8-2014

# Sleep-Related Arousal and Spontaneous Movement Properties in Methadone-Exposed Neonates: A Videographic Assessment On the First or Second Postnatal Night

Hira Shrestha

# SLEEP-RELATED AROUSAL AND SPONTANEOUS MOVEMENT PROPERTIES IN METHADONE-EXPOSED NEONATES: A VIDEOGRAPHIC ASSESSMENT ON THE FIRST OR SECOND POSTNATAL NIGHT

By Hira Shrestha

Thesis Advisor: Dr. Marie J Hayes

An Abstract of the Thesis Presented
in Partial Fulfillment of the Requirements for the
Degree of Master of Arts
(in Psychology)
August 2014

Prenatal substance exposure such as alcohol, nicotine, and opiates is known to modulate autonomic regulatory function during sleep, and to decrease arousability and spontaneous movements (SM). SM during sleep may reflect a protective mechanism for immature patterns of arousals. Neurodevelopmental compromise in sleep and arousal systems may underlie sudden infant death syndrome (SIDS) risk in which infants expire during sleep. Previous studies from our laboratory found abnormal patterns of neonatal arousal, sleep fragmentation, and deficits in sleep-related SM in infants with prenatal alcohol exposure. In this study, prenatal exposure to methadone was hypothesized to disrupt the development of sleep and arousal neural circuitry, which have been found for other high-risk samples. Neonatal Abstinence Syndrome (NAS) is a common consequence of prenatal methadone exposure that may appear within 24 - 72 hours post-birth, and is known to disrupt sleep due to hyperarousability. As a secondary hypothesis, the neonatal age (day 1 or 2 of life) was expected to affect infant sleep and arousal

outcomes in methadone-exposed neonates particularly on day 2 when NAS symptoms increase. Additionally, single nucleotide polymorphism (SNP) in the catechol-O-methyl-transferase (*COMT*) gene was found to associate with the severity of NAS in our previous study. NAS severity has been associated with sleep disorders. Therefore, the second hypothesis of this thesis study is that the minor allelic variants (AG/GG) of the *COMT* gene previously identified as protective of NAS severity may also associate with better sleep organization and more robust SM than the carriers of the AA genotype. Rural, disadvantaged Caucasian mothers and infants (N=58 dyads: methadone=37, comparison=21) were recruited from multiple narcotic treatment sites and prenatal clinic at Eastern Maine Medical Center (EMMC). Mothers were interviewed to determine demographics, psychiatric status, and substance abuse history during the 3$^{rd}$ trimester. Bi-weekly maternal urinalysis screens and neonatal meconium were applied to verify co-morbid alcohol, tobacco, and other drug use. Finnegan scores determined symptoms of withdrawal in opioid exposed newborns. Videosomnographic recordings of behavioral states were collected in the newborn nursery of EMMC overnight, and recordings between 2400-0500h were analyzed for frequency and duration of sleep, wake, arousal, and SM. Saliva samples for genetic study was collected using Oragene$^{TM}$ kits. Results from behavioral state analysis (n=50) showed that methadone-exposed neonates were significantly hyper-aroused and crying more on both day 1 and 2 of life ($p<.05$); and both the frequency and duration of these parameters increased significantly in the methadone-exposed neonates on day 2 of life, as expected. In the genetic study (n=20), neonates with NAS protective AG/GG genotypes showed better behavioral sleep, fewer arousals, and robust SM than infants with NAS risk AA genotype ($p<.05$). These findings support

evidence of sleep fragmentation in the exposed neonates that is exacerbated by the passage of time since birth when withdrawal symptoms compound the intensity of sleep disturbance and infant distress. Consistent with other findings from other SIDS-risk samples, these findings indicate that arousal and SM regulation may be disrupted in methadone-exposed neonates, suggesting that prenatal methadone may increase risk for SIDS.

# SLEEP-RELATED AROUSAL AND SPONTANEOUS MOVEMENT PROPERTIES IN METHADONE-EXPOSED NEONATES: A VIDEOGRAPHIC ASSESSMENT ON THE FIRST OR SECOND POSTNATAL NIGHT

By

Hira Shrestha

B.A. Tribhuwan University, 2002

B.S. Husson University, 2009

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Arts

(in Psychology)

The Graduate School

University of Maine

August 2014

Advisory Committee:

Marie J Hayes, Professor of Psychology, Advisor

Alan Rosenwasser, Professor of Psychology

Cynthia Erdley, Professor of Psychology

## LIBRARY RIGHTS STATEMENT

In presenting this thesis in partial fulfillment of the requirements for an advanced degree at the University of Maine, I agree that the Library shall make it freely available for inspection. I further agree that permission for "fair use" copying of this thesis for scholarly purposes may be granted to the Librarian. It is understood that any copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Signature:

Date:

## THESIS ACCEPTANCE STATEMENT

On behalf of the Graduate Committee for Hira Shrestha I affirm that this manuscript is the final and accepted thesis. Signatures of all committee members are on file with the Graduate School at the University of Maine, 42 Stodder Hall, and Orono, ME 04469.

Marie J. Hayes, Professor of Psychology, Advisor August 15, 2014

## ACKNOWLEDGEMENTS

This thesis would not have been possible without the expert guidance and support of my advisor Dr. Marie Hayes, and the rest of the committee, Dr. Alan Rosenwasser and Dr. Cynthia Erdley. I am forever indebted for their generosity and would like to extend my sincerest gratitude to them. I would especially like to thank Dr. Hayes for allowing me a chance to be acquainted with the depth of her knowledge and expertise, and making every effort to make sure of my success along this journey even during the moments when I was discouraged to continue. I will always revere her as my mentor.

The former graduate colleagues, Dr. Jonathan Paul, Dr. Beth Logan, Deborah Morrison, and Dr. Nicole Heller, of the Hayes Lab are my role models. They have inspired me in so many ways to move forward in my academic career. I am very grateful for all the love and guidance they have offered me during their stay at the Umaine and also remotely.

This thesis study involved hours of coding and re-coding of lengthy videos of sleeping neonates, which was only possible due to the commitment of the research assistants, and also pots and pots of coffee. I would particularly like to thank Zakiah Lee-Meeks, Katrina Daigle, Jason Horr, Gabrielle Stone, Emily Lavoie, Mariah Bundy, and Alexandria McVicar who spent many hours in the lab coding videos, harvesting and entering data, and helping out the lab with administrative tasks. I am also thankful to Amanda Dickey and Chelsea Folger for their company and their contribution to data collection.

I would also like to thank my national and international graduate colleagues for all the encouraging words, movies, delicious cuisine, parties, and emotional supports they offered me throughout my graduate life. I admire their intellect, hard work, and kindness that greatly inspired me to complete this degree.

My especial thanks to Sean Coleman for always being my rock during this process, cooking meals and making coffee for me any time of the day, believing in me, reminding me that this is temporary and that everything is going to be fine at the end. Without his love and endless support, I would not have been able to complete this thesis and I am very blessed to have him in my life. Additionally, I would like to thank John Coleman for being a 'Dad' in a foreign land and unconditionally loving me. My deepest appreciation and love to Sean and his entire family for providing me a home away from home. Thanks to my sisters in Nepal, especially Muga Shrestha and Moti Shrestha for keeping me entertained via Skype and telephone while I was stressed out.

I would also like to thank the faculty and staff of the psychology department for providing me with an opportunity to expand my knowledge of psychology as a science, and allowing me to explore my options while I was in the program. I consider this thesis a cumulative effort of all of them because every bit of direct or indirect input I received as a graduate student was essential for the completion of this thesis. Additionally, I would like to thank the Office of International Programs for making international students like me feel safe and welcomed at the University of Maine. Many thanks go to Dr. Jennifer Goldenberg for her kind care and support since my days at Husson University; and to Lorene Stevens and Susan Niles for always guiding me in administrative tasks with big smiles on their faces.

This project would not have been a success without the cooperation of many individual organizations. I would like to thank the National Institute of Health and the University of Maine for the funding support for this research; the Acadia Hospital, Discovery House of Bangor, Metro Clinic, the Maine office of Women, Infants and Children, and Eastern Maine Medical Center for the recruitment and follow-up of participants. I would like to extend my sincerest appreciation for the cooperation and support that the nursing staffs of the Grant 7 and 8 of EMMC continue to provide to this study.

Finally, I would like to acknowledge everybody who are not mentioned here but have been supportive of my effort to graduate. The Hayes Lab will always remain in my memory as a place where I learnt everything I needed to move forward in my career. I am genuinely grateful for the opportunity.

## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| ACh | Acetylcholine |
| ANOVA | Analysis of Variance |
| ANS | Autonomic Nervous System |
| AS | Active Sleep |
| BA | Brief Arousal |
| BDI-II | Beck Depression Inventory – Second Edition |
| BM | Bangor Metro |
| BSID-III | Bayley Scale of Infant Development – Third Edition |
| BSIQ | Brief Infant Sleep Questionnaire |
| cAMP | Cyclic adenosine monophosphate |
| CCN | Continuing Care Nursery |
| CI | Confidence Interval |
| CNS | Central Nervous System |
| COMT | Catechol-O-methyl transferase |
| CRH | Corticotrophin Releasing Hormone |
| DA | Dopamine |
| DH | Discovery House |
| DHHS | Department of Health and Human Services |
| DNA | De-ribonucleic Acid |
| DOPAC | Dihydroxyphenylacetic Acid |
| DOR-OP | Delta opioid receptor |
| DRD4 | Dopamine Receptor D4 |
| DSM-IV | Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition |

| | |
|---|---|
| DT-MRI | Diffusion Tensor MRI |
| EEG | Electroencephalography |
| EMMC | Eastern Maine Medical Center |
| ERP | Event Related Potential |
| FA | Full Arousal |
| FAEE | Fatty Acid Ethyl Esters |
| FAS | Fetal Alcohol Syndrome |
| FPC | Family Practice Center |
| GI | Gastrointestinal Tract |
| GLM | General Linear Model |
| HIAA | Hydroxyindoleacetic Acid |
| HIPAA | Health Insurance Portability and Accountability Act |
| HPA | Hypothalamic Pituitary Adrenal |
| IBI | Inter-bout Interval |
| IQ | Intelligence Quotient |
| IRB | Institutional Review Board |
| IS | Indeterminate State |
| KOR-OP | Kappa opioid receptor |
| LOS | Length of Stay |
| MAST | Michigan Alcoholism Screening Test |
| MecSTAT-7 | Meconium Toxicology Screens |
| MMT | Methadone Maintenance Therapy |
| MOPEG | 3-methoxy-4-hydroxyphenylglycol |
| MOR-OP | Mu Opioid Receptor |

| | |
|---|---|
| mPFC | Medial Pre-frontal cortex |
| mRNA | Messenger Ribonucleic Acid |
| NAc | Nucleus Accumbens |
| NAc | Nucleus Accumbens |
| NAS | Neonatal Abstinence Syndrome |
| NE | Norepinephrine |
| NGF | Nerve Growth Factor |
| NI | Nurse Intervention |
| NICU | Neonatal Intensive Care Unit |
| NNNS | Neonatal Intensive Care Unit Network Neurobehavioral Scales |
| NOR-OP | Nociception receptor |
| NREM | Non-Rapid Eye Movement |
| NTP | Narcotics Treatment Program |
| ONDCP | Office of National Drug Control Policy |
| OWLS | Oral and Written Language Scales |
| PNS | Peripheral Nervous System |
| PPVT – III | Peabody Picture Vocabulary Test – Third Editions |
| QS | Quiet Sleep |
| QVF | Quantity Variability Frequency |
| RAS | Reticular Activation System |
| REM | Rapid Eye Movement |
| RSA | Respiratory Sinus Arrhythmia |
| SAMHSA | Substance Abuse and Mental Health Services Administration |
| SCL-90R | Symptom Checklist 90-Revised |

| | |
|---|---|
| SES | Socioeconomic Status |
| SIDS | Sudden Infant Death Syndrome |
| SM | Spontaneous Movements |
| SNP | Single Nucleotide Polymorphism |
| SPSS | Statistical Package for the Social Sciences |
| SWS | Slow Wave Sleep |
| TACE | Tolerance Annoyed Cut-down Eye-Opener |
| TWEAK | Tolerance Worried Eye-Opener Amnesia Cut-down |
| UDS | Urinary Drug Analysis |
| VEP | Visual Evoked Potential |
| VTA | Ventral Tegmental Area |
| WIC | Women, Infant, and Children Program |
| WISC-III | Wechsler Intelligence Scale for Children |

# CHAPTER 1

# INTRODUCTION

Misuse of prescription opiates such as Oxycontin, Percocet, and Vicodin has increased in the past decade and is alarming because a high percentage of the opiate dependent population consists of women of childbearing age (i.e., 18 to 34 years old) which has resulted in a sharp increase in the number of newborns with prenatal opiate exposure (Office of National Drug Control Policy, 2011). These newborns are at high risk for developing neonatal abstinence syndrome (NAS), which is a marker for stress abstinence likely to surface within 24 to 72 hours postpartum (Jones, Kaltenbach, Heil, Stine, Coyle et al., 2010). Maine is identified as one of the states with the highest opiate addiction, according to a recent report by the Substance Abuse and Mental Health Services Administration (SAMHSA, 2012), thus making the prescription opiate abuse a major concern of the state of Maine. Our laboratory has investigated pregnancy and early development of methadone-exposed infants born in Maine by assessing cognition and learning (Logan, Hayes, Brown, Tisher, Paul, & Krishnan, 2009), maternal depression and infant temperament (Heller, 2012), variations in the degree of NAS in relation to maintenance therapy type (Pritham, Paul, & Hayes, 2012) and genetics (Wachman, Hayes, Brown, Paul, Harvey-Wilkes et al., 2013). This thesis study will investigate signs of potential neural circuitry deficits in utero by analyzing the sleep-wake continuity and arousal regulation of methadone-exposed neonates within the first 2 nights of life before NAS appears.

NAS is characterized by central nervous system (CNS) hyperirritability and dysfunction in the autonomic nervous system, gastrointestinal tract, and respiratory

system, and one major characteristics of this condition is sleep disruption due to hyperarousability (Finnegan & Kaltenbach, 1992). Proper sleep regulation is essential especially during the neonatal period when maximum neural proliferation or expansion of neural network occurs, particularly during rapid eye movement (REM) sleep (Mirmiran, Maas, & Ariagno, 2003). An infant's inability to maintain sleep quality has been associated with sudden infant death syndrome (SIDS) (Kinney, 2005; Sawaguchi, Franco, Kadhim, Mori, Ito, Taki, Sawaguchi, & Kahn, 2014).

SIDS has been associated with prenatal neural insult in sleep areas of the developing brain in infants who have had prenatal alcohol exposure (Kinney, 2005). Our laboratory has reported that prenatal exposure to alcohol was associated with increased sleep fragmentation, defined as increased arousals during sleepand also suppressed SM during sleep (Troese, Fukumizu, Sallinen, Gilles, Wellman, Paul, & Hayes, 2008). This pattern of sleep disturbance is associated with the development of sleep deprivation. In a study of premature infants who have a significantly higher than average risk of SIDS, our laboratory has found that arousals and awakenings eventually decrease with increasing days of sleep fragmentation, as would SMs (Hayes, Akilesh, Fukumizu, Gilles, Sallinen et al, 2007). Hence, SIDS risk groups such as premature births, and infants with prenatal exposure to alcohol were both associated with poor sleep quality, increases and eventual decreases in arousals and awakenings, and suppressed activation of SM. This thesis study is designed to examine another prenatal exposure, the synthetic opioid methadone, which has similarly been associated with increased SIDS rates (Hunt, 2008). The thesis study will investigate the characteristics of sleep quality, sleep-related arousals, and SM

patterning in neonates who have been prenatally exposed to the opioid methadone taken daily by the mother for opioid dependence.

### 1.1. Neuropharmacology of Opioid Addiction

The next section will examine the fundamentals of opioid addiction and dependence to provide background for neonatal dependence in NAS. Koob (2008) describes drug addiction as a disorder governed by motivation, which is modulated by the reward circuitry of the brain. This process begins with elevated motivation to experience the hedonic or rewarding influence of the drug (impulsivity) followed over time by the desire to avoid the anhedonia (i.e., lack of pleasure) and unpleasant consequences of withdrawal (compulsivity). According to Koob, common motivation-driven cyclical characteristics of addiction include craving, tolerance, dependence, withdrawal, and relapse; and these processes are mediated by the mesoaccumbens dopamine (DA) system, in which mesolimbic DA is projected from the ventral tegmental area (VTA) primarily to the nucleus accumbens (NAc), as well as the medial prefrontal cortex (mPFC), amygdala, and ventral pallidum. Repeated exposure to drugs eventually alters this neurocircuitry causing both synaptic-level and systems-level plasticity.

Neuroadaptation is the transformation of reward to a habitual state from drug related activation of this system. In this thesis, it is proposed that early developmental changes in drug-related reward circuitry might reduce children's reward level by setting a higher bar for eliciting a hedonic effect. This change results in the activation of aversive, or anti-reward, circuitry (Koob, 2008). Koob's theory of the underlying mechanism of addiction indicates that withdrawal symptoms frequently occur as an aftermath of drug dependence. Among drugs with high potency for dependence, are opiates.

Opioid prescription medications leading to dependence and methadone replacement therapy is the background of mothers in the current study in which the developing fetus is exposed to opioids through maternal use during pregnancy. Maternal opioid medications, such as methadone, affect the developing fetus through placental transfer and membrane changes that improve opioid absorption during the last trimester. Fetuses exposed to opioids after thirty-four weeks gestational age are more likely to develop an abstinence syndrome than those born earlier (Barr, McPhie-Lalmansingh, Perez, & Riley, 2011). In infants, withdrawal is a similar process as that observed in adults. Therefore, prenatal exposure to drugs of abuse followed by neonatal withdrawal may pose significant neuroadaptive consequences to the developing brain.

Opioids are psychoactive chemical substances that can exert analgesic and rewarding effects by binding to opiate receptors, which are distributed widely in different areas of the brain. In a recent review, Waldhoer, Bartlett, and Whistler (2004) reported that opioid receptors are primarily located in the central (CNS) and peripheral nervous system (PNS) as well as in the gastrointestinal tract (GI).

Four major types of opiate receptors have been identified: mu (MOR-$OP_3$), delta (DOR-$OP_1$), kappa (KOP-$OP_2$), and nociception receptors (NOP-$OP_4$), according to Stein, Schäfer, and Machelska (2003) as well as Fine (2004). These authors further specify the locations and respective functions of these receptors. The primary locations of DOR-$OP_1$ are the pontine nuclei, amygdala, olfactory bulbs, and deep cortex of the brain. This receptor has two subtypes – $delta_1$ and $delta_2$ – and is believed to be responsible for eliciting analgesia, antidepressant effects, convulsion effects, and physical dependence. It is also suspected to play a role in mu-opioid receptor-mediated respiratory depression or

hypoventilation. KOR-$OP_2$ is found in the hypothalamus, periaqueductal gray, and claustrum of the brain, and also in the substantia gelatonisa of the spinal cord. The primary functions of these receptors are analgesia, anticonvulsant effects, dissociative and deliriant effects, diuresis, dysphoria, miosis or pupil constriction, neuroprotection, and sedation. Three currently identified subtypes of MOR-$OP_3$ are $mu_1$, $mu_2$, and $mu_3$. These receptors are located in the areas of brain such as cortex, thalamus, striosomes, periaqueductal gray, and rostral ventromedial medulla, in the substantial gelatinosa of spinal cord, in the intestinal tract, and the peripheral sensory neurons. The primary functions of these receptors are subtype-specific such that $mu_1$ functions to elicit analgesia and physical dependence; $mu_2$, is responsible for respiratory depression, miosis, euphoria, reduced GI motility, and physical dependence; and $mu_3$ for vasodilation. The NOP-$OP_4$ receptors are located in the areas of brain namely cortex, amygdala, hippocampus, septal nuclei, habenula, and hypothalamus, and in the spinal cord.

These endogenous opioid systems are crucial in modulating the sensation of pain and pleasure (Fine, 2004). Prolonged, repeated exposure to narcotics derived from nature as well as synthetic opioids may compromise this system such that the body becomes sensitized to the effects of opioids and requires higher levels of opioids to elicit an adequate amount of effect. This could result in the development of opiate dependence. Therefore, the effect of synthetic opioids is associated with a rewarding, pain-free, or even pleasant experience (Fine, 2004; Stein et al., 2003; Waldhoer et al., 2004).

**1.1.1. Neonatal Outcomes of Prenatal Exposure to Opioids.** Prenatal exposure to substances of abuse such as opioids has been associated with less optimal neonatal outcomes. Opiate addiction is typically polydrug abuse where multiple drugs are used.

The most common co-occurring substances are alcohol, tobacco, and marijuana. The sociodemographic population at greatest risk for opiate addiction is more likely to use other drugs of abuse, especially tobacco, alcohol and marijuana (Hayes, Brown, Hofmaster, Davare, Parker, & Raczek, 2002). Patrick et al. (2012) report that infants with prenatal opioid exposure show significant likelihood for respiratory diagnoses, low birth weight, feeding difficulties and seizures. In a recent report, Negrato and Gomes (2013) found that low-birth weight was likely a consequence of intrauterine insults such as maternal substance abuse. Low-birth weight is associated with health complications such as breast and testicular cancer, osteoporosis, cardiac hypertonicity, lack of alertness, and instability of mood in adulthood. These findings in combination with the consistent prevalence of low-birth weight among opiate-exposed infants are some of the examples of concern that make the investigation of prenatal opiate exposure-related developmental outcomes crucial to the health of at-risk children.

Prenatal exposure to opiates, often in combination with cocaine, has been associated with early life neurodevelopmental adversities including infant mortality such as Sudden Infant Death Syndrome or SIDS (Burns, Conroy, & Mattick, 2010). Conradt, Sheinkopf, Lester, Tronick, LaGasse et al. (2013) found prenatal opioid exposure to be associated with the highest level of heart rates and the lowest level of respiratory sinus arrhythmia (RSA) which indicate suboptimal autonomic functioning. Janssen, DiPietro, Elko, Williams, Milio, and Velez (2012) have studied RSA during fetal life. RSA is a variation in heart rate that occurs naturally during a breathing cycle such that heart rate increases during inhalation and slows down during exhalation. Such a pattern of heart rate is termed heart rate variability or HRV. In the same study, the opiate-exposed fetuses post

birth showed more non-optimal reflexes and stress/abstinence signs, poorer quality of movements and increased hypertonicity during a sustained visual task using the NICU Intensive Care Unit Neurobehavioral Scale (NNNS; Lester, 2004). These results indicate that autonomic nervous system (ANS) dysfunction is of concern in infants born with prenatal exposure to opiates and cocaine, and the developmental outcomes of these infants in early life is neurobehaviorally poorer than comparison samples.

**1.1.2. Treatment of Opioid Addiction During Pregnancy**. A current standard of care for treating opiate dependence during pregnancy is the narcotic treatment program (NTP). Clinically controlled substitutes such as buprenorphine or methadone are used to prevent opioid drug seeking and to promote harm reduction during pregnancy (Patrick et al., 2012). For this thesis, all mothers in the methadone-exposed group were enrolled in the NTP and maintained on methadone.

Lugo, Satterfield, and Kern (2005) state that methadone is a lipophilic, synthetic opioid that is a $\mu$-opioid receptor agonist, a weak NMDA agonist, and inhibitor of the production of cyclic adenosine monophosphate (cAMP) at the cellular level. Methadone accumulates at tissue binding sites, and is released into plasma during redistribution. This contributes to a significantly longer half-life (24 hours) or duration of influence compared to its counterparts in addition to being cost effective, which makes it an ideal substance for opiate replacement therapy (Jones et al., 2010). Methadone is valued for its qualities to exhaust cravings for opiates for a relatively longer period of time, stop physiological withdrawal symptoms, and prevent the euphoric effect associated with illicit opioid abuse (Kreek, 2000; Ward, Drover, Hammer, Stemland, Kern et al.,2014). Methadone is also considered safer, as proposed by Jones, O'Grady, Malfi, and Tuten (2008), for pregnant

opiate-dependent women and their fetus in comparison to continued prescription painkiller or heroin abuse because the treatment provides them with consistency in daily opiate doses as opposed to irregular binge and withdrawal cycles that are characteristics of street narcotic abuse. Another advantage of being in treatment, as mentioned by Jones et al., is that the use of other substances of abuse is monitored through random drug screens that occur at least monthly. The pharmacological testing performed by Affiliated Laboratories, Inc., of Bangor, Maine included urinary drug screens (UDS) for benzodiazepines, cocaine, methylphenidate, cocaine metabolites, cannabinoids, lysergic acid diethylamide, buprenorphine (subutex), methadone and methadone metabolites, oxycodone, barbiturates, cotinine, and other drugs during pregnancy and postnatally for the methadone-exposed group mother of the present study. Also, screening for alcohol was done by using breathalyzers at the time of UDS. However, it is not always possible to trace in-utero alcohol exposure through theUDS alone. Therefore, an exposure to alcohol was also ascertained through careful clinical interview with standard methods that were included in this thesis.

Nanovskaya, Nekhayeva, Hankins, and Ahmed (2008) report that the placenta is permeable to maternal use or consumption of substances such as alcohol, tobacco, and opioids and that the rate of fetal exposure to opioids is the highest during the 3$^{rd}$ trimester when the placental barrier becomes highly permeable, thus, increasing the concentration of penetrated substances in the fetal environment. The underlying mechanism of this increased permeability during the 3$^{rd}$ trimester is purported to result from the readiness of the fetus to absorb as many nutrients as possible from maternal blood in order to ramp up the growth process in preparation for the upcoming birth. On the other hand, the same

mechanism also may cause the maternal dose of methadone to permeate the placental barrier more readily, and this may result in the fetus becoming an additional consumer of the maternal dose, which may cause an earlier-than-usual experience of withdrawal in mothers. Therefore, Logan, Brown, and Hayes (2013) suggest a careful maternal dose management to be recommended to the methadone maintained mothers especially during the 3rd trimester of gestation by following the most practiced paradigm (i.e., split-dose as opposed to once-a-day dose) in order to increase methadone half-life and avoid potential repeated fetal withdrawal cycles that may cause severe neurobehavioral consequences including fetal mortality. Besides all the advantages of methadone treatment for opiate dependence during pregnancy, effects of prenatal exposure to methadone on the development of humans from fetal stage throughout the lifespan are relatively unknown. The following section presents some of the neurodevelopmental outcomes of prenatal exposure to methadone.

**1.1.3. Neurodevelopmental Consequences of Methadone Exposure.** In order to fully comprehend the actual effect of methadone on neonates, taking into consideration the outcomes of both animal and human studies are equally importance because while animal studies ideally provide an insight into specific biobehavioral effects of methadone exposure, human studies present an opportunity to investigate those isolated effects in association with environmental factors to broaden our understanding of the implication of prenatal methadone exposure. Therefore, this thesis study attempted to look into potential neurodevelopmental impact of methadone exposure by examining the sleep state organization of the exposed neonates.

