Spring 5-2015

Allelic Variants of OPRM1, COMT And ABCB1 On Pre-Withdrawal Sleep-Wake Regulation In The Opioid Exposed Neonate

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ALLELIC VARIANTS OF OPRM1, COMT AND ABCB1 ON PRE-WITHDRAWAL SLEEP-WAKE REGULATION IN THE OPIOID EXPOSED NEONATE

By

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A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
(Biology)

The Honors College

University of Maine

May 2015

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ACKNOWLEDGEMENTS

This thesis would not have been possible without the guidance and expertise of Dr. Marie Hayes, my advisor. In addition to her dedication and hard work I must extend my thanks to Dr. Alan Rosenwasser, Dr. François Amar, Dr. Len Kass and Dr. Jordan Labouff for their commitment to helping me produce this thesis to fulfill my Honors curriculum. Their time and energy put into my thesis cannot be repaid with words.

My graduate-level colleague Hira Shrestha, was my primary role-model throughout the thesis process. After spending countless hours with her in the lab during the summer of 2014 coding videos and learning the ropes of the laboratory environment, I am extremely grateful for all of the knowledge she passed on to me. In addition to her guidance I have received a copious amount of assistance from Katrina Daigle, a colleague, throughout my thesis process. There have been many instances where I have needed her immediate help, and she has always come right to my side to help me finish any task I need to complete.

Without the help from Ella Sullinski, another colleague, I know that I would not have been able to complete all of my work. Her help using our statistical software SPSS has been phenomenal and without her help, my results would have been disastrous. I am also thankful for Margaret Stavros and Brooke Kimball for their company and their contribution to the rest of the lab.

My largest thanks goes out to my family, whom have been by my side throughout my whole college career. Without my mother and grandparents I do not think I would be able to complete this thesis or be where I am today. I am forever grateful that I know my family is just a phone call away.

I would like to extend my thanks out to everyone who has made this study possible, if not listed above. Without the commitment of many organizations such as Eastern Maine Medical Center, University of Maine Psychology Department, the Acadia Hospital, and many more, this study would not be as successful as it is today.

ABSTRACT

Neonatal Abstinence Syndrome (NAS) is a neonatal medical condition of prenatal opioid withdrawal, secondary to prenatal exposure. NAS increases mortality and morbidity through seizure risk, and excessive sympathetic autonomic tone; which affects respiration and dysregulates sleep and feeding. Our laboratory has recruited more than 200 pregnant women who are in treatment for opiate dependence with methadone maintenance treatments. We have found that NAS severity is modulated by the presence of allelic variants of OPRM1 118A>G (μ-opiate receptor) and COMT 158 A>G (catechol-o-methyl transferase) genes, revealing a positive correlation between minor alleles of these two genes and severity reflected in length of hospitalization and treatment.

In neonates, in this thesis, it is predicted that SNPs protective for NAS will be associated with improved sleep-wake regulation, including movement arousals (MAs), periodic movement bursts during sleep, whereas those infants without the protective alleles may show increased sleep fragmentation and resulting sleep deprivation, providing psychobiological markers of neurodevelopmental risk. In this thesis, I propose that the allelic variants of OPRM1 118A>G, and COMT, that have been associated with NAS severity, will similarly associate with early markers of withdrawal in sleep and arousal before withdrawal has begun. Further, we had examined several genes from the ABCB1 cassette, MDRa, MDRb, MDRc, which are associated with multi-drug resistance (Levran, et al., 2008), for relation to NAS severity. Although there had been no prior research on the ABCB1 cassette (Wachman et al., 2013), I examined MDR genes as well for potential association with pre-withdrawal sleep and wake.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ANS	Autonomic Nervous System
AS	Active Sleep
BA	Brief Arousal
cAMP	Cyclic adenosine monophosphate
CI	Confidence Interval
CNS	Central Nervous System
COMT	Catechol-O-methyl transferase
CRH	Corticotrophin Releasing Hormone
DA	Dopamine
DHHS	Department of Health and Human Services
DNA	De-ribonucleic Acid
DRD4	Dopamine Receptor
EMMC	Eastern Maine Medical Center
FA	Full Arousal
FAS	Fetal Alcohol Syndrome
HIPAA	Health Insurance Portability and Accountability Act
IRB	Institutional Review Board
IS	Indeterminate State
LOT	Length of Treatment
MDRa	SNP C3435>T
MDRb	SNP G2677>T

MDRc	SNP C1236>T
mRNA	Messenger Ribonucleic Acid
NAS	Neonatal Abstinence Syndrome
NI	Nurse intervention
NICU	Neonatal Intensive Care Unit
NREM	Non-Rapid Eye Movement
OPRM1 118A>G	Opiate Receptor μ1
PNS	Peripheral Nervous System
SES	Socioeconomic Status
SIDS	Sudden Infant Death Syndrome
SM	Spontaneous Movements
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences

CHAPTER ONE
INTRODUCTION

According to the World Health Organization, an estimated 2 million people are dependent on opioid medications, which accounts for epidemic rises in opioid-associated mortality (Hayes and Brown, 2014). Similarly, there has been a concomitant rise in the abuse of opiates during pregnancy (SAMHSA, 2010; Hayes and Brown, 2012). According to research conducted by the Maternal Lifestyle Study, 2.3% of pregnancies involve heroin or methadone exposure. Methadone maintenance treatments are the standard of care for opiate dependent pregnant women (Jones, 2008). In 60-80% of newborns, opioid withdrawal or Neonatal Abstinence Syndrome (NAS), requires opioid pharmacological treatment and weaning (Patrick, 2012). NAS withdrawal complications can be life threatening, e.g. seizures, brainstem dysregulation, respiratory sinus arrhythmia, or poor sleep and feeding (Hudak, 2012). NAS severity is affected by allelic variants of the OPRM1 118A>G (opiate receptor μ1) and COMT (catechol-o-methyl-transferase) genes, which have been linked to brain circuitry for opioid pain effectiveness, addiction and psychiatric disease (Wachman et al., 2013).

Addiction is a neuroadaptation process that is progressive, but can proceed rapidly when significant quantities of opioid compounds (e.g. as prescription drugs or heroin) are consumed over a period of months or years. All of the mothers in this study suffered from an addiction to opioid narcotics and required treatment with methadone throughout their pregnancies. Addiction is considered a condition that results when a person ingests a substance or engages in an activity that activates neural pathways associated with dopamine. But, when this act is continued, it may become compulsive, reflecting neuroadaptive changes in the CNS. In this psychiatric state, the person is dependent on

the drug and is suffering from addiction. The user may not be aware that their behavior is out of control. This is a neurobiological state in which the brain will adapt to the presence of a drug, so that the drug no longer gives the same initial effect and is defined as tolerance. In the absence of drug, the addicted organism will experience withdrawal when the drug is discontinued. In opiate and other drug addictions such as alcohol, the person in withdrawal seeks the drug to relieve symptoms and decrease psychological distress. Mothers in the present study are monitored carefully to insure that they do not experience withdrawal, which has been hypothesized to cause fetal harm or demise in opioid addicted women not in treatment. The present study explores the relationship of genetic factors in the pre-withdrawal period and examines allelic differences in the mother and baby to NAS severity. Differences have been found for OPRM1 and COMT, as described above by Dr. Hayes' lab (Wachman et al., 2013). My study examined these and other gene variants on sleep in relation to the pre-withdrawal status of the newborn using an overnight sleep study on day 0 or day 1 post-birth.

