which means that there are limits to the euphoric effects, this is more important in adults, so there is a decrease chance that it will be abused for its hallucinogenic effects (Coe, Lofwall, & Walsh, 2019).

In the clinical settings it is also rare that the professionals take into account whether the baby is male or female and whether that will impact how they react to the medication. There is no current research on sex differences in NOWS babies being treated with buprenorphine. There are studies that do show that adult women are more likely to develop a substance use disorder when taking prescription opioids compared to men and there are higher rates of opioid use in women of a reproductive age compared to people who are older and younger (NIDA, 2022). Women are also more likely to relapse and return to opioid use (NIDA, 2022). But, it has been found in rodent models that male rodents are more likely to be born with NOWS when the dam is injected with morphine compared to the female rodents (Borrelli et al., 2021). In a different study conducted by Bobzean et al., researchers found that male rats experienced a more severe opioid withdrawal compared to the female rats (Bobzean et al., 2019). They believe this to be due to a difference in thermoregulatory response between males and females due to the morphine induced hypothermia that resulted in "wet-dog shake," which is a withdrawal behavior (Bobzean et al.,

2019). Females also were found to have a slower rate of decrease in withdrawal behaviors which they describe as being related to an enhanced HPA responses in earlier phases of morphine withdrawal compared to females, which would also explain the increase in metabolic symptoms in their behaviors (Bobzean et al., 2019). Due to the differences found in multiple empirical articles, one of the current goals of the study is to find if there are significant sex differences in postnatal morphine withdrawal behaviors.

Purpose of Study

The purpose of this study is to determine if buprenorphine could be a possible treatment option for babies born with NOWS. In addition, it is of interest to determine if there are sex differences in the withdrawal behaviors following perinatal opioid exposure, and the extent to which there would be sex differences the effects that buprenorphine has on the pups. In order to answer this question, ⅔ separate litters of pups will be injected with morphine and the other ⅓ with saline for seven days, starting postnatal day 3 (p3). This period of development is consistent with the third trimester in humans and was chosen to model prenatal opioid exposure without having to treat the dams, which might have necessitated maternal opioid withdrawal treatment or cross-fostering to ensure that the behaviors witnessed in the pups was due to opioid withdrawal, and not deficits in maternal care (Bakhireva et al., 2022). On p10 and p11, each pup will be analyzed for withdrawal symptoms from the morphine injections using a modified finnegan scale. The next day (p12), the buprenorphine (or saline) injections will start, and will last for 8 days. During treatment, daily observations of withdrawal-related behaviors will continue. Treatment and observations will terminate on P20, and sex will be determined on P22 as it is

easiest to measure during this time. Based on supporting data on the clinical success of buprenorphine it is hypothesized that (1) buprenorphine will significantly minimize the severity of the withdrawal symptoms in the NOWS pups; (2) there will be sex differences in the withdrawal behaviors; (3) there will be sex differences in the behavior and symptoms from the treatment of withdrawal with buprenorphine.

Methods

Subjects

Three female Sprague-Dawley rat dams will be housed individually on a 15:9 light:dark cycle with *ad libitum* access to food and water. The day of birth will be considered p0. Rat pups will remain undisturbed until p3 when two thirds of each litter will receive morphine injections, while the other third will receive saline. The goal will be to have at least 12 pups per group: morphine+buprenorphine; morphine+saline; saline+saline, resulting in a minimum desired N of 36 rats. Since the sex of the rats will not be known until well into the treatment periods, determination of sex will occur at weaning on p21, and will be applied retroactively to the behavioral data. The goal will be to have roughly equal numbers of male and female rats in each group, though this is based on probability alone. Rats will be monitored daily throughout the experiment, and pups will be weaned into same-sex groups on p21. All pups will be housed with the dams until p21. All procedures will be approved by the Allegheny College Animal Research Committee..