Due to the complexity of human substance exposure in-utero resulting from a highly frequent trend of poly-drug abuse among opiate-addicted pregnant women, it is not easy to replicate an animal model of methadone exposure, and the results may face translational controversies. However, carefully designed studies such as the one by Hutchings, Zmitrovich, Church, and Malowany (1992) using mouse pups showed dose-dependent neurobehavioral consequences of methadone exposure in those mouse- pups at 22 days of age where high dose pups demonstrated less restfulness, disrupted rhythmicity, and poor state regulation as shown by increased state lability indicating dose-specific neurodevelopmental abnormalities in comparison to low-dose methadone and non-methadone pups.

A classic study by Guo, Enters, McDowell, and Robinson (1990) primarily investigated the effect of methadone on the neurotransmitters cholinergic, noradrenergic, dopaminergic, and serotonergic neurons, which are crucial for optimal psychophysiological regulation in vertebrates. An osmotic minipump was used to administer maternal methadone. The rats were impregnated naturally by placing male and female rats together in the same cages. On the $8^{th}$ day of gestation, two groups of pregnant rats were subcutaneously administered either methadone or sterile water. The methadone-exposed pups were postnatally injected with naloxone in order to confirm methadone dependence. Then 4-day old pups were sacrificed and analyzed for the neurotransmitters of interest. Results indicated sex-related differences in the brain region-specific neurotransmitter content. The most important finding of this study was the similar reduction of striatal acetylcholine content in both male and female pups suggesting that prenatal methadone exposure may impair the stress regulatory system in

infants of both genders equally. These researchers further demonstrated the effect of prenatal methadone exposure on central cholingergic neuronal activity, delays in the development and expression of striatal cholinergic neurons, and similar effects also on the dopamine, norepinephrine, and serotonin systems.

Robinson and Kolb (1997) expanded their approach to examine the impact of perinatal exposure to methadone on neurotransmitters more closely by using ratio of neurotransmitters to their corresponding metabolites during both perinatal and postnatal exposure to methadone. Pregnant rats were implanted with osmotic minipumps containing either methadone hydrochloride (initial dose, 9 mg/kg/day) or sterile water on day 7 of gestation. Their offspring were cross-fostered so that they were exposed to methadone prenatally and/or postnatally. Then those pups were analyzed for dopamine (DA), norepinephrine (NE), serotonin (5-HT), and their metabolites in relation to their metabolites (i.e., 3,4-dihydroxyphenylacetic acid (DOPAC) for DA; 3-methoxy-4-hydroxyphenylglycol (MOPEG) for NE; and 5-hydroxyindoleacetic acid (5-HIAA) for 5-HT) on day 21. The results indicated gender specific differences in the disruptions of the dopaminergic, noradrenergic, and serotonergic activity in a brain region. Males exposed to methadone postnatally showed reduction in the ratio of the DA metabolite DOPAC to DA in the frontal cortex, and this effect was not observed in the striatum. The ratio of MOPEG to NE in the hippocampus was increased significantly in males exposed to methadone prenatally. A slight increase in the striatal and parietal cortical 5-HT metabolite 5-HIAA was observed in rats exposed to methadone postnatally but there was no interaction between the ratios of 5-HT to its metabolite. The authors concluded that the effects of methadone exposure might be dependent on the developmental stage at

which exposure occurred and not so much on the neonatal withdrawal per se, and that changes in activity of these three neurotransmitter systems may contribute to the effect of perinatal methadone on the activity of other neurons.

Putting their findings into perspective, Robinson (2000) consolidated a review of contemporary animal models of opioid exposure including methadone and reported that even though opiate replacements such as buprenorphine or methadone are considered non-teratogenic (i.e., not harmful to fetus) and preventative of repeated cycles of in utero withdrawals, there is consistency in the finding of disruption of cholinergic system development, particularly in the striatum of infant animals prenatally exposed to opioids. Such effect has been deemed surpassable with an increase in postnatal cholinergic neuronal activity, but even though acetylcholine (ACh) content and the expression of choline acetyltransferase protein and mRNA are reduced in the early postnatal period by prenatal opioid exposure in the rats, the activity of the cholinergic neurons remains disrupted, with a large increase in ACh turnover rate suggesting that the chances of overcoming cholinergic system deficit is low. One potential mechanism involved in this phenomenon is related to atypical changes in the expression of nerve growth factor (NGF), a neurotrophin known to influence the development of cholinergic neurons, which has been found to be reduced by opioid exposure (Wu, Hung, Shen, Chen, Chang et al., 2014). It is proposed that similar mechanisms are at work in methadone-exposed infants leading to abnormal cell growth and migration, increasing the risk for neurodevelopmental deficits.

Unlike animal models, human exposure to maternal use of methadone is much more complicated due to the consistently documented issue of co-occurring multidrug use

in addition to clinical methadone. Therefore, the study of the nature of prenatal exposure to substances of abuse must rely on urinalysis screens that are merely a snapshot of recent exposure for most substances, with the exception of cannabis and opioids, which can persist for several weeks after ingestion (Katz & Fanciullo, 2002) Methadone dose and dose change throughout pregnancy is determined through the medical records but additional use is documented through random, monthly urinalysis. Since alcohol exposure related to maternal use is known as a profound teratogen (Riley, Infante, & Warren, 2011), determining dosage and timing of exposure is particularly difficult. State of the art method in this area relies on maternal self-report using well-established alcohol use instruments.

Functional consequences of prenatal exposure to opioids is evaluated through neonatal neurological (imaging and motor testing), neurobehavioral [NICU Network Neurobehavioral Scales (NNNS)] and neurocognitive (EEG, and event related potential or ERP) assessments, and longitudinal postnatal behavioral observations to investigate the effect of methadone, and such methods bring along controversies regarding potential confounding variables. With an increase in the NAS incidence in the past decade, there is a concern that prenatal methadone exposure may increase NAS severity (Jones et al., 2010) and may compromise both pre and postnatal development, the latter primarily related to CNS consequences of prolonged withdrawal, and iatrogenic effects of replacement opioid medications.

Fetal neurobehavior can be viewed as an early marker of the developing nervous system, and any in utero insult such as opioid exposure to this system may represent a threat to the neural development of the fetus (Nijhuis, 2003). Even though opiate

replacement therapy is a safer substitute for narcotic addiction, controversies about whether they are safe for fetal neurodevelopment continue. Therefore in an attempt to investigate the nature of impact buprenorphine or methadone may have on fetal neurobehavior, Janssen et al. (2011) recorded fetal heart rate (FHR) and fetal movement of 17 opioid-dependent pregnant women maintained on replacement therapy with either buprenorphine or methadone. This demographically similar sample was drawn from the Johns Hopkins Bayview Medical Center-based multidisciplinary treatment care facility called the Center for Addiction and Pregnancy. Data were recorded at different time points (i.e., 24, 28, 32, and 36 weeks) of gestation by using ultrasound for heart rate and actigraphy for movement. The results showed that acceleration of fetal heart rate (a measure to assess HR variability) during 24 and 28 weeks of gestation was significantly greater for fetuses in the buprenorphine group with no acceleration at all for the methadone-exposed group during the recording period. Additionally, the methadone-exposed group showed significantly suppressed fetal movement in comparison to the buprenorphine group suggesting that methadone may have adverse neurobehavioral consequences in growing fetuses.

In another study, Coyle, Salisbury, Lester, Jones, Lin, Graf-Rohrmeister, and Fischer (2012) assessed the effect of methadone versus buprenorphine exposure on neonatal neurobehavior on 39 full-term infants at 3, 5, 7, 10, 14-15 and 28-30 postpartum days by using the Neonatal Intensive Care Unit (NICU) Network Neurobehavioral Scale (NNNS), a standard measure of infant neurobehavioral development. The results revealed in favor of buprenorphine with buprenorphine-exposed infants showing significantly fewer stress-abstinence signs, less hypertonia, better self-regulation, less excitability, less

over-arousal, and less handling need to maintain a quiet alert state in comparison to prenatally methadone-exposed infants (Coyle et al., 2012). Additionally, the infants who began NAS treatment at an older age demonstrated significantly higher self-regulation scores, and the least amount of average excitability and hypertonia. The results suggest that methadone may be less optimal for neonatal neurobehavioral development.

In the post-neonatal period, prenatal opiate exposure seems to pose a threat to neuronal growth as demonstrated by Hu, Sheng, Lokensgard, and Peterson (2002), who report that prenatal opiate exposure is associated with increased programmed cell death (i.e., apoptosis) in human microglia and neurons. Neuronal apoptosis is known to be important for the learning process and it functions by allowing for unused neurons to die so that active neurons get proper environment to migrate to specific locations according to the type of learning that has occurred. However, excessive increase of neuronal apoptosis could indicate deficits in the brain white matter that may interfere with appropriate learning processes, resulting in cognitive and behavioral dysfunctions. Expanding this finding, Walhovd, Watts, Amlien, Woodward, and Woodward (2012) examined the early cerebral connective tract development of infants born to methadone-maintained mothers and comparison infants by using diffuser tensor imaging with an assumption that the methadone-exposed infants (n=15) show signs of lower neural tract maturation than comparison infants (n=7). The outcome variable was determined based on the mean diffusivity where higher diffusivity was marked as a sign of less maturation and myelination. All infants were scanned within 13 to 44 days post-birth by using a General Electric Signa HDx 3T MR scanner, and all scanned sets were checked for motion artifacts (i.e., EEG data resulted from unwanted movements during scanning), and

the researchers analyzed the voxel-based statistical analysis of the mean diffusivity by using the tract-based spatial statistics and permutation based statistics (Walhovd et al., 2012). Diffusion tensor MRI (DT-MRI) is a non-invasive method to map the characteristics of soft tissue in which the MRI or magnetic resonance imaging signal to the random molecular motion of water molecules (diffusion) is sensitized by adding 'diffusion encoding gradients' to a standard MR pulse sequence (Jones & Leemans, 2011). The corpus callosum as well as eight major white matter areas was defined as areas of interest, and inferior longitudinal fasciculus and superior longitudinal fasciculus were selected as tracts of interest for analyzing the outcome difference. In order to match the hypothesis of this study, higher level of diffusivity was expected in infants exposed to methadone because high diffusivity has been associated with a sharp decrease in the brain maturation as well as demyelination or decrease in myelination in both human and animal studies. Supporting their hypothesis, the results of this study demonstrated that the methadone-exposed infants tended to have higher diffusivity of the brain neural tracts than the comparison group. These results were similar to many other human and animal studies indicating delay or deficit in brain maturational process as a result of prenatal insult, particularly opiate exposure as discussed in the article. The significance of this study relies on identifying early markers of neural alterations that may be predictive of cognitive and behavioral problems observed in existing studies pertaining to prenatal opioid exposure (Walhoved et al., 2012).

Children with prenatal exposure to substances also tend to have difficulties with attention in the later years. To investigate early detection of attention problems in methadone-exposed infants, a study by Paul, Logan, Krishnan, Heller, Morrison et al.,

(2014) from our lab used a subset of a sample from an ongoing longitudinal study to assess infants' habituation to auditory stimuli within 4-15, 16-32, and 33-120 days of postnatal age. Habituation to novel stimuli is one of the markers for cognitive development as it indicates an infant's efficiency for learning new information, and is measured by using the auditory oddball paradigm [a method used in electrocephalogram (EEG) that involves the presentation of frequent auditory stimuli with a randomly distributed novel high-pitched auditory stimulus presented occasionally throughout the testing period to examine variability in the brain waveforms in response to frequent and random stimuli] with primary focus on the amplitude and latency of peak-2 (P2) in response to novel stimuli. Results revealed that there were no group differences in the P2 amplitude in the older groups as opposed to younger groups, indicating that postnatal environmental buffers may moderate possible adverse consequences of methadone in the long run, and the group difference observed during the youngest age range could have resulted from the neonatal abstinence syndrome (NAS).

Another major pediatric health concern associated with prenatal exposure to methadone is impairment of visual development. Hamilton, McGlone, MacKinnon, Russell, Bradnam, and Mactier (2010) reported case studies of 20 of their pediatric patients who were exposed to methadone in utero and referred to them for visual anomaly. The authors used the measure of ophthalmic and orthoptic examination and visual electrophysiology as seemed appropriate on an individual basis. Additionally, the children's case history and maternal urine toxicology were obtained for cross analysis. The results revealed ophthalmic abnormalities including reduction in acuity (95%), nystagmus (70%), delayed visual maturation (50%), strabismus (30%), refractive errors

(30%), and cerebral visual impairment (25%) (Hamilton et al., 2010). Of the 20 patients, 60% had abnormal visual electrophysiology and one-fourth of the children showed neurodevelopmental abnormalities associated with the visual anomaly. Most importantly, 79% of the nystagmus cases were treated for neonatal abstinence syndrome (NAS). These results indicate that infants with in utero exposure to methadone are at high risk for visual abnormalities, and NAS-treated babies are potential targets for nystagmus.

McGlone, Hamilton, McCulloch, Boulton, Bradnam, Weaver, and Mactier (2013) expanded on the visual development of methadone-exposed infants in their study by using flash visual evoked potentials (VEPs), an EEG method that elicits brain waveforms upon the presence of flashed visual stimuli. The researchers recorded flash VEPs from infants within 3 days of birth from 100 infants prenatally exposed to methadone, and 50 demographically matched comparison infants. The classification of VEP morphology consisted of mature, typical, or immature, which were categorized based on the amplitudes and implicit times of the major waveform components. Maternal history, maternal and infant urine, and meconium toxicology were used to determine drug exposure. The results revealed that maternal methadone-exposed infants were more likely to form immature waveform and the peaks of such waveforms were smaller in overall amplitude. More importantly, this effect persisted even after controlling for birth weight, cigarette smoking, and excess in utero alcohol exposure indicating that methadone exposure may affect visual anomaly in children and may need earliest possible attention to their visual health. All these short- and long-term postnatal outcomes aside, the biggest concern considering prenatal methadone exposure is NAS, which is experienced by many methadone-exposed neonates.

**1.1.4. Neonatal Abstinence Syndrome (NAS).** One persistent consequence of prenatal methadone exposure is NAS, which surfaces within 24-72 hours post birth (Finnegan et al., 1991). NAS is a constellation of stress abstinence symptoms resulting from a physiological dependence to maternal use of opiates. In the clinical protocol described in a report to The Committee on Drugs and The Committee on the Fetus and Newborn by Hudak, Tan, Frattarelli, Galinkin, Green et al. (2012), NAS is characterized to have symptoms of tremors, irritability, sleeping problems, high-pitched crying, hypertonicity, poor feeding, stuffy nose, sneezing, yawning, gastro-intestinal dysregulation, dehydration, sweating and seizures, which together are indicators of CNS immaturity. NAS also poses a risk of neuromaturational deficits as manifested in the form of seizures, poor weight gain, dehydration and general dysregulation because of its long course of three to seven weeks with residual symptoms for six months or longer (Hayes & Brown, 2012). About 50% to 70% of these infants need pharmacological intervention (i.e., clonidine and/or phenobarbital) based on the severity of symptoms determined by the Finnegan Neonatal Abstinence Severity Index (Janssen et al., 2013). However, comparatively milder abstinence syndromes not requiring replacement medications have been reported with prenatal exposure to cocaine, nicotine, sedatives, barbiturates, SSRIs, etc. during pregnancy (reviewed in Janssen et al., 2012).

Methadone-exposed infants tend to show variability in the profile of NAS - some exposed neonates go home in 5 days whereas many stay for longer than 3 weeks (Jones et al., 2010). In the clinical research, therefore, the length of hospital stay (LOS) is used as a benchmark to assess the severity of NAS (Patrick et al., 2012). A few known causes of such variability appear to be maternal methadone dose and gestational exposure to

benzodiazepines (Pritham, Paul, & Hayes, 2012); higher overall drug dose before delivery and gestational age (i.e., shorter gestation = shorter LOS) (Liu, Jones, Murray, COOK, & Nanan, 2010); and allelic variants in the *mu* opioid receptor type 1 (*OPRM1*) and catechol-O-methyl-transferase (*COMT*) genes (Wachman et al., 2013). In the genetic study by Wachman et al. (2013), single nucleotide polymorphism (SNP) in the *OPRM1* and *COMT* genes predicted NAS severity where AA genotypic variation in both genes were associated with a higher severity of NAS and AG or GG genotypes served as protective factors. Revisiting Koob's theory of tolerance, tolerance results from an enhanced physical ability to intake a substance without having withdrawal symptoms, thus, reinforcing the reward system of the substance user to use more, which is a risk factor for addiction. If that is true, then perhaps the neonates with the minor allelic variants of the *COMT* SNP might be at risk for addiction due to enhanced tolerance to opiates.

Taken together, it may be speculated that deficits in the CNS and ANS maturational process associated with prenatal opiate and potentially comorbid substance exposure. One way to investigate this possibility is by assessing sleep organization during the neonatal period. The association of prenatal substance exposure to sleep, wake, and arousal deficits has been established through previous studies in our lab in which perinatal alcohol appeared to mediate the effect of methylxanthine on arousal regulation of preterm apneic neonates (Hayes et al., 2007).

### 1.2. Ontogenic and Biological Bases of Sleep and Wake

Sleep is a ubiquitous phenomenon among vertebrates. The duration and intensity of sleep are regulated by the interaction of homeostatic processes, in which the requirement

for sleep builds during waking and is relieved by sleep, and circadian rhythm. According to Stern, Parmalese, Akiyama, Schultz, & Wenner, (1969), this rhythm determines the timing of the sleep/wake cycle according to an internal signaling system such as the suprachiasmatic biological clock and external signals such as the light-dark cycle. Circadian rhythm follows a diurnal sleep-wake pattern with 24-hour periodicity and this periodicity is characterized by sustained wakefulness during the daylight and sustained nocturnal sleep. Most adults' sleep cycles follow this pattern and the onset and offset of the sleep cycle is determined by an individual's biological clock. For example, de Benedictis, Larson, Kemp, Barston, and Segal (2007) suggest that individuals who are prone to waking up early in the morning are likely to continue waking up at the same time every day even if slightly sleep deprived. In mammals, sleep/wake regulation is conducted in the basal forebrain, which is located adjacent to preoptic and supraoptic levels of the hypothalamus and includes cholinergic cells as well as glutamatergic and GABAergic neurons. Despite an ongoing debate on the optimal amount of sleep for an adult, a common understanding is that a healthy adult sleeps about 7-8 hours a night for optimal functioning during the day. However, multiple factors may play a role in the timing, duration, and rhythmicity of sleep. The typical nocturnal sleep cycle in adults consists of the oscillation of Rapid Eye Movement (REM) and Non-rapid Eye Movement (NREM) sleeps, which generally repeat every 90 minutes (Roehrs, Carskadon, Dement, & Roth, 2005). In an overview of the neurobiology of REM and NREM sleep, McCarly (2007) characterizes REM sleep or paradoxical sleep as the stage when low amplitude, high frequency EEG activity is present, muscle tone is extremely suppressed, and the rapid ocular saccades occur. Most neuronal activity and dreaming are believed to occur

during this stage. McCarly further describes NREM sleep to have specific EEG oscillation called spindles (waxing and waning within the 12-15Hz sigma band frequency range that lasts at least 0.5 seconds), delta wave (1-3Hz), and slow rhythms. In humans, NREM sleep stage undergoes three different stages. The first stage (N1), also called drowsy sleep, is characterized by oscillation of EEG activity from alpha waves having frequency of 8-13Hz to theta waves of 4-7Hz, some muscle activity, and the slow rolling of eyes with opening and closing moderately. Consciousness of the environment starts to deplete. This stage typically lasts for about 1-7 minutes. The second stage (N2) is characterized by the appearance of sleep spindles and K-complexes ranging from 12-14Hz EEG wave frequency and lasts for 10-25 minutes. K-complexes are the largest EEG frequency waves that consist of a large positive peak followed by another large negative peak. Metcalf, Mondale, & Butler (1971) mention that these peaks do not appear in infants until the age of 6 months. McCarly further describes that muscular activity decreases even more at this stage of NREM sleep and consciousness of the environment disappears completely. About 45-55% of adult sleep comprises this stage. The N2 gradually develops into the last stage (N3) of NREM sleep. This stage is called deep sleep or slow-wave-sleep (SWS), which is characterized by 20-50% presence of the EEG delta frequency waves of 0-4Hz and lasts for 20 to 40 minutes in the first cycle. In this stage, the sleeper does not respond to many environmental stimuli. A typical adult sleep pattern follows four or five cycles of N1 – N2 – N3 – N2 – REM per night. The majority (~80%) of the first half of the nocturnal sleep cycle comprises NREM sleep and the most of the second half is spent on REM sleep.

Unlike adults, the sleep-wake cycles of neonates and infants are much shorter, and a newborn tends to awake every 3-4 hours for feeding and physiological needs. This pattern continues until the first few months with the majority of their time (12-18 hours a day) spent asleep, and about two-thirds of this sleep time is spent on active or REM sleep (Roehrs, et al., 2005). In a clinical review of fetal and neonatal sleep and circadian rhythm, Mirmiran et al. (2003) propose that the origin of sleep and circadian rhythm may develop during the last 10 weeks of gestation, and the sleep-wake pattern during the fetal period is comparable to preterm infants at 30-40 post-conceptional ages. Major characteristics of infant sleep include Active Sleep (AS) that is equivalent to REM sleep in adults, Quiet Sleep (QS) similar to adult's NREM, and indeterminate sleep (IS) that has the elements of both AS and QS. Mirmiran et al. (2003) report in a review that AS and QS could be differentiated as early as 32 weeks of gestational age even though the majority of sleep cycle during this time is spent on IS. Scher, Johnson, and Davis (2005) expanded this hypothesis through an EEG sleep study on a subset of 33 preterm infants at 25-30 weeks post-conceptional ages by recording over 2 to 3 hours of the sleep period. The results produced by Polysomnography data revealed that the majority of these neonates showed a sleep cyclic pattern of 68 $\pm$ 19 minutes per bout, and the variability ranged from 37-100 minutes per bout of sleep. Another noteworthy finding of this study included individual variability in the cycle length but not in the state cyclicity, indicating that onset of sleep cycle development is potentially in progress as early as 25 weeks of gestation and the determining factor of the quality of sleep could be individually different. Despite all the possible individual variability, it has been well understood that continuous maintenance of sleep and circadian rhythm is essential for optimal daily

functioning and prolonged deprivation of sleep caused by sleep disorders or some biological condition has been known to have a cumulative effect on the psychobiology of an individual.

**1.2.1. Sleep Deprivation and Consequences**. Although the neurobiological phenomenon of sleep has been studied through research using both humans and animals, most of the theories of sleep biology are derived from animal studies, and the assumptions pertaining to sleep-related brain plasticity have been formed based on the deprivation model, which is measured through behavioral observation as well as histology (Hayes et al., 2002). The deprivation model refers to the method that assumes an adverse outcome when the outcome is preceded by the deprivation of stimulus. In sleep related brain development, for example, research primarily focuses on depriving subjects of certain stages of sleep (e.g., REM) by presenting external stimuli (e.g., visual, auditory, physical), and compares its impact on the relative brain region between deprived and non-deprived subjects.

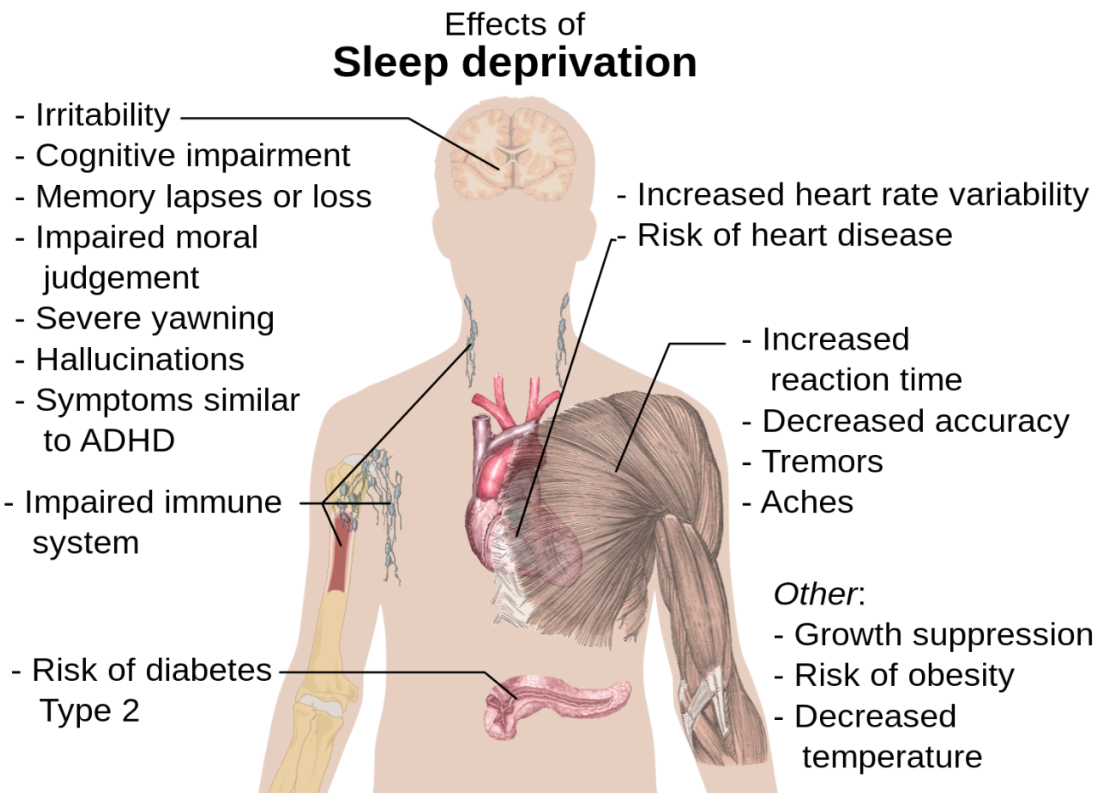


Figure 1.1. Potential adverse psychobiological effects of sleep deprivation.

http://en.wikipedia.org/wiki/Sleep

Orzeł-Gryglewska (2010) suggests that prolonged sleeplessness can result in impairment of perception, lack of concentration, difficulty with vision, decrease in reaction time, diminished schematic thinking that is responsible for decisions-making, and problems with optimal emotion regulation that can manifest as deteriorated interpersonal responses and increased aggressiveness. Sleep deprivation was also associated with the presence of micro-episodes of sleep during wakefulness that lead to lower efficiency of task performance and increased number of performance errors, as well as poor memorizing. These findings indicate that sleep deprivation may alter brain circuitry involving cognitive, emotional, and physiological functioning, thus placing an

individual with sleep deprivation at risk for adversities including a threat to personal well-being (e.g., automobile accidents, aggressive outburst).