1.1 NAS AND GENES:

Naturally occurring single nucleotide polymorphisms (SNPs) in genes often produce profound effects on the functioning of proteins. SNPs are DNA sequence variations that occur commonly within a population, and a single nucleotide in the genome will differ between members of the biological species. They are biological markers, and help to locate genes that could be associated with diseases. It has been found that SNPs can be associated with an individual's responses to certain drugs. OPRM1 118 AG risk allele occurs at a rate of approximately 12% in the Caucasian ethnicity and is linked with to a risk for substance abuse. The μ-opioid receptor is a

member of the G protein coupled receptor family. This group of receptors sense molecules outside of the cell, and activate signal transduction pathways and cellular responses. Approximately 40% of all modern medicinal drugs target these G protein coupled receptors. The mechanism behind these receptors starts with external signaling in the form of a ligand that creates the conformational change that leads to the activation of a G protein. One ligand category that can bind to the G protein is opioid peptides. Though we do not know entirely how the signal transduction occurs, it is thought that the molecule exists in equilibrium between active and inactive, and there is a possibility that the binding of the ligands will shift the equilibrium state towards the active state.

The COMT 158A>G SNP has a minor allele frequency of approximately 50% in whites and has been associated with responses to pain and morphine dosage requirements in adults (Wachman, 2013). Variations in the COMT gene have also been previously linked with disorders such as schizophrenia, anxiety and drug abuse. Drugs that cause addiction increase the brain's dopaminergic transmission, and the COMT enzyme plays a critical role in dopamine inactivation. A shorter length of treatment (LOT) was found by Wachman, Hayes and colleagues, (Wachman et al, 2013) with a reduction in COMT enzyme activity due to an increase in levels of circulating catecholamine and led to an improved stress tolerance.

Another gene complex, termed the ABCB1 cassette is involved in drug processing leading some SNPs to be associated with faster or slower metabolic degradation of opioids and other drugs. Methadone is a substrate of the transporter P-glycoprotein (P-gp) which is encoded by the ABCB1 (MDR1: Multi Drug Resistance Protein 1) gene. These genes has been found to have significant variations in allele frequencies among various

populations, and a few variants have been shown to be associated with P-gp expression, drug response and disease susceptibility. When homozygous to the T allele (TT) are present, lower in-vivo duodenal P-gp expression occurs (in comparison with CT or CC). Gene duplications have been associated with rapid metabolism and non-functional variants associated with poorer metabolism. Rapid metabolizers are often born with a propensity towards early withdrawal (Levran et al., 2008).

In a previous study conducted by Wachman et al. (2013) to determine whether SNPS in the OPRM1, ABCB1 and COMT genes are associated with length of hospital stay and the need for treatment of NAS. Infants were enrolled at Tufts Medical Center and affiliated nurseries (Brockton Hospital, Melrose Wakefield Hospital, Lowell General Hospital) and Eastern Maine Medical Center. Eligibility criteria had included maternal prescribed methadone or buprenorphine exposure in utero for at least 30 days before delivery. The result, using the genetics statistical approach called the dominant model (e.g. in which group one is associated with homozygous variants for the major allele, termed AA, and group two is composed of collapsed groups of heterozygotes and homozygotes for the minor allele ie. AG/GG), found that infants in group two with the minor G allele, either OPRM1 AG or GG, had a shortened length of stay and were less likely to receive NAS treatment than infants who did not have the G allele, designated OPRM1 AA genotype. For COMT minor alleles (group two) shortened length of stay and significantly less treatment (with 2+ medications) was observed than the dominant alleles (group one). Overall, this study concluded that the minor variants in the OPRM1 and COMT genes were associated with a shorter length of stay and less of a need for pharmacological treatment.

An additional study done by Wachman et al. (2014) shows that methylation in OPRM1 may also affect responsiveness to opioids. The SNP polymorphism in the μ-opioid receptor can affect the activity of the receptor, and alter the sensitivity to β-endorphins and the potency of post-synaptic cellular activity. These findings are associated with previous findings by Wachman et al., and the increased length of stay in newborns. If newborns with higher methylation in OPRM1 have more severe withdrawal, it may be because methylation of OPRM1 down-regulates opiate receptors and decreases the sensitivity of opiate receptors to replacement medications leading to slower recovery. This is hypothesized by Wachman (2014) to be an epigenetic consequence of high exposure to opioids prenatally.

In this thesis it is imperative to uncover the association between NAS, and the SNPs labeled as protective. It is predicted that these protective SNPs will be associated with improved sleep-wake regulation including movement arousals (periodic movement bursts during sleep), whereas those infants without the protective alleles may show an increased sleep fragmentation pattern, which will result in sleep deprivation (decrease in the frequency and duration of arousal parameters following sleep onset and decreased spontaneous movement duration) providing psychobiological markers of neurodevelopmental risk.

1.2 SLEEP PROPERTIES AND NAS:

It is known that newborns that are exposed to opioids prenatally show impairment in autonomic arousal-regulatory tone (Jansson, 2011). One measure of changes in autonomic tone during sleep are movement arousals (MA), high frequency cycles of

sleep-related, spontaneous movements (SM). Vecchierini and Navalet (2002) state that the process of differentiating awakenings from arousal is based on the polysomnographic and behavioral state criteria in which awakening is believed to occur when behavioral markers such as quiet or restless, open eyes, grimaces, movements and occasional cry is present. In our lab's sleep studies, MAs are depressed in high-risk-for-SIDS groups, e.g. prenatal alcohol and tobacco exposure (Troese et al., 2008); high-risk premature infants (Hayes et al, 2007). There is a sleep pattern known as the "cascade of disrupted sleep" which many of our infants display. The first stage is sleep organization measured immediately post birth for sleep/wake states, state-dependent brief and full arousals and state independent primitive arousals (with sleep related spontaneous movements). If prenatal insult occurs (ie. opioid exposure) then we next see signs of sleep fragmentation (high arousals). This includes frequent awakenings, excessive crying, decreased active sleep and increased arousals. This state can lead to sleep deprivation (low arousals), a consequence of sleep fragmentation. This leads to decreased frequency and duration of arousal parameters, following sleep onset and decreased spontaneous movement duration further leading to disrupted CNS arousal properties and posing a potential risk for SIDS. Movement arousals are primitive movements, protective from SIDS.