Materials

Drugs

Morphine (Sigma-Aldrich) will be dissolved in a saline solution at a concentration of 0.1 mg/ml, and administered subcutaneously in the scruff at a dose of 1 mg/kg in a 1 ml/kg injection volume twice a day (Zhang et al., 2022). Buprenorphine (Sigma) will be dissolved in a saline solution at a concentration of 0.05 mg/kg, and administered subcutaneously in their scruff at a dose of 1 mg/kg in a 1 ml/kg injection volume once a day (Zhang et al., 2022). Saline will be administered at a concentration of 0.1 mg/ml twice a day in their scruff (Zhang et al., 2022).

Apparatus

Each pup will be monitored for withdrawal symptoms placed individually in a clear cage with one heating pad per cage to reduce their stress response. A timer will be used for monitoring each pup as they are observed for five minutes each. A printout of the Finnegan scale along with a clipboard will be used to record the observations made.

Procedure

Pregnant dams will be monitored twice daily during the last few days of gestation (G18-21) to determine the day of birth, which will be denoted p0. On p1, each pup will be weighed, and morphine rats will each get different variations of marks with sharpie on hind and front paws (control rats will have no markings). Twice daily morphine (or saline) injections, once at 8:00 a.m. and once at 8:00 p.m., will last from p3-p9; this is based on how long it will take to reach tolerance to the morphine-exposed (Oliviera de et al., 2017). This also mimics the third trimester in humans, which is when the fetus is most susceptible to NOWS and will ensure the safety of the pup as by p3 they will learn to nurse and will be big enough for injections (Yen et al., 2019).

In between injections, in the afternoon at 4:30 p.m., each pup will be monitored individually in a clear cage for withdrawal symptoms based on 5 minutes using the modified Finnegan scale (Appendix 1).

On p10, 24 hours after the last morphine injection, rats will be observed for morphine withdrawal-related behaviors using a modified Finnegan scale. It will assess behaviors such as wall climbing, scratching, and tremors and will be ranked from 1-3 with one being mild to 3 being severe. After the session the pups will be placed back with their mother in the housing cages. On p11, 24 hours after the last observation on p10, withdrawal-like behavior observations will be recorded again into the modified Finnegan scale to ensure that there are not any traces of morphine from the last injection on p9.

On p12, buprenorphine (or saline) injections will begin, and will last until p19 (8 days total). Half of the morphine pups will receive a subcutaneous injection of buprenorphine, and half will receive saline; the same will be true for the control-treated rats. Injections will occur only once a day in the evening, and each pup will be monitored for withdrawal behaviors using the same procedure described above. On p19 the last injection of buprenorphine will be given in the evening and 24 hours later, on p20, the morphine+buprenorphine, morphine+no buprenorphine, and the saline groups will all be observed for withdrawal behaviors from buprenorphine and morphine with the same procedure conducted on p10 and p11. All observations will be terminated on p21. On P22 sex determination will be conducted by measuring the distance between anus and genitals on each pup. They will be marked female or male on their Finnegan scale sheet which will be analyzed to determine if there are sex differences in withdrawal and treatment with buprenorphine.

Statistical Analysis

Separate 3 x 2 factorial ANOVAS with early life opioid exposure; morphine alone, morphine+buprenorphine, vehicle (saline), and sex (male vs. female). Between-subjects variables were conducted for the withdrawal symptoms from the modified Finnegan scale; head shakes, straub tail, rolling, wall climbing, stretching, moving paws/fluttering, vocalization, tremors, and excoriation. The between-subject variables will be measured by the severity of each of the symptoms/behaviors. The significance level will be set to $\underline{\alpha} < 0.05$, and Bonferroni post-hoc tests will be performed where appropriate. Analyses will be conducted using Prism and JASP software.

Results

For statistical analysis ANOVA tests were performed to determine if there were significant differences between the independent variables:treatment during both morphine exposure (saline vs. morphine), and post-withdrawal treatment (saline+saline, morphine+saline, morphine+buprenorphine), and sex (male vs. female). Assignment to either the male or female group was done post-hoc as the sexes of the rats were now known during treatment. An ANOVA test was specifically used because the goal is to find if specific treatments either increased or decreased Finnegan withdrawal scores between groups.