In infants, the consequences of sleep deprivation are even greater because sleep deprivation has been found to lead to longer-than-usual duration of sleep and decreased latency to REM sleep (Franco, Seret, Van Hees, Scaillet, Vermeulen et al., 2004) as well as decreased sleep-related arousal that is believed to be a 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 consequent arousal deficits, Franco et al. (2004) induced sleep deprivation and arousals on fourteen healthy infants of age 6–18 weeks arousals in a sleep laboratory. These infants underwent polygraphic recording during a morning nap and an afternoon nap, and were sleep-deprived for 2 hours before being allowed to fall asleep. Sleep deprivation was achieved by keeping the infants awake for as long as possible beyond their habitual bedtimes. The results revealed that most sleep characteristics were similar for the normal and sleep-deprived conditions, except that the duration of the naps increased, whereas the latency of REM sleep and the density of body movements decreased. During such sleep, these infants needed more-intense auditory stimuli for arousal compared with normal nap sleep. Additionally, sleep deprivation was associated with a significant increase in the frequency of obstructive sleep apnea episodes, especially during REM sleep, which is another risk factor for SIDS (Hunt & Hauck, 2006).

In order to determine the characteristics of arousal from sleep of infants who eventually died of SIDS, Kato et al. (2003) monitored sixteen infants some days or weeks before they succumbed to SIDS. The comparison of polygraphic sleep recordings of those

infants with matched control infants showed that the cortical arousals in SIDS infants were significantly less frequent than in the control infants during both REM and non-REM sleep ($p = 0.039$) in both frequency ($p = 0.017$) and duration ($p = 0.005$) measures. Interestingly, the infants who later died of SIDS had more frequent cortical activation between 9:00 P.M. and 12:00 A.M. ($p = 0.038$), and fewer cortical arousals between 3:00 and 6:00 A.M. ($p = 0.011$). The results suggest that a deficient arousal processes in infants may pose a risk of SIDS in infants.

The interaction among sleep deprivation, arousals, and SIDS appears rather complex though because 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 (Hayes et al., 2007, 2008). Therefore based on the 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. Therefore it is important to understand the underlying mechanisms as well as the risk factors associated with these sleep components.

**1.2.2. Arousals and SM in Sleep.** Sleep state organization is 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 criteria have been controversial due to the lack of standard operational definition that can be applied across studies. 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 are present. Arousal in general is also defined as a physiological and psychological state of being awake or reactive to stimuli, which is achieved through a symphonic process of the reticular activating system in the brain stem, the autonomic nervous system, and the endocrine system stimulation leading to increases in blood pressure and heart rate that trigger sensory alertness, mobility, and readiness to respond (Robinson, 2000). A locomotive feature called SM during sleep is another measure to assess arousal quality during sleep in neonates. It is a sleep state dependent movement pattern predicted to occur periodically in a 3-5 minute window. SMs, as adapted from Prechtl (1990), is defined as writhing bodily movements, characterized according to amplitude and speed, which may be small-to-moderate or slow-to-fast, extension of the extremities (particularly the arms), and exhibiting an elliptical motion, making such movements similar in writhing quality. SMs have been believed to be a "primitive" physiological arousal system that offers autonomic protection in sleep during the neonatal and early infancy period when arousal systems are immature (Hayes et al., 1993). SMs, thus, serve a potential primitive and protective arousal regulatory mechanism and their effect is to restore airway openness and to up-regulate cardiorespiratory cycles of the medullary circuitry (Hayes et al., 2007). Franco, Kato, Richardson, Yang, Montemitro et al. (2010) state that the primary function of arousal from sleep is to allow sleep to continue in the face of endogenous or exogenous stimuli that normally elicit responses during wakefulness and also permit awakening. Thus, arousal bears an adaptive mechanism.

**1.2.3. Substance Exposure, Sleep, and Arousal System.** Abnormal sleep architecture including sleep apnea is common in substance dependence and withdrawal.

The medullary circuitry modulates autonomic tone, sleep, and arousal systems and develops quickly in the first few months of postnatal life. However, infants exposed to high levels of neurotoxic substances, including opiates, alcohol, and tobacco, often exhibit neural circuitry abnormalities particularly in the brainstem region (Kinney, 2005). As infant gestation advances, an emerging coupling mechanism of cardiac and somatic functioning becomes increasingly prominent and is considered a function of innervation of the autonomic nervous system (DiPietro, 2001). Reduced baseline heart rate and depressed respiration have been found in methadone-exposed infants compared to non-exposed neonates along with significant differences in fetal cardiac-somatic coupling (movement-heart rate), fetal motility, and the variability of heart rate patterns in methadone-exposed neonates (Janssen et al., 2009), which suggest that disparities in fetal neurobehavior are likely to cause postnatal autonomic dysregulation.

Endogenous opiates are known to modulate autonomic regulatory function, particularly respiration during sleep, and decrease arousability in adults (Kruger, 1995). In infants, of the many psychophysiological symptoms of neonatal withdrawal, pediatric sleep dysregulation appears to be a common concern, which may potentially surface due to abnormalities in the serotonergic system (5-HT) of the medulla oblongata (Harper, Kinney, Fleming, & Thach, 2000). Medulla oblongata is a brainstem region that is primarily responsible for modulation of cardio-respiration and arousals under homeostatic stress or the physiological challenges presented to maintain homeostasis similar to infants exposed to high levels of neurotoxic substances, including opiates, alcohol, and tobacco, who often exhibit circuitry abnormalities (Kinney et al., 2003; Kinney, 2005). Such a deficiency is believed to pose a risk for Sudden Infant Death Syndrome (SIDS)

particularly if infants are prenatally exposed to substances of abuse, according to Jones et al. (2010) who further emphasize that opiate-exposed infants are at even higher risk for SIDS compared to other conditions such as prematurity, alcohol and/or nicotine exposure, and sleep apnea.

While there has been general consensus on the potential sleep-related developmental risks associated with opioids, the effect of in-utero exposure to methadone as an exogenous opioid on sleep-related arousal regulatory systems has not been studied yet. However, in adults methadone users, the most commonly reported neurobehavioral problem associated with methadone use is insomnia or the lack of sleep; and in pregnant methadone users, this problem appears to exacerbate during the 3$^{rd}$ trimester when mothers are likely to increase methadone dose due to increased permeability of the placenta that makes the fetus an additional consumer of the maternal dose and causes the mother to withdraw before 24 hours (Nanovskaya, Nekhayeva, Hankins, & Ahmed, 2008). During this same period, fetuses are also supposed to be adapting to maternal sleep-wake patterns such that if the mother is suffering from insomnia, so is the fetus (Janssen et al., 2011), which could result in an abnormal sleep organization or sleep dysregulation in newborns (Symanski et al., 2002).

Sleep dysregulation has been associated with increased sleep fragmentation, which is characterized by increased frequency and duration of wakefulness following sleep onset and decreased active sleep (Troese et al., 2008), the phenomenon previously found by Hayes et al. (2007) to be responsible for sleep debt or sleep deprivation, and is likely to decrease the frequency and duration of arousal parameters following sleep onset indicating a deficient arousal regulatory system. Among these outcomes, for example,

prematurity seems to add to the degree of risk for sleep disorders as demonstrated in a study by Hayes et al. (2007) on apneic pre-terms who tended to exhibit poor cardiorespiratory integrity especially during periods of sleep, which indicates that prematurity could pose a cardiorespiratory challenge during sleep and impact the sleep architecture of such infants. Since prematurity is one of the neonatal outcomes associated with opioid exposure, this finding adds to the hypothesis of this thesis that perhaps an exposure to exogenous opiates such as methadone could result in similar sleep-related neurodevelopmental abnormalities in neonates as early as from birth.

Since the arousal system is emergent developmentally at birth, this thesis examined neonatal sleep architecture as early as first or second postnatal night to determine whether sleep and arousal features differ in neonates with prenatal opioid exposure (i.e., maternal methadone) compared to non-exposed, demographically similar infants. Quantitative deficits in arousals and wake state were hypothesized to reflect neurobehavioral instability in opioid-exposed infants in the pre-withdrawal period (Days 1 or 2 of life). Additionally, it was secondarily hypothesized that opioid-exposed infants may show more sleep fragmentation, e.g., shorter sleep periods as well as excessive arousability and irritability on day 2 than day 1 of life due to the development of opiate withdrawal. In addition, this study also explored the genetic factor that may contribute to the expression of behavioral states and SM.

### 1.2.4. Pharmacogenomics of Methadone in Sleep Wake and Arousal Regulation.

As mentioned earlier, methadone primarily works as a μ-opioid receptor (*OPRM1*) agonist meaning that it binds to and activates the *OPRM1* receptors. One of the many recurrently reported side effects of methadone treatment appears to be sleep

disturbance, particularly insomnia, which calls for a close investigation of the pharmacogenomics of such an association. A group of Taiwanese researchers (Wang, Tsou, Chen, Chen, Ho et al., 2012) found an association of SNP in the *OPRM1* gene to the change-in-libido and insomnia side effects sometimes ($p<0.009$). The insomnia finding was observed in methadone patients with the *OPRM1* polymorphisms who had a positive urine morphine test and who did not use benzodiazepine hypnotics indicating that SNPs in opiate receptor genes may predict disturbed sleep regulation in adults.

One of the adverse outcomes of prenatal exposure to opiates has been sleep disorder in pediatric populations and this association appears to be influenced by the severity of NAS (O'Brien & Jeffery, 2002). In a collaborative effort of Tufts Medical Center and Eastern Maine Medical Center, Wachman et al. (2013) along with our lab investigated the effects of single-nucleotide polymorphisms in the mu-opioid receptor type 1 (*OPRM1*) and catechol-o-methyltransferase (*COMT*) genes on NAS severity. Both of these genes have been linked to neural circuitry for addiction, including opiate and psychiatric risks. The dominant genetic model was used, which takes into account the homozygotes for the major allele (e.g., AA) in one group while the collapsed heterozygotes and homozygotes for the minor allele (e.g., AG/GG) in another group to compare group differences in outcome variables, which in this study, were length of hospital stay and need for NAS treatment in methadone-exposed neonates. The results revealed that the infants with the *OPRM1* 118A>G AG/GG and the *COMT* 158A>G AG/GG genotypes had shortened LOS and less need for NAS treatment compared to the carriers of AA genotype, suggesting that the polymorphism in the *COMT* gene may play a role in the variability of withdrawal severity in methadone-exposed neonates.

One explanation proposed in this study for the observed results is that the variant allele may cause an increase in binding affinity with beta-endorphin and a decrease in protein expression for the *OPRM1* gene (Bond, Laforge, & Tian, 1998). Similarly, *COMT* gene transcribes COMT enzyme, one of the functions of which is to degrade or inactivate catecholamines such as dopamine, epinephrine, and norepinephrine at the postsynaptic cleft (Grossman, Emanuel, & Budarf, 1992). SNP in the *COMT* gene results in a valine to methionine mutation at position 158 (Val158Met) rs4680 (Lotta, Vidgren, Tilgmann, Ulmanen, Melen, et al., 1995). The Val variant catabolizes dopamine about four times more and faster than methionine (Lachman, Morrow, Shprintzen, Veit, Parsia, Faedda et al., 1996). However, the overexpression of Met variant in the brain (Zhu, Lipsky, Xu, Ali, Hyde, Kleinman et al., 2004) consequently decreases the enzyme activity by about 40% (Chen, Lipska, Halim, Matsumoto, Melhem et al., 2004) and results in an increased dopaminergic stimulation of the post-synaptic neuron due to a higher synaptic dopamine level following neurotransmitter release. So the SNP in this gene may cause reduction in the enzyme activity and increase the levels of catecholamines, which may result in an improved stress tolerance in these infants. Taken together, since NAS severity is associated with sleep disorders, and SNP in the *COMT* gene is associated with the NAS severity, this thesis study explored to see if there may be an association between the allelic variation of the *COMT* gene and sleep organization in neonates. Only methadone-exposed neonates were assessed for this part of the study because the neonates in comparison group did not have genetic data.

### 1.3. Covariates: Periconceptional Absolute Alcohol Consumption

Alcohol is a known teratogen that severely affects fetal growth (Goh, Hutson, Lum, Roukema, Gareri, Lynn et al. (2010). However, alcohol consumption during pregnancy is not easy to detect due to the underreporting of such cases in general self-reported methods (Baña, Tabernero, Pérez-Muñuzuri, López-Suárez, Dosil et al., 2014; Hayes et al., 2002; Troese et al., 2008). Therefore, meconium bioassay is used frequently for assessing prenatal alcohol exposure. Bana et al. (2014) employed the techniques of gas chromatography/mass spectrometry and liquid chromatography/tandem mass spectrometry to detect drugs and markers of chronic consumption of alcohol in meconium to assess this phenomenon. A prospective study was performed during a period of 10 months among 110 infants assessing anthropometry, neuromuscular development and determination of toxic substances in urine and meconium. Meconium analysis was conducted to identify fatty acid ethyl esters (FAEEs) and ethyl glucuronide (Etg), which are the known meconium biomarkers for prenatal alcohol exposure (Goh et al., 2010). Participants were surveyed regarding the obstetric history, toxic habits, and employment status. The results of early detection markers analyzed in meconium (FAEE >1000 ng/g and/or Etg >50 ng/g meconium), showed that 34.65% of pregnant women consumed alcohol during pregnancy, and 17% were positive for both markers. Among the positive cases, 50% of those exceeding a FAEE's value of 5000 ng/g in meconium had low birth-weight children. Notably, only 4.5% of this sample had reported occasionally drinking alcohol. These findings indicate that pregnancy-related drinking is highly likely to be under-reported and may complicate the investigation of isolated substance exposure in humans.

Extensive research involving both animal and human samples has demonstrated that prenatal alcohol exposure results in injury to the developing brain and adverse developmental consequences such as fetal alcohol syndrome (FAS), growth retardation, CNS anomalies, developmental impairment of cognitive processing (Green et al., 2007), and Due to the likelihood of under-reported incidents of alcohol use during pregnancy particularly in the high-risk population (Guo et al., 2010), studying ethanol exposure as a possible contributing co-morbid factor to opiates in population is warranted.

In our previous study, Hayes et al. (2007) found lifetime history of alcohol consumption to be associated with sleep deprivation and lower sleep related arousals in apneic preterm neonates who were treated for apnea with caffeine or methylxanthine, a standard treatment protocol for pediatric sleep apnea. Additionally another of our study by Troese et al. (2008) also found prenatal alcohol to be associated with sleep fragmentation in otherwise healthy infants. Prenatal exposure to alcohol was found to lead to poor sleep in another study by Pesonen, Räikkönen, Matthews, Heinonen, Paavonen et al. (2009) that assessed 8-year-old children with sleep disorders through actigraphic assessments for 7 consecutive nights and found that children exposed prenatally to alcohol had a 2.9-fold (95% CI: 1.1 to 7.6) increased risk for having short sleeps and 3.6-fold (95% CI: 1.3 to 10.0) increased risk for low sleep efficiency, which are markers of sleep fragmentations. Importantly, these associations were not confounded by sex, gestational length, prenatal and perinatal complications, asthma, allergies, or parental socioeconomic status, and body mass index at 8 years.

In this thesis sample (n=58), the amount of alcohol consumed per binge before pregnancy (i.e., AA/binge) was significantly higher ($p = 0.006$) for mothers in the

methadone group. Even though this measure was not directly reported for the current pregnancy, it was important to note that the pattern of historic alcohol abuse is a reliable predictor of under-reported alcohol use during pregnancy (Bana et al., 2014; Hayes et al., 2002). The effects of prenatal alcohol, therefore, provide a model for evaluation of the impact of prenatal exposure in this study.

## 1.4. Hypotheses

Based on the studies and arguments presented above the following hypotheses were proposed and tested in this thesis study.

**1.4.1. Hypothesis 1: Behavioral States Integrity.** Quantitative deficits in arousals and wake state are hypothesized to reflect neurobehavioral instability in opioid-exposed infants in the pre-withdrawal period. To test whether sleep, arousal, and SM features differ in neonates with prenatal opioid exposure (i.e., maternal methadone treatment for dependence; n=31) compared to non-exposed, demographically similar infants (n=19), all-night, sleep studies, using videosomnography, were conducted on day 1 or 2 of life.

A secondary exploratory hypothesis was proposed that opioid-exposed infants might show more sleep fragmentation (e.g., shorter sleep periods, excessive arousal and cry) on day 2 than on day 1 of life due to the possible development of opiate withdrawal, which often begins at or around 48 hours post-birth. No differences in the behavioral state parameters were expected between day 1 and 2 in the outcomes of comparison infants. A 2 (infant age) x 2 (exposure groups) analysis of variance (ANOVA) model was used to test the interaction. Day 1 (methadone-exposed = 18; comparison = 8) and day 2

(methadone-exposed = 13; comparison = 11) infants were compared for group differences.

### 1.4.2. Hypothesis 2: Genetic Corollaries of Behavioral States and SM.

Preliminary work from our laboratory has shown that withdrawal severity in NAS-treated infants differs based on single nucleotide polymorphisms (SNPSs) or allelic variants in the *COMT* (catechol-o-methyl-transferase) gene with minor allelic variants (AG or GG) showing shorter LOS and a fewer medication than the carriers of the major allelic variant (AA; Wachman et al., 2013). In this study, it was hypothesized that minor allelic genotypes of the SNPs in the *COMT* gene of opioid-exposed neonates would show better sleep organizational characteristics measured by sleep-wake, and SM parameters in the pre-withdrawal sleep study. For this hypothesis, 20 of the 35 methadone-exposed neonates were examined through saliva samples (Genotek OGR-250 kit with CS-1) for the alleles of the *COMT* gene and associations among sleep-related variables (SM, sleep, wake, and arousal parameters) in the early post-birth, pre-withdrawal period.

# CHAPTER 2

# METHOD

## 2.1. Participants

A subset of Caucasian (>98%) mother-infant dyads (N=58; total 116) from our longitudinal study (grant number: DA024806-01A2) of the University of Maine funded by National Institute of Health (NIH) was extracted for this thesis study. Participants consisted of pregnant women receiving the methadone maintenance treatment (MMT) program pre- or post-conception along with their neonates (N=37 mother-infant dyads; 74 total). Mothers were recruited during the 3$^{rd}$ trimester of pregnancy from three sites in Bangor: the Acadia Hospital's Narcotics Treatment Program (NTP), Bangor Discovery House, and Metro Treatment of Maine. A socio-demographically matched comparison group of mothers and infants (N=21 mother-infant dyads; 42 total) was also recruited. Both methadone-exposed and comparison groups were similar in demographic characteristics including alcohol and tobacco use except for the opiate use.

**2.1.1. Recruitment Sites.** The majority of methadone-maintained expecting mothers were recruited from the Narcotics Treatment Program (NTP) at the Acadia Hospital, as well as the Discovery House Clinic and Metro Treatment of Maine in Bangor, Maine. Acadia Hospital served as the primary recruitment site for methadone-maintained women, as it is the largest MMT in Penobscot County, serving over 600 patients, of which 300 are female and 15% are pregnant at a given time.

Participants in the comparison group were recruited from the Family Practice Center (FPC) and Women Infant and Children (WIC) office in Bangor, Maine. The FPC

provides services to over 30,000 outpatients annually, and has been demonstrated in pilot data to be similar demographically and psychologically to the NTP sample in both preliminary (Heller et al., 2013; Logan et al., 2012) and historical (Hayes et al., 2002) data. Importantly, the FPC and WIC population have been shown in our laboratory to be similar to the NTP sample with respect to alcohol and tobacco use.

**2.1.2. Inclusionary and Exclusionary Criteria.** Participants were pre-screened for initial eligibility criteria based on their age, socioeconomic status, stage of pregnancy, and present and past substance use. Inclusionary criteria required all prospective participants to be English speaking pregnant women between the ages of 18 and 40 years who, if enrolled into the Narcotics Treatment Program, were maintained on methadone (suboxone disqualified), and were diagnosed according to DSM-IV as opiate-dependent at the time of MMT entry. Consents to access pregnancy and birth-related medical records were obtained by collecting HIPAA waiver from mothers on behalf of themselves and their neonates. Use of tobacco products or alcohol in either sample did not disqualify, neither did continued illicit drug use during pregnancy or following birth in the methadone-exposed sample. Mothers with serious medical condition (e.g., cancer, diabetes) or psychopathology were excluded from the study as such conditions may adversely impact infant outcomes, and results may not be comparable to the rest of the sample. Other exclusionary criteria included preterm labor (<35 weeks gestational age), congenital fetal malformation, or severe obstetrical complications during delivery that compromised infant survival. In addition, infants must not have scored higher than 8 in the Finnegan scores, a diagnostic tool of neonatal abstinence syndrome (NAS) used by NICU nationwide, on the night of neonatal data collection. Infants were expected to be in

a stable environment such as the newborn nursery on the night sleep data was recorded. If the first night video was not usable, the data collection occurred the second night with a pre-established assumption that NAS symptoms start appearing within or after 48 hours of birth. Any data collected beyond 48 hours for the methadone-exposed group, therefore, was not included in this analysis.

**2.1.3. Institutional Review Board Approval.** This project acquired an approval from the University of Maine's Institutional Review Board (IRB) as well as Eastern Maine Medical Center's IRB. The study fulfilled all requirements for Health Insurance Portability and Accountability Act (HIPAA) compliance.

**2.1.4. Risks and Discomforts.** There were no physical risks involved with participating in this study. A little psychological risk was anticipated during the maternal interview process. Therefore, the participants were adequately informed ahead of time, and encouraged to let the interviewer know of any discomforts they experienced while answering certain items of the assessment tools. If need arose, referral to psychological services was available to such participants. No such incidence has happened to this date.

**2.1.5. Benefits.** No direct benefits to either the mother or infant were expected as a result of participating in this study. Participants were made aware of the purpose of the study and the main objective of their participation, which was to contribute to new research about how the developing brain works. Upon request, summaries of individual assessment or results of overall findings were provided. Mothers were encouraged to share the results with their children's pediatrician.

**2.1.6. Confidentiality.** All records were kept private and separate from identifiable information in a double-locked filing cabinet accessible only to the essential study personnel. All data were coded with a unique identifier to protect confidentiality. This study was further protected by a Certificate of Confidentiality from the National Institutes of Health. With this Certificate, study staff cannot be forced to give identifying information to any federal, state, or local civil, criminal, administrative, legislative, or other proceedings, even with a court subpoena. The researchers would use this certificate to resist any demands for information that would identify study participants, although no such circumstances arose.

**2.1.7. Compensation.** For this part of the project, participants received a total sum of $175.00 compensation upon completion of the entire protocol. Of the total amount, $100 was awarded upon completion of the maternal interview during the 3rd trimester, and $75 for assessments conducted during the newborn hospital stay that included overnight recording of the neonatal sleep, actigraphic data collection (data not included in this thesis), and mother-infant saliva collection for genetic study. Saliva specimens were also collected during the follow-up visits in case of an inability to collect them during the hospital stay.

**2.1.8. Voluntary Participation.** Participants were informed during the initial interview that their participation to the research is voluntary meaning they have the rights to decide whether they want to participate in the study or withdraw from it any time during the course of this study. This aspect of the study was emphasized repeatedly during any other follow-up visits in order to make sure that the participants fully understood their rights as research participants.

## 2.2. Materials and Measures

Both mothers and infants were assessed using multiple IRB-approved tools for this thesis project.

**2.2.1. Maternal Assessments.** The purpose of the maternal assessment was to screen for any pregnancy or pre-pregnancy drug and alcohol history, and to collect sociodemographic and psychological status at the time of enrollment.

***2.2.1.1. Drug and Alcohol Use Measurement***. A semi-structured clinical interview was conducted to quantify licit and illicit drug use that occurred before or during the current pregnancy. Quantity-Variability-Frequency (QVF; Jacobson, Jacobson, Sokol, Martier, & Ager, 1993) method was used to measure all current and past substance use in which, the mother was asked to report use of various substances retrospectively (before conception) and currently (during the 3rd trimester), by detailing the quantity, frequency, and variability of substance use during a typical week. Pregnant participants were asked to describe to the interviewer how many drinks, what size drink, and what type of drink they would consume on a typical Friday night, and these questions were repeated for other days of the week. Participants were then asked the same series of questions about their alcohol use during pregnancy. These participants were also asked if they sometimes drank more than 5 glasses of beer, wine, or liquor as an assessment of binge drinking, followed by questions to determine the frequency of binge drinking episodes within the past year from the date of interview. They were also asked if they had any alcohol dependence treatment history and for how long.

In addition to the QVF, the Michigan Alcoholism Screening Test (MAST; Selzer, 1971) was also administered, which is a structured interview consisting of 25 yes/no questions assessing both current and retrospective problematic alcohol consumption. They include questions regarding participation in Alcoholics Anonymous, arrests for drunk driving or drunken behavior, hospital admissions related to drinking, and loss of employment or significant others as a result of drinking. The MAST has a good reliability for detecting both lifetime history of problem drinking as well as recent past and current use of alcohol (Zung, 1982). A clinical score of five affirmative answers was chosen from the MAST as a cutoff indicative of problem drinking, as was recommended by the developer (Selzer, 1971). The T-ACE (Russell, 1994), a 4-question screening tool assessment of tolerance to alcohol also derived from the MAST, and the TWEAK (Russell, 1994), a 5-question screening tool assessing alcohol dependence, were also administered.

Additional alcohol use variables were calculated following a completion of the interview. Alcohol use variables were calculated by determining the amount of alcohol content in the type of alcohol the participant drank. Absolute alcohol (AA) was determined using an algorithm based on the type of alcohol (e.g., beer, wine, and liquor are assigned weighted multipliers), size of the container (e.g., number of ounces per drink), and the number of drinks consumed during a sitting. Then AA was calculated by multiplying the number of drinks by the number of ounces, the product of which was then multiplied by the weighted multiplier for the type of drink. The AA estimate was then used to derive other variables related to alcohol consumption, including average AA per day (AA/D), AA per drinking day (AA/DD), and AA per binge (AA/binge).

Following the attainment of alcohol information, participants were asked if they had ever smoked marijuana. If so, they were asked to provide the age at which they first used marijuana. They were then asked to determine the age at which their period of heaviest use occurred as well as the frequency of use during that time period. Participants were asked if they had used marijuana since becoming pregnant or before they knew they were pregnant, and if so, asked to describe the frequency of use during pregnancy. This series of questions was repeated for each of the opiates (heroin, methadone, oxycodone, hydrocodone, codeine), cocaine and crack cocaine, barbiturates, sedatives and tranquilizers (e.g., Valium, Xanax), amphetamines (including prescription psychostimulants), methamphetamines, antidepressants, hallucinogens, and inhalants. Participants were also asked at what age they began smoking, the number of cigarettes per day currently smoked, the number of cigarettes per day they smoked at the beginning of their pregnancy, and whether or not they had ever tried to quit. Copies of these tools are attached in Appendix A.

***2.2.1.2. Hollingshead Four-Factor Index of Social Status.*** Hollingshead 4-factor Index of Social Status (Hollingshead, 1975) is a measure of socioeconomic status that contains questions about general demographic information as well as data on education and occupation of the participant and her partner. Education level and the type of occupation are assigned point values (education = 1 to 7; occupation = 1 to 9). For the weighted scores, education values were multiplied by 3 and occupation values multiplied by 5; and the weighted scores were then added for the participant and her partner individually before averaging them. The average was not computed if only one member

of the family worked. For families in which neither partner worked, the score belonging to the individual with the most education was utilized.

This measure was chosen because it is considered a more accurate portrayal of socioeconomic status than income alone. Scores on this scale ranged from of 8 to 66 with higher scores indicating better SES. The index was validated utilizing the 1970 US Census such that the median years of school completed by the participants were correlated by .83 and the occupation scores by .85. Additionally, median income earned correlated by .78 and .67 with the occupation scores for men and women, respectively (Hollingshead, 1975). Though unpublished, over 5800 articles have cited this index.

***2.2.1.3. Peabody Picture Vocabulary Test – 3rd Editions (PPVT-III).*** The PPVT-III is a measure of verbal ability, which was chosen for its relatively short duration, ease of administration, and validated use as a rough estimate of IQ (Dunn & Dunn, 1997). The test consists of seventeen sets of twelve items each. For adults, the exam begins with set thirteen, item number 145. Each item consists of a verb or noun to be matched with one of the four pictures provided on the item page. The test administrator orally provides a word to the participant and scores the right or wrong answer on the record form. The assessment takes approximately fifteen minutes to administer and is intended for individuals between the ages of 2 and 90+ years. The basal rule for this test is one or fewer items wrong in the first age-appropriate set in order to proceed to the forward sets. The discontinuation of test occurs upon eight or more incorrect answers in a single set. This measure produces a raw score, standard score, stanine, percentile rank, normal curve and age equivalent score. For this study, the standard score was used as an estimate of IQ that can range from 40 to 160 with higher scores indicating better verbal ability. This

measure has internal consistencies between .92 to .98, split-half reliability of .86 to .97, alternate-form reliability of .88 to .96, and test-retest reliability of .91 to .94. The established validity of verbal ability of PPVT-III correlation ranges between .69 to.74 with the OWLS scales, .91 with the WISC-III VIQ, .89 with the KAIT Crystallized IQ, and .81 with the K-BIT Vocabulary (Dunn & Dunn, 1997).

***2.2.1.4. Depression and Anxiety Measures.*** For the general assessment of pregnancy-related depression, this study used the *Beck Depression Inventory – 2nd Edition* (BDI-II; Beck, Steer, & Brown, 1996). This widely used twenty-one item self-report tool assesses depressive symptoms. Participants are expected to accurately identify and circle the number next to a statement that best describes their emotional or cognitive state on each item over the past two weeks including the day of assessment. Total score ranges from 0 to 63 with higher scores indicating greater depressive symptoms severity. Even though the BDI-II does not have a cutoff score to indicate clinical depression, the following guidelines are often used to interpret the total scores: minimal range 0-13, mild depression 14-19, moderate depression 20-28, and severe depression 29-63; an internal consistency for psychiatric patients is .86 and for non-psychiatric patients is .81 (Smarr, 2003).

*Symptoms Checklist 90-Revised* (SCL-90-R; Derogotis, 1983) is a screening tool used for assessing a global distress in the areas of obsessive-compulsive thought, somatization, depression, anxiety, hostility, paranoid ideation, phobic, interpersonal sensitivity, and psychoticism. The symptoms are divided into three global indices: global severity index (GSI), positive symptom distress, and positive symptom. The questionnaire consists of ninety symptoms evaluated using a Likert scale (0=*not at all* to

4=*extremely*), and participants are asked to rate how much they have been bothered by each symptom over the past week including the day of assessment. The raw score derived from the participants' responses is then converted to a t-score. This measure rendered an assessment of global psychological well being beyond the depressive symptoms assessed by the BDI-II. Copies of these tools are attached in Appendix B.

**2.2.2. Infant Assessments.** Behavioral, medical, and demographic information regarding the infants was collected at various time points using the following methods.

***2.2.2.1. Infant Biomarkers.*** Infant biomarkers of substance exposure were assessed during the hospital stay. Nurses from the NICU collected meconium samples from the diaper in the first few days of life according to the established protocol, i.e., nurses collect approximately 5 gm of fecal material obtained from the first bowl movements of the neonate (typically one diaper is sufficient), scrape the material from the diaper, and store it in a specified standard label container to later send it to the Affiliated Laboratories, Bangor, ME for screening of cocaine, opiates, cannabinoids, amphetamines, and phencyclidine (PCP). This is a standard procedure for opiate-exposed infants in the NICU at EMMC.

***2.2.2.2. Finnegan Neonatal Abstinence Scoring System.*** This scoring system for NAS (Kaltenbach & Finnegan, 1986) has been exclusively used as a standard measurement of autonomic symptoms of withdrawal during the postnatal period. A score was assigned every four hours by EMMC nursing staff during the infant's hospital stay. The study staff later obtained the daily scores from the medical record of the neonates after they were discharged from the hospital. A copy of this tool is attached in Appendix C.

***2.2.2.3. Videography.*** Infant sleep, arousal, and SM linked to emergent arousal regulation and wake-sleep transitions (Giganti, Hayes, Akilesh, & Salzarulo, 2002; Symanski, Hayes, & Akilesh, 2002) were monitored through an overnight videographic sleep recording of neonates during the first or second postnatal night by using a SONY DCR-SR82 camcorder that produced time-locked, digital, infrared videographic data of maximum of 13 hours per neonate.

***2.2.2.4. Behavioral States.*** Behavioral categories for coding neonatal sleep, wake, arousal, and SM were derived from conventional methods used in previous research (e.g., Giganti et al., 2002; Symanski et al., 2002). To insure a fine-grained analysis of behavioral arousal coding, types of arousal were analyzed using the following categories: brief arousal (10-30 seconds), full arousal (>30 seconds and < 1 minute), and awakening (≥1 minute) which we have found to be representative of neonatal wake and sleep-sate arousal behaviors. In order to assess sleep state arousal, it is essential to define the time criteria for sleep as well. For this study, sleep was considered stable if a sleep bout lasted longer than a minute following an onset. Within each sleep bout, behavioral observation of SM bouts (if they occurred) were coded when the event lasted for greater than or equal to 5 seconds (≥5 seconds) post onset, and a latency of >5 seconds post offset was required to determine inter bout interval as well as quiescence periods. Research Assistants blind to the infant's group status were trained to code behavioral states based on the Developmental Neuroscience Laboratory's training system beginning with familiarization with the behavioral state coding methods of our laboratory for neonatal state coding (Giganti et al., 2002; Hayes, Akilesh, Gilles, Fukumizu, Sallinen, et al., 2007; Symanski et al., 2002;). Inter-observer reliability of >0.7 was established by using the Kappa

coefficient method for each behavioral state: brief arousal, full arousal, wake, transition, sleep, and SM separately.

**2.2.3. Medical Record Access.** Maternal medical records pertaining to current pregnancy and delivery, and infant medical records from birth up to 1-month of age were harvested after obtaining HIPAA consent from the participant during the interview. Medical record data obtained included the date of induction into MMT, psychiatric diagnoses, current and historical methadone doses, results of random UDS, date of entry into prenatal care, and prescription drug use during pregnancy. Labor and delivery measures were also obtained. Infant birth parameters such as gestational age, sex, delivery type, birth weight and length, head circumference as well as onset of NAS symptoms, pharmacological treatment for NAS, Finnegan scores, hearing screen results, and feeding data were collected.

**2.2.4. Saliva Samples.** All DNA samples were collected using Oragene kits either during the hospital stay or at the post discharge follow-up clinics of the same study. Mothers were asked to spit in the Oragene GR-500 kit tube and the infant sample was collected using an OG-250 DNA collection kit with CS-1 sponges. The maternal kit contained a tube with marks for saliva quantity measurement and a lid with solution to preserve the saliva. The infant kit included five cotton swabs, a pair of scissors, and storage kit with solution to preserve the DNA from saliva and buccal cells. These kits were ideal for this study as they were designed to store the collected specimen in the room temperature for as long as 5 years.

## 2.3. Study Design and Procedure

Participants were divided into exposed and non-exposed comparison groups. Group 1 (n=37) comprised pregnant women who had been enrolled into a methadone maintenance program (MMT) before or during the current pregnancy. Group 2 (n=21) comprised a sociodemographically similar comparison mother-infant dyad group recruited from the Family Practice Clinic as well as the Women, Infant, and Children (WIC) office in Bangor, ME during the 3$^{rd}$ trimester; or at the time of birth while mother and infant were hospitalized at the EMMC. Majority of the comparison mothers were using nicotine during pregnancy. Therefore, cigarette smoking has been added in the demographic characteristics of this sample.

**2.3.1. Participant Recruitment Protocol.** Prospective pregnant women were pre-screened for the eligibility for the study based on the maternal inclusionary and exclusionary criteria by graduate assistants or the PI either by phone or through a face-to-face interaction. Prospective participants had to be Caucasian pregnant women from low-income households with no known pregnancy complications at the time of prescreening and no opioids present in the urine analysis. The exposure group participants had to meet all these criteria in addition to being enrolled into the MMT.

**2.3.2 Maternal Interview.** Once the pregnant women met eligibility criteria and agreed to participate in the study, a semi-structured interview was performed at our lab space in Bangor, ME to collect maternal self-reported information regarding any prenatal use of drug and alcohol. In addition, sociodemographic and physiological statuses of the participants during the 3rd trimester of pregnancy were also collected. Informed consents

for both mother and infant were obtained during this visit. Consent for HIPAA-approved medical record access pertaining to current pregnancy and delivery for mother, and birth-related information for infants was obtained. Maternal information on sociodemographic background (Hollingshead), IQ (PPVT-III), depression (BDI-II), and anxiety (SCL-90R) were also collected during this visit. The collected data were scored by our graduate assistants in the lab at the University of Maine, and double scored by trained undergraduate research assistants. All original files were de-identified and stored in a double-locked cabinet in the PI's office. The interview process took approximately one hour and 15 minutes per participant and the participants were compensated with a $100 cash or equivalent gift card at the end of this visit.

**2.3.3. Markers of Maternal Substance Use and Infant Exposure.** Besides an extensive prenatal maternal interview about a possible drug and alcohol use during this pregnancy, infant exposure to maternal substance use was also measured through neonatal meconium toxicology screens (MecSTAT-7) and urine toxicology screens upon admission to the NICU and maternal urine toxicology screens after delivery. This measure was useful for detecting possibly undetected poly drug use during pregnancy.

**2.3.4. Neonatal Sleep Data (24-48 h Post-birth Pre-withdrawal Periods).** During the interview, information pertaining to possible delivery methods and tentative due date was obtained from the mother. Our study team started contacting the newborn nursery at the EMMC at least two weeks prior to due date twice a day to ensure no missed opportunity of pre-withdrawal neonatal data collection. Mothers were also requested to alert the study team about the birth within 12 hours post-birth if it occurred before the due date. Cardiorespiratory monitoring was initiated immediately at birth in

study participants and NAS scoring was initiated upon the coding of the first Finnegan score >8. With a purpose to collect quality videographic data, parents were requested/encouraged to house their neonates in the newborn nursery on the night of videographic data collection. However, parental wish was equally respected, which resulted in some variability in the location of recording such as mother's room, newborn nursery, or continuing care nursery (CCN). If the first night of data collection was not successful, they were collected on the second night. Hence data resulted in the recording time of 24-48 postnatal hours.

The study team went to the newborn nursery around the time of evening shift change in order to avoid family visiting hours and to personally assure that the night nurse for the participant was fully informed of the study conditions. A SONY DCR-SR8 camera was set up on a sturdy tripod next to the infant's bassinet with the camera view focused to cover at least the upper half of the body, and the recordings of nocturnal sleep period (midnight to 0500) were collected, which formed the basis for sleep, arousal and SM analyses. Midnight to 5AM was deemed the most optimal time as the environment tended to be much quieter with significantly less interruption occurring. Nurses were asked to use cluster care (i.e., disturb infants only as needed and for feeding during the night recording period). Finnegan scores were tabulated by the attending nurse following the feed and were inclusive of the previous 4h-interval as was normally done. If scores suggested active abstinence (>8), the departmental directive required scoring every 2 h. The main purpose of the Finnegan score in this study was to determine that the infant's status was pre-withdrawal during the recording period.

***2.3.4.1. Videographic Data Processing.*** Each baby had approximately 13 hours of video, which was the maximum capacity of the camera memory card. The camera and other equipment were collected the next morning from the newborn nursery at EMMC and brought to our University lab to download the video in the external hard drives. If the first night data was spoiled, data was collected the second postnatal night using the same procedure as the first night. Once the download was complete, the time stamp for midnight was calculated using an Excel spreadsheet and the segment from midnight to 5 AM (0000-0500) was trimmed for behavioral state analysis.

***2.3.4.2. Behavioral States Coding.*** Trained and group-blinded undergraduate RAs coded and double-coded the videos using our lab computers. Coding criteria for sleep, wake, arousal, and SM were adapted from our previous studies mentioned below.

***2.3.4.2.1. Coding Protocol.*** The following coding criteria were derived from Giganti et al. (2002); Hayes et al. (2007); and Troese at el. (2008), with primary reference to Behavioral State Criteria stated in Tohman et al. (1985).

***Sleep.*** Sleep is generally divided into active and quiet types depending on the behavioral and physiological characteristics. For this sample, however, it was not possible to separate the types of sleep due to the nature of the videographic data that was influenced by environmental factors in the newborn nursery. Therefore, the sleep was coded when an infant's eyes were shut; motor activity was either low or sporadic with muscle tone remaining low. Occasional mouthing and grimacing behaviors as well as SM may or may not have occurred. These characteristics were expected to persist longer than a minute (>60 secs) to be coded as sleep in this sample. Otherwise, the sleep-like behavior was coded as either indeterminate or whatever state the baby was in before the

initiation of such a behavior. Even though an identification of respiratory variability is important in characterizing behavioral states, it was difficult to detect respiration in this sample as the babies were swaddled for the most part of the videos. Therefore, the quality of respiration was not included while characterizing the sleep and wake states.

***Wake.*** Wakefulness is an alternate state of sleep. The process of awakening mostly involves - but may or may not be preceded and followed by - transition events. For this study, wakeful state was coded when the infant's eyes were mostly open, focused, and may have been scanning the environment. This state was expected to include crying and fussing with high or low motor activity tracked by regular respiration. Sometimes the baby's eyes were not expected to be open or just one eye would have been open due to the environmental factors such as lighting and position. In such a scenario, the coder had to take into account other behavioral characteristics that met the criteria for a wake state. These characteristics, if continued for longer than a minute (>1 minute) was considered a wake state. The event with these characteristics, if lasted for less than a minute, was counted as an arousal.

***Sleep State Arousals.*** Arousal was coded when it occurred within sleep bouts, and had, in addition to partial or full eye opening, all or either of the characteristics such as general body movements, increased heart rate, occasional grimacing, and cry or fuss. The neonate was usually expected to return to sleep state after this event but sometimes was intervened by a nurse especially if the neonate was crying or fussing. Depending on the duration of the event, arousal was divided into two categories: brief arousal for which, these characteristics had to last from 10 to 30 seconds, and for full arousal, greater than 30 seconds up to 60 seconds.

***Nurse Intervention.*** For our sample, nurse intervention was characterized by the presence of an adult hands/arms/body in the camera view for longer than or equal to 5 seconds (>5 secs) touching the baby, which may or may not have initiated or caused a change of state. Nurse intervention was coded for two categories: (1) within state intervention: an adult hand or body appeared in the camera view for at least 5 seconds to medicate or adjust the baby when the baby was within a stable sleep or wake state; (2) state changing intervention: an adult hand or body appeared in the camera view for at least 5 seconds, touched the neonate, and caused a state change (e.g., sleep to wake).

***Transition.*** Transition event was expected to precede or follow sleep and wake states, and could include any of the characteristics such as SM (sleep-to-wake transition), yawn, drowse, and/or grimaces. SM or grimaces that occurred during a sleep bout and did not lead to the baby's awakening were not coded as transition. Transition was considered ended when the baby achieved a stable state (i.e., awake or sleep) according to the states criteria.

***Indeterminate.*** A state was coded indeterminate if the neonate was showing signs of mixed states characterized by oscillation between sleep- or wake-like states for at least 10 seconds without any distinctive transition to a different state occurring. For example, if a neonate was drowsing and/or grimacing with excessive head and body movements in the middle of a sleep or wake bout that looked like a transition but the neonate failed to transition to the next state and instead retained the preceding state, such a bout was coded as an indeterminate state, which is also named disorganized sleep in a study by Field, Diego, Hernandez-Reif, Figueiredo, Schanberg, and Kuhn (2007).

***Cry.*** Frequency and duration of cry utterances were measured. Even though cry behavior bears a range of characteristics (LaGasse, Neal, & Lester, 2005), only the vocalized cries were recorded in this study due to methodological constraints. Cry events were recorded with 5 seconds of forgiving period between two bouts. For example, if two or more cry bouts occurred with less than a 5-second-gap in between, the whole set of bursts was coded as one whole bout. Cry has similar physiological characteristics to arousals or awakenings with an exception of difference in the pitch of vocalization (LaGasse et al., 2005). Therefore, the cry bouts that occurred within a sleep bout that met the duration criteria for brief arousal, full arousal, or awakening were also added in calculating the frequency and duration of arousal and awakenings.

***No baby***. In overnight videographic data, one issue we faced was losing sight of the neonates because they might have been taken away from camera view for multiple reasons such as for feeding, changing, or cuddling (if the neonate was distressed). In this study, the primary target length of video data for each neonate was 5 hours (2400-0500h) but many of the videos in the current sample had sections in which the infant was taken away from camera view or the camera was shut off for during parts of the recording period. This issue was problematic in consistently calculating the average duration and frequency of events that occurred within the 5-hour period. Therefore, the length of each video clip during which the neonate was removed from the camera view for two or more minutes was coded as "no baby," and the total duration of the segment with missing data was subtracted from total video duration of each neonate before obtaining the average frequency and duration measures.

**2.3.4.3. SM Coding.** After isolating the sleep bouts of neonates from midnight to 5 AM video segments, the sleep bouts were coded by our group-blinded RAs for SM bout frequencies and durations using an Excel template developed in our Developmental Neuroscience lab. The criteria for coding SM were adopted from our previous lab works (e.g., Hayes & Mitchell, 1998; Hayes, Plante, Kumar, & Delivoria-Papadopoulos, 1993; Hayes, Plante, Fielding, Kumar, & Delivoria-Papadopolos, 1994) and also from Prechtl (2001). The characteristics included neonatal writhing gross motor movements with a variable sequence of arm, leg, neck, and trunk movements within a sleep bout to last greater than or equal to five seconds (≥5) with a 5-second of forgiving time in between two bouts. If two such bouts occurred within less than 5 seconds, it was considered one whole bout. Quiescence was coded when the neonate remained immobile between offset of one SM bout and the onset of subsequent SM bout within a single sleep event duration for greater than or equal to 5 (≥5) seconds with the exception of occasional twitches or mouth movements (e.g., Hayes et al., 1994). Additionally, inter-bout interval (IBI) was calculated to gauge the lapse of time between two or more bout onsets within single sleep event duration. The observational coding task for the RA's involved identifying and recording on the coding template the onset and offset of each SM bout. The template then rendered the total duration and frequency of SM bouts. The template was also formatted to compute the frequency and duration of quiescence and IBI. The outputs were imported to an SPSS file and further computed for the average bout duration, frequency per sleep bout, and proportion to total sleep time spent on each of the variables (i.e., SM, Quiescence, and IBI).

**2.3.5. Genetic Data Processing.** Since neonates tend to have dry mouth, the inside of their cheeks were swabbed and the swab heads were then secured in the OGR-250 kit for assaying. Mothers were asked to spit in the tube provided by the OGR-500 kit and the tube was securely tightened after the saliva was mixed with the storage fluid. These specimens were stored at room temperature before sending to Tufts Medical Center for DNA extraction. Once the specimen arrived Tufts Medical Center Clinical and Translational Research Center Core Laboratory for processing, DNA was isolated and the regions of interest were genotyped for the following five main SNPs: 118A>G (rs1799971, dbSNP database; assay C_8950074_1) within the *OPRM1* gene; 3435C>T (rs1045642, dbSNP; assay C_7586657 _20), 2677G/T/A (rs2032582, dbSNP; assays C_11711720C_30 and C_11711720D_40), and 1236C>T (rs1128503, dbSNP; assay C_7586662 _10) within the *ABCB1* gene; and 158A>G (rs4680, dbSNP; assay C_25746809_50) within the *COMT* gene, using established Taqman technology (Wachman et al., 2013). For this thesis study, only 158A>G (rs4680, dbSNP; assay C_25746809_50) within the *COMT* gene was analyzed using the dominant model (AA vs AG/GG).

## 2.4. Data Analysis

**2.4.1. Calculation of Event Frequency and Duration of Behavioral States and SM Parameters.** For the analysis of behavioral states and SM parameters, the most commonly used method in our lab is the calculation of frequency and duration of individual states, the averages of which are then analyzed for overall analysis. However, every neonate presents with a variable set of behavioral patterns as well as the amount of time spent on each behavior. In addition to these obstacles, in the videographic data of

this study, the neonates were not always positioned in the most desired way, and were removed from the camera view at times that resulted in the fragmentation of total video length and some missing video duration. In order to adjust for such variability in the duration of total time per neonatal study, the total duration of "no baby" segments were individually subtracted before computing the average frequency, average event duration, and proportion of total time spend on an event for each observed behavior per neonate.

***2.4.1.1 Average Event Duration Measure***. Average event duration of each observed behavior was obtained by dividing the total duration of each event or state by the total frequency of corresponding event or state for each neonatal video.

$$\frac{\textit{Sum of Event Duration}}{\textit{Sum of Event Frequency}} = \textit{Average Duration (Sec)/event}$$

A combined average duration of full and brief arousal was also computed as a composite measure of arousal in order to control for the variability in arousal parameter across sample due to two separate arousal measures used in this study. The composite average duration was computed by dividing the sum of total raw durations of brief and full arousals by the sum total of raw frequencies of both measures for individual neonates.

$$\frac{\textit{Sum of BA and FA Raw Durations}}{\textit{Sum of BA and FA Raw Bout Frequency}} = \textit{Composite Arousal Duration}$$

***2.4.1.2. Average Frequency Measure***. For the frequency measure of behavioral states, the raw sum of an event frequency total divided by total coded hour that rendered a frequency of a state or an event per hour.

$$\frac{\textit{Sum of Raw Event Frequency}}{\textit{Coded Video Duration (Hour)}} = \textit{Average Frequency/Hr}$$

For the average frequency of SM and arousal parameters, total sum of the raw frequency of SM, IBI, quiescence, BA, and FA bouts were individually divided by total sleep bouts per neonate which provided an 'average frequency per sleep bout' measure for SM and arousal parameters.

$$\frac{\textit{Sum of Raw Event Frequency}}{\textit{Sum of Total Sleep Event Frequency}} = \textit{Average Frequency/Sleep Bout}$$

***2.4.1.3. Proportion Measure.*** Proportion of total coded duration spent on an event was obtained for behavioral state parameters in order to measure how much of the total time a neonate expressed a certain behavior. To obtain this measure, the duration sum of sleep, awakening, nursing intervention, transitioning, and indeterminate state was divided by total coded video duration in seconds individually for each neonatal sleep study.

$$\frac{\textit{Sum of Event Durations (Seconds)}}{\textit{Total Coded Video Duration (Seconds)}} = \textit{Proportion of Event Duration}$$

The proportion measure of brief arousal, full arousal, as well as SM parameters was derived by replacing the denominator of the above equation with the sum of total sleep duration in seconds.

$$\frac{\textit{Sum of Event Durations (Seconds)}}{\textit{Total Coded Sleep Duration (Seconds)}} = \textit{Proportion of Event Duration}$$

**2.4.2. Statistical Analysis.** Data was analyzed using IBM Statistical Package for the Social Sciences - Version 21 (IBM-SPSS-21) for Windows. Measures of maternal and infant demographic characteristics as well as maternal drug and alcohol use were analyzed for group differences in methadone and comparison by using independent sample t-tests for continuous variables and chi-square for categorical variables. Each state/behavior was categorized into average duration, frequency per hour/sleep bout, and proportion of total coded time/sleep time categories (e.g., average sleep bout duration, frequency of sleep per hour, and proportion of total sleep).

All behavioral state and SM measures were analyzed by using multivariate full factorial general linear mode (GLM). The independent variables were contrasted for difference. Factor interactions were displayed in means for overall interaction, between-factor interaction, as well as within-factor interactions. Main effects were computed with confidence interval adjusted to LSD at 5% significance level. Homogeneity of variance was analyzed using Levine's test. After performing an exploratory analysis using Pearson's correlations and independent sample t-tests, AA/binge was found to be

significantly different ($p = 0.006$) between the two exposure groups. Therefore AA/binge was added in the model as a covariate.

Hypothesis 1 was tested using the abovementioned model on behavioral state parameters (frequency and duration measures of sleep, wake, arousal, cry, nursing intervention, transition, and indeterminate state) and SM parameters (frequency and duration measures of SM, non-movement quiescence, and IBI).

For Hypothesis 2, allelic variants in the *COMT* gene were identified and divided into AA and AG/GG groups according to the dominant model (e.g., Wachman et al., 2013) and used as a two level factor in the multivariate full factorial GLM to test whether polymorphisms in the *COMT* gene would influence the expression of SM and behavioral states parameters.

# CHAPTER 3

# RESULTS

## 3.1. Demographic Characteristics

Table 3.1 shows the summary of total participants used per study for this thesis. The sleep study included 58 mother-infant dyads, of which 37 dyads were in the methadone-exposed group and 21 were in the comparison group. Of the 58, a subset of 50 (methadone-exposed=31, comparison=19) dyads comprised the sample for behavioral state analysis, 38 (methadone-exposed=25, comparison=13) for SM analysis, and 20 dyads (methadone-exposed =20) for the genetic study.

Table 3.1. Distribution of Sample

| | Methadone | | | Comparison | | | |
|---|---|---|---|---|---|---|---|
| Study Category | Day 1 | Day 2 | n | Day 1 | Day 2 | n | Total |
| Sleep Studies | 21 | 16 | 37 | 9 | 12 | 21 | 58 |
| Genetics Studies | 13 | 7 | 20 | n/a | n/a | n/a | 20 |
| Spontaneous Movements | 18 | 7 | 25 | 8 | 5 | 13 | 38 |
| Behavioral State Analysis | 18 | 13 | 31 | 8 | 11 | 19 | 50 |

Summary of the distribution of total sleep study cases across groups (i.e. methadone and comparison) and study categories (i.e. genetic studies, spontaneous movements, and behavioral state analysis).

The 58 dyads were further divided into infant age categories - day 1 and day 2 - based on neonatal age at the time of sleep recording. The methadone-exposed group had 21 neonates in the day 1 group and 16 neonates in the day 2 groups; and the comparison group had 9 neonates in the day 1 group and 12 neonates in the day 2 group. Altogether, this sample consisted of 30 one-day old neonates and 28 two-day olds.

**3.1.1. Maternal Demographics and Substance Use.** All mothers were assessed during the 3$^{rd}$ trimester of pregnancy for periconceptional (i.e., before the knowledge of

pregnancy) use of alcohol, tobacco and other substances of abuse. Mothers also provided lifetime use maximum estimates of substances of use.

Table 3.2. 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 3.2 shows group differences in maternal demographic characteristics that were obtained by using parametric measure (independent sample T-test) for the dependent variables or nonparametric measure (chi-square) for the independent variables as deemed appropriate. Maternal demographic characteristics such as mother's age at infant birth, socioeconomic status (SES), verbal IQ (PPVT-III), prenatal depression (BDI-II), global anxiety index (SCL-90-GSI), and number of cigarettes smoked per day during pregnancy were compared between groups. Results revealed that the mothers from both methadone-exposed and comparison groups were similar in age, SES, and PPVT-III, but significant group differences were observed for BDI-II [$t(54) = -2.29$; $p = 0.026$] and SCL-90R (GSI) [$t(54) = -3.14$; $p = 0.003$] measures. Most of the mothers across groups smoked cigarettes during pregnancy (mean number of cigarettes/day: comparison = 2.50; methadone-exposed = 9.20) but the methadone-exposed group mothers smoked

significantly more cigarettes per day than the comparison mothers [$t(55) = -4.15$; $p = 0.00$].

Table 3.3. Maternal Drug and Alcohol Use Pre-pregnancy

| | Comparison | | | Methadone | | | | |
|---|---|---|---|---|---|---|---|---|
| Pre-Pregnancy Alcohol Use | n | Mean | SD | n | Mean | SD | *t* | *p* |
| MAST score | 20 | 2.90 | 3.65 | 36 | 5.47 | 4.66 | -2.13 | 0.038 |
| TWEAK score | 20 | 2.55 | 2.63 | 36 | 3.89 | 2.76 | -1.77 | *ns* |
| T-ACE score | 20 | 1.40 | 1.50 | 36 | 2.06 | 1.72 | -1.43 | *ns* |
| AA/day (in ounces) | 20 | 1.94 | 2.29 | 36 | 4.00 | 4.52 | -1.50 | *ns* |
| AA/DD (in ounces) | 20 | 1.05 | 1.82 | 36 | 2.33 | 2.52 | -1.90 | *ns* |
| AA/Binge (in ounces) | 20 | 1.36 | 1.94 | 36 | 3.91 | 4.51 | -2.89 | 0.006 |
| Binge (# of times/week) | 20 | 5.94 | 4.59 | 29 | 7.59 | 5.50 | -2.19 | 0.033 |
| Lifetime Drug Use (freq/week) | | | | | | | | |
| Marijuana | 20 | 2.08 | 2.86 | 36 | 4.23 | 3.22 | -2.57 | 0.01 |
| Other Opiates | 20 | 1.88 | 2.87 | 36 | 6.58 | 1.23 | -6.98 | 0.00 |
| Cocaine | 20 | 0.01 | 0.06 | 36 | 1.90 | 2.90 | -3.91 | 0.00 |
| Sedatives | 20 | 0.48 | 1.63 | 36 | 2.93 | 3.09 | -3.89 | 0.00 |
| Antidepressants | 20 | 1.40 | 2.87 | 36 | 3.31 | 3.46 | -2.21 | 0.03 |

Pattern of periconceptional alcohol use and lifetime drug use showed overall higher score for methadone group with significant difference in all drug use measures. Alcohol use history measured in the MAST and frequency of binges per week pre-pregnancy were different based on group. MAST = Michigan Alcoholism Screening Test (historical and recent past alcohol abuse); TWEAK (index of alcohol withdrawal); T-ACE (index of alcohol tolerance); AA = absolute alcohol; DD = drinking day

The maternal sample was also compared for lifetime and periconceptional use of drugs and alcohol by applying QVF measures for assessing frequency of use/week, quantity of use each time and variability in the pattern of use per week. The maternal samples were compared for pre-pregnancy lifetime use of drug and periconceptional use of alcohol prior to the awareness of pregnancy and during pregnancy. The results, shown in Table 3.3, reveal no group differences in TWEAK, T-ACE, AA/day, and AA/DD, although the M group had non-significant but higher scores on these measures. The two groups differed significantly for the following periconceptional alcohol measures: MAST scores [$t(54) = -2.13$; $p = 0.038$], binge frequency per week [$t(54) = -2.19$; $p = 0.033$],

and AA/binge [$t(54) = -2.89$; $p = 0.006$]. Specifically, the methadone-exposed group scored higher than the comparison group on these measures. Mothers were also assessed for lifetime use of other licit and illicit drugs. Group differences were analyzed for cannabinoid use, other opioids besides methadone, cocaine, sedatives, and antidepressants because both groups had some history of using these particular substances out of all that were assessed. As shown in Table 3.3, the methadone-exposed group showed significantly higher lifetime use of other licit and illicit drugs than the comparison group [marijuana: $t(54) = -2.57$, $p = 0.014$; other opiates: $t(54) = -6.98$, $p = 0.000$; cocaine: $t(54) = -3.91$, $p = 0.000$; sedatives: t(54) = -3.89; $p = 0.000$; antidepressants: $t(54) = -2.21$; $p = 0.032$].

**3.1.2. Infant Demographics.** Table 3.4. shows neonatal health parameters such as length of hospital stay (LOS), gestational age (GA), birth weight (BW), and birth length (BL), head circumference at birth (HC), and sex for all babies as well as NAS treatment status for the methadone-exposed neonates only.

Table 3.4. Neonatal Characteristics

| | Methadone (n = 37) | | Comparison (n = 21) | | | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | $t/\chi^2$ | *p* |
| Gestational Age (Weeks) | 38.62 | 1.89 | 39.62 | 1.28 | 2.15 | 0.036* |
| Birth Weight (kg) | 3.14 | 0.60 | 3.46 | 0.42 | 2.16 | 0.035* |
| Birth Length (cm) | 50.44 | 3.13 | 51.36 | 4.19 | 0.94 | *ns* |
| Head Circumference (cm) | 33.89 | 1.71 | 34.26 | 1.19 | 0.88 | *ns* |
| Length of Hospital Stay (days) | 17.47 | 13.12 | 2.31 | 0.87 | -5.27 | 0.000** |
| Sex of the baby (% Male) | 57% | -- | 95% | -- | -- | -- |
| NAS Treatment (% Treated) | 40% | -- | n/a | -- | -- | -- |

Infant demographic characteristics were measured by Gestational Age, Birth Weight, Birth Length, Head Circumference, Length Of Hospital Stay, and Sex for all neonates; and NAS treatment rate for methadone-exposed neonates only. Neonatal outcomes differed only in Gestational Age and Birth Weight. * <0.05, ** <0.005

The results revealed overall higher scores for the comparison group in LOS [$t(55) = 5.27$; $p = 0.00$], GA [$t(55) = 2.16$; $p = 0.04$], and BW [$t(55) = 2.16$; $p = 0.04$]. LOS was expected to be significantly different between groups because while comparison group neonates go home by 2-3 days, methadone-exposed neonates must stay for 5 days minimum and the LOS may prolong if they experience NAS. Even though GA and BW were significantly different between the two groups, these variables did not have an effect on the neonatal behavioral state outcomes (>.05) in the analysis of covariance.

### 3.2. Sleep Study

The sleep study for this thesis included 58 mother-infant dyads. The data was analyzed for the frequency and duration of behavioral state categories (i.e., sleep, wake, cry, transition, nursing intervention, brief arousal, full arousal, and indeterminate) and primitive arousal categories (i.e., SM, quiescence, and inter-bout interval). Data was analyzed using multivariate full factorial general linear model (GLM) with exposure group and infant age (day 1 or 2) as independent variables to test Hypothesis 1 concerning behavioral state and quantitative arousal measures. As shown in Table 3.3, AA/binge was used as a covariate in this and all GLM analyses as it differed significantly between exposure groups in our sample and is an established teratogen. For Hypothesis 2, restricted to methadone-exposed neonates, the frequency and duration parameters of arousal and SM were examined using GLM model with the allelic variants of the *COMT* gene (using the dominant model of allelic variants AA, AG/GG) as the independent variable and AA/binge as the covariate.

**3.2.1. Hypothesis 1: Behavioral States.** It was expected in Hypothesis 1 that the methadone-exposed group neonates would show quantitative deficits in wake and arousal

regulation in relation to the Comparison group. Secondarily, it was hypothesized that infant's age on the day of recordings (day 1 or 2) would affect neonatal behavioral outcomes. The direction of deficit was expected to be greater for the methadone-exposed group on day 1 but increased on day 2 because of the fact that neonates with methadone exposure could be experiencing early signs of NAS on day 2 of life and they might be more prone to manifesting signs of sleep fragmentations. Hypothesis 1 included 50 (comparison = 19, methadone-exposed = 31) sleep studies for behavioral state analysis, one per neonate. Table 3.5 summarizes the results. Behavioral state analysis revealed significant findings for arousal and cry parameters using the GLM model described above. A main effect of exposure group was observed for the average duration of brief arousal [$F(1,44) = 4.601$, $p = 0.038$], as well as duration of the composite arousal measures [$F(1,44) = 5.262$, $p = 0.027$]. Post hoc analyses revealed that the methadone-exposed group showed significantly more arousability than the comparison group reflected in both brief and composite arousal measures ($p < 0.05$). A main effect of infant age at recording (day 1 or 2) was also found, showing increased frequency of brief arousals [$F(1,44) = 4.920$, $p = 0.032$], proportion of full arousals [$F(1,44) = 4.301$, $p = 0.044$], and frequency of full arousal [$F(1,44) = 4.476$, $p = 0.040$] on day 2 of life.

An interaction of exposure group and infant age was observed on the proportion of full arousal measure [$F(1,44) = 4.411$, $p = 0.041$], explained by the methadone-exposed group showing a dramatic increase in arousability on day 2 ($p<.05$), not observed in the Comparison group and interpreted as related to withdrawal.

Table 3.5. Infant Age, Exposure Group, and AA/Binge on Behavioral State Parameters

| | Main Effect | | |
|---|---|---|---|
| | Infant Age | Exposure Group | Age x Exposure |
| Arousal and Behavioral States | | | |
| Cry (proportion) | | | $F(1,44) = 9.293$, $p = 0.004$ |
| Cry (freq/hr) | | | $F(1,44) = 11.135$, $p = 0.002$ |
| BA (avg bout duration) | | $F(1,44) = 4.601$, $p = 0.038$ | |
| BA (ave freq/sleep event) | $F(1,44) = 4.920$, $p = 0.032$ | | |
| FA (proportion) | $F(1,44) = 4.301$, $p = 0.044$ | | $F(1,44) = 4.411$, $p = 0.041$ |
| FA (ave freq/sleep event) | $F(1,44) = 4.476$, $p = 0.040$ | | |
| Ave Composite Arousal Duration | | $F(1,44) = 5.262$, $p = 0.027$ | |

Frequency and duration measures for cry were relative to total duration of the coded video per neonate, and arousal measures were related to the total duration of sleep events. The results were derived from the multivariate full factorial general linear model with AA/binge as a covariate, and infant age and exposure group as independent variables (AA/binge: Mean = 2.7882). FA = Full Arousal, BA = Brief Arousal

For infant cry, an age by exposure group interaction was found for relative frequency [$F(1,44) = 11.135$, $p = 0.002$] and duration [$F(1,44) = 9.293$, $p = 0.004$] measures. These results showed that the methadone-exposed group had significantly higher sleep-related arousals ($p < 0.05$) as well as cry frequency ($p < 0.05$) and duration ($p < 0.05$) compared to comparison neonates. Interestingly, the direction of change in the duration and frequency of cry parameters was reversed in both methadone-exposed and comparison groups such that even though the methadone-exposed group had higher overall cry scores, comparison neonates were crying more on day 1 while methadone-exposed neonates showed an accelerated level of irritability characterized by increased cry on day 2, which supports the secondary hypothesis of this thesis study that methadone-exposed neonates are probably at risk for increased sleep fragmentation on

day 2 due to potentially compromised sleep regulatory systems in utero. Such hyperarousability in the methadone-exposed neonates may indicate that neonates were withdrawing earlier than 48 hours. All other behavioral state parameters were not significantly different between groups.

Since the methadone-exposed group showed an elevated rate of arousability and irritability on day 2 of life, the behavioral states outcomes were further analyzed for the methadone-exposed neonates only in lieu of genetic corollaries in an attempt to understand the mechanism of primitive arousals in depth. Additionally, the effect of genetics on the outcomes of SM measures was also explored.

**3.2.2. Hypothesis 2: Genetic Corollaries of Arousal and SM.** Neonatal SM and arousal parameters were explored further to test Hypothesis 2 with a prediction that polymorphism in the *COMT* gene would have a protective effect on neonates at the presentation of homeostatic challenges during sleep. The directionality of outcome was predicted to be higher for neonates with minor allelic variants (i.e., AG/GG) of the *COMT* SNP genotypes. Of the 20 exposed neonates that had genetics data, 19 had the SM data and 15 had the behavioral state data. Three in each of the behavioral states group and SM group neonates had the AA genotype; and 16 in SM group and 12 in the behavioral states group had the AG/GG genotypes. Analysis of full factorial multivariate GLM was performed with SM and arousal parameters as dependent variables and AA/binge as a covariate. The independent variable was the genotypes of *COMT* SNP gene that was divided into two groups: AA genotype as one group, and AG and GG genotypes collapsed into the second group. The genotypes in the second group are collapsed in order to

minimize the variability of sample distribution between groups as G allele is believed to occur less frequently in humans.

The results revealed a main effect of *COMT* alleles on SM duration [$F(1,16) = 6.978$, $p = 0.018$], quiescence duration [$F(1,16) = 8.706$, $p = 0.009$], and IBI duration [$F(1,16) = 10.048$, $p = 0.006$]. The directionality of effect was as predicted such that infants with the protective allelic variants (AG/GG) of the *COMT* SNP had significantly higher SM in relation to neonates with the AA genotype. In the behavioral state measures, a between-subject effect of *COMT* genotypes showed that neonates with AG/GG genotype had lower average composite arousal duration [$F(2,12) = 9.824$, $p = 0.003$], mean duration of full arousal [$F(2,12) = 6.373$, $p = 0.013$], mean cry duration [$F(2,12) = 9.383$), $p = 0.004$]; increased sleep frequency [$F(2,12) = 12.198$, $p = 0.001$] and mean duration [$F(2,12) = 4,784$, $p = 0.030$] and proportion of sleep time [$F(2,12) = 4.628$, $p = 0.032$]; increase in the frequency of awakening [$F(2,12) = 4.662$, $p = 0.032$], and decrease in the total awake time [$F(2,12) = 4.628$, $p = 0.032$] compared to neonates with AA genotype. These results are further discussed in chapter 4.

# CHAPTER 4

# DISCUSSION

Early markers of sleep disturbances indicate congenital neuronal deficits in the arousal regulatory systems in the brainstem region. One of the functions of the brainstem is cardiorespiratory regulation. Abnormality in this brain region as a consequence of prenatal alcohol-exposure has been associated with SIDS (Athanasia, Karavasiliadou, & Styliadis, 2011; Kinney, 2005). The purpose of this thesis study was to investigate whether prenatal exposure to methadone, and other potentially under-reported substances such as alcohol, could impact the neurodevelopmental integrity of a neonate that could result in poor sleep quality as well as a deficit in the sleep-related arousal regulatory systems. It was hypothesized that such deficits, if any, should manifest as early as 24 to 48 hours of life before the withdrawal from prenatal exposure. Finally, it was proposed as an exploratory hypothesis that by day 2, impaired sleep may be present in the methadone-exposed group due to the development of NAS. The role of a genetic contribution to sleep-wake, arousal, and SM was examined in hypothesis 2 in the methadone-exposed neonates only. The polymorphism in the *COMT* gene, which our lab has shown affects NAS severity, was found to also affect pre-withdrawal, neonatal sleep and sleep movements

## 4.1. Behavioral States, SM, and Genetic Corollaries

In the Hypothesis 1, the methadone-exposed neonates were expected to have arousal deficits in relation to the non-exposed comparison group neonates. We also explored the effect of infant age (day 1 or 2) in these outcomes with an expectation that the methadone-exposed neonates would show more sleep disruption, arousals, and cry

bout on day 2 of life. Additionally, in the Hypothesis 2, the influence of a polymorphism in the *COMT* gene on the sleep organization of the methadone-exposed neonates was explored with an expectation to see that the carriers of the minor allelic variants would have better sleep organization and robust SM compared to the carriers of the major allele/This hypothesis relates to the findings by Wachman et al., (2013) that showed better NAS neonatal outcomes for the carriers of the G allele.

**4.1.1. Outcome Measures, Infant Age, and Exposure Group**. One of the major findings of this study was that the methadone-exposed group had an overall higher level of arousability than the comparison group,. This result suggests that the sleep deficit is related to sleep fragmentation (i.e., increased arousals per sleep bout), as we found with alcohol-exposed infants (e.g., Hayes et al., 2007), rather than the deficit in arousal that is seen once sleep fragmentation has led to sleep deprivation (e.g., Troese et al., 2008). This association was influenced by infant age such that 2-day old neonates from the methadone-exposed group, but not the comparison group, were more arousable and irritable on day 2. The behavioral states of the comparison group remained relatively stable on both days.

The hypothesis about arousal deficit due to sleep deprivation was influenced by the findings from our previous studies as well as from the findings that revealed that the neonates who succumbed to SIDS were suffering from an arousal deficiency (e.g., Kato, Franco, Groswasser, Scaillet, Kelmanson et al., 2003), and that SIDS cases observed in alcohol exposed infants were associated with an abnormality in the brainstem serotonin system (Kinney et al., 2005), one of the functions of which is to regulate sleep and arousal. Even though arousal deficit was not observed in the present study, significant

signs of sleep disruption in the form of sleep fragmentation characterized by hyper-arousability and crying during the night was seen in the methadone-exposed group but not in the comparison group. Sleep fragmentation is the precedence for sleep deprivation and arousal deficits as a function of sleep debt over time (Hayes et al., 2007).

The level of arousals showed main effects of group in which the methadone-exposed group was higher than the comparison group, and age, in which both groups were higher on day 2. A significant interaction of group and age was also found that showed that the arousal measures were similar across days for the comparison group, but elevated for the methadone-exposed neonates on day 2. These findings do not clearly support a pre-withdrawal brain injury in the methadone-exposed neonates. Nonetheless, sleep fragmentation is clearly present in the methadone-exposed neonates by day 2 of life. Hence, early in the post birth period, sleep fragmentation sets the conditions for sleep deprivation as the methadone-exposed infants enter the withdrawal period after day 2. The prediction of arousal deficit in the methadone-exposed neonates at birth was not found. However, the findings from this study do indicate that sleep fragmentation in the methadone-exposed neonates is evident as a main effect of group. In early infancy, the excessive propensity to arouse from sleep has been associated with poor sleep quality (e.g., sleep disruptions and insomnia), which is known to impair daytime alertness and performance in adults (Franco et al., 2010). Therefore, it is predicted that excessive arousals due to sleep fragmentation in the methadone-exposed neonates will be exacerbated through the withdrawal period, and the amount of arousals would likely decrease to below the level of comparison neonates due to cumulative sleep debt during the post withdrawal period.

Hyperarousability and irritability that are generally observed in the NAS neonates during NNNS assessments (Jones et al., 2010), our observation of a comparatively higher rate of arousal and cry in the methadone-exposed group ($p<.05$) on day 2 of life may have also resulted from the fact that withdrawal symptomatology for exposed neonates may be surfacing earlier than expected by the physicians and nurses as reflected in the standard NICU protocol which states that, on average, neonates take about 24 to 72 hours to withdraw from the pre-delivery maternal dose of methadone (Hudak et al., 2012; Logan et al., 2013). If this is the case, it provides evidence that neonates are suffering withdrawal much earlier than is evident in the Finnegan withdrawal scoring method. The methadone-exposed neonates may be challenged in terms of neurodevelopmental adaptation to the environment for basic functions besides sleep such as feeding and social interactions with the mother that is believed to begin immediately after birth (Fifer, Byrdd, Kakua, Eigsti, Islerb et al., 2010). Fifer et al. (2010) suggest that neonates must adjust their physiology and behavior to the specific demands of the novel postnatal environment as fast as possible in order to insure survival. When neonatal sleep is interrupted by excessive arousals and cry, the amount of restorative sleep is consequently shortened leading to a compromised stress regulatory system. The hyperarousability and irritability during the night-time sleep period observed in the methadone-exposed neonates in the present study suggest that neonatal withdrawal may be evident by using sleep quality as an important marker of withdrawal distress. Currently, sleep is one of the ten markers of withdrawal, as is crying, but is a minor contributor to the assessment of withdrawal severity in the Finnegan score. Earlier pharmacological intervention may be afforded if sleep quality is seen as a marker for developing withdrawal. In future

analyses, the relationship between Finnegan scores and sleep fragmentation for individual exposed neonates in this study will be examined.

**4.1.2. Maternal Psychiatric Health.** Mothers in the methadone-exposed group had significantly elevated psychiatric symptoms as indicated by the scores on depression and anxiety measures during the third trimester ($p < .05$), which is known to compromise the hypothalamic-pituitary-adrenal (HPA) axis of the fetus over time and potentially permanently affect stress regulation in their offspring (Oberlander, Warburton, Misri, Aghajanian, & Hertzman, 2006). Lifelong comorbidity of lower SES, addiction, depression, and anxiety is well documented. In this study sample, it is possible that the mother who suffered opiate addiction may have also suffered from elevated depression and anxiety throughout the pregnancy and even before pregnancy; and the impact of maternal psychiatric disease may have created an adverse epigenetic influence on their offspring (Hein, Rauh, Engel, Häberle, Dammer, Voigt et al., 2014). Fetal exposure to the combination of pharmacological and psychological stressors prenatally may compromise the child's long-term ability to modulate stress, and may affect adaptation over time. So, it is possible that neonates were born exposed to excessive maternal stress through psychiatric disease, maternal methadone, and other illicit substances, which are less often seen in women in treatment, but are more common than in non-exposed comparison infants.

**4.1.3. *COMT* SNPS Genotype and Sleep Organization.** The results for genetic corollaries of SM and behavioral state parameters revealed that the carriers of protective G allele (e.g., AG/GG genotypes) had comparatively more robust SM, less arousal and cry, and increased sleep frequency and duration compared to neonates with the carriers of

risk allele (e.g., AA genotype) indicating that the presence of G allele, previously shown to be responsible for shorter NAS (e.g., Wachman et al., 2013), may also serve as a protective factor against sleep disruptions.

Similar findings were observed in another study conducted by Gruber, Grizenko, Ben, Gauthier, de Guzman, and Joober (2006) who investigated whether underlying mechanism of sleep would be modulated by SNPs of the *COMT* gene of sleep-disordered children. Thirty-four children aged 7-12 years who had a clinical diagnosis of ADHD were assessed using actigraphic recordings. The results revealed that the children who were carriers of the AA genotype showed poorer sleep regulation than those who carried the AG/GG genotypes indicating that mutation in the *COMT* gene may influence pediatric sleep organization as was observed in our study. These findings may provide a partial explanation for the underlying mechanisms that may influence sleep state organization of the methadone-exposed neonates in the present study.

COMT is an enzyme that deactivates dopamine, epinephrine, and norepinephrine post-synaptically. Perhaps, the polymorphism in the gene producing this enzyme may result in a decrease in the enzyme action on these catecholamines, thus, allowing excess neurotransmitters to be available. Such increased availability of dopamine and epinephrine may provide some advantage against neonatal opiate withdrawal in neonates and mitigate the severity of NAS symptoms. For example, Wichers, Aguilera, Kenis, Krabbendam, Myin-Germeys, Jacobs et al. (2007) studied 621 women to assess an association between the subjective wellbeing and the polymorphism in the *COMT* gene. They found that the ability of the participants to experience reward increased with the number of G (Met) alleles. More interestingly, individuals with the val/val (AA) genotype

experienced similar amounts of subjective well-being from a ‘very pleasant event’ as met/met (AG/GG) individuals did from a ‘bit pleasant event’ indicating that the carriers of AA genotype may have a diminished sense of well-being or perhaps the AG/GG carriers may have an enhanced sensitivity to feel well. The findings suggest that the SNP in the *COMT* gene may create a neurobiological environment that is more suitable for producing positive affect as well as an increased ability to cope with stress. Future work on the role of *COMT* SNPs in NAS is ongoing in our laboratory, but the sleep health advantage in sleep continuity and robust sleep movements found for G carriers in my study expands our understanding of the impact of polymorphisms in genes of addiction in newborn withdrawal.

**4.1.4. Perinatal Alcohol Effect.** All significant outcomes observed in this study were present in covariance with the total alcohol content that the mother used per binge cycle before pregnancy (i.e., AA/binge). It is well known that alcohol is teratogenic (Guo et al., 2010). Despite all the known fetal adversities, moderate consumption of alcohol has recently been argued as safe during pregnancy in a large epidemiological study (Humphriss, Hall, May, Zuccolo, & Macleod, 2013). Nonetheless, in an investigation of moderate alcohol exposure on gene expression, Rosenberg, Wolff, El-Emawy, Staples, Perrone-Bizzozero, and Savage (2010) conducted micro-array analysis of more than 28,000 genes in alcohol-exposed pups and found that the expression of 304 known genes was altered two-fold or greater in the placenta from ethanol-consuming dams compared with controls and about 76% of these genes were repressed in ethanol-exposed placentas. The observed gene expression changes involved proteins associated with central nervous system development, organ morphogenesis, immunological responses, endocrine

function, ion homeostasis, and cardiovascular development, suggesting that even small amounts of alcohol, that is most likely to go unreported (Baña et al., 2010; Hayes et al., 2002), may alter the expression of a large number of placental mRNAs. Methadone-dependent mothers are suffering from addiction and typically have a history of higher alcohol intake than mothers who are not diagnosed with addiction. Hence, it is likely that the mothers with opioid addiction and history of alcohol use may exert a compromised epigenetic effect on their fetus by altering the ways in which their fetuses receive methadone.

**4.1.5. Primary Implications**. Considering the significance of a burden NAS poses on neonates, parents, care-providers, as well as policy-makers, a proper pharmacological and clinical management of this condition is crucial. This thesis study was conducted to examine pre-withdrawal status of sleep and arousal systems in a controlled study. In the course of that effort, sleep fragmentation was found to be greater in methadone-exposed infants, and that this impact was due to the likely emergence of withdrawal on day 2. Further, amongst methadone-exposed neonate, polymorphisms in the *COMT* gene were critical in predicting the level and severity of sleep fragmentation. It is believed that the sleep-wake results are valuable in this regard because the sleep parameters are likely early neurobehavioral markers of NAS that have not been reported to date. Care-providers at the pediatric care facilities as well as the parents of such neonates can benefit from such information by increasing their awareness of the at-risk neonates' behavioral patterns during sleep and by taking extra caution while observing their neonates. Additionally, the duration and intensity of cry, arousals, and SM patterns

observed during the pre-withdrawal period can be utilized in developing a protocol for individualized treatment plan of the methadone-exposed neonates.

Since the results found in this thesis were present in covariance with pre-pregnancy consumption of alcohol (particularly binge), an accelerated effort to detect a possible exposure of alcohol during pregnancy seems important. Similarly, a sustainable support system for psychiatric conditions such as depression and anxiety particularly among opioid dependent pregnant mothers could reduce the harm of methadone exposure to some extent by allowing the developing fetus a relatively less adverse environment. Finally, it would also be useful to identify additional genetic corollaries of NAS that may be specific to certain symptoms, such as gastrointestinal, disrupted sleep continuity, and other physiological as well as psychobiological symptoms to better predict the course of NAS illness for the individual infant.

In relation to the outcomes of the comparison group, it is important to note that the neonates in this group are considered high-risk as well because of their mothers' low SES and other maternal risk factors associated with poverty such as tobacco exposure and maternal use of other substances such as marijuana. These risk factors influence prenatal as well as postnatal outcomes of these neonates. Hence, the differences and similarities observed in the outcomes of the comparison group are generalizable to a public health population where health insurance is typically public, but are less generalizable to a community sample inclusive of all income levels. Normative samples have been studied by our group, which has established benchmarks for sleep parameters including deficits in arousal and SM in typical neonates (Hayes et al., 1993, 1994, 1998, 2007) that serve as a comparison for future work. The findings for the comparison group in the present study

are comparable to our work with normative samples. The sleep-wake organization findings with the methadone-exposed neonates are similar to other high-risk groups with prematurity, alcohol or other prenatal exposures, with the exception of the identification in the opiate sample of early opiate withdrawal on day 2.

### 4.2. Methodological Strengths and Limitations

The biggest strength of this thesis study is that it is the first human study assessing arousals and SM of the methadone-exposed newborns as early as day 1 or 2 of life. Another strength of this study is the identification of genetic corollaries that provides new insight into risk and protective SNPs for sleep integrity in the early postnatal period. Research in clinical samples presents significant risk of confounding factors as alternative explanations for group findings. In order to minimize confounding influences, the demographic characteristics of both methadone-exposed and non-exposed comparison groups were matched as closely as possible through standardization of recruitment sites and strategies across exposure groups, careful interview for psychiatric and substance abuse patterns including urinalysis for all participants and similarities in age, SES and mode of insurance. This resulted in the methadone-exposed group and non-exposed comparison group having common backgrounds with the exception of opioid dependence and the treatment with methadone. As a result, we were able to significantly reduce the confounding factors such as maternal age, education, SES, ethnicity. Nonetheless, important differences existed in periconceptual alcohol binge history, lifetime drug and alcohol history, and psychiatric health during the pregnancy, discussed in more detail below. Neonatal outcomes were differences in gestational age and birth weight but not in other growth parameters.

There were significant group differences in maternal characteristics such as depression and anxiety as measured by BDI-II and SCL-90R, and tobacco consumption, which, despite my and our lab's efforts, were not controlled in this study due to sample size and could not be added to model development. With regard to prenatal alcohol measures, several alcohol measures were significantly different between the methadone-exposed and comparison groups. However, absolute alcohol per binge episode "before the knowledge of pregnancy" was used as the alcohol covariate because it showed a stronger association with exposure group. Therefore, the general linear model proposed for this study was constrained to covary alcohol exposure because of our earlier work, but future modeling may improve the solution, particularly for maternal depression and anxiety markers as described above. Another noted methodological limitation of this study was the inability of the examiner to control the quality of overnight neonatal video data, which resulted in variability in the total coded time across the sample thus warranting adjustment for lost data. Neonates were moved away from the camera frequently, and the camera was occasionally turned off accidentally at night. Additionally, babies were not facing the camera while sleeping in some cases, and this made it more difficult to determine the behavioral states of these neonates.

Furthermore, despite a continuous effort of the investigator to train the coders over several months in depth, it is also to be noted that the measures used to code the videos for both behavioral states and SM parameters still require additional confirmation through intra-class correlation. Significant computational effort was applied to automate and streamline data collection accuracy to reduce error but there may still be some

undetected inter-coder biases that may have influenced the results. For future work, these potential biases must be assessed.

In regard to the outcomes pertaining to arousals and cry during sleep, one possible explanation for such high arousability in the methadone-exposed group may be the fact that cry events within sleep that met the duration criteria for arousals were also calculated as arousals besides separately being calculated for cry events. Crying has similar underlying physiology as wake and arousals, and is thought of as a sub-state of wake except it involves higher-than-normal acoustic characteristics (LaGasse et al., 2005). Therefore, it was justified that neonatal cry occurring within a sleep state should be considered an arousal or a wake event depending on the duration. However, since the methadone-exposed group had higher cry bouts and, hence, appeared more aroused. Future work will further examine the duration measures of arousal in relation to cry features, which have been found to be disrupted in cocaine-exposed newborns (Zeskind, & Lester, 2001).

Sample distribution across groups was another limitation of this study. Even though the project included a total of 58 participants with sleep studies, the sample was not evenly distributed for the methadone-exposed (n=37) and comparison (n=21) groups. Our lab often has more exposed infant sleep studies as comparison infants are more difficult to recruit and typically healthier so have less inter-individual differences than exposed samples. Also, for hypothesis 2, the genetic study was small with only 20 participants, of which only 4 were the carriers of AA genotype, and may have impacted the outcomes. Even though the ration of allelic distribution was similar to the distribution of Wachman et al. (2013), the statistical method they used was more robust due to sample

size. I have more genetic and sleep study participants in process that I will be able to add to my dataset in the future. It is also important to note that the infant age in this study was cross-sectional, and the differences in outcomes observed on day 1 and 2 included different neonates in each group. Therefore, the results may have been influenced by individual differences of neonates in each group. Longitudinal assessment of sleep is suggested for the future studies.

The group differences in maternal depression and anxiety were not included due to power constraints, and represent another limitation of this study. The association of depression and anxiety to sleep disruption is well documented, and maternal depression during pregnancy has been associated with neonatal sleep organization (Field, Diego, Hernandez-Reif, Figueiredo, Schanberg, & Kuhn, 2007). In their study, Field et al. (2007) assessed 253 women for prenatal depression and sleep disturbances during the 2nd and 3rd trimester, and observed their newborns during sleep. The depressed women reported more sleep disturbances, higher anxiety, and elevated anger as well as elevated norepinephrine and cortisol levels during both trimesters. The newborns of the depressed mothers showed lowered deep sleep, more time in indeterminate (disorganized) sleep, were more active and cried/fussed more. These symptoms indicate disrupted sleep that may eventually lead to sleep deprivation. Given that these findings are similar to what was observed in our results, and that our methadone-exposed group participants had significantly higher depression and anxiety, it is possible that controlling for the depression and anxiety measures may influence the outcomes of this study and need to be included in future analysis.

Gender of the neonates was another major factor that was not included in the final analysis of this study. In this sample, 57% of in the methadone-exposed group and 95% in the comparison group consisted of males. The gender difference in the comparison group was significantly different (p=< .005). Given the findings of this study pertains to SIDS risk, it is important to note that SIDS is more prevalent in males with consistent findings of 50% male excess (Mage & Donner, 2014). Additionally, males and females have different overall physiological profiles that may play a role in the expression of the behavioral measures assessed in this study. For example, Kraemer (2000) summarizes in his review why males are more at risk than female counterparts from as early as conception. According to his review, "fragile males" are more vulnerable than females even from conception, and male mortality is greater than female mortality throughout life. Therefore, gender difference in the exposed neonates is an important uncontrolled variable that may provide important results for the model of prenatal injury, SIDS risk and sleep development proposed in my study.

Lastly, it is worth noting that the genetic corollaries of behavioral states and SM were only available and assessed for the methadone-exposed group. The comparison group genetic data, if available, could have provided us with an opportunity to assess an interaction between the study groups and genetic variants in predicting the outcomes of interest, thus broadening our understanding of an underlying genetic factors affecting sleep organization.

### 4.3. Conclusion

This thesis study investigated the hypothesis that prenatal methadone exposure may compromise neurodevelopment that may be indexed by analyzing sleep integrity and

arousal properties during the pre-withdrawal period. Excessive increase in the arousability and cry as well as a deficit in the average duration of SM measure were observed in the methadone-exposed neonates, Additionally, the severity of such deficit was further enhanced by genetic factors such that the exposed neonates carrying minor G allele of the *COMT* gene, who are known to experience less severe NAS, showed comparatively less arousability and irritability, more robust SM, and longer duration of uninterrupted sleep than neonates without a G allele. Based on these observations, it is hypothesized that the methadone-exposed neonates show poorer sleep integrity characterized by sleep fragmentation, and show this difference from non-exposed, comparison infants on day 2 of life. Hence, methadone-exposed neonates may be experiencing sleep fragmentation related to opiate withdrawal earlier than 48 hours of life. Finnegan scores measure stress abstinence, e.g., tremors, sweating, and diarrhea, which generally not elevated during the sleep studies. Sleep fragmentation may represent an early marker of NAS and may similarly adversely affect neonatal brain development if not treated promptly. Therefore, to avoid such adversities, appropriate individually modified treatment and management of NAS is recommended to reduce the potential harm of prenatal methadone exposure. Disrupted sleep evidenced in this study may also indicate a neurodevelopmental anomaly in the arousal regulatory system modulated primarily by the medulla oblongata in the brainstem. The deficit in this area and an altered arousal regulation have been linked to SIDS, thus, indicating that the reported SIDS risk associated with prenatal exposure to opiates may be reflected in sleep fragmentation in the neonatal period that is also associated with prenatal alcohol exposure and the emergence of NAS.

## REFERENCES


Athanasia, E., Karavasiliadou, S., & Styliadis, I. (2011). The factors contributing to the risk of sudden infant death syndrome. *Hippokratia*, *15*(2), 127.

Barr, G. A., Zmitrovich, A., Hamowy, A. S., Liu, P. R., Wang, S., & Hutchings, D. E. (1998). Neonatal withdrawal following pre- and postnatal exposure to methadone in the rat. *Pharmacology, Biochemistry and Behavior*, *60*(1), 97-104. doi:10.1016/S0091-3057(97)00596-0

Beck, A. T., Steer, R. A., & Brown, G. K. (1996). *Beck Depression Inventory manual*. 2nd edition. The Psychological Corporation, San Antonio, TX.

Brooks M. (2014). Grim stats for opioid-related deaths, prescribing in US. *Medscape Medical News* [serial online]. Retrieved July 16, 2014 from http://www.medscape.com/viewarticle/827741.

Burns, L., Conroy, E., & Mattick, R. P. (2010). Infant mortality among women on a methadone program during pregnancy. Drug and Alcohol Review, *29*(5), 551-556.

Chan, A. W. K., Pristach, E. A., Welte, J. W., & Russell, M. (1993). Use of the TWEAK test in screening for alcoholism/heavy drinking in three populations. *Alcoholism: Clinical and Experimental Research, 17*(6), 1188-1192.

Chen, J., Lipska, B. K., Halim, N., Ma, Q. D., Matsumoto, M., Melhem, S., et al. (2004). Functional Analysis of Genetic Variation in Catechol-O-Methyltransferase (COMT): Effects on mRNA, Protein, and Enzyme Activity in Postmortem Human Brain. *The American Journal of Human Genetics*, *75*(5), 807-821.

Conradt, E., Sheinkopf, S. J., Lester, B. M., Tronick, E., LaGasse, L. L., Shankaran, S., ... & Hammond, J. A. (2013). Prenatal substance exposure: neurobiologic organization at 1 month. *The Journal of Pediatrics*, *163*(4), 989-94.

Coyle, M. G., Salisbury, A. L., Lester, B. M., Jones, H. E., Lin, H., Graf-Rohrmeister, K., & Fischer, G. (2012). Neonatal neurobehavior effects following buprenorphine versus methadone exposure. *Addiction, 107*(S1), 63-73.

de Benedictis, T., Larson, H., Kemp, G., Barston, S., & Segal, R. (2007). *Understanding Sleep, Sleep Needs, Cycles, and Stages*. Retrieved May 5, 2014 from http://www.helpguide.org/life/sleeping.htm

DiPietro, J. A. (2001). Fetal neurobehavioral assessment. In L. Singer, P. Zeskind (Eds.), *Biobehavioral assessment of the infant* (pp. 43-80). New York, NY: Guilford Press.

Dunn, L. M., & Dunn, L. M. (1997). Examiner's manual for the PPVT-III Peabody picture vocabulary test: Form IIIA and Form IIIB. AGS.

Dunn, K. E., Sigmon, S. C., Reimann, E. F., Badger, G. J., Heil, S. H., & Higgins, S. T. (2010). A contingency-management intervention to promote initial smoking cessation among opioid-maintained patients. *Experimental and Clinical Psychopharmacology, 18*(1), 37.

Field, T., Diego, M., Hernandez-Reif, M., Figueiredo, B., Schanberg, S., & Kuhn, C. (2007). Sleep disturbances in depressed pregnant women and their newborns. *Infant Behavior and Development*, *30*(1), 127-133.

Fifer, W. P., Fingers, S. T., Youngman, M., Gomez-Gribben, E. & Myers, M. M.(2009). Effects of alcohol and smoking during pregnancy on infant autonomic control. *Developmental Psychobiology, 51*(3), 232-242.

Fine, P. G. (2004). Opioid insights: Opioid-induced hyperalgesia and opioid rotation. *Journal of Pain and Palliative Care Pharmacotherapy, 18*(3), 75-79.

Finnegan, L. P., Connaughton Jr., J. F., Kron, R. E., & Emich, J. P. (1975). Neonatal abstinence syndrome: Assessment and management. *Addictive Diseases, 2*(1-2), 141-158.

Finnegan LP, Kaltenbach K. (1992). Neonatal abstinence syndrome. In Hoekelman RA, Friedman SB, Nelson NM, et al., (2nd ed.), *Primary Pediatric Care* (1367-78). St. Louis: Mosby.

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 deprivation. *Pediatrics*, *114*(2), e192-e197.

Goh, Y., Hutson, J. R., Lum, L., Roukema, H., Gareri, J., Lynn, H., & Koren, G. (2010). Rates of fetal alcohol exposure among newborns in a high-risk obstetric unit. *Alcohol, 44*(7-8), 629-634. doi:10.1016/j.alcohol.2010.02.008

Green, C. R., Munoz, D. P., Nikkel, S. M., & Reynolds, J. N. (2007). Deficits in eye movement control in children with fetal alcohol spectrum disorders. *Alcoholism: Clinical and Experimental Research, 31*(3), 500-511. doi: 10.1111/j.1530-0277.2006.00335.x

Grossman, M. H., Emanuel, B. S., & Budarf, M. L. (1992). Chromosomal mapping of the human catechol-O-methyltransferase gene to 22q11. 1→ q11. 2. *Genomics*, *12*(4), 822-825.

Gruber, R., Grizenko, N., Schwartz, G., Ben Amor, L., Gauthier, J., de GUZMAN, R. O. S. H. E. R. R. I. E., & Joober, R. (2006). Sleep and COMT polymorphism in ADHD children: preliminary actigraphic data. *Journal of the American Academy of Child & Adolescent Psychiatry*, *45*(8), 982-989.

Guo, H., Enters, E., McDowell, K., & Robinson, S. (1990). The effect of prenatal exposure to methadone on neurotransmitters in neonatal rats. *Developmental Brain Research, 57*(2), 296-298.

Habeck, H., Odenthal, J., Walderich, B., Maischein, H. M., & Schulte-Merker, S. (2002). Analysis of a zebrafish VEGF receptor mutant reveals specific disruption of angiogenesis. *Current Biology, 12*(16), 1405-1412.

Hamilton, R., McGlone, L., MacKinnon, J. R., Russell, H. C., Bradnam, M. S., & Mactier, H. (2010). Ophthalmic, clinical and visual electrophysiological findings in children born to mothers prescribed substitute methadone in pregnancy. *British Journal of Ophthalmology, 94*(6), 696-700.

Harper, R. M., Kinney, H. C., Fleming, P. J., & Thach, B. T. (2000). Sleep influences on homeostatic functions: implications for sudden infant death syndrome. *Respiration Physiology, 119*(2), 123-132.

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

Hayes, M. J., Akilesh, M. R., Fukumizu, M., Gilles, A. A., Sallinen, B. A., Troese, M., & Paul, J. A. (2007). Apneic preterms and methylxanthines: Arousal deficits, sleep fragmentation and suppressed spontaneous movements. *Journal of Perinatology, 27*(12), 782-789.

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.

Hayes, M.J., Brown, E., Hofmaster, P.A., Davare, A.A., Parker, K.G., & Raczek, J.A. (2002) Prenatal alcohol intake in a rural, Caucasian clinic. *Family Medicine,* 34:120-125Hu, S., Sheng, W. S., Lokensgard, J. R., & Peterson, P. K. (2002). Morphine induces apoptosis of human microglia and neurons. *Neuropharmacology, 42*(6), 829-836.

Hayes, M. J., & Mitchell, D. (1998). Spontaneous movements during sleep in children: Temporal organization and changes with age. *Developmental Psychobiology*, *32*(1), 13-21.

Hayes, M. J., Plante, L. S., Fielding, B. A., Kumar, S. P., & Delivoria-Papadopolos, M. (1994). Functional analysis of spontaneous movements in preterm infants. *Developmental Psychobiology*, *27*(5), 271-287.

Hayes, M. J., Plante, L., Kumar, S. P., & Delivoria-Papadopoulos, M. (1993). Spontaneous motility in premature infants: Features of behavioral activity and rhythmic organization. *Developmental Psychobiology*, *26*(5), 279-291.

Hein, A., Rauh, C., Engel, A., Häberle, L., Dammer, U., Voigt, F., et al. (2014). Socioeconomic status and depression during and after pregnancy in the Franconian Maternal Health Evaluation Studies (FRAMES). *Archives of Gynecology and Obstetrics*, *289*(4), 755-763.

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.

Humphriss, R., Hall, A., May, M., Zuccolo, L., & Macleod, J. (2013). Prenatal alcohol exposure and childhood balance ability: findings from a UK birth cohort study. *BMJ open*, *3*(6).

Hutchings, D. E., Zmitrovich, A., Church, S., & Malowany, D. (1992). Methadone during pregnancy: The search for a valid animal model. *Annali dell'Istituto superiore di sanità, 29*(3), 439-444.

Jacobson, S. W., Jacobson, J. L., Sokol, R. J., Martier, S. S., Ager, J. W. (1993). Prenatal alcohol exposure and information processing ability. *Child Development*, 64, 706-1721.

Jansson, L. M., Di Pietro, J. A., Elko, A. A., Williams, E. L., Milio, L. L., & Velez, M. M. (2012). Pregnancies exposed to methadone, methadone and other illicit substances, and poly-drugs without methadone: A comparison of fetal neurobehavior and infant outcomes. *Drug and Alcohol Dependence, 122*(3), 213-219. doi:10.1016/j.drugalcdep.2011.10.003

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., Jansson, L. M., O'Grady, K. E., & Kaltenbach, K. (2013). The relationship between maternal methadone dose at delivery and neonatal outcome: Methodological and design considerations. *Neurotoxicology and Teratology*, *39*, 110-115. doi:10.1016/j.ntt.2013.05.003

Jones, D. K., & Leemans, A. (2011). Diffusion tensor imaging. In *Magnetic resonance neuroimaging* (pp. 127-144). Humana Press.

Jones, H. E., Kaltenbach, K., Heil, S. H., Stine, S. M., Coyle, M. G., Arria, A. M., et al. (2010). Neonatal abstinence syndrome after methadone or buprenorphine exposure. *New England Journal of Medicine, 363*(24), 2320-2331.

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*(4), 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), 1298-1303.

Katz, N., & Fanciullo, G. J. (2002). Role of urine toxicology testing in the management of chronic opioid therapy. *The Clinical Journal of Pain*, *18*(4), 76-82.

Kinney, H. C. (2005). Abnormalities of the brainstem serotonergic system in the sudden infant death syndrome: A review. *Pediatrics Developmental Pathology, 8*(5), 507-24.

Kinney, H. C., Randall, L. L., Sleeper, L. A., Willinger, M., Belliveau, R. A., Zec, N., et al. (2003). Serotonergic brainstem abnormalities in Northern Plains Indians with the sudden infant death syndrome. *Journal of Neuropathology & Experimental Neurology, 62*(11), 1178-1191.

Kraemer, S. (2000). The fragile male. *BMJ*, *321*(7276), 1609-1612.

Lachman, H. M., Morrow, B., Shprintzen, R., Veit, S., Parsia, S. S., Faedda, et al. (1996). Association of codon 108/158 catechol‐O‐methyltransferase gene polymorphism with the psychiatric manifestations of velo‐cardio‐facial syndrome. *American Journal of Medical Genetics*, *67*(5), 468-472.

LaGasse, L. L., Neal, A. R., & Lester, B. M. (2005). Assessment of infant cry: Acoustic cry analysis and parental perception. *Mental Retardation and Developmental Disabilities Research Reviews, 11*(1), 83-93.

Lester, B. M., & Tronick, E. (2004). *NICU Network Neurobehavioral Scale (NNNS) manual*. Paul H Brookes Pub CO

Liu, A. J., Jones, M. P., Murray, H., COOK, C. M., & Nanan, R. (2010). Perinatal risk factors for the neonatal abstinence syndrome in infants born to women on methadone maintenance therapy. *Australian and New Zealand Journal of Obstetrics and Gynaecology*, *50*(3), 253-258.

Logan, B. A., Brown, M. S., & Hayes, M. J. (2013). Neonatal abstinence syndrome: Treatment and pediatric outcomes. *Clinical Obstetrics and Gynecology, 56*(1), 186-192.

Logan, B. A., Hayes, M. J., Brown, M. S., Tisher, P., Paul, J. A., & Krishnan, R. (2009). Maine's high-risk infants and maternal health and wellbeing: The Maine infant follow-up project. *Maine Policy Review, 18*(1), 60-67.

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I., & Taskinen, J. (1995). Kinetics of human soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry*, *34*(13), 4202-4210.

Lugo, R. A., Satterfield, K. L., & Kern, S. E. (2005). Pharmacokinetics of methadone. *Journal of Pain and Palliative Care Pharmacotherapy, 19*(4), 13-24.

Mage, D. T., & Donner, E. M. (2014). Is excess male infant mortality from sudden infant death syndrome and other respiratory diseases X‐linked?. *Acta Paediatrica*, *103*(2), 188-193.

Matsumoto, M., Weickert, C. S., Akil, M., Lipska, B. K., Hyde, T. M., Herman, M. M., et al. (2003). Catechol-O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. *Neuroscience, 116*(1), 127-137.

McCarley, R. W. (2007). Neurobiology of REM and NREM sleep. *Sleep Medicine, 8*(4), 302-330.

McGlone, L., Hamilton, R., McCulloch, D. L., Boulton, R., Bradnam, M. S., Weaver, L. T., & Mactier, H. (2013). Neonatal visual evoked potentials in infants born to mothers prescribed methadone. *Pediatrics, 131*(3), e857-e863.

Mental Health Services Administration (2012). Results from the 2011 national survey on drug use and health: summary of national findings. *NSDUH Series H-44, HHS Publication No (SMA)*, 12-4713.

Messinger, D. S., Bauer, C. R., Das, A., Seifer, R., Lester, B. M., Lagasse, L. L., et al. (2004). The Maternal Lifestyle Study: Cognitive, motor, and behavioral outcomes of cocaine-exposed and opiate-exposed infants through three years of age. *Pediatrics, 113*(6), 1677-1685.

Metcalf, D. R., Mondale, J., & Butler, F. K. (1971). Ontogenesis of spontaneous k-complexes. *Psychophysiology*, *8*(3), 340-347.

Mirmiran, M., Maas, Y. G., & Ariagno, R. L. (2003). Development of fetal and neonatal sleep and circadian rhythms. *Sleep Medicine Reviews, 7*(4), 321-334.

Nanovskaya, T. N., Nekhayeva, I. A., Hankins, G. D., & Ahmed, M. S. (2008). Transfer of methadone across the dually perfused preterm human placental lobule. *American Journal of Obstetrics and Gynecology, 198*(1), 126-e1.

Nanovskaya, T., Nekhayeva, I., Karunaratne, N., Audus, K., Hankins, G. D., & Ahmed, M. S. (2005). Role of P-glycoprotein in transplacental transfer of methadone. *Biochemical Pharmacology, 69*(12), 1869-1878.

Negrato, C. A., & Gomes, M. B. (2013). Low birth weight: causes and consequences. *Diabetoogyl Metabolic Syndrome*, *5*(1), 49-57.

Oberlander, T. F., Warburton, W., Misri, S., Aghajanian, J., & Hertzman, C. (2006). Neonatal outcomes after prenatal exposure to selective serotonin reuptake inhibitor antidepressants and maternal depression using population-based linked health data. *Archives of General Psychiatry*, *63*(8), 898-906.

O'Brien, C. M., & Jeffery, H. E. (2002). Sleep deprivation, disorganization and fragmentation during opiate withdrawal in newborns. *Journal of Paediatrics and Child Health, 38*(1), 66-71.

Office of National Drug Control Policy, US Executive Office of the President, & United States of America. (2011). FY 2012 Budget and Performance Summary: Companion to the Drug Control Strategy.

Orzeł-Gryglewska, J. (2010). Consequences of sleep deprivation. *International Journal of Occupational Medicine and Environmental Health, 23*(1), 95-114.

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

Paul, J., Logan, B., Krishnan, R., Heller, N., Morrison, D., Pritham, U., Tisher, P.W., Troese, M., Brown, M., & Hayes, M. (2014). Development of auditory event-related potentials in infants prenatally exposed to methadone. *Developmental Psychobiology, 56*(5), 1119-1128.

Prechtl, H. F. (1990). Qualitative changes of spontaneous movements in fetus and preterm infant are a marker of neurological dysfunction. *Early Human Development, 23*(3), 151-158.

Pritham, U. A., Paul, J. A., & Hayes, M. J. (2012). Opioid dependency in pregnancy and length of stay for neonatal abstinence syndrome. *Journal of Obstetric, Gynecologic, & Neonatal Nursing, 41*(2), 180-190.

Rainnie, D. G., Bergeron, R., Sajdyk, T. J., Patil, M., Gehlert, D. R., & Shekhar, A. (2004). Corticotrophin releasing factor-induced synaptic plasticity in the amygdala translates stress into emotional disorders. *The Journal of Neuroscience, 24*(14), 3471-3479. doi:10.1523/JNEUROSCI.5740-03.2004

Riley, E. P., Infante, M. A., & Warren, K. R. (2011). Fetal alcohol spectrum disorders: An overview. *Neuropsychology Review, 21*(2), 73-80.

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

Robinson, T. E., & Kolb, B. (1997). Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. *The Journal of Neuroscience, 17*(21), 8491-8497.

Roehrs, T., Carskadon, M. A., Dement, W. C., & Roth, T. (2005). Daytime sleepiness and alertness. *Principles and Practice of Sleep Medicine. 4th ed. Philadelphia, PA: Elsevier/Saunders*, 39-50.

Rosenberg, M. J., Wolff, C. R., El-Emawy, A., Staples, M. C., Perrone-Bizzozero, N. I., & Savage, D. D. (2010). Effects of moderate drinking during pregnancy on placental gene expression. *Alcohol, 44*(7), 673-690.

Sarfi, M., Martinsen, H., Bakstad, B., Røislien, J., & Waal, H. (2009). Patterns in sleep–wakefulness in three-month old infants exposed to methadone or buprenorphine. *Early Human Development*, *85*(12), 773-778.

Sastry, B. R., & Janson, V. E. (1995). Smoking, placental function and fetal growth. In *Placental Toxicology* (pp. 45 - 80). Boca Raton, FL: CRC Press.

Sawaguchi, T., Franco, P., Kadhim, H., Mori, T., Ito, S., Taki, T., Sawaguchi, A. & Kahn, A. (2014). Sudden Infant Death Syndrome from the Perspective of Arousal Deficiency. In *Sudden Infant Death Syndrome* (pp. 81-105). Springer Japan.

Scher, M. S., Johnson, M. W., & Holditch-Davis, D. (2005). Cyclicity of neonatal sleep behaviors at 25 to 30 weeks' postconceptional age. *Pediatric Research, 57*(6), 879-882.

Stein, C., Schäfer, M., & Machelska, H. (2003). Attacking pain at its source: new perspectives on opioids. *Nature Medicine*, *9*(8), 1003-1008.

Stern, E., Parmelee, A. H., Akiyama, Y., Schultz, M. A., & Wenner, W. H. (1969). Sleep cycle characteristics in infants. *Pediatrics, 43*(1), 65-70.

Stone, K. C., LaGasse, L. L., Lester, B. M., Shankaran, S., Bada, H. S., Bauer, C. R., & Hammond, J. A. (2010). Sleep problems in children with prenatal substance exposure: The Maternal Lifestyle Study. *Archives of Pediatrics & Adolescent Medicine, 164*(5), 452-456.

Symanski, M. E., Hayes, M. J., & Akilesh, M. K. (2002). Patterns of premature newborns' sleep–wake states before and after nursing interventions on the night shift. *Journal of Obstetric, Gynecologic, & Neonatal Nursing, 31*(3), 305-313.

Troese, M., Fukumizu, M., Sallinen, B. J., Gilles, A. A., Wellman, J. D., Paul, J. A.,& Hayes, M. J. (2008). Sleep fragmentation and evidence for sleep debt in alcohol-exposed infants. *Early Human Development, 84*(9), 577-585.

Tsang, D., Ho, K., & Wen, H. (1986). Effect of maternal methadone administration on the development of multiple forms of monoamine oxidase in rat brain and liver. *Brain Research, 391*(2), 187-192.

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., et al. (2013). Association of OPRM1 and COMT single-nucleotide polymorphisms with hospital length of stay and treatment of neonatal abstinence syndrome. *JAMA, 309*(17), 1821-1827.

Waldhoer, M., Bartlett, S. E., & Whistler, J. L. (2004). Opioid receptors. *Annual Review of Biochemistry, 73*(1), 953-990.

Walhovd, K. B., Watts, R., Amlien, I., & Woodward, L. J. (2012). Neural tract development of infants born to methadone-maintained mothers. *Pediatric Neurology, 47*(1), 1-6.

Wang, S. C., Tsou, H. H., Chen, C. H., Chen, Y. T., Ho, I. K., Hsiao, C. F., et al. (2012). Genetic polymorphisms in the opioid receptor mu1 gene are associated with changes in libido and insomnia in methadone maintenance patients. *European Neuropsychopharmacology, 22*(10), 695-703.

Ward, R. M., Drover, D. R., Hammer, G. B., Stemland, C. J., Kern, S., Tristani-Firouzi, M., et al. (2014). The pharmacokinetics of methadone and its metabolites in neonates, infants, and children. *Pediatric Anesthesia, 24*(6), 591-601

Wichers, M., Aguilera, M., Kenis, G., Krabbendam, L., Myin-Germeys, I., Jacobs, N., ... & van Os, J. (2007). The catechol-O-methyl transferase Val158Met polymorphism and experience of reward in the flow of daily life. *Neuropsychopharmacology*, *33*(13), 3030-3036.

Wong, C. S., Lee, Y. J., Chiang, Y. C., Fan, L. W., Ho, I. K., & Tien, L. T. (2014). Effect of prenatal methadone on reinstated behavioral sensitization induced by methamphetamine in adolescent rats. *Behavioral Brain Research, 258*, 160-165.

Wu, C. C., Hung, C. J., Shen, C. H., Chen, W. Y., Chang, C. Y., Pan, H. C., et al. (2014). Prenatal buprenorphine exposure decreases neurogenesis in rats. *Toxicology Letters, 225*(1), 92-101.

Zhu, G., Lipsky, R. H., Xu, K., Ali, S., Hyde, T., Kleinman, J., et al. (2004). Differential expression of human COMT alleles in brain and lymphoblasts detected by RT-coupled 5′ nuclease assay. *Psychopharmacology*, *177*(1-2), 178-184.

Zeskind, P. S., & Lester, B. M. (2001). Analysis of infant crying. *Biobehavioral Assessment of the Infant*, 149-166.

Zung, B. J. (1982). Evaluation of the Michigan Alcoholism Screening Test (MAST) in assessing lifetime and recent problems. *Journal of Clinical Psychology*, *38*(2), 425-439.

# APPENDIX A: Drug And Alcohol Measurement Tools

Michigan Alcohol Screening Test

Rev.August 27, 1995

CURRENT ALCOHOL AND DRUG USE

ID:_____
INTERVIEWER:_____/_____
DATE:_____/_____/_____

Respondent
___1=Mother
___2=Father
___3=Child's grandmother (name:_____)
___4=Child's aunt (name:_____)
___5=Other relative (name:_____/relationship:_____)
___6=Other non-relative (name:_____)
___7=Foster parent (name:_____)
___8=Adoptive parent (name:_____)

Sex of respondent: M F

I. Smoking

1. Do you currently smoke (regular) cigarettes? ____Yes ____No

2. How many cigarettes do you smoke in one day? ___ ___.___

II. MAST

Now, I am going to ask you about your drinking. I realize these questions are personal and may be difficult to answer honestly. I want to emphasize that this information is confidential. First, I will ask about how alcohol affects you. Just answer 'yes' or 'no' as honestly as you can. Remember that this information is strictly confidential.

1. Do you feel you are a normal drinker:..... Y N
   IF RESPONDENT DENIES USE OF ALCOHOL: How long has it been since you drank?___._years
   ____ never drank

2. Have you ever awakened the morning after some drinking the night before and found that you could not remember a part of the evening before?..... Y N

3. Does your spouse (or parents) ever worry or complain about your drinking..... Y N

4. Can you stop drinking without a struggle after 1 or 2 drinks?..... Y N

5. Do you ever feel bad about your drinking?..... Y N

6. Do friends or relatives think you are a normal drinker?..... Y N

7. Do you ever try to limit your drinking to certain times of the day or certain places?..... Y N

8. Are you always able to stop drinking when you want to?..... Y N

9. Have you ever attended a meeting of Alcoholics Anonymous?..... Y N

10. Have you gotten into fights when drinking?..... Y N

ID:___
2

11. Has drinking ever created problems with you and your spouse?..... Y N

12. Has your spouse (or other family member) ever gone to anyone for help about your drinking? Y N

13. Have you ever lost friends or girlfriends/boyfriends because of drinking?..... Y N

14. Have you ever gotten into trouble at work because of drinking?..... Y N

15. Have you ever lost a job because of drinking?..... Y N

16. Have you ever neglected your obligations, your family, or your work for two or more days in a row because you were drinking?..... Y N

17. Do you ever drink before noon?..... Y N

18. Have you ever been told you have liver trouble? (Cirrhosis?)..... Y N

19. Have you ever had delirium tremens (DTs), severe shaking, heard voices, or seen things that weren't there after drinking?..... Y N

20. Have you ever gone to anyone for help about your drinking?..... Y N

21. Have you ever been a patient in a hospital because of drinking?..... Y N

22. Have you ever been a patient in a psychiatric hospital or on a psychiatric ward of a general hospital where drinking was a part of the problem?..... Y N

23. Have you ever been seen at a psychiatric or mental health clinic, or gone to a doctor, social worker, or clergy man for help with an emotional problem in which drinking had played a part?..... Y N

24. Have you ever been arrested, even for a few hours, because of drunk behavior?..... Y N

25. Have you ever been arrested for drunk driving or driving after drinking?..... Y N

TWEAK AND T-ACE

1. Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover? Y N

2. Have you ever felt you ought to cut down on your drinking? Y N

3. Have people annoyed you by criticizing your drinking? Y N

4. How many drinks does it take to make you feel high? ___ ___.___

   (IF ASKED: "before first beginning to feel the effects of alcohol")

   a. When you say (number of drinks), what are you referring to? Beer, wine, liquor? _____

   b. How much would one drink be for you? ___ ___.___oz.

5. How many drinks can you hold? ___ ___.___

   (IF ASKED: "before passing out or falling asleep")

ID:___
3

III. Current Drinking

1. Now I am going to ask you about your current drinking. I want to know on the average what and how much you usually drink during a typical week (within the past year). Let's start with a typical weekend. Think of what you usually do during the weekend; that is, stay home, go out, have friends over, whatever. Consider if you usually drink while you do this. On a typical Friday, what do you do? (pause) Do you go out? (pause) Do you go to a party? (pause) Do you have friends over? (pause) Do you relax in front of TV with a beer? (pause) If so, how much? (pause) A jumbo (40 or 32 oz.)? How about during the day? When you drink on Friday, how much (wine, beer, shots) do you drink? Do you have anything with it like wine, wine cooler, brandy...? How about on Saturday? Anything during the day? Saturday night? How about Sunday? Do you have anything to drink during the week? Let's start with Monday, etc.

| | Regular Beer or Zima | | Malt Liquor or Ice Beer | | Wine | | Liquor* | | Wine cooler | |
|---|---|---|---|---|---|---|---|---|---|---|
| DAY | Num drinks | oz/ drink | Num drinks | oz/ drink | Num drinks | oz/ drink | Num drinks | oz/ drink | Num drinks | oz/ drink |
| Fri | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Sat | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Sun | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Mon | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Tues | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Wed | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Thurs | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |

*Please note if straight liquor or mixed drinks.

Possible size of containers:
Beer is available in 8 oz. glasses, 12 oz. cans or bottles, 16 oz. cans or bottles, and also available in the following sizes: 14.9 oz. 21.6 oz.,
22 oz (or 1 pt. 6 oz.), 24 oz., 40 oz., 45 oz. (4 x 5's), or 64 oz. Bottles.
Zima is available in both a 12 oz. size and a 1 pt. 6 oz. size.
A glass of wine is 6 oz.
A cooler is 12 oz.
A standard shot is 1.5 oz.
A pint is 16 oz.
A 1/5th is 26 oz.

2. Was there a time since (child's) birth when you drank more heavily than you currently drink? ___Yes ___No (Go to Question 3)

a. When was that? . From _____ years ago until _____ years ago.

b. How much did you drink during a typical week at that time? On a typical Friday night, what might you be doing? etc.

| | Regular Beer or Zima . | | Malt Liquor or Ice Beer | | Wine | | Liquor* | | Wine cooler | |
|---|---|---|---|---|---|---|---|---|---|---|
| DAY | Num drinks | oz/ drink | Num drinks | oz/ drink | Num drinks | oz/ drink | Num drinks | oz/ drink | Num drinks | oz/ drink |
| Fri | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Sat | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Sun | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Mon | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Tues | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Wed | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |
| Thurs | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ | ___ |

*Please note if straight liquor or mixed drinks.

Possible size of container:
Beer is available in 8 oz. glasses, 12 oz. cans or bottles, 16 oz. cans or bottles, and also available in the following sizes: 14.9 oz. 21.6 oz., 22 oz (or 1 pt. 6 oz.), 24 oz., 40 oz., 45 oz. (4 x 5's), or 64 oz. Bottles.
Zima is available in both a 12 oz. size and a 1 pt. 6 oz. size.
A glass of wine is 6 oz.
A cooler is 12 oz.
A standard shot is 1.5 oz.
A pint is 16 oz.
A 1/5th is 26 oz.

3. Do you *sometimes* drink 5 or more glasses of beer, wine, or liquor?
NOTE: If respondant regularly drinks the equivalent of 5 or more drinks/day, ask:
"What is the most you drink on a single occasion?" and continue with a. and b. below.
_____Yes _____No (Go to question 4)

If YES:

a. How often have you done so during the past month? _____times

During the past 12 months (including the past month)? _____times

Since (child's) birth (including the past 12 months)? _____times

b. How much do you usually drink when you drink heavily?
(Record only what R would drink on a single occassion. If she describes more than one drinking pattern, record the heaviest)

| | number of drinks | oz/drink |
|---|---|---|
| Beer or Zima | | |
| Malt liquor or ice beer | | |
| Wine | | |
| Liquor* | | |
| Wine cooler | | |

*Please note if straight liquor or mixed drinks.

4. Have you ever gone for help or treatment for your drinking? Yes No
(List below or write none)
How many times have you gone for help or treatment for your drinking?

FOR EACH, ASK: When? Where did you go? Was this for detox only?

| Dates | Help or Treatment | Detox only |
|---|---|---|
| | | Y N |
| | | Y N |
| | | Y N |
| | | Y N |

5. How old were you when you first started drinking?
(excluding first sips) _____years old or _____never drank

IV. DRUGS

I'm going to ask you about drug use. I want to remind you that the questions I am asking are completely confidential and will only be used for research purposes. Have you ever taken any of the following drugs?

1a. Have you ever used marijuana (pot) or hashish? ____Yes ____No

b. IF YES: How old were you when you first used (drug)?
Did you use it when you were pregnant with (child)?

c. Have you used it in the past 12 months? IF YES, how often? Once a week? Twice a week?

| | Age when started | During pregnancy | Past 12 Months |
|---|---|---|---|
| Marijuana | _____.__ | Yes / No | __ __.__ days/wk, mo., 12 mos. |

d. For how many years altogether have you used marijuana (pot) or hashish? __ __.__ yrs.

e. If there is no current use: Was there a time since (child) was born that you used (drug)?

If there is current use: Was there a time since (child) was born that you used (drug) more frequently than during the past 12 months? If there is more than one period of time since child was born where drug use was more frequent than current use, record the period of heaviest use.

| | Days/ month | OR | Num occasions | Years ago | until | Years ago |
|---|---|---|---|---|---|---|
| Marijuana (pot) or hashish | __ __.__ | | __ __ | ____ | | ____ |

2. Heroin, methadone, or other opiates (e.g., Codeine, Percodan, Dilaudud)? ____Yes ____No

b. IF YES: How old were you when you first used (drug)? Did you use it when you were pregnant with (child)?

c. Have you used it in the past 12 months? IF YES, how often? Once a week? Twice a week?

| | Age when started | During Pregnancy | Past 12 Months |
|---|---|---|---|
| Total heroin | ___.___ | Yes / No | __ __.__ days/ wk, mo., 12 mo. |
| Heroin shooting | ___.___ | Yes / No | __ __.__ days/ wk, mo., 12 mo. |
| Heroin snorting | ___.___ | Yes / No | __ __.__ days/ wk, mo., 12 mo. |
| Methadone | ___.___ | Yes / No | __ __.__ days/ wk, mo., 12 mo. |
| Codeine | ___.___ | Yes / No | __ __.__ days/ wk, mo., 12 mo. |
| Tylenol 3 or 4 | ___.___ | Yes / No | __ __.__ days/ wk, mo., 12 mo. |
| Other opiates (specify:_____) | ___.___ | Yes / No | __ __.__ days/ wk, mo., 12 mo. |

d. (IF USES MORE THAN ONE OPIATE): All in all, how many days per month do you use heroin, methadone, or other opiates? ___.___ days per month.

e. For how many years have you used heroin, methadone, or other opiates? ___.___ yrs.

f. If there is no current use: Was there a time since (child) was born that you used (drug)?

If there is current use: Was there a time since (child) was born that you used (drug) more frequently than during the past 12 months? If there is more than one period of time since child was born where drug use was more frequent than current use, record the period of heaviest use.

| | Days/ month | OR | Num occasions | Years ago | until | Years ago |
|---|---|---|---|---|---|---|
| Heroin (total) | __ __.__ | | __ __ | ____ | | ____ |
| Heroin-shooting | __ __.__ | | __ __ | ____ | | ____ |
| Heroin-snorting | __ __.__ | | __ __ | ____ | | ____ |
| Methadone | __ __.__ | | __ __ | ____ | | ____ |
| Codeine | __ __.__ | | __ __ | ____ | | ____ |
| Tylenol 3 or 4 | __ __.__ | | __ __ | ____ | | ____ |
| Other (specify:_____) | __ __.__ | | __ __ | ____ | | ____ |

ID:___
8

3. a. Have you ever used cocaine or crack? ____Yes ____No

b. IF YES: How old were you when you first used (drug)?
Did you use it when you were pregnant with (child)?

c. Have you used it in the past 12 months? IF YES, how often? Once a week? Twice a week?

| | Age when started | During Pregnancy | Past 12 months |
|---|---|---|---|
| Total cocaine | ___.___ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Cocaine shooting | ___.___ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Cocaine snorting | ___.___ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Crack (free-basing) | ___.___ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Other (specify:_____) | ___.___ | Yes / No | __ __.__ days/wk, mo., 12 mo. |

d. (IF USES MORE THAN ONE FORM OF COCAINE): All in all, how many days per month do you use cocaine or crack?
_____.___ per month.

e. For how many years have you used cocaine or crack? ____.___ yrs.

f. If there is no current use: Was there a time since (child) was born that you used (drug)?

If there is current use: Was there a time since (child) was born that you used (drug) more frequently than during the past 12 months? If there is more than one period of time since child was born where drug use was more frequent than current use, record the period of heaviest use.

| | Days/ month | OR | Num occasions | Years ago | until | Years ago |
|---|---|---|---|---|---|---|
| Cocaine (total) | __ __.__ | | __ __ | ____ | | ____ |
| Cocaine-shooting | __ __.__ | | __ __ | ____ | | ____ |
| Cocaine-snorting | __ __.__ | | __ __ | ____ | | ____ |
| Crack | __ __.__ | | __ __ | ____ | | ____ |
| Other (specify:_____) | __ __.__ | | __ __ | ____ | | ____ |

ID:___
9

4. a. Have you ever used barbiturates (e.g., Seconal, "reds"), sedatives, tranquilizers (e.g.,Valium, Librium, Xanax)?

____Yes ____No

b. IF YES: How old were you when you first used (drug)?
Did you use it when you were pregnant with (child)?
c. Have you used it in the past 12 months? IF YES, how often? Once a week? Twice a week?

| | Age when started | During Pregnancy | Past 12 months |
|---|---|---|---|
| Total barbiturates | ____.____ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Barbiturates oral | ____.____ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Barbiturate shooting | ____.____ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Sedatives/tranquilizers (specify____) | ____.____ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Other depressants (specify:_____) | ____.____ | Yes / No | __ __.__ days/wk, mo., 12 mo. |

d. (IF USES MORE THAN ONE TYPE OF DEPRESSANT): All in all, how many days per month do you use barbiturates, sedatives, or tranquilizers? ___.___ per month.

e. For how many years have you used barbiturates, sedatives, or tranquilizers? ___.___ yrs.

f. If there is no current use: Was there a time since (child) was born that you used (drug)?

If there is current use: Was there a time since (child) was born that you used (drug) more frequently than during the past 12 months? If there is more than one period of time since child was born where drug use was more frequent than current use, record the period of heaviest use.

| | Days/ month | OR | Num occasions | Years ago | until | Years ago |
|---|---|---|---|---|---|---|
| Barbiturates (total) | __ __.__ | | __ __ | ____ | | ____ |
| Barbiturates-oral | __ __.__ | | __ __ | ____ | | ____ |
| Barbiturates-shooting | __ __.__ | | __ __ | ____ | | ____ |
| Sedatives/tranquilizers (specify:_____) | __ __.__ | | __ __ | ____ | | ____ |
| Other depressants (specify:_____) | __ __.__ | | __ __ | ____ | | ____ |

ID:___
10

5. a. Have you ever used amphetamines (e.g.,"speed," Dexadrine, "Bennies," "Black Beauties"), methamphetamines (e.g., Methedrine, Crystal or Crystalmeth) or antidepressants (e.g., prozac)? ___Yes ___No

b. IF YES: How old were you when you first used (drug)?
Did you use it when you were pregnant with (child)?

c. Have you used it in the past 12 months? IF YES, how often? Once a week? Twice a week?

| | Age when started | During pregnancy | Past 12 months |
|---|---|---|---|
| Total amphetamines/methamphetamines | ____.__ | Yes / No | __ __.__ days/wk, mo, 12 mo |
| Amphetamine oral | ____.__ | Yes / No | __ __.__ days/wk, mo, 12 mo |
| Amphetamine shooting | ____.__ | Yes / No | __ __.__ days/wk, mo, 12 mo |
| Methamphetamine shooting | ____.__ | Yes / No | __ __.__ days/wk, mo, 12 mo |
| Methamphetamine snorting | ____.__ | Yes / No | __ __.__ days/wk, mo, 12 mo |
| "Ice" smoking | ____.__ | Yes / No | __ __.__ days/wk, mo, 12 mo |
| Antidepressants (specify:_____) | ____.__ | Yes / No | __ __.__ days/wk, mo, 12 mo |
| Other stimulants (specify:_____) | ____.__ | Yes / No | __ __.__ days/ wk, mo., 12 mo. |

d. (IF USES MORE THAN ONE TYPE OF STIMULANT): All in all, how many days per month do you use amphetamines, methamphetamines, or antidepressants? __.__ per month.

e. For how many years have you used amphetamines, methamphetamines, or antidepressants? __.__ yrs.

f. If there is no current use: Was there a time since (child) was born that you used (drug)?

If there is current use: Was there a time since (child) was born that you used (drug) more frequently than during the past 12 months? If there is more than one period of time since child was born where drug use was more frequent than current use, record the period of heaviest use.

| | Days/month OR Num occasions | Years ago until Years ago |
|---|---|---|
| Amphetamines or methamphetamines (total) | __ __.__ __ __ | ____ ____ |
| Amphetamines-oral | __ __.__ __ __ | ____ ____ |
| Amphetamines-shooting | __ __.__ __ __ | ____ ____ |
| Methamphetamines-shooting | __ __.__ __ __ | ____ ____ |
| Methamphetamines-snorting | __ __.__ __ __ | ____ ____ |
| "Ice" | __ __.__ __ __ | ____ ____ |
| Anti-depressants: (specify:(_____) | __ __.__ __ __ | ____ ____ |
| Other stimulants (specify:_____) | __ __.__ __ __ | ____ ____ |

ID:___
11

6. a. Have you ever used hallucinogens (e.g., LSD, PCP, mescaline) ___Yes ___No

b. IF YES: How old were you when you first used (drug)?
Did you use it when you were pregnant with (child)?

c. Have you used it in the past 12 months? IF YES, how often? Once a week? Twice a week?

| | Age when started | During pregnancy | Past 12 months |
|---|---|---|---|
| LSD | ___.___ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| PCP | ___.___ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Mescaline | ___.___ | Yes / No | __ __.__ days/wk, mo., 12 mo. |
| Other hallucinogens (specify:_____) | ___.___ | Yes / No | __ __.__ days/wk, mo., 12 mo. |

d. (IF USES MORE THAN ONE HALLUCINOGEN): All in all, how many days per month do you use hallucinogens?___.___ per month.

e. For how many years have you used hallucinogens? ___.___ yrs.

f. If there is no current use: Was there a time since (child) was born that you used (drug)?

If there is current use: Was there a time since (child) was born that you used (drug) more frequently than during the past 12 months? If there is more than one period of time since child was born where drug use was more frequent than current use, record the period of heaviest use.

| | Days/ month | OR | Num occasions | Years ago | until | Years ago |
|---|---|---|---|---|---|---|
| LSD | __ __.__ | | __ __ | ___ | | ___ |
| PCP | __ __.__ | | __ __ | ___ | | ___ |
| Mescaline | __ __.__ | | __ __ | ___ | | ___ |
| Other hallucinogens (specify:_____) | __ __.__ | | __ __ | ___ | | ___ |

ID:___
12

7. a. Have you ever used inhalants (e.g., glue, solvents)? ___Yes ___No

b. IF YES: How old were you when you first used (drug)?
Did you use it when you were pregnant with (child)?

c. Have you used it in the past 12 months? IF YES, how often? Once a week? Twice a week?

| | Age when started | During pregnancy | Past 12 months |
|---|---|---|---|
| Glue | ___.___ | Yes / No | ___ ___.___ days/wk, mo., 12 mo. |
| Solvents | ___.___ | Yes / No | ___ ___.___ days/wk, mo., 12 mo. |
| Other inhalants (specify:_____) | ___.___ | Yes / No | ___ ___.___ days/wk, mo., 12 mo. |

d. (IF USES MORE THAN ONE INHALENT): All in all, how many days per month do you use inhalants?
___.___ per month.

e. For how many years have you used inhalants? ___.___ yrs.

f. If there is no current use: Was there a time since (child) was born that you used (drug)?

If there is current use: Was there a time since (child) was born that you used (drug) more frequently than during the past 12 months? If there is more than one period of time since child was born where drug use was more frequent than current use, record the period of heaviest use.

| | Days/ month | OR | Num occasions | Years ago | until | Years ago |
|---|---|---|---|---|---|---|
| Glue | ___ ___.___ | | ___ ___ | ____ | | ____ |
| Solvents | ___ ___.___ | | ___ ___ | ____ | | ____ |
| Other inhalants (specify:_____) | ___ ___.___ | | ___ ___ | ____ | | ____ |

8. How much would you say you spend on drugs during a typical month (over the past 12 months)? $____.00

9. Have you gone for help or treatment for drug abuse? (List below or write None.) FOR EACH, ASK: When? Where did you go? Was this for detox only?

Drug Treatment History

| Dates | Help or Treatment | Detox only? |
|---|---|---|
| | | Y N |
| | | Y N |
| | | Y N |
| | | Y N |
| | | Y N |

## AA Scoring Sheet

1. AA/D= $\frac{\text{SUM OF WEEK'S DRINKS}}{7}$

_____ × _____ × _____ = _____ = _____.
TOTAL # OF DRINKS × TOTAL OUNCES × ALC. CONTENT 7

2. AA/DD= $\frac{\text{SUM OF ABSOLUTE ALCOHOL ON DRINKING DAY}}{\text{\# OF DAYS OF DRINKING}}$

_____ × _____ × _____ = _____ = _____.
TOTAL # OF DRINKS × TOTAL OUNCES × ALC. CONTENT # OF DD'S

3. AA/BINGE = $\frac{\text{ABSOLUTE ALCOHOL PER EVENT}}{\text{\# OF BINGE DRINKING DAYS IN WEEK}}$ (Applies to binge days only)

_____ × _____ × _____ = _____ = _____.
TOTAL # OF DRINKS × TOTAL OUNCES × ALC. CONTENT # OF DD'S

4. AA/MAX (HIGHEST CONSUMPTION DAY) = ABSOLUTE ALCOHOL

_____ × _____ × _____ = _____
TOTAL # OF DRINKS × TOTAL OUNCES × ALC. CONTENT

**QVF Scoring**

**ID:** _____

**Tobaccco:**

Smoker? Y / N

How many cigarettes per day? _____

Age began smoking _____

Have you ever quit? Y / N

**Current Drinking:**

Is there any current drinking? Y / N

AA/day _____

AA/drinking day _____

**Past Drinking:**

AA/day _____

AA/drinking day _____

**Binge Drinking:**

Binge drinker? Y / N

Binge drinking during pregnancy? Y / N
How often? _____

Binge drinking before pregnancy? Y / N
How often? _____

AA/Binge _____

How old when first started drinking? _____

Did you ever quit drinking? _____

# MAST, TWEAK, T-ACE WORKSHEET

| File ID____ | | | | | |
|---|---|---|---|---|---|
| | | | | | |
| MAST | Y | N | SCORE | | |
| 1 | 0 | 1 | | | |
| 2 | 1 | 0 | | | |
| 3 | 1 | 0 | | | |
| 4 | 0 | 1 | | | |
| 5 | 1 | 0 | | | |
| 6 | 0 | 1 | | | |
| 7 | 0 | 1 | | | |
| 8 | 0 | 1 | | | |
| 9 | 1 | 0 | | | |
| 10 | 1 | 0 | | | |
| 11 | 1 | 0 | | | |
| 12 | 1 | 0 | | | |
| 13 | 1 | 0 | | | |
| 14 | 1 | 0 | | | |
| 15 | 1 | 0 | | | |
| 16 | 1 | 0 | | | |
| 17 | 1 | 0 | | | |
| 18 | 1 | 0 | | | |
| 19 | 1 | 0 | | | |
| 20 | 1 | 0 | | | |
| 21 | 1 | 0 | | | |
| 22 | 1 | 0 | | | |
| 23 | 1 | 0 | | | |
| 24 | 1 | 0 | | | |
| 25 | 1 | 0 | | | |
| | | TOTAL: | | | |
| | | | | | |
| | | MAST 2 → | | | |
| | YES/PTS. | NO | SCORE | TWEAK | T-ACE |
| 1 | 1 | 0 | | | |
| 2 | 1 | 0 | | | |
| 3 | 1 | 0 | | | |
| 4 | >2 is 2 pts. | 0 | | | |
| 5 | >5 is 2 pts. | 0 | 0 | | 0 |
| | | TOTALS: | | | |

# APPENDIX B: Finnegan Scoring Sheet

**NEONATAL ABSTINENCE SCORING SYSTEM**

| Modified Finnegan Neonatal Abstinence Score Sheet[1] | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **System** | **Signs and Symptoms** | **Score** | **AM** | | | | | | | | | | | | **PM** | | | | | | | | | | **Comments** |
| **Central Nervous System Disturbances** | Excessive high-pitched (or other) cry < 5 mins | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| | Continuous high-pitched (or other) cry > 5 mins | 3 | | | | | | | | | | | | | | | | | | | | | | | |
| | Sleeps < 1 hour after feeding | 3 | | | | | | | | | | | | | | | | | | | | | | | |
| | Sleeps < 2 hours after feeding | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| | Sleeps < 3 hours after feeding | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Hyperactive Moro reflex | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| | Markedly hyperactive Moro reflex | 3 | | | | | | | | | | | | | | | | | | | | | | | |
| | Mild tremors when disturbed | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Moderate-severe tremors when disturbed | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| | Mild tremors when undisturbed | 3 | | | | | | | | | | | | | | | | | | | | | | | |
| | Moderate-severe tremors when undisturbed | 4 | | | | | | | | | | | | | | | | | | | | | | | |
| | Increased muscle tone | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Excoriation (chin, knees, elbow, toes, nose) | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Myoclonic jerks (twitching/jerking of limbs) | 3 | | | | | | | | | | | | | | | | | | | | | | | |
| | Generalised convulsions | 5 | | | | | | | | | | | | | | | | | | | | | | | |
| **Metabolic/ Vasomotor/ Respiratory Disturbances** | Sweating | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Hyperthermia 37.2-38.3C | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Hyperthermia > 38.4C | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| | Frequent yawning (> 3-4 times/ scoring interval) | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Mottling | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Nasal stuffiness | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Sneezing (> 3-4 times/scoring interval) | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Nasal flaring | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| | Respiratory rate > 60/min | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Respiratory rate > 60/min with retractions | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| **Gastrointestinal Disturbances** | Excessive sucking | 1 | | | | | | | | | | | | | | | | | | | | | | | |
| | Poor feeding (infrequent/uncoordinated suck) | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| | Regurgitation (≥ 2 times during/post feeding) | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| | Projectile vomiting | 3 | | | | | | | | | | | | | | | | | | | | | | | |
| | Loose stools (curds/seedy appearance) | 2 | | | | | | | | | | | | | | | | | | | | | | | |
| | Watery stools (water ring on nappy around stool) | 3 | | | | | | | | | | | | | | | | | | | | | | | |
| | **Total Score** | | | | | | | | | | | | | | | | | | | | | | | | |
| | **Date/Time** | | | | | | | | | | | | | | | | | | | | | | | | |
| | **Initials of Scorer** | | | | | | | | | | | | | | | | | | | | | | | | |

1. Finnegan LP. Neonatal abstinence syndrome: assessment and pharmacotherapy. In: Nelson N, editor. Current therapy in neonatal-perinatal medicine. 2 ed. Ontario: BC Decker; 1990.

The NAS score sheet lists 21 symptoms that are most frequently observed in opiate-exposed infants. Each symptom and its associated degree of severity are assigned a score and the total abstinence score is determined by totalling the score assigned to each symptom over the scoring period.

## Key points

- The first abstinence score should be recorded approximately two hours after birth or admission to the nursery (baseline score). This score reflects all infant behaviour up to the first scoring interval time point.
- Following the baseline score all infants should be scored at 4-hourly intervals, except when high scores indicate more frequent scoring.
- The score sheet allows for 2-hourly scoring over the 24-hour period.
- A new sheet should be started at the beginning of each day.
- Scoring is dynamic. All signs and symptoms observed during the scoring interval are included in the point-total for that period.
- If the infant's score at any scoring interval is ≥ 8, scoring is increased to 2-hourly and continued for 24 hours from the last total score of 8 or higher.
- If the 2-hourly score is ≤ 7 for 24 hours then 4-hourly scoring intervals may be resumed.
- If pharmacotherapy is not needed the infant is scored for the first 4 days of life at 4-hourly intervals.
- If pharmacotherapy is required the infant is scored at 2- or 4-hourly intervals, depending on whether the abstinence score is less than or greater than 8 throughout the duration of therapeutic period.
- If after cessation of pharmacotherapy the score is less than 8 for the following 3 days, then scoring may be discontinued.
- If after cessation of pharmacotherapy the score is consistently 8 or more, then scoring should be continued for the following 4 days (minimum) to ensure that the infant is not likely to develop late onset of withdrawal symptoms at home following discharge.

## Guide to assessment and scoring[2, 3]

The neonatal abstinence syndrome scoring system was designed for term babies on four-hourly feeds and may therefore need modification for preterm infants. In a term infant scoring should be performed 30 minutes to one hour after a feed, before the baby falls asleep.

If necessary the infant should be awakened to elicit reflexes and behaviour, but if the infant is woken to be scored then diminished sleep after scoring should not be recorded. A crying infant should be soothed and quietened before assessing muscle tone, Moro reflex and respiratory rate.

| | |
|---|---|
| High-pitched cry | Score 2 if high-pitched at its peak, 3 if high-pitched throughout. Infant is scored if crying is prolonged, even if it is not high-pitched.[2] |
| Sleep | This is a scale of increasing severity and a term infant should receive only one score from the three levels of severity. A premature infant on 3 hourly feeds can sleep for 2½ hours at most. Scoring should thus be 1 if the baby sleeps less than 2 hours, 2 if less than 1 hour and 3 if the baby does not sleep between feeds.[2] |
| Moro reflex | The Moro or startle reflex is a normal reflex of young infants and occurs when a sudden loud noise causes the child to stretch out the arms and flex the legs. Score if the infant exhibits pronounced jitteriness (rhythmic tremors that are symmetrical and involuntary) of the hands during or at the end of a Moro reflex. Score 3 if jitteriness and clonus (repetitive involuntary jerks) of the hands and/or arms are present during or after the initiation of the reflex. |

| | |
|---|---|
| Tremors | This is a scale of increasing severity and an infant should only receive one score from the four levels of severity. Undisturbed refers to the baby being asleep or at rest in the cot.[2] |
| Increased muscle tone | Score if excessive or above-normal muscle tone or tension is observed - muscles become "stiff" or rigid and the infant shows marked resistance to passive movements, e.g. if the infant does not experience any head lag when being pulled to the sitting position; or if there is tight flexion of the infant's arms and legs (unable to slightly extend these when an attempt is made to extend and release the supine infant's arms and legs).[4] |
| Excoriation | Excoriations (skin abrasions resulting from constant rubbing against a surface that is covered with fabric such as bed linen). Score only when excoriations first appear, increase or appear in a new area.[2] |
| Myoclonic jerks | Score if involuntary muscular contractions which are irregular and exceedingly abrupt (usually involving a single group of muscles) are observed.[4] |
| Generalised convulsions | In the newborn infant generalised seizures or convulsions are often referred to as tonic seizures. They are most commonly seen as generalised activity involving tonic extensions of all limbs, but are sometimes limited to one or both limbs on one side. Unusual limb movements may accompany a seizure. In the upper limbs these often resemble "swimming" or "rowing". In the lower limbs, they resemble "pedalling" or "bicycling." Other subtle signs may include eye staring, rapid involuntary movements of the eyes, chewing, back arching, and fist clenching.[4] |
| Sweating | Score if sweating is spontaneous and is not due to excessive clothing or high room temperature[4] |
| Hyperthermia | Temperature should be taken per axilla. Mild pyrexia (37.2-38.3ºC) is an early indication of heat produced by increased muscle tone and tremors. |
| Yawning | Score if more than 3 yawns observed within the scoring interval.[2, 4] |
| Mottling | Score if mottling (marbled appearance of pink and pale or white areas) is present on the infant's chest, trunk, arms, or legs.[4] |
| Nasal stuffiness | Score if the infant sounds congested; mucous may be visible.[4] |
| Sneezing | Score if more than 3 sneezes observed within the scoring interval.[2, 4] |
| Nasal flaring | Score only if repeated dilation of the nostrils is observed without other evidence of lung or airways disease.[4] |
| Respiratory rate | Respirations are counted for one full minute. Score only if >60 per minute without other evidence of lung or airways disease.[2] Score 2 if respiration involves drawing in of the intercostal muscles (retractions). |
| Excessive sucking | Score if hyperactive/disorganised sucking, increased rooting reflex, or attempts to suck fists or thumbs (more than that of an average hungry infant) are observed.[3, 4] |
| Poor feeding | Score if the infant demonstrates excessive sucking prior to feeding, yet sucks infrequently during a feeding taking a small amount of breast milk or formula, and / or demonstrates an uncoordinated sucking reflex (difficulty sucking and swallowing).[3] Premature infants may require tube feeding and should not be scored for poor feeding if tube feeding is expected at their gestation.[2] |
| Regurgitation | Score if at least one episode of regurgitation is observed even if vomit is contained in the mouth.[4] |
| Loose/watery stools | Score if loose (curds/seedy appearance) or watery stools (water ring on nappy around stool) are observed. Check the nappy after the examination is completed if not apparent during the examination.[4] |

## References


1. Finnegan LP. Neonatal abstinence syndrome: assessment and pharmacotherapy. In: Nelson N, editor. Current therapy in neonatal-perinatal medicine. 2 ed. Ontario: BC Decker; 1990.
2. Royal Women's Hospital Drug Information Centre. Newborn Emergency Transport Service (Victoria). Neonatal handbook. Carlton, Vic: Royal Women's Hospital; 2004.
3. Finnegan LP, Kaltenbach K. Neonatal abstinence syndrome. In: Hoekelman RA, Friedman SB, Nelson N, Seidel HM, editors. Primary pediatric care. 2 ed. St Louis: C V Mosby; 1992. p. 1367-78.
4. Lester BM, Tronick EZ, Brazelton TB. The Neonatal Intensive Care Unit Network Neurobehavioral Scale Procedures. Pediatrics. 2004;113(3 Pt 2):641-67.

# APPENDIX C: Maternal Anxiety and Depression Tools

*Study* _____ *ID* _____

*Date* __ __ /__ __ /__ __ __ __

## Symptom Checklist 90-R

Below is a list of problems and complaints that people sometimes have. Please read each one carefully and **enter the number** that best describes how much you were bothered by that problem during the past week.

**Please enter only ONE.**

**FOR THE *PAST WEEK*, HOW MUCH WERE YOU BOTHERED BY:**

| | Not At All | A Little Bit | Moderately | Quite A Bit | Extremely |
|---|---|---|---|---|---|
| 1. Headaches | 0 | 1 | 2 | 3 | 4 |
| 2. Nervousness or shakiness inside | 0 | 1 | 2 | 3 | 4 |
| 3. Unwanted thoughts, words, or ideas that won't leave your mind | 0 | 1 | 2 | 3 | 4 |
| 4. Faintness or dizziness | 0 | 1 | 2 | 3 | 4 |
| 5. Loss of sexual interest or pleasure | 0 | 1 | 2 | 3 | 4 |
| 6. Feeling critical of others | 0 | 1 | 2 | 3 | 4 |
| 7. The idea that someone else can control your thoughts | 0 | 1 | 2 | 3 | 4 |
| 8. Feeling others are to blame for most of your troubles | 0 | 1 | 2 | 3 | 4 |
| 9. Trouble remembering things | 0 | 1 | 2 | 3 | 4 |
| 10. Worried about sloppiness or carelessness | 0 | 1 | 2 | 3 | 4 |
| 11. Feeling easily annoyed or irritated | 0 | 1 | 2 | 3 | 4 |
| 12. Pains in heart or chest | 0 | 1 | 2 | 3 | 4 |
| 13. Feeling afraid in open spaces or on the streets | 0 | 1 | 2 | 3 | 4 |
| 14. Feeling low in energy or slowed down | 0 | 1 | 2 | 3 | 4 |
| 15. Thoughts of ending your life | 0 | 1 | 2 | 3 | 4 |
| 16. Hearing words that others do not hear | 0 | 1 | 2 | 3 | 4 |
| 17. Trembling | 0 | 1 | 2 | 3 | 4 |
| 18. Feeling that most people cannot be trusted | 0 | 1 | 2 | 3 | 4 |
| 19. Poor appetite | 0 | 1 | 2 | 3 | 4 |
| 20. Crying easily | 0 | 1 | 2 | 3 | 4 |



*SCL DMU v1 03/28/2003*

*Study* _____ *ID* _____

*Date* __ __ /__ __ /__ __ __ __

**FOR THE *PAST WEEK*, HOW MUCH WERE YOU BOTHERED BY:**

| | Not At All | A Little Bit | Moderately | Quite A Bit | Extremely |
|---|---|---|---|---|---|
| 21. Feeling shy or uneasy with the opposite sex | 0 | 1 | 2 | 3 | 4 |
| 22. Feeling of being trapped or caught | 0 | 1 | 2 | 3 | 4 |
| 23. Suddenly scared for no reason | 0 | 1 | 2 | 3 | 4 |
| 24. Temper outbursts that you could not control | 0 | 1 | 2 | 3 | 4 |
| 25. Feeling afraid to go out of your house alone | 0 | 1 | 2 | 3 | 4 |
| 26. Blaming yourself for things | 0 | 1 | 2 | 3 | 4 |
| 27. Pains in lower back | 0 | 1 | 2 | 3 | 4 |
| 28. Feeling blocked in getting things done | 0 | 1 | 2 | 3 | 4 |
| 29. Feeling lonely | 0 | 1 | 2 | 3 | 4 |
| 30. Feeling blue | 0 | 1 | 2 | 3 | 4 |
| 31. Worrying too much about things | 0 | 1 | 2 | 3 | 4 |
| 32. Feeling no interest in things | 0 | 1 | 2 | 3 | 4 |
| 33. Feeling fearful | 0 | 1 | 2 | 3 | 4 |
| 34. Your feelings being easily hurt | 0 | 1 | 2 | 3 | 4 |
| 35. Other people being aware of your private thoughts | 0 | 1 | 2 | 3 | 4 |
| 36. Feeling others do not understand you or are unsympathetic | 0 | 1 | 2 | 3 | 4 |
| 37. Feeling that people are unfriendly or dislike you | 0 | 1 | 2 | 3 | 4 |
| 38. Having to do things very slowly to insure correctness | 0 | 1 | 2 | 3 | 4 |
| 39. Heart pounding or racing | 0 | 1 | 2 | 3 | 4 |
| 40. Nausea or upset stomach | 0 | 1 | 2 | 3 | 4 |
| 41. Feeling inferior to others | 0 | 1 | 2 | 3 | 4 |
| 42. Soreness of your muscles | 0 | 1 | 2 | 3 | 4 |
| 43. Feeling that you are watched or talked about by others | 0 | 1 | 2 | 3 | 4 |
| 44. Trouble falling asleep | 0 | 1 | 2 | 3 | 4 |

*Study* _____ *ID* _____

*Date* __ __ /__ __ /__ __ __ __

**FOR THE *PAST WEEK*, HOW MUCH WERE YOU BOTHERED BY:**

| | Not At All | A Little Bit | Moderately | Quite A Bit | Extremely |
|---|---|---|---|---|---|
| 45. Having to check and double-check what you do | 0 | 1 | 2 | 3 | 4 |
| 46. Difficulty making decisions | 0 | 1 | 2 | 3 | 4 |
| 47. Feeling afraid to travel on buses, subways, or trains | 0 | 1 | 2 | 3 | 4 |
| 48. Trouble getting your breath | 0 | 1 | 2 | 3 | 4 |
| 49. Hot or cold spells | 0 | 1 | 2 | 3 | 4 |
| 50. Having to avoid certain things, places, or activities because they frighten you | 0 | 1 | 2 | 3 | 4 |
| 51. Your mind going blank | 0 | 1 | 2 | 3 | 4 |
| 52. Numbness or tingling in parts of your body | 0 | 1 | 2 | 3 | 4 |
| 53. A lump in your throat | 0 | 1 | 2 | 3 | 4 |
| 54. Feeling hopeless about the future | 0 | 1 | 2 | 3 | 4 |
| 55. Trouble concentrating | 0 | 1 | 2 | 3 | 4 |
| 56. Feeling weak in parts of your body | 0 | 1 | 2 | 3 | 4 |
| 57. Feeling tense or keyed up | 0 | 1 | 2 | 3 | 4 |
| 58. Heavy feelings in your arms or legs | 0 | 1 | 2 | 3 | 4 |
| 59. Thoughts of death or dying | 0 | 1 | 2 | 3 | 4 |
| 60. Overeating | 0 | 1 | 2 | 3 | 4 |
| 61. Feeling uneasy when people are watching or talking about you | 0 | 1 | 2 | 3 | 4 |
| 62. Having thoughts that are not your own | 0 | 1 | 2 | 3 | 4 |
| 63. Having urges to beat, injure, or harm someone | 0 | 1 | 2 | 3 | 4 |
| 64. Awakening in the early morning | 0 | 1 | 2 | 3 | 4 |
| 65. Having to repeat the same actions such as touching, counting, washing | 0 | 1 | 2 | 3 | 4 |
| 66. Sleep that is restless or disturbed | 0 | 1 | 2 | 3 | 4 |
| 67. Having urges to break or smash things | 0 | 1 | 2 | 3 | 4 |
| 68. Having ideas or beliefs that others do not share | 0 | 1 | 2 | 3 | 4 |

*Study* _____ *ID* _____

*Date* __ __ /__ __ /__ __ __ __

**FOR THE *PAST WEEK*, HOW MUCH WERE YOU BOTHERED BY:**

| | Not At All | A Little Bit | Moderately | Quite A Bit | Extremely |
|---|---|---|---|---|---|
| 69. Feeling very self-conscious with others | 0 | 1 | 2 | 3 | 4 |
| 70. Feeling uneasy in crowds, such as shopping or at a movie | 0 | 1 | 2 | 3 | 4 |
| 71. Feeling everything is an effort | 0 | 1 | 2 | 3 | 4 |
| 72. Spells of terror or panic | 0 | 1 | 2 | 3 | 4 |
| 73. Feeling uncomfortable about eating or drinking in public | 0 | 1 | 2 | 3 | 4 |
| 74. Getting into frequent arguments | 0 | 1 | 2 | 3 | 4 |
| 75. Feeling nervous when you are left alone | 0 | 1 | 2 | 3 | 4 |
| 76. Others not giving you proper credit for your achievements | 0 | 1 | 2 | 3 | 4 |
| 77. Feeling lonely even when you are with people | 0 | 1 | 2 | 3 | 4 |
| 78. Feeling so restless you couldn't sit still | 0 | 1 | 2 | 3 | 4 |
| 79. Feelings of worthlessness | 0 | 1 | 2 | 3 | 4 |
| 80. Feeling that familiar things are strange or unreal | 0 | 1 | 2 | 3 | 4 |
| 81. Shouting or throwing things | 0 | 1 | 2 | 3 | 4 |
| 82. Feeling afraid you will faint in public | 0 | 1 | 2 | 3 | 4 |
| 83. Feeling that people will take advantage of you if you let them | 0 | 1 | 2 | 3 | 4 |
| 84. Having thoughts about sex that bother you a lot | 0 | 1 | 2 | 3 | 4 |
| 85. The idea that you should be punished for your sins | 0 | 1 | 2 | 3 | 4 |
| 86. Feeling pushed to get things done | 0 | 1 | 2 | 3 | 4 |
| 87. The idea that something serious is wrong with your body | 0 | 1 | 2 | 3 | 4 |
| 88. Never feeling close to another person | 0 | 1 | 2 | 3 | 4 |
| 89. Feelings of guilt | 0 | 1 | 2 | 3 | 4 |
| 90. The idea that something is wrong with your mind | 0 | 1 | 2 | 3 | 4 |

Roche

V 0477

# Beck Depression Inventory

CRTN: _____ CRF number: _____

Page 14 patient inits: _____

**Baseline**

BDI-II®

Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

**Instructions:** This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

**1. Sadness**

0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad or unhappy that I can't stand it.

**2. Pessimism**

0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

**3. Past Failure**

0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

**4. Loss of Pleasure**

0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

**5. Guilty Feelings**

0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

**6. Punishment Feelings**

0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

**7. Self-Dislike**

0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

**8. Self-Criticalness**

0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

**9. Suicidal Thoughts or Wishes**

0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

**10. Crying**

0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

Subtotal Page 1

Continued on Back

THE PSYCHOLOGICAL CORPORATION®
Harcourt Brace & Company
SAN ANTONIO
Orlando • Boston • New York • Chicago • San Francisco • Atlanta • Dallas
San Diego • Philadelphia • Austin • Fort Worth • Toronto • London • Sydney



0154018392
NR15645

**11. Agitation**

0 I am no more restless or wound up than usual.

1 I feel more restless or wound up than usual.

2 I am so restless or agitated that it's hard to stay still.

3 I am so restless or agitated that I have to keep moving or doing something.

**12. Loss of Interest**

0 I have not lost interest in other people or activities.

1 I am less interested in other people or things than before.

2 I have lost most of my interest in other people or things.

3 It's hard to get interested in anything.

**13. Indecisiveness**

0 I make decisions about as well as ever.

1 I find it more difficult to make decisions than usual.

2 I have much greater difficulty in making decisions than I used to.

3 I have trouble making any decisions.

**14. Worthlessness**

0 I do not feel I am worthless.

1 I don't consider myself as worthwhile and useful as I used to.

2 I feel more worthless as compared to other people.

3 I feel utterly worthless.

**15. Loss of Energy**

0 I have as much energy as ever.

1 I have less energy than I used to have.

2 I don't have enough energy to do very much.

3 I don't have enough energy to do anything.

**16. Changes in Sleeping Pattern**

0 I have not experienced any change in my sleeping pattern.

1a I sleep somewhat more than usual.

1b I sleep somewhat less than usual.

2a I sleep a lot more than usual.

2b I sleep a lot less than usual.

3a I sleep most of the day.

3b I wake up 1–2 hours early and can't get back to sleep.

**17. Irritability**

0 I am no more irritable than usual.

1 I am more irritable than usual.

2 I am much more irritable than usual.

3 I am irritable all the time.

**18. Changes in Appetite**

0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.

1b My appetite is somewhat greater than usual.

2a My appetite is much less than before.

2b My appetite is much greater than usual.

3a I have no appetite at all.

3b I crave food all the time.

**19. Concentration Difficulty**

0 I can concentrate as well as ever.

1 I can't concentrate as well as usual.

2 It's hard to keep my mind on anything for very long.

3 I find I can't concentrate on anything.

**20. Tiredness or Fatigue**

0 I am no more tired or fatigued than usual.

1 I get more tired or fatigued more easily than usual.

2 I am too tired or fatigued to do a lot of the things I used to do.

3 I am too tired or fatigued to do most of the things I used to do.

**21. Loss of Interest in Sex**

0 I have not noticed any recent change in my interest in sex.

1 I am less interested in sex than I used to be.

2 I am much less interested in sex now.

3 I have lost interest in sex completely.

_____ Subtotal Page 2

_____ Subtotal Page 1

_____ Total Score

## BIOGRAPHY OF THE AUTHOR

Hira Shrestha was born and raised in Bhojpur, a small village in the hills of eastern Nepal. Her family migrated to Kathmandu, the capital city of Nepal when she was14 years old. She completed her education up to class 10 (School Leaving Certificate; SLC) from Vijaya Smarak High School in Dillibazar, Kathmandu, Nepal in 1993, and an Intermediate of Arts (2 years) degree with English concentration in 1995 from Kanya Multiple College of Dillibazar, an affiliated campus of Tribhuwan University, Nepal. She completed her Bachelor's of Arts (3 years) degree in English Literature with Culture and Sociology minor in 2003. Hira was a teacher in several private high schools as well as a non-academic English language instructor during her stay in Kathmandu until she decided to pursue her second Bachelor's degree in Psychology with Clinical Mental Health track (MHRT- Certified) from Husson University, Bangor, Maine in the Fall of 2006. She graduated *Magna Cum Laude* in December 2009, completed her practicum internship at the Acadia Hospital in Bangor under the supervision of Dr. David Prescott, and then joined the Bangor branch of the Developmental Neuroscience Lab of the University of Maine under the leadership of Professor Marie Hayes who is also Hira's mentor and advisor. Hira started her graduate degree in Developmental Psychology with Professor Hayes in the Fall of 2011 after a short visit to her family in Nepal. She is a candidate for the Master of Arts degree in Developmental Psychology from The University of Maine in August 2014.