Although not addressed directly in this thesis, it is noteworthy that when exposed to exogenous opioids, the human adult sleep regulatory system is compromised (Hartwell, 2008). During the third trimester, fetuses begin to entrain their biological clocks to their mother's sleep patterns. Prenatal opioid exposure may delay fetal development leading to sleep deficit from abnormal sleep cycles of the infant post birth (Jansson, 2011); alternatively, early withdrawal may also disrupt sleep patterns. The

hypothesis of my thesis incorporates our prior findings that the minor alleles of OPRM1 and COMT (rs1799971 dbSNP database) are associated with milder NAS (Wachman, 2013), defined by shorter length of stay and less pharmacological replacement drug, and thereby, deemed "protective." In our recent study it was found that opioid exposed infants with a more severe NAS phenotype (i.e. longer length of stay and two or more medication to stabilize withdrawal) had an increase in DNA methylation in the OPRM1 (Wachman et al., 2014). This result suggests that in addition to NAS severity based on allelic differences in OPRM1, there may be epigenetic changes during exposure in utero that result in methylation of OPRM1 gene expression, and, perhaps, decreases sensitivity to opioid replacement therapy during withdrawal.

1.2.1 SLEEP FRAGMENTATION AND DEPRIVATION

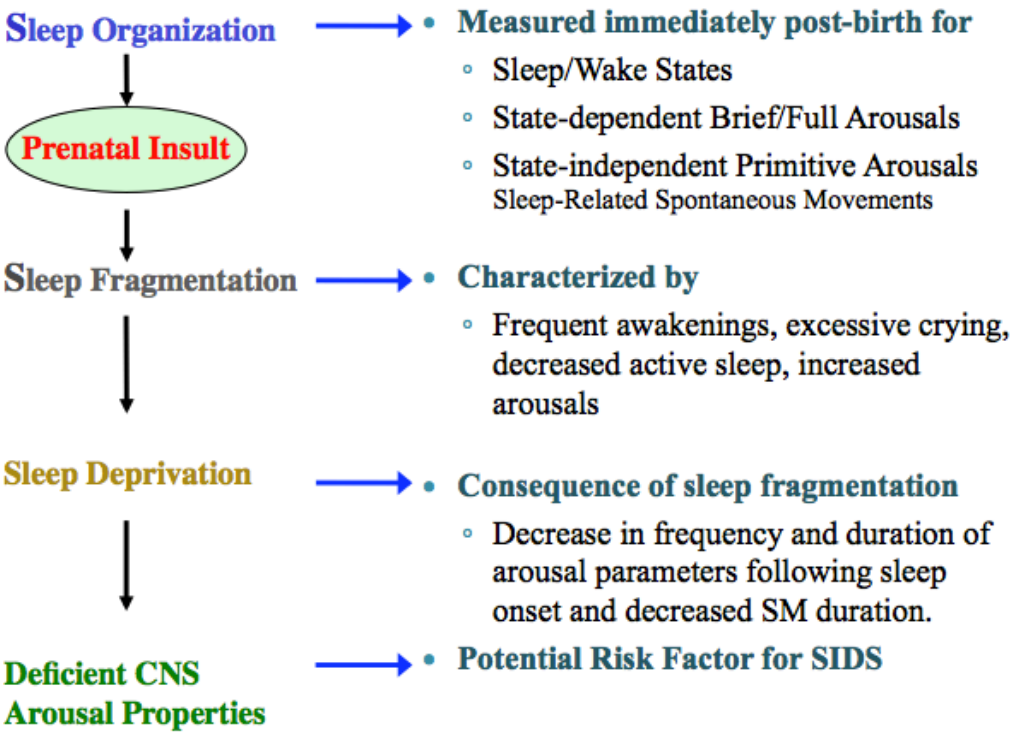

Figure 1.1: Cascade of Disrupted Sleep

In figure 1.1 we can analyze the cascade of disrupted sleep, and its measurements. Sleep organization is measured immediately post birth in the neonate. The sleep cycle is recorded and calculations made for sleep/wake states, state-dependent brief and full arousals, state independent primitive arousals (also known as spontaneous movements). When an infant experiences prenatal insult such as pre-term labor, methadone exposure and other factors (such as tobacco use, alcohol, anti-depressants) the infant typically will experience sleep fragmentation. Fragmentation is characterized by frequent awakenings, excessive crying, decreased active sleep and an increase in arousals. If sleep fragmentation is not properly resolved, then sleep deprivation is likely to occur. Sleep fragmentation includes a decrease in frequency and duration of arousal parameters following sleep onset and decreased spontaneous movement duration. Sleep fragmentation and deprivation are key factors in Central Nervous System deficiencies and are a potential risk factor for SIDS.

While most theories of sleep biology and the concept of deprivation are derived from animal studies, the assumptions pertaining to sleep-related brain plasticity have been formed based on the deprivation model, which is measurable by use of behavioral observation and histology (Hayes, 2002). The model of deprivation assumes an adverse outcome when the outcome is preceded by the deprivation of stimulus. Psychobiological effects of sleep deprivation are potentially adverse and are comprised of many different factors. Some of these effects include: irritability, cognitive impairment, memory lapses, impaired moral judgment, yawning, hallucinations, ADHD symptoms, impaired immune system, a risk of diabetes, increased heart variability, decreased accuracy, tremors, aches, growth suppression and many more.

In infants the consequences of sleep deprivation are even greater than in adults, because sleep deprivation has been found to lead to longer-than-usual durations of sleep and decreased latency to REM sleep (Franco, Seret, Van Hees, Scaillet, Vermeulen et al., 2004) in addition to decreased sleep-related arousal, which is believed to be a high risk factor for SIDS in infants (Kato, Franco, Groswasser, Scaillet, Kelmanson, Togari, & Kahn, 2003). In order to measure the level of sleep deprivation and the subsequent arousal deficits, Franco et al. induced sleep deprivation and arousals on fourteen healthy infants ages 6-18 weeks in a sleep laboratory. The infants underwent polygraphic recording during a morning and afternoon nap and were sleep deprived for two hours before being allowed to fall asleep. Deprivation was achieved by keeping infants awake for as long as possible before their usual nap times. The results showed that most sleep characteristics were similar for normal and sleep deprived conditions except that the duration of total nap increased, and latency of REM sleep and density of body movements saw a decrease. During sleep these infants needed more intense auditory stimuli for arousal; compared with a normal nap.

The interactions among sleep deprivation, arousals and SIDS is extremely complex. While arousal deficit due to sleep deprivation is associated with SIDS, excessive arousability during sleep leads to sleep fragmentation, which is the predecessor of sleep deprivation (Troese et al., 2007) Therefore, based on several documented associations among sleep fragmentation, deprivation, arousal deficit and SIDS, it appears that while optimal amount of arousal is adaptive, hyper- or hypo- arousability may pose a risk for adverse post-natal outcomes. This is extremely important to understanding the mechanism and risk factors associated with SIDS.

1.3 HYPOTHESES:

In the proposed study, I hypothesize that movement arousals (MA) based on spontaneous movements (SM) and sleep-wake behavioral states will be impaired with infants with allelic variants known to affect NAS.

Hypothesis 1. Overall this project will be used to compare the sleep-wake and SM parameters of opiate exposed infants possessing OPRM1 and COMT SNPs that affect NAS severity. It is predicted that SNPs protective for NAS will share and be associated with sleep-wake regulation, while those associated with the more severe NAS phenotype will display sleep-wake dysregulation during all night videographic and actigraphic sleep studies.

Hypothesis 2. Our preliminary data (Mariah Bundy, Biology/pre-med, Capstone, 2014) has shown that newborns with the COMT protective allele have more normative SMs and less impairment of SM vigor. NAS infants with one of the protective or risk alleles of COMT or OPRM1 are predicted to have SM patterns that reflect both neurodevelopmental and pre-withdrawal status on days 0-1 of life.

CHAPTER TWO
METHODS

In our larger longitudinal study, our lab has collected data on rural, disadvantaged mothers who are dependent on methadone and demographic controls (N= 200). All mothers are recruited from three local methadone treatment clinics and all infants are birthed at Eastern Maine Medical Center. For this study, 19 newborns who were > 36 weeks gestational age were used. Mothers who were dependent on methadone were recruited during the third trimester. Eligibility criteria include maternal prescribed methadone exposure *in utero* for a minimum of 30 days, singleton pregnancies, and infants who were medically stable after delivery. This study has been approved by the University of Maine and Eastern Maine Medical Center institutional review boards with written informed consent and compensation for participation of $175 for the perinatal assessments described below.

2.1 PARTICIPANTS:

We collected a subset of Caucasian (>98%) mother-infant dyads (N=19; total of 200) from our lab's longitudinal study at the University of Maine with funding from the National Institute of Health (NIH, #DA024806). Our participant demographics consisted of women that were pregnant and concurrently receiving methadone maintenance treatments (MMT). The mothers were recruited through a variety of programs during their third trimester of pregnancy from sites throughout the Bangor area. These sites included the Bangor Discovery House, Metro treatment of Maine and Acadia Hospital Narcotics Treatment Programs. The comparison group, whose sleep data are not included in this study, contained mothers with low socioeconomic status, which were recruited from Family Practice Center and Woman Infant and Children, also located in Bangor.

Both groups contained similar demographic characteristics including alcohol and tobacco use with the exception of opiate use for the comparison group. For the remainder of this thesis, the study group is restricted to sleep studies of 19 methadone dependent mother-infant dyads.

In the 3rd trimester, a semi-structured clinical interview of alcohol tolerance and dependence, socioeconomic status and depression and urinalysis and infant meconium document opioid and other drug exposures, that is used to quantify exposures. Both groups underwent a pre-screening process, which was used to evaluate the drug and alcohol history of the pregnant mother and also gain various demographics on age, ethnicity, socioeconomic status and mental health state of the mother.

The infants also underwent assessments post-birth in which behavioral state, genetics and medical records were collected after various HIPPA acts had been signed by the parent in order to learn more about each individual infant throughout various time points. The first testing was done in the hospital where infant biomarkers of substance exposure were assessed and recorded during initial hospital stay after birth. The nurses follow a protocol to take fecal matter samples and send them to Affiliated Laboratories in Bangor, ME for a toxic substance screening looking for particularly cocaine, opiates, cannabinoids, amphetamines and PCP.

Throughout the neonates hospital stay they undergo *Finnegan Neonatal Abstinence Scoring System* to look for symptoms of NAS (Kaltenbach & Finnegan, 1986). This scoring system lists 21 symptoms that are most frequently experienced in opiate-exposed infants. The symptoms are associated with a degree of severity and a

'score'. In the present study, all infants were pre-withdrawal; hence, do not score in the withdrawal range.

2.2 SLEEP STUDY PROTOCOL:

On Day 0 or 1, a sleep study was scheduled in all pre-enrolled mother-infant dyads. A Sony DCR-SR82 digital camcorder with a 60GB hard drive is used. It is a low light camera, which can record up to 40 hours of video for determination of behavioral state (described below) and sleep related spontaneous movements (SM) also termed movement arousals (MAs). The camera is set up for a maximum of thirteen hours, but the quietest periods occur between 2400-0500 hours and this period is standardized across newborns as the coding period. In addition to video coding, movements were also recorded using Minimitter Actiwatch AW-64 device. This device is a wrist-sized watch containing piezoelectric accelerometers. Previous tests confirmed use on pediatric populations on the leg to provide raw data for SM analyses at 10Hz to be analyzed by standardized software. The actigraphic data will not be incorporated into this thesis.

Analyzing the videos was automated using XL script by pre-defined criteria for behavioral units, which yielded coded information for frequency, duration and sequence (utilizing a Dell XPS 710 computer with a 3.0GHz Intel Core 2 CPU). Sleep-wake and behavioral state changes were measured over a 5-hour period from 2400-0500 in the neonatal nursery or the mother's room. Infants were not moved once the sleep study was begun at approximately 2000 hours. All of our videos are coded in the laboratory, by research assistants, who are trained to specific video coding criterion, in order to follow standard inter-rater reliability methods. In order to ensure proper coding techniques, all research assistants are withheld information regarding the infant's status, making the

study double-blinded. All video coding for each sleep study was repeated with independent observers and Kappa coefficients (0.68-90) met published criterion for acceptance. Over the course of the past year I have coded over 50 sleep-wake state videos, and 30 spontaneous movement videos. However, not all of these videos included genetic data, see results.

With the infant sleep and arousal coding we look at various behavioral states of the infant. In order to have consistency in the coding technique between each individual there were various parameters set in place in order to ensure consistencies.

2.3 AROUSALS:

In order to assess infant arousals, a brief arousal (10-30”), full arousal (>30”<1’), awakening (≥1’) were used. In order to assess sleep state, timing criteria were also put in place. Sleep bouts were defined as sleep behavioral unit that lasted longer than 1 minute following the onset of sleep. We were able to code SM bouts if the event lasted longer than or equal to five seconds. There was a latency requirement post SM offset of <5” of the spontaneous movement, or the movement was considered one continuous movement. Research assistants were blind to the infant’s status and were trained to code behavioral states based on the system, described more fully below.

2.3.1 SM: The characteristics of spontaneous movement were gross motor movements with the addition of arm, leg, neck and full body movements during the time of a sleep bout. SM had to last a minimum of five seconds, with a five second pause permitted. SM offset was determined with the end of one spontaneous bout and more than five second between the first and second bout within one sleep cycle. Inter-rater

reliability was established by using the Kappa coefficient method for each behavioral state: brief arousal, full arousal, wake, transition, sleep and spontaneous movements.

Though arousal criteria have been controversial due to the lack of standard operational definition to be applied across studies, sleep state organization is considered an essential marker of CNS and ANS maturity, common features of which, according to Hayes et al. (2007), include presence of arousals and SM during sleep periods in a cyclical fashion. Arousal is generally is defined as a physiological and psychological state of being awake or reactive to stimuli, achieved through the symphonic process of the reticular activating system within the brain stem, ANS and endocrine system stimulation. Arousal typically leads to increases in blood pressure and heart rate that will trigger sensory alertness, mobility and readiness to respond (Robinson, 2000**)**.

SM occurs throughout sleep and is a measure that can assess arousal quality during sleep in neonates. SM is a state-dependent movement pattern that is predicted to occur periodically in a 3-5 minute window. SMs are characterized as writhing bodily movements and sub-characterized according to amplitude and speed. SMs are believed to be a 'primitive' physiological arousal system that offers autonomic protection in sleep during the neonatal and early infancy period, when the arousals are immature (Hayes et al., 1993). SMs serve as a potential primitive and protective arousal regulatory mechanism and their effect is to restore airway openness and upregulate cardiorespiratory cycles of the medullary circuitry.

2.4 BEHAVIORAL STATE CODING:

The following coding criteria were derived from Giganti et al. (2002); Hayes et al. (2007); and Troese at el. (2008).

Table 1. Behavioral States Coding Criteria

Behavioral State	Coding Criteria
Sleep	Sleep states are composed as two different types. These are known as active sleep (REM) and quiet sleep (NREM). Each state is based on EEG and behavioral states, and each state is comprised of its own behavioral and physiological characteristics. This study did not classify into two separate types of sleep. We coded the videos simply for when the infant's eyes were shut, motor activity was low, and muscle tone was low. This state must last for a minimum of sixty seconds.
Spontaneous Movements	Throughout the sleep cycle, bursts of spontaneous movements occur every 3-5 minutes. A spontaneous movement is defined as a cluster of movements during sleep that is >5 seconds and can include occasional mouthing and grimacing behaviors in addition to movements of the limb, trunk and head.
Wake	Wake is the alternative state for sleep. The process of this state change involves transition states before and after the wake state. The infants must show signs of wake and have eyes open for > 1 minute to be coded as a wakeful state. Typically the infant is scanning the surroundings and is focusing on the environment. Sometimes the state of wake includes crying or other motor movements (to be coded separately)
Indeterminate	When a behavioral state is mixed between sleep and wake states but does not meet the criteria for either state.
Transition	These events are expected to come before or after sleep and wake states. They include characteristics such as spontaneous movements, yawn and drowse. Transition was considered to occur when the baby successfully transitioned from one state to the next.
Cry	Cry was measured based on vocalized cries that were heard through the audio of our recording equipment. If there were multiple cries, then the bout between them had to be more >5 seconds to be considered individual cries.
Nurse Intervention	Occasionally in our study it was difficult to see the baby due to an adult in the line of the camera's sight. This was coded as Nurse Intervention if it occurred longer than five seconds. Nurse intervention often caused changes in the babies behavioral state as well, and this was taken into consideration.
No Baby	Often the neonates were taken away for various activities such as feeding, changing, vitals and health check-ups. Despite our efforts to limit interruption of the video by working with the mother and

	nursing staff, because the infants were between 24-48 hours of age, this aspect of experimental control was important. If the baby was taken away for two or more minutes, this segment was coded as 'no-baby' and this segment of the video is considered to be missing data and is not coded into our calculations of frequency or duration.

A second category of sleep behavior is known as movement arousals (MAs), which are coded from sleep-related spontaneous movements (SMs) which occur in bursts every 3-5 minutes and do not disrupted sleep, but are similarly impaired in high risk samples such as infants exposed to prenatal alcohol (Troese et al., 2008). Arousals were coded as described previously.

2.5 DNA COLLECTION:

Saliva samples were collected during the hospital stay. In addition to infant DNA, we collected mother's DNA through saliva by having them spit into an Oragene GR-500 kit tube. The infant's saliva was collected using the infant version, OG-250 DNA collection kits with CS-1 sponges. Separated by 30 minutes from a feed, the infant's gum line was swabbed using five cotton swabs and placed in the storage kit to preserve the DNA. The kits can be stored at room temperature for as long as five years post-collection.

Infant DNA is collected in the newborn period in the first few days of life. Exploration of genotype SNP and methylation analyses were conducted for OPRM1 and COMT and the ABCB1 cassette genes. The protocol for collecting DNA from saliva is by gently swabbing the inside of the gum line, tongue and cheek of the infant's mouth with 5 swabs from the newborn saliva kit (*Genotek*^{TM}, Kanata, Ontario, Canada). When swabbing the infant's cheek the sponge has been placed between the gums and the inner

cheek. The swab is rolled against the cheek for a maximum of 15 seconds with precautions set in place to not scrape the mouth. The sponge of each swab is snipped off into a collector and capped where Oragene solution will release and preserve the DNA. Genetic SNP and methylation findings will be compared with Polyvideosomnographyic data from our ongoing longitudinal data on pre-withdrawal neonatal sleep and genetic information gathered over the last several years will be used in addition to new data collected for use and coding as well.

2.6 DNA PROCESSING:

The specimens were sent to Tufts Medical Center and the Clinical and Translational Research Center Genetics Core Laboratory for processing. The DNA was isolated and there were five main SNPs evaluated.

1. OPRM1 118A>G (rs1799971, dbSNP database; assay C_8950074_1)
2. ABCB1: 3435C>T (rs1045642, dbSNP; assay C_7586657 _20),
3. ABCB1: 2677G/T/A (rs2032582, dbSNP; assays C_11711720C_30 and C_11711720D_40)
4. ABCB1: 1236C>T (rs1128503, dbSNP; assay C_7586662 _10)
5. COMT: 158A>G (rs4680, dbSNP; assay C_25746809_50)

DNA isolation for genotyping was done using established Taqman technology (Wachman et al., 2013). These results were returned to our lab for comparison with the behavioral state measures. The methylation results weren't included in this report.

2.7 STATISTICS:

Using the dominant model, genes were examined as the major homozygous allele vs. the minor allele/s. This created 2 groups to contrast with sleep-wake measures and SM. Group 1 is associated with homozygous variants for the major allele (ie. AA), and Group 2 is associated with collapsed heterozygotes and homozygotes for the minor allele (ie. AG/GG). I was able to analyze 19 sleep studies for comparison to the dominant model gene groups and used separate t-test to compare sleep-wake states and SM parameters with OPRM1, COMT, and 3 of the ABCB1 genes. Statistical significance was defined as $p<.1$ without multiple comparison correction. This approach was used because of the pilot nature of the study, and bias was assumed to avoid the Type II error.

CHAPTER 3
RESULTS

Throughout the analysis of my results, I was able to use 17 sleep studies, which had corresponding genetic data on mother and infant and an infant pre-withdrawal from the nocturnal period of day 0 or 1 of life.

The infants' prenatal exposure and pregnancy quality were evaluated through a maternal interview in the 3rd trimester. Although not presented here, meconium (infant fecal material immediately post-birth) data were consistent with maternal peri-pregnancy (i.e. retrospective to before knowledge of pregnancy and during pregnancy drug and alcohol use/abuse) and psychological state reported in the structured interview. Table 1 shows maternal demographics of SES, intelligence estimate (measured by the PPVT), prenatal depression and psychiatric status. My sample is a subset (n=19) of the methadone group. As shown, a comparison group is similar in age, SES and PPVT measures, but has significantly higher rates of depression, psychiatric symptoms and use of tobacco. In the cohort described in Table 1, a subset of methadone exposed neonates who had both sleep studies and genetics data were used (N=19).

Table 2. Maternal Demographic Characteristics

	Comparison			Methadone				
	n	Mean	SD	N	Mean	SD	*t*	*p*
Maternal Age (in years)	21	24.62	2.92	36	26.44	3.78	-1.9	ns
Socioeconomic Status (SES)	20	26.4	8.52	36	23.6	9.18	1.12	ns
PPVT-III	20	96.55	8.71	33	95.94	6.15	0.3	ns
Prenatal BDI-II	20	10.6	9.76	36	16.31	8.43	-2.29	0.026*
SCL-90 (GSI)	20	52.2	10.86	36	60.5	8.66	-3.14	0.003*
Cigarettes/day	20	2.5	3.54	35	9.2	6.68	-4.15	0.000*

Maternal Demographics. SES = Socioeconomic Status; PPVT-III = Peabody Picture Vocabulary Test – 3rd Edition (verbal intelligence); BDI-II = Beck Depression Inventory – 2nd Edition (depressive symptoms); SCL-90R (GSI) = Symptoms Checklist 90 – Revised (global screen for psychological health); *p<.05, **p<.005

Table 3. Infant Demographic Characteristics

	n	Mean	SD
Sex (% Female)	8	42%	
Length of Stay (days)	19	17.2	15.2
Gestational Age	19	15.2	1.9
Weight (kg)	19	3.3	0.5
Length (cm)	19	51.2	2.5
Head Circumference (cm)	19	34.4	1.7
Delivery Method (%vaginal)	12	68%	

Table 3. Infant demographics for n=19 infants with sleep-wake and genetics data.

All infants reported herein were full-term (>38 weeks gestational age) and had had normal vaginal or caesarian delivery and were admitted in the pre-withdrawal period to the low risk neonatal nursery or were "rooming in" with their mothers.

The sleep-wake results are organized by each genotype according to the dominant model. This model consists of two groups separated by the presence or absence of the minor allele (example: Group 1: AA; Group 2: AG or GG with the minor allele). Groups were contrasted with sleep-wake and SM measures separately for each allelic gene pattern identified in the infant. Although not hypothesized, some statistically significant findings were uncovered for the mother's SNPS and are reported as well.

3.1 OPRM1.

Table 2 presents the results of the t-test comparisons between Groups 1 and 2 for behavioral state and SM parameters. No results were found for the association of sleep-wake or MA measures and infant genotype group using the dominant model. As shown in Table 2, for the mother's DNA, infants in Group 2 (protective minor alleles, Wachman et al., 2013) had significantly increased frequency of awakenings ($p<.04$), sleep-wake

transitions ($p<.004$), and number of sleep bouts ($p<.059$), all calculated as a proportion of the total observation window.

Behavioral Unit	Gene	Values	St. Dev	n
	OPRMMOM			
Ratio of total awakening frequency to total clip (per hour)	*Group 1*	0.574	0.155	15
	Group 2	1.765	0.434	2
	P Value	0.041		
Ratio of total sleep frequency to total clip (per hour)	*Group 1*	1.031	0.391	15
	Group 2	1.885	1.558	2
	P Value	0.059		
Ratio of total transition frequency to total coded clip (hour)	*Group 1*	0.353	0.233	15
	Group 2	1.100	0.707	2
	P Value	0.004		
	MDRaMOM			
Ratio of spontaneous movements to sleep duration	*Group 1*	0.122	0.016	4
	Group 2	0.041	0.031	10
	P Value	0.000		
	MDRbMOM			
Average quiescence duration	*Group 1*	97.440	13.980	5
	Group 2	185.780	78.300	8
	P Value	0.032		5
Average IBI	*Group 1*	114.780	11.390	8
	Group 2	202.690	73.040	
	P Value	0.023		
	MDRcMOM			
Average duration of sleep	*Group 1*	2812.220	851.270	3
	Group 2	3281.910	2199.990	8
	P Value	0.073		
Average quiescence duration	*Group 1*	97.440	13.980	5
	Group 2	174.970	80.100	9
	P Value	0.057		
Average IBI	*Group 1*	114.780	11.390	5
	Group 2	191.280	76.400	9
	P Value	0.049		

Behavioral Unit	Gene	Values	St. Dev	n
	MDRaINF			
Ratio of total awakening frequency to total clip (per hour)	*Group 1*	1.277	1.216	4
	Group 2	0.562	0.363	12
	P Value	0.077		
Proportion of total coded time awake	*Group 1*	0.258	0.204	4
	Group 2	0.089	0.090	12
	P Value	0.032		
Proportion of total coded time transitioning	*Group 1*	0.040	0.038	4
	Group 2	0.014	0.013	12
	P Value	0.048		
	MDRbINF			
Average brief arousal	*Group 1*	4.330	10.614	6
	Group 2	12.070	12.564	9
	P Value	0.024		
Average full arousal	*Group 1*	7.666	18.779	6
	Group 2	33.805	21.853	9
	P Value	0.033		
	MDRcINF			
Ratio of total sleep frequency to total clip (per hour)	*Group 1*	0.238	0.180	5
	Group 2	0.094	0.097	12
	P Value	0.099		
Average full arousal	*Group 1*	9.200	20.570	5
	Group 2	29.188	23.353	12
	P Value	0.118		
	COMTINF			
Ratio of spontaneous movements to sleep duration	*Group 1*	0.005	0.006	3
	Group 2	0.066	0.041	16
	P Value	0.025		

Table 4. This table shows the relationships between genotypes and behavioral states using t-tests and the dominant model. Group one and Group two values are displayed with associated p-values listed below.

3.2 COMT.

Infant SNPs for the protective minor allele (Group 2; Wachman et al., 2013) were associated with improved vigor in the MA system, measured by the rate of SM bouts per hour As shown in Figure 1, the frequency of SM is greater in Group 2 than Group 1 ($p<.025$). No other relationships to sleep measures were found for infant or maternal DNA.

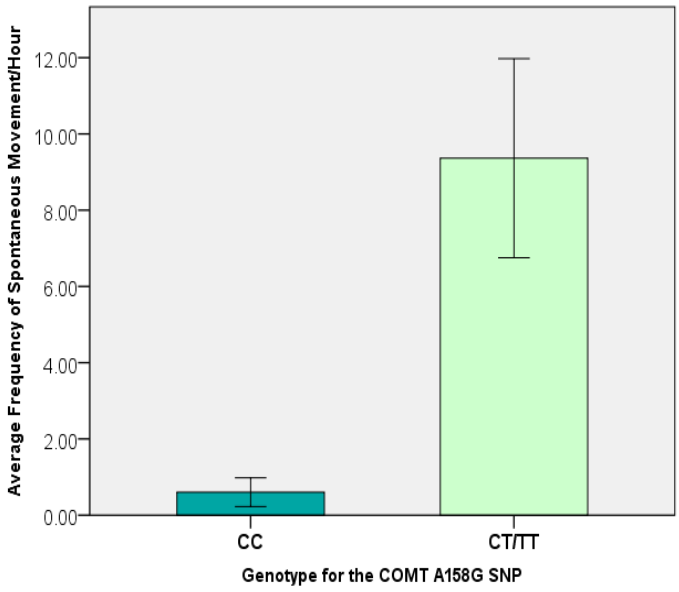

Figure 3.1 Genotype for the COMT A158G SNP with Average Frequency of Spontaneous Movements per hour. Here we see a higher frequency of SM in Group 2 with the minor T allele (CT/TT) than Group 1 (CC).

3.3 ABCB1 CASSETTE: (including MDR a,b,c genes)

As described in the introduction, p-glycoprotein, the main enzyme in methadone pharmacokinetics is more active in the minor SNPs of the cassette genes.

An important result for infant MDRb was associated with an increase in brief arousal ($p<0.024$) and full arousals ($p<0.033$) in Group 2. As well, infant MDRc is associated with increased full arousal ($p<0.01$) and decreased sleep ($p<0.099$) in Group 2 when compared to Group 1. These results suggest that Group 2 in both MDRb and MDRc are comprised of infants with sleep fragmentation, defined as increased arousals and wake state during a sleep period, relative to Group 1.

Group 2 results for MDRb and MDRc show evidence for sleep disorder (both fragmentation and deprivation). Consistent with a sleep disorder effect, maternal DNA for both MDRb and MDRc from maternal DNA was associated with a decrease in SM measures. As shown for mean values in Table 2, Group 2 is associated with longer average interburst interval (IBI: time between the onset of SM bursts) ($p<0.023$) suggesting that there is a suppression of SM in Group 2 vs. Group 1. However, SM frequency and duration were not different which would be expected by the IBI change.

MDRa infant DNA was associated with decreased wakefulness ($p<0.032$), and concomitant decrease in sleep-wake transition time ($p<0.048$) in Group 2. Interestingly, in maternal DNA, MRDa was associated with decreased relative frequency of SM bouts, our measure of MAs during sleep. These results suggest a more severe phenotype for fast metabolism consistent with sleep deprivation that follows sleep fragmentation temporally in our prior studies.

CHAPTER FOUR
DISCUSSION

This thesis project explored the relationship between all night sleep studies conducted in the first few days of life in prenatally methadone exposed newborns and the genetic profile for key genes that have been linked to processing of opioids by the CNS. The hypothesis was to examine whether allelic variants associated with NAS severity would be associated with sleep disorder, particularly, increases of arousal or wake state, and intrusion into sleep (sleep fragmentation) or decreases in arousal or wake state from typical rates (sleep fragmentation). A significant limitation of my findings is that genotype information for mothers and newborns with matched sleep studies was far less than would be optimal to establish these relationships. I was able to use a total of 19 mother-infant dyads with sleep and infant and maternal genetic data, so the pilot results should be interpreted with caution.

4.1 OPRM1:

Though I was unable to find an association for sleep-wake or MA measures using the genetic dominant model, we found that infants whose *mother* carried the protective minor alleles (Group 2) had rates of awakenings, sleep-wake transitions, and total number of sleep bouts that were significantly lower than in Group 1. Group 2's sleep-wake behavior was consistent with our normative data, and is also consistent with the milder NAS phenotype where newborn patterns of sleep-wake regulation are preserved. Group 1 did not show the typical pendulum from sleep to arousal or wake behavior, and this group had shown the more severe NAS phenotype in our earlier study. This result suggests that Group 1 infants may already be showing sleep deprivation (which is reflected by less

arousals). For example, the mean frequency of awakenings/hour is lower in the risk group (0.5 awakenings/hour), matching consistently with sleep deprivation or neurodevelopmental delay from the prenatal environmental conditions, compared to Group 1 showing higher rates. Of notes, this also suggests that maternal genotyping may help to predict the NAS phenotype before withdrawal is diagnosed with the Finnegan scores.

4.2 COMT:

Group 2 infants with SNPs that included the protective minor allele (Wachman et al., 2013) had sleep findings that were associated with an improvement of vigor in the primitive MA system, which was measured in this study by sleep-related spontaneous movements. Protective COMT SNPs are associated with a milder NAS phenotype and in this pilot study were associated with a robust MA profile. In studies in our laboratory, the MA system has been shown to be reflective of optimal functioning during sleep, and hypothesized as a protective mechanism for SIDS. High-risk for SIDS subgroups (e.g. prenatal alcohol exposure, apnea of prematurity, prenatal tobacco exposure) have been found to have reduced MA during sleep. Further, this pattern was preceded by sleep fragmentation (increased arousal intrusion into sleep) that converts, in our prior studies of high risk infants, to sleep deprivation (characterized by decreased arousals during sleep). It is important for infants to have 1-2 brief to full arousals/hour of sleep and to exhibit robust MA every 3-5 minutes during sleep (Hayes, 2002).

4.3 ABCB1 CASSETTE:

I was able to find that both MDRb and MDRc in *maternal* DNA are associated with changes in several infant SM measures consistent with decreases in the primitive MA system. As discussed in the introduction, minor alleles of these genes are associated with increased p-glycoprotein degradation of opioid, including methadone, and are associated with a faster metabolism of methadone, consistent with shorter latency to enter NAS withdrawal.

In reference to infant MDRb, infant DNA was associated with an increase in brief arousal and full arousals with the minor allele in Group 2. For MDRc, infant DNA with the minor allele, Group 2, showed increases in full arousal, and a decrease in total sleep. These results suggests that infant minor alleles for MDRb and MDRc are associated with sleep fragmentation, which is consistent with maternal DNA for these same genes in the suppression of MA system that is suppressed for both sleep fragmentation and sleep deprivation (Hayes, 2002). Sleep fragmentation is observed clinically with NAS and may reflect the 'fast metabolizer' phenotype that is associated with the minor allele in adults.

MDRa infant DNA was associated with a decrease in wakefulness and a concomitant decrease in state transitions for infants with the minor allele in Group 2. Interestingly, SM bout frequency during sleep was depressed when MDRa *maternal* DNA was cross-examined with infant sleep. These results are suggesting that minor allele status in the infant and mother has features of sleep deprivation (e.g. decreased arousals and SM, and increased sleep). Sleep deprivation, or low arousal/wake including MA, reflects post-fragmentation sleep disorder, which is more severe, but consistent with

withdrawal, and in the same direction as findings for MDRb and MDRc, which showed sleep fragmentation.

It is well known that sleep fragmentation, characterized by increased intrusion of wakefulness and arousal periods into sleep, leads to poorer sleep consolidation (Touchette, et al., 2005). Once sleep fragmentation is well established, sleep deprivation effects can be observed. During sleep deprivation, arousals during sleep are decreased and MAs (as measured by SM measures) are similarly depressed. The latter is a known protective system that reflects sleep integrity and autonomic regulatory processes during sleep.

In summary, the clearest findings related to the ABCB1 cassette genes that argue that infants with the minor alleles (group 2) represent a "fast metabolizer" phenotype that may reflect rapid withdrawal onset. In our earlier work (Wachman et al., 2013) we did not find a role for the ABCB1 cassette and associated SNPS in the severity for NAS. However, these findings suggest that a careful inspection of NAS scoring in the pre-withdrawal period and time to treatment may confirm this hypothesis.

The utility of maternal DNA for predicting infant sleep regulation, and implications for neurodevelopment and/or latency of opioid withdrawal was found for OPRM1 and genes in the ABCB1 cassette, which may provide important information on the infant withdrawal phenotype before birth. OPRM1 maternal minor alleles predicted improved sleep regulation, which is consistent with the milder NAS phenotype, although we did not replicate the infant findings from our early work, likely related to the low sample size in the current study. For infant DNA, minor alleles of COMT were associated

with improved MA profile, which is similarly consistent with better sleep regulation and in our earlier study, a milder NAS phenotype.

In short, the results of my pilot study suggest that addiction (OPRM1 and COMT) and metabolism (ABCB1 cassette) allelic profile of both infants and their mothers may provide important information regarding infant opioid withdrawal and clinical course. In combination with early assessment of infant sleep-wake regulation, gene-function relationships relevant to withdrawal are apparent prior to the emergence of frank clinical signs assessed by the Finnegan score, and may aid in identifying which infants may need early opioid replacement to improve their recovery course.

Though the study had a small N, there is importance of creating this pilot study. A pilot study is also known as a feasibility study, used to pre-test a particular research technique to determine if it is appropriate for a full-scale study. Pilot studies typically provide insight for other researchers and can spark discussions and birth new studies. The value of this study is primarily the identification of early markers of pre-withdrawal; poor sleep quality and poor neurodevelopment. This knowledge can facilitate the withdrawal process for neonates early and reduce the risk for SIDS.

There are areas that merit further review, such as sample size, gender of the infant in relationship to drug response, metabolism, etc. While reviewing numbers it is important to note that not all infants needed pharmacological treatment for NAS. Eight of nineteen infants in this study did not receive pharmacological treatment in this study, and this lack of treatment would merit further review. Also, there are important limitations to my study that should be mentioned. First, this was a pilot study and many of the findings may not hold up with a larger sample and more stringent statistical tests. Also, I was not

able to control for potential group differences in prenatal exposure to other factors such as comorbid drug use and maternal depression and psychiatric status. Other studies have shown that maternal psychological health to compromise fetal neurodevelopment through CNS stress pathways (Glover et al, 2011). Future work from our group will aim to expand on these preliminary findings.

REFERENCES

Franco, P., Seret, N., Van Hees, J. N., Scaillet, S., Vermeulen, F., Groswasser, J., & Kahn, A. (2004). Decreased arousals among healthy infants after short-term sleep

Giganti, F., Hayes, M. J., Akilesh, M. R., & Salzarulo, P. (2002). Yawning and behavioral states in premature infants. *Developmental Psychobiology*, *41*, 289–296.

Hartwell, Leland et al. From Genes to Genomes. Boston: McGraw-Hill Higher Education, 2008. Print.

Hayes, M.J., Akilesh, M.R., Fukumizu, M., Gilles, A.A., Sallinen, B.A., Troese, M., & Paul, J.A. (2007). Apneic preterns and methylxanthines: Arousal deficits, sleep fragmentation, and supporesed spontaneous movements.Journal of Perinatology, 27, p. 782-789

Hayes, M. J., & Brown, M. S. (2012). Epidemic of prescription opiate abuse and neonatal abstinence. *JAMA,* 307(18), 1974-1975

Hayes, M. J. (2002). Methodological issues in the study of arousals and awakenings during sleep in the human infant. *Advances In Consciousness Research,38*, 23-46

Hudak, M. L., Tan, R. C., Frattarelli, D. A., Galinkin, J. L., Green, T. P., Neville, K. A., et al. (2012). Neonatal drug withdrawal. Pediatrics, 129(2), 540-560.

Jansson, L. M., DiPietro, J. A., Velez, M., Elko, A., Williams, E., Milio, L., ... & Jones, H. E. (2011). Fetal neurobehavioral effects of exposure to methadone or buprenorphine. Neurotoxicology and teratology, 33(2), 240-243.

Jones, H. E., O'Grady, K. E., Malfi, D., & Tuten, M. (2008). Methadone maintenance vs methadone taper during pregnancy: Maternal and neonatal outcomes. The American Journal on Addictions, 17(5), 372-386.

Kaltenbach, K., & Finnegan, L. P. (1986). Neonatal abstinence syndrome, pharmacotherapy and developmental outcome. *Neurobehavioral Toxicology & Teratology, 8(3), 353-355*

Kato, I., Franco, P., Groswasser, J., Scaillet, S., Kelmanson, I., Togari, H., & Kahn, A. (2003). Incomplete arousal processes in infants who were victims of sudden death. *American Journal of Respiratory and Critical Care Medicine*,*168*(11),

Levran, O., K. O'hara, E. Peles, D. Li, S. Barral, B. Ray, L. Borg, J. Ott, M. Adelson, and M. J. Kreek. "ABCB1 (MDR1) Genetic Variants Are Associated with Methadone Doses Required for Effective Treatment of Heroin Dependence." *Human Molecular Genetics* 17.14 (2008): 2219-227.

Patrick, S. W., Schumacher, R. E., Benneyworth, B. D., Krans, E. E., McAllister, J. M., & Davis, M. M. (2012). Neonatal abstinence syndrome and associated health care expenditures: United States, 2000-2009. JAMA, 307(18), 1934-1940.

Robinson, S. E. (2000). Effect of prenatal opioid exposure on cholinergic development. *Journal of Biomedical Science, 7*(3), 253-257. doi:10.1159/000025456

Touchette, É., Petit, D., Paquet, J., Boivin, M., Japel, C., Tremblay, R. E., & Montplaisir, J. Y. (2005). Factors associated with fragmented sleep at night across early childhood. *Archives of pediatrics & adolescent medicine*, *159*(3), 242-249.

Troese, M., Fukumizu, M., Sallinen, B.J., Gilles, A.A., Wellman, J.D., Paul, J.A., Brown, E.R., Hayes, M.J. (2008). Sleep fragmentation and evidence for sleep debt in alcohol-exposed infants. Early Human Development, 84, p. 577-585.

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES. "Results From the 2010 National Survey on Drug Use and Health: National Findings." *PsycEXTRA Dataset* (2011): n. pag. Web.

Vecchierini, M., & Navelet, Y. (2002). Arousals and awakenings in infancy: Evaluation for clinical context. In P. Salzarulo, G. Ficca (Eds.) , Awakening and sleep-wake cycle across development (pp. 213-232). Amsterdam, Netherlands: John Benjamins Publishing Company.

Wachman, E. M., Hayes, M. J., Brown, M. S., Paul, J., Harvey-Wilkes, K., Terrin, N., & Davis, J. M. (2013). Association of OPRM1 and COMT Single-Nucleotide Polymorphisms With Hospital Length of Stay and Treatment of Neonatal Abstinence Syndrome SNPs and Outcomes in Neonatal Abstinence Syndrome. JAMA, 309(17),

Wachman, E. M., Hayes, M. J., Lester, B. M., Terrin, N., Brown, M. S., Nielsen, D. A., & Davis, J. M. (2014). Epigenetic Variation in the Mu-Opioid Receptor Gene in Infants with Neonatal

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