Observed Withdrawal Behaviors

Morphine Exposure

A 2x2 factorial ANOVA test was performed for differences between the Finnegan

withdrawal score in the morphine exposed rats and the vehicle exposed rats (saline) 2 days after the cessation of 7 days of morphine (or saline) injections (Figure 1.A). The main effect is significant in the withdrawal scores of the morphine exposed pups [$F(1,31)=33$; $p<0.0001$]. There is no significant difference found in the main effect of sex *[F(1,31)= 0.0; p=0.9206]*. There is also no significant difference found between the treatment and sex interaction [*F(1,31)=0.8; p=0.3582*]. The post hoc analysis found that the marginal mean (mm) for morphine treated is (*mm=7.874)* and saline treated is *(mm=1.917),* which signifies that there is a difference between the Finnegan scores of the two treatment groups (Figure 1.B.). The marginal means for the morphine treated females is *(mm=8.600*) and in males is *(mm=7.147)*. The marginal means for the saline treated groups in females is *(mm=1.500)* and in males is *(mm=2.333)*.

A 2x2 factorial ANOVA was run to determine the differences between the withdrawal scores in the morphine+saline group over the two observation periods (Figure 1.A.). There is no significance between sex and time [*F(1,12)=0.02224; p=0.8839*]. There is also no significance in sex specifically [*F(1,12)=2.841; p=1177*]. There is no significance found in time specifically [*F(1,12)=0.4843; p=0.4997*]. No significant differences were found in the subjects [*F(12, 12)=0.5437; p=0.8476*]. The Finnegan withdrawal scores are consistent through both periods and are not significantly different from each other.

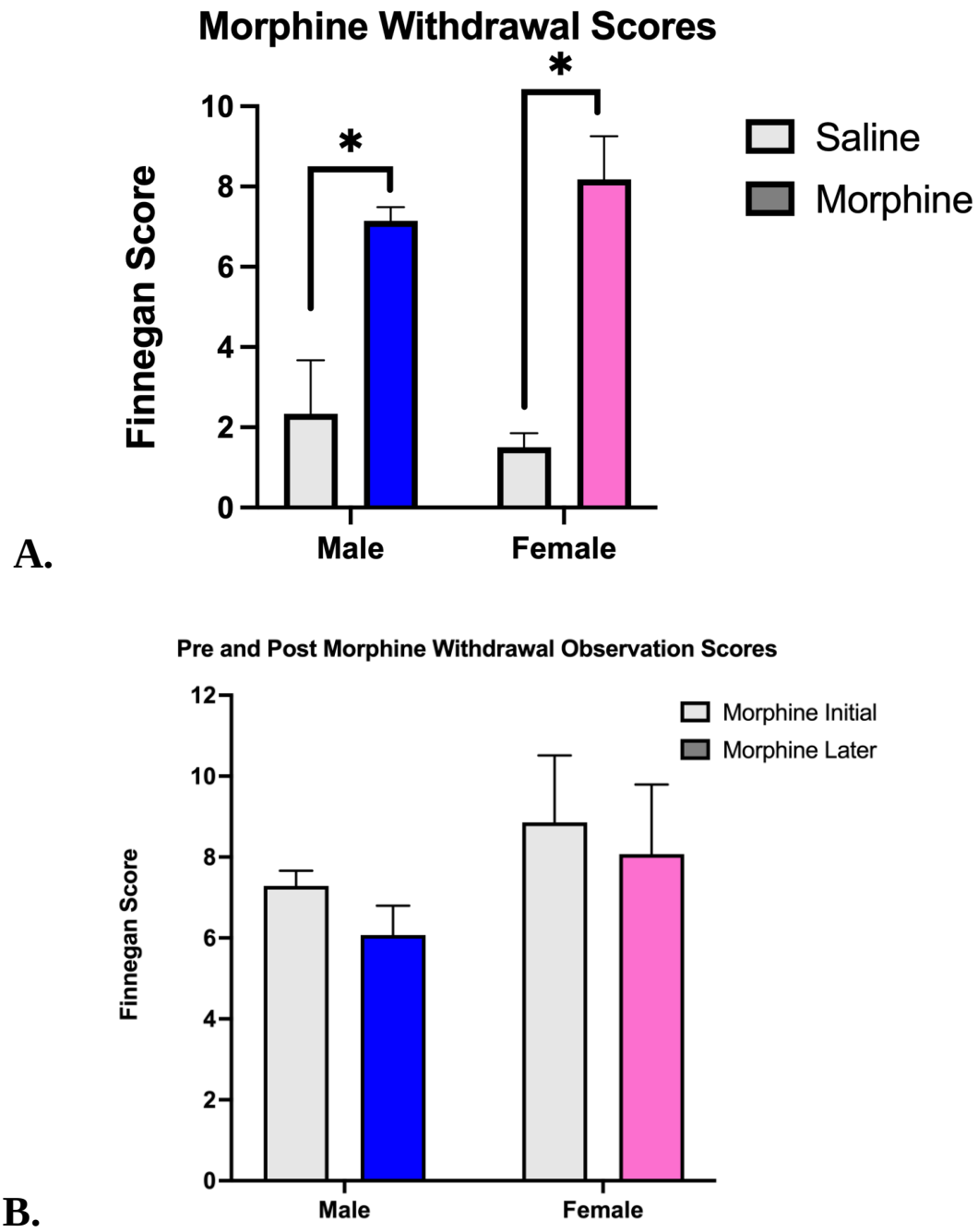

Figure 1. Error bars in both graphs represent the standard error of mean. The x axis represents sex differences and the y axis represents average Finnegan score for both. The Finnegan scores for morphine are represented in the blue and pink bars and vehicle (saline) group in gray in both graphs.**(A)** This bar graph shows the differences between male and female pups in their withdrawal scores from treatment with either morphine or saline after the initial observation period (female morphine n=11; male morphine n=17)(female saline n=4, male saline n=3). The

stars above the bars signifies that there is a significant difference ($p>0.05$) between the groups. **(B)** This bar graph represents the change in average Finnegan withdrawal scores over both observation periods (p10+p11 and p20+p21)(female morphine initial n=7; male morphine initial n=7)(female morphine later n=7, male morphine late n=7).

Buprenorphine Exposure

A 3x2 factorial ANOVA test was performed for differences between the Finnegan withdrawal score in the morphine exposed rats, vehicle exposed rats (saline), and the morphine exposed rats treated with buprenorphine 2 days after the cessation of 8 days of saline or buprenorphine injections (Figure 2). It was found that there is a significant main effect found between the three treatment groups [*F(2,29)=9.5; p=0.0007*] (Figure 2). There was no significant difference found between the withdrawal scores between male and female pups [*F(1,29)=1.2; p=0.2730*]. There was also no significant difference found between the different interactions [*F(2,29)=0.5; p=0.5832*]. To determine which levels of the treatment variable were different from one another, separate one-way ANOVAs were run in each sex. There was no significant difference found between the treatment groups for the female specific pups [*F(2,12)=3.377;p=0.0686*]. There was a significant difference found between treatment groups for the male specific pups [*F(2,17)=9.693; p=0.0016*]. The post hoc results show that there was a difference between the morphine+saline group and the saline and morphine+buprenorphine groups as both are significant.

The marginal means from the post hoc show that there is not a significant difference between sex [females (*mm=4.514);* males *(mm=4.079)*]. The marginal means also show that there is a significant difference in treatment groups [morphine+buprenorphine *(mm=3.500);* morphine+saline *(mm=6.244)*; saline *(mm=3.146)*]. The marginal means also shows that there is no significance between sex and treatment due to the similarities in their values

[morphine+buprenorphine female *(mm=3.500)* male *(mm=3.500)*; morphine+saline female *(mm=6.417)* males *(mm=6.071)*; saline female *(mm=3.625)* male *(mm=2.667)*].

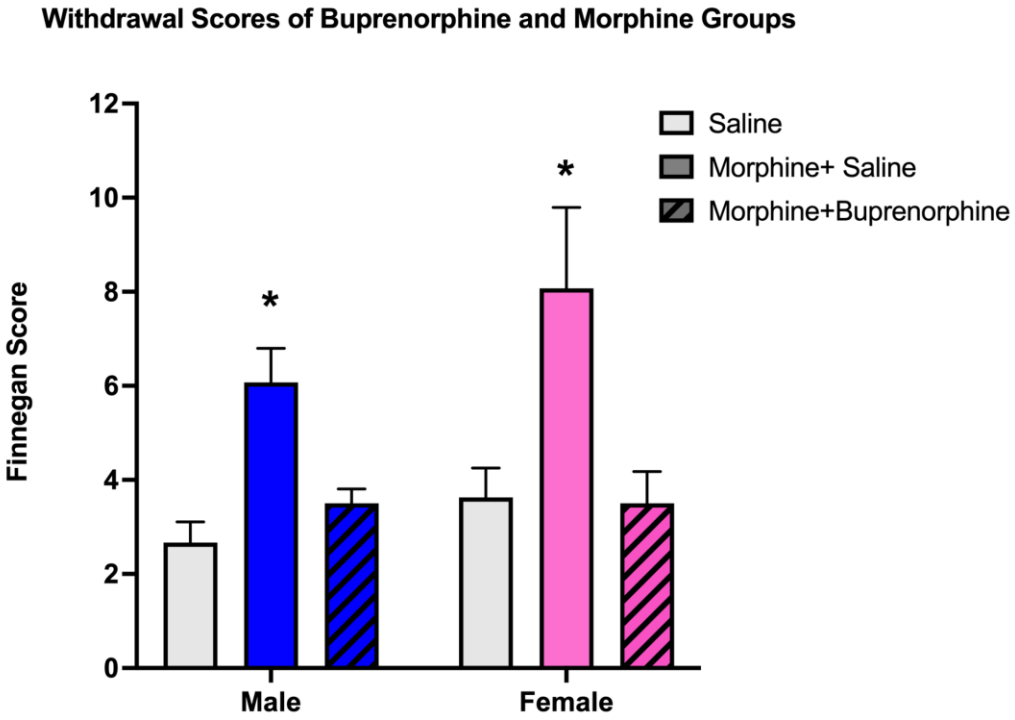

Figure 2. This bar graph depicts the differences in Finnegan withdrawal scores between the male and female pups and their treatment groups. The error bars represent standard error of mean. The stars represent significant effects within the groups (*=p>0.05), (female buprenorphine+morphine n=4, male buprenorphine+morphine n=10)(female morphine+saline n=7; male morphine+saline n=7)(female saline n=4, male saline n=3). The x axis signifies the sex differences and the y axis represents the average Finnegan score. The female morphine treated bars (Finnegan scores) are pink and the morphine+buprenorphine treatment bar is pink with stripes. The male morphine treated bars (Finnegan scores) are blue and the morphine+buprenorphine treatment bar is blue with stripes. All the saline bars are gray.

Discussion

The purpose of this study was to observe the effects that buprenorphine has on neonatal opioid withdrawal syndrome behaviors and if there are significant differences between the withdrawal scores specifically for males and females. Due to buprenorphine's extensive use in adults and its ability to significantly decrease withdrawal symptoms this project mimicked its use

in newborn subjects. The statistical analysis demonstrates that buprenorphine decreased the average Finnegan withdrawal, therefore minimizing the severity of the withdrawal symptoms . These results are consistent with the hypothesis, morphine-treated rats had higher Finnegan Scale withdrawal scores compared to saline-treated controls, demonstrating that this dosing regimen led to measurable and significant withdrawal. When these morphine-treated rats were later treated with buprenorphine, they had significantly lower Finnegan scores compared to the morphine group treated with saline, to the extent that their scores were similar to the control group. These specific results support the hypothesis that buprenorphine will significantly decrease the severity of the withdrawal symptoms in the NOWS pups. The pups that did not receive buprenorphine for the second injections were found to have consistent withdrawal scores throughout both observation periods. There was no significance found from the analysis that implied that the withdrawal behaviors either increased or decreased. This was also consistent with the statistical analysis that also shows that there was no significant difference in the Finnegan withdrawal scores between males and females. What this means is that sex does not impact how high or low the Finnegan withdrawal scores are and there was no correlation between the two. The graph from Figure 2 show that the scores from the morphine+buprenorphine, morphine+saline, and saline+saline groups were similar for males and females and there was not a drastic difference between the values and does not support the hypothesis that there would be sex differences in withdrawal behaviors. These results are also consistent with Figure 1.B. as there are no significant differences between sex and the initial or later observations, which means that sex did not have an influence on how severe the withdrawal behaviors were over time.

Induction of NOWS

Through statistical analysis the morphine was seen to create a significant increase in withdrawal behaviors such as tremors and fluttering. What this signifies is that the morphine worked to induce neonatal opioid withdrawal syndrome postnatally due to the higher average Finnegan score compared to the group that received only saline. This is consistent with the study conducted by Oliviera de et al., specifically on how long it takes for pups to gain a dependence to morphine (Oliviera de et al., 2017). The researchers injected the pups for 7 days in order to study their neuromotor development (Oliviera de et al., 2017). In the current study, inducing NOWS postnatally in rat pups was performed by administering morphine to a portion of each litter in order to create a dependence to the drug. Administering the pup postnatally instead of prenatally was more ethical due to the lack of withdrawal treatment that the dam would receive after delivering the litter. The reason for not treating the dam is to ensure that there would not be any outside variables affecting the withdrawal of the pups, more specifically, so that the pups do not ingest any of the drug through nursing. This also carries over into the withdrawal behaviors that the dam might experience that would result in harm to her pups such as increased aggression, which is seen in this article written by Piccin and Contarino, that states that even after 49 days of withdrawal from opiates there was an increase in aggression and attack behavior (Piccin & Contarino, 2020). Due to this research it was deemed more ethical and safe to inject the pups individually after they are born on p3. In the current study in order to determine if withdrawal was occurring a Finnegan score sheet was used with 0 signifying the behavior was not occurring and 3 meaning that the behavior is severe. Using a statistical analysis it was supported that

morphine led to more severe Finnegan scores compared to the control group. When running analysis on data based off of the initial morphine scores it was found that the withdrawal behaviors did not have a significant increase or decrease during the second observation period. In all, the withdrawal in the morphine group was very apparent and stayed consistent over the two observation periods.

Buprenorphine as a possible treatment for NOWS

There were significant differences found between the different treatment groups. More specifically, this shows that the morphine had a significant impact on the withdrawal behaviors. This led to higher finnegan withdrawal scores, with the most consistent behavior noted being tremors and wall climbing. Based on the withdrawal scores over the four days total, when treated with buprenorphine, the scores went down drastically specifically for tremors. In Figure 2 the values are very similar between the saline group and the buprenorphine+morphine group, which shows that the buprenorphine decreased the withdrawal scores back to the baseline of pups that did not receive any treatment This aligns with the study performed by Kraft et al, where they found that buprenorphine decreased the withdrawal behaviors in babies diagnosed with neonatal abstinence syndrome (Kraft et al., 2017). They found that there was a significant effect of buprenorphine on the time they were in the hospital and on the withdrawal effects based on their finnegan score. What the current study found is that even after the study ceased treatment and observations that the withdrawal behaviors consistently decreased in the buprenorphine+morphine group. This suggests that buprenorphine not only works to diminish the symptoms but also prevents the behaviors from appearing again in the future. This is also similar

to what Devlin et al. found, which is that “buprenorphine was the optimal medication with respect to reducing LOT (length of treatment) (Devlin et al., 2022). Along with this they found that the duration of buprenorphine treatment was shorter compared to the commonly used methadone (Devlin et al., 2022). Even with the extensive research that has already been done on buprenorphine it is currently not approved for the treatment of NOWS and there is no form commercially available for pediatricians to prescribe (Devlin et al., 2022). Based on the current study buprenorphine decreased the Finnegan withdrawal scores significantly, and based on the Finnegan score sheets, completely diminished tremors in the pups that had experienced 2 or 3s in the first observation period. Phenobarbital is used most commonly to treat tremors in babies diagnosed with NOWS, and due to the current findings, buprenorphine could potentially be used instead of phenobarbital in these situations. In fact, buprenorphine also decreased the withdrawal scores in other behaviors such as wall climbing and head shakes, so it could almost replace phenobarbital entirely in a clinical setting. As stated in the introduction, this might be helpful due to the negative impacts that phenobarbital has on cognitive development in the future (Quinlan et al., 2018). The groups treated with morphine and buprenorphine also had a mortality rate of 0, which supports that it is a safe option. The pups gained weight regularly and had similar growth as the saline group such as hair growth, vocalization, eye opening,etc. The results of this current study support that buprenorphine could be a safe option for treating NOWS behaviors.

Sex differences in NOWS and its Treatment

There were no significant differences found between the treatment groups of males and females. What this means is that the withdrawal scores were consistent between males and

females and one sex did not have a more severe withdrawal period compared to the other. This could be due to the inability to determine their sex at p1 so that treatment groups do not have an equal number of females and males. This is because it is difficult to determine the sex accurately at that age because of the underdeveloped testes in males and the space between the anus and vagina being too short to determine if the pup is a female. This led to litter 3 having all of buprenorphine+morphine treated pups being males and all of the controls in that litter were females. This could also explain why there was no significance between the treatment groups in females when the individual one-way ANOVA tests were run. Overall there were less females in litters 1 and 3 which could have potentially impacted the statistical results of significance between treatment groups. Literature suggests that sex differences in neonatal opioid withdrawal syndrome need to be further studied due to the lack of research and variance in clinical reports (Madurai et al., 2024). What they mean by variance is that there are clinicians that report that there is no significance in the severity of withdrawal in males compared to females, and other clinicians that state that males are more likely to be born with NOWS (Madurai et al., 2024). In a study written by Unger et al., they describe that male babies with neonatal abstinence syndrome (NAS) had larger birth weight and head circumference, but there was no difference in the development of NAS between males and females (Unger et al., 2011). In a different study conducted by Borrelli et al. they found that there was an increased sensitivity to NOWS in male rats but there was no significant increase in NOWS in females compared to the male rats (Borrelli et al., 2021). Overall based on empirical evidence there is not a significant correlation between the development of NOWS and sex, but males may be more sensitive to developing it prenatally when exposed during the third trimester (Borrelli et al., 2021)(Unger et al., 2011). The

current study applies to these findings because there was no significant interaction between sex and treatment for any of the groups, which aligns with both the Borrelli and Unger studies.

Limitations

Because of the insignificant relation between sex and treatment, it did not support either of the original hypotheses that there will be sex differences in the withdrawal behaviors and in the symptoms from the treatment of withdrawal with buprenorphine. Based on other studies and literature there is limited knowledge on the sex differences specifically in neonatal opioid withdrawal syndrome. Specifically in the studies mentioned in the paragraph above, Borrelli and Unger, it is stated that there were no significant findings in either human or rodent studies for the interaction of sex and treatment group. This could potentially be because of maternal opioid exposure, when it happens, which type of opioid, or if the mother was receiving treatment for opioid use disorder (Lotsch, 2005). More specifically, no matter the sex that the baby is born, the environment prenatally ultimately has the larger impact on the extent of the opioid withdrawal behaviors. Relating that to the current study each dam produced a different sized litter and sex was not able to be determined until p22 due to the sexual organs not fully developing yet. Because of this, there was no way to make sure there was an equal amount of female and male pups in each treatment group, which could partially explain why there is significance in treatment in males and not females. On p22 it was determined that all of the buprenorphine treated pups in litter 3 were all males, which could impact the significance of the treatment groups in the females in litter 3.

In the saline treatment group it was difficult to keep a consistent n as many of the pups in the saline group were the “runts” and had small body weights and ceased to grow compared to the other pups. Due to this in litter 1 there were 5 pup fatalities, not caused by morphine as they were all controlled, therefore it made it more difficult to keep a consistent number of control rats but also in each sex. Because of the sudden decrease in litter size it was hard to keep a consistent sample size. For example, as mentioned before there was a very limited amount of control pups so it may be harder to determine the baseline of what no withdrawal behaviors look like.

Future Direction and Research

A larger sample size is recommended for future studies and research on NOWS in postnatal pups. It is important to note that there is no control in how many pups the dams deliver, so breeding more litters can prepare researchers for a larger size in case of pup mortality or low litter sizes. A larger sample size will make the study less individualized and allow for larger treatment groups. Therefore, if there are certain outliers or pup fatalities it will not have as great an impact on the results as it would in a smaller sample size. It will also allow the data to be more concise and focus on the treatment as a group and a whole instead of each individual pup. Each individual pup should still be focused on during the observation period, especially when deciding on environments to perform these observations. In the current study clear cages were used, but it was observed that the pups could see their own reflections and see the other pups which could impact their behaviors such as wall climbing and vocalization. They may be more likely to vocalize when they see another pup or scratch at the wall to get closer.

Besides observation, researchers should further investigate the possible effects that buprenorphine has on cognitive and behavioral development throughout childhood and adolescent periods. It would be beneficial to compare it to phenobarbital and methadone to measure if there are similarities in how it impacts learning and motor. This has yet to be studied due to the lack of approval for buprenorphine to be used in clinical settings for babies with NOWS. Due to it not being approved for prescription, it would also be beneficial to study if there is a possibility that there is an increased chance of opioid use disorder as adolescents and adults. This has also not been studied before due to the inability of pediatricians to use buprenorphine in clinical settings, but this possible research could influence if buprenorphine would be ethical to prescribe if there is an increased chance of developing opioid use disorder later in life.

Conclusion

The results from this current study support that buprenorphine decreases the severity of withdrawal in pups exposed to morphine postnatally. There was a significant decrease in Finnegan withdrawal scores over the two day observation period, and based on the data sheets, diminished certain behaviors such as tremors. Another pattern that is significant is the development of NOWS after injections of morphine for 7 days, which the analysis shows an increase in Finnegan scores compared to the saline control group. This supports that hypothesis that morphine would have a significant effect on withdrawal due to the significant increase in Finnegan scores. An aspect of this that is not significant is sex as the scores are consistent for both males and females and there is not a significant relation between sex and treatment groups. This is consistent in the literature that describes that there is no correlation between the severity

of NOWS and the development of it between males and females. There is further research that should be done to study its effects in the development of adolescents and adults and also if larger sample sizes could potentially impact the significant differences between sex and treatment groups. This current study supports that buprenorphine has the potential to be a treatment option for babies born with NOWS due to it limiting Finnegan scores, diminishing certain behaviors, and there are no negative effects on physiological health.

Appendix 1

MODIFIED FINNEGAN SCALE

DATE:

START NEW OBSERVATION PAGE FOR EACH DAY!

SIGNS/SYMPTOMS	SCORE	TIME	TIME
Head shakes			
Straub tail			
Rolling			
Wall Climbing			
Stretching			
Moving paws/fluttering			
Vocalization			
Tremors			

Excoriation			

*SCORE IS RATED FROM 0 TO 3 WITH 0 BEING NONEXISTENT AND 3 BEING SEVERE

Definitions

Headshakes: lateral and rotary motions of head
Straub tail: Erect tail of 45 to 90 degrees
Rolling: Turning body over 360 degrees at least once
Wall Climbing: Placing both forepaws on the cage wall
Stretching: Elongation of extremities
Moving paws/fluttering: Continuous movement of paws towards face
Vocalization: Audible sounds
Tremors: shaking of the whole body
Excoriation: scratching of the skin

Scale adapted from (Richardson et al., 2006)

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