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Neonatal Abstinence Syndrome: Neurobehavior at 6 Weeks of Age in Infants With or Without Pharmacological Treatment for Withdrawal

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Abstract

Use and abuse of prescription opioids and concomitant increase in Neonatal Abstinence Syndrome (NAS), a condition that may lead to protracted pharmacological treatment in more than 60% of infants, has tripled since 2000. This study assessed neurobehavioral development using the NICU Network Neurobehavioral Scale in six-week old infants with prenatal methadone exposure who did (NAS+; n=23) or did not (NAS-; n=16) require pharmacological treatment for NAS severity determined by Finnegan Scale. An unexposed, demographically similar group of infants matched for age served as comparison (COMP; n=21). NAS+, but not NAS- group, had significantly lower scores on the regulation ($p<.01$) and quality of movement ($p<.01$) summary scales than the COMP group. The NAS+ and NAS- groups had higher scores on the stress-abstinence scale than the COMP group ($p<.05$). NAS diagnosis (NAS+) was associated with poorer regulation and quality of movement at six weeks of age compared to infants without prenatal methadone exposure from the same demographic.

Keywords

Methadone; Neonatal Abstinence Syndrome (NAS); NICU Network Neurobehavioral Scales (NNNS); Neurobehavioral Development

Use and abuse of prescription opioids (e.g., Oxycontin, Percocet, Vicodin) has increased dramatically among individuals of childbearing age (CDC, 2012; Patrick, Davis, Lehman & Cooper, 2015). Rates of neonatal abstinence syndrome (NAS), a condition that may lead to protracted hospitalization in more than 60% of infants, have more than tripled since 2000, as has the cost of NAS treatment (Patrick, Schumacher, Benneyworth, Krans, McAllister, & Davis, 2012). Prevention and treatment of opioid use disorder during pregnancy, and development of new strategies to treat neonatal opioid withdrawal, has become a national health concern (Bagley, Wachman, Holland and Brogly, 2014; Hayes & Brown, 2012; Kraft, Stover & Davis, 2016; Logan, Hayes & Brown, 2013).

Methadone maintenance treatment is the accepted standard of care for opioid use disorder during pregnancy (Bart, 2012; Jones et al., 2008). Methadone is a long-acting, μ -opioid receptor agonist that reduces drug craving, mitigates physiological withdrawal symptoms, and limits the intoxication associated with opioid abuse (Joseph, Stancliff & Langrod, 2000). Beneficial outcomes for pregnant women include increased retention in drug treatment programs and receiving prenatal care earlier than opioid-dependent women not in treatment (Bart, 2012; Burns, Mattick, Lim & Wallace, 2006, Jones et al., 2008). Prenatal exposure to methadone maintenance treatment ensures regularity in the dose and interval of fetal opioid exposure during pregnancy in contrast to street narcotic use which is associated with binge and withdrawal episodes and fetal loss (Jones et al., 2008; Kaltenbach, Berghella, & Finnegan, 1998).

Prenatal exposure to opioids like methadone results in a withdrawal illness termed Neonatal Abstinence Syndrome (NAS) which is a constellation of symptoms that include, but are not limited to, high-pitched, inconsolable crying, gastro-intestinal distress, tremors, feeding and sleeping difficulties, hypertonia, sweating, and/or sneezing due to the absence of opioids in the neonate's system (Kocherlakota, 2014). NAS is initially treated with non-pharmacological methods (i.e. swaddling, low-lighting, quiet room, use of pacifiers, Kangaroo care), and if the symptoms do not show improvement or worsen as indicated by standard ratings of the Finnegan scoring tool (Hudak, Tan, The Committee on Drugs and The Committee on Fetus and Newborn, 2012), pharmacological interventions (e.g. morphine, methadone) may be used (Kocherlakota, 2014). Some factors contributing to NAS severity and need for pharmacological intervention include type of prenatal exposure (e.g. methadone, morphine, heroin), neonatal and maternal genetic variation and epigenetic changes in opioid metabolism (Kraft et al., 2016; Wachman et al., 2013; Wachman et al., 2014), as well as comorbid prenatal exposures to tobacco and benzodiazepines (Huestis, Schroeder & Jones, 2014; Logan et al., 2013; Pritham, Paul & Hayes, 2012).

A series of older studies examined the performance of infants with prenatal methadone exposure and subsequent withdrawal on infant neurobehavioral and developmental outcomes using the Neonatal Behavioral Assessment Scale (NBAS; Brazelton & Nugent, 1995, also referred to as the Brazelton Neonatal Behavioral Assessment Scale, BNBAS; Brazelton, 1973) and Bayley Scales of Infant Development (BSID; Bayley, 1969) with some consistent results. On the second day of life, several studies found that neonates with prenatal methadone exposure had poorer performance in orientation, motor maturity, cuddliness, and

state control as well as more irritability and tremulousness than non-substance exposed comparison infants (Chasnoff, Hatcher, Burns, 1982; Strauss, Lessen-Firestone, Starr, & Ostrea, 1976; Strauss, Starr, Otsrea, Chavez, & Stryker, 1975). At a one-month follow-up, Ramer & Lodge (1975) reported that infants with prenatal methadone exposure had poorer alertness and visual orientation and following when compared to normative data for the NBAS. Though the BSID findings are mixed, particularly with regard to the Mental Development Index (MDI), several studies consistently found poorer scores on the Motor Development Index (PDI) for infants with prenatal methadone exposure when compared to non-opioid exposed infants (see Kaltenbach, 1996 for review).

In sum, historical work has identified poorer performance on the NBAS and Bayley Scales in prenatal methadone-exposed samples vs. non-exposed samples. There is evidence of consistent findings with regard to poorer motor movement functioning among infants with prenatal methadone exposure. Motor delay has also been found to be associated with prenatal alcohol exposure (Kalberg et al., 2006; Landgren, Svensson, Stromland & Gronlund, 2010; Mattson, Crocker & Nguyen, 2011), a companion misuse in patients with opioid addiction that is often not examined. Careful measurement and analysis of comorbid prenatal exposure to alcohol, tobacco, and other substances were not consistently considered in all the aforementioned studies.

Advances in technology have created opportunities for studying the effects of prenatal methadone exposure on fetal physiological and behavioral responses. In fetal monitoring studies, methadone had transient, but disruptive effects on movement, movement-cardiac coupling and behavioral variability (Jansson et al., 2011; Salisbury et al., 2012). Additionally, a daughter measure to the NBAS, the NICU Network Neurobehavioral Scales (NNS; Lester & Tronick, 2004, see description below), was developed to study neurobehavioral outcomes in at-risk infants. Recent work has examined neurobehavioral differences between infants who require pharmacological intervention for NAS and those who do not. Like the historical results, there is considerable variability within the more recent findings. At three days of age, infants who needed pharmacological treatment for NAS showed poorer habituation to stimuli, more arousal and excitability, and higher hypertonicity than newborns who did not need pharmacological intervention (Velez, Jansson, Schroeder & William, 2009). At two weeks of age, pharmacologically-treated newborns have been reported to have poorer quality of movement, higher excitability, and more lethargy than non-pharmacologically-treated newborns (Jones, O'Grady, Johnson, Velez & Jansson, 2010).

To date, most studies examined the effects of prenatal methadone exposure and NAS in urban samples which have different environmental stressors than rural populations. Additionally, recent controlled studies of prenatal opioid exposure did not employ demographically similar comparison groups that share economic and socioemotional background features in the postnatal environment (e.g. Jansson et al., 2011; Jones et al., 2010; Salisbury et al., 2012; Velez et al., 2009). Finally, findings uncovered using the NNS by Jansson and colleagues during the early neonatal period (Jones et al., 2010; Velez et al., 2009) have not examined neurobehavioral functioning after resolution of NAS following discharge from the hospital.

Like previous recent research, this study examined prenatal methadone exposure, NAS severity and treatment effects on neurobehavioral performance in infants who were, or were not, pharmacologically-treated following opioid weaning and hospital discharge. Our cohort is rural, Caucasian, and from the Northeast which may confer different gene-environment consequences than African-American, Hispanic, and Caucasian cohorts from large urban areas. A demographically similar comparison group without opioid exposure with low socioeconomic status, and tobacco, alcohol, and marijuana polysubstance use typical of our regional pattern was used (Hayes, Brown, Hofmaster, Davare, Parker & Raczek, 2002; Jacobson & Jacobson, 2000). From this sample, we aimed to identify neurobehavioral differences among prenatal methadone-exposed newborns who did and did not require treatment and a demographically similar comparison cohort. Rather than assessing neurobehavioral outcomes in the immediate neonatal period, infants were assessed at six weeks of age after which time they were weaned from medications for treatment of NAS (if needed) and were discharged from the hospital.

Method

Participants

This IRB-approved study was part of a larger, longitudinal study examining the effects of prenatal methadone exposure and subsequent treatments for NAS conducted at Eastern Maine Medical Center (EMMC) and the University of Maine Developmental Laboratory. Participants were rural, economically disadvantaged mother-infant dyads (N=60). Thirty-nine women receiving methadone maintenance treatment at conception or before 20 weeks gestational age were recruited from Acadia Psychiatric Hospital and two other privately-owned, accredited, comprehensive treatment programs in the Bangor, Maine area. Approximately 200 methadone-exposed infants are born at the study hospital every year. The largest narcotics treatment program participating with this study served approximately 900 patients with 15% of those patients being pregnant at any giving time. The three participating narcotics treatment programs had similar rates of pregnant patients. These rates are consistent with national averages which suggest that rates of pregnant women admitted into treatment who admit prescription opioid abuse range between 2 to 28% (Martin, Longinaker, & Terplan, 2014).

Comparison mothers (n=21) were recruited from the Women, Infants and Children (WIC) program and the Bangor and EMMC's public health clinics during the third trimester of pregnancy. All women were English-speaking and between the ages of 18 and 40. None of the women in the study had serious medical conditions (e.g. cancer, gestational diabetes), pregnancy complications, or were being treated with buprenorphine. Caesarian sections were not exclusionary criteria for participation. All prenatal care and deliveries occurred at EMMC. To ensure ecological validity, we did not exclude women who used nicotine, alcohol, marijuana, or prescribed psychiatric medications from participating in the study.

Inclusion was limited to infants with gestational age greater than or equal to 37 weeks, five-minute Apgar scores greater than 6, birthweight and length between 10th and 90th percentile as determined by infant medical records, and absence of congenital abnormalities or serious perinatal illness (e.g. sepsis, gestational diabetes, thyroid disease). Data from one infant born

at 34 weeks postconceptional age was excluded. NICU admission for treatment of infant NAS symptoms occurred in some cases but was not exclusionary.

Infant electronic medical records were used to follow the pharmacological treatment of NAS in the postnatal period. Pediatrics protocol required pharmacological intervention for infants scoring greater than or equal to 8 on the Finnegan Scoring tool for three consecutive scoring sessions or 12 or above at any point during the hospital stay (Hudak et al., 2012). Need for pharmacotherapy in response to NAS symptoms determined placement into the pharmacologically-treated infant group (NAS+; N=23) or the non-pharmacologically-treated group (NAS-; N=16). The decision to start pharmacotherapy was made by the attending physician. Methadone was administered to all infants requiring pharmacotherapy. In approximately 25% of NAS+ cases, a 2nd line drug, clonidine was prescribed when opioid weaning was unsuccessful. Two infants in the NAS+ group were prescribed phenobarbital in addition to clonidine to control NAS symptoms.

Instruments

Hollingshead four-factor index of social status—This measure of socioeconomic status assigns weighted point values to education and occupation levels for both the mother and her partner (if applicable). In cases where only one member of the family worked or the mother did not have a partner, only one individual's score was used and the average was not computed. For families in which neither partner worked, the score belonging to the individual with the most education was utilized. Hollingshead scores range from 8 to 66 with higher scores indicating higher socioeconomic status (Hollingshead, 1975).

Peabody Picture Vocabulary Test – 3rd edition (PPVT-III)—The PPVT-III is a measure of verbal ability that was administered to mothers during the third trimester visit. For this study, the standard score was used for analyses. This score can range from 40 to 160 with higher scores indicating better verbal ability (Dunn & Dunn, 1997).

Quantity-Variability-Frequency (QVF)—The QVF is a commonly used method of guided interview that determines alcohol, tobacco, marijuana, opioid, cocaine/crack, barbiturate, sedative, tranquilizer, amphetamine, methamphetamine, antidepressant, hallucinogen, and inhalant use (Jacobson, Jacobson & Sokol, 1994; Jacobson, Jacobson, Sokol, Martier & Ager, 1993) that was administered to mothers during the third trimester visit. Drug (illicit and licit) and alcohol use was assessed by asking if the participant used a particular substance (coded as “yes” or “no”), at what age she started using that drug, and if she used that drug at any point during her pregnancy (even before she knew she was pregnant; coded as “yes” or “no”). If the participant stated that she used a substance, she was then asked the number of days per week the substance was used at the time of heaviest use and during pregnancy (if applicable), the approximate amount of that substance she used per day during the period of heaviest use and during pregnancy (if applicable), and number of years the substance was used (Cahalan, Cisin & Crossley, 1969; Lester et al., 2002). Absolute alcohol intake was calculated from this information (pre-pregnancy and pregnancy intake rates) by multiplying the number of weekly drinks by the total ounces consumed in each drink by the alcohol content (.04 for beer, .02 for wine and .4 for liquor).

Electronic medical records—Mothers signed Health Insurance Portability and Accountability Act (HIPAA) forms for herself and the newborn. Essential research staff had access to mother and infant medical records at the delivery hospital and from treatment clinics. Variables of interest included maternal methadone dose, labor and delivery notes, infant birth parameters, infant pharmacological treatment, and infant length of stay.

NICU Network Neurobehavioral Scale (NNNS)—The NNNS exam is intended to measure neurobehavioral development in at-risk infants. The 30-minute assessment involves observation of and ordered introduction of various stimuli to the infant by a certified examiner trained to administer and score infant performance (see Lester & Tronick, 2004). The NNNS is interpreted using 11 summary scores: habituation, attention, handling, quality of movement, arousal, regulation, reflexes, tone, excitability, lethargy, and stress/abstinence.

The summary scores of interest for this study were regulation, quality of movement, stress/abstinence, and attention. Regulation is defined as “Capacity to organize motor activity, physiology, and state during the examination and to respond to cuddling, consoling, and negative stimuli...” and has a range of scores between 1 and 9. Quality of movement is defined as, “Measure of motor control including smoothness, maturity, lack of startles and tremors...” and has a range of scores between 1 and 9. Stress/abstinence is defined as, “Amount of stress and abstinence signs observed during examination...” and ranges in score between 0–1 (Lester & Tronick, 2004, p. 694).

The NNNS summary scores have internal consistency (Fink, Tronick, Olson, & Lester, 2012) and predictive validity of early childhood behavioral outcomes (Liu et al., 2010). The NNNS certification process ensures inter-examiner reliability (Lester & Tronick, 2004). Neurobehavioral status of high-risk newborns (30–46 weeks gestational age), particularly those prenatally exposed to substances of abuse such as methamphetamines, cocaine, and opioids as well as polysubstance use has been measured using the NNNS (e.g. Conratt et al., 2013; Lester, et al., 2002; LaGasse et al., 2011) making this an appropriate measure for infants with prenatal methadone exposure.

Procedure

This report summarizes findings from a third trimester maternal interview and a six-week postnatal follow-up appointment with mothers and infants. Participants were recruited by brochures available at recruitment sites at the hospital and narcotics treatment programs. Interested pregnant women were consented during the third trimester interview. After consent was obtained, participants completed a brief demographic form from which the Hollingshead Four Factor Index of Social Status was calculated. The PPVT was then administered followed by the QVF. At the conclusion of the visit, the participant received \$100 cash or \$100 gift card for completing the maternal assessments.

At 6 weeks, mothers and infants returned to the laboratory for NNNS testing. All infants had been discharged from the hospital for 2–5 weeks and were no longer receiving pharmacological treatment for NAS (if applicable). For the six-week postnatal follow-up (average infant age = 41.6 days, SEM $\pm$ 1.9), mothers and infants met with research staff in a private room at Acadia psychiatric hospital or at the WIC program. Mother were seated

at an office desk and asked to complete several paper-and-pencil surveys as part of the larger longitudinal study. While mothers completed survey assessments, research staff conducted the NNNS using a dedicated bassinette under warm conditions with lowered ambient light. The NNNS was completed by certified NNNS examiners who received training at Brown University and the Women and Infants Hospital in Providence, RI (see Lester & Tronick, 2004). Examiners were blind to infant treatment status. At the conclusion of testing and maternal assessments, mothers received a \$50 gift card.

Statistics & Design

One-way ANOVAs with Bonferroni post-hoc corrections were used to compare group means (COMP, NAS–, and NAS+) for maternal demographic data, infant birth parameter data, and infant NNNS summary scales outcomes. χ^2 analyses were used to compare groups on categorical variables (e.g. pregnancy SSRI & benzodiazepine use, infant sex). ANCOVAs were used to examine maternal and infant characteristics that differed between groups as possible covariates contributing to neurobehavioral development beyond prenatal exposure and treatment status alone.

Results

Maternal Demographic and Substance Use Characteristics

Maternal characteristics were derived from data collected at the third trimester maternal interview. Pregnancy use data for cigarettes, marijuana, non-prescription opioids, prescribed SSRIs, prescribed benzodiazepines, and pre-pregnancy alcohol use are presented in Table 1. Pre-pregnancy alcohol use is presented instead of pregnancy alcohol use in this report due to few self-reports of alcohol use during pregnancy. Pre-pregnancy alcohol use rates have been found to be linearly related to pregnancy alcohol use in this population (Hayes et al., 2002). Though opioid use disorder was the defining maternal group characteristic, two women in the comparison group self-reported infrequent nonprescription opioid use, but did not have opioid use disorder diagnoses. Their infants' birth parameters fell within the normal range of scores for infants in the comparison group, and thus, these data were included in the analyses. Similar rates of maternal nonprescription opioid use (in addition to methadone maintenance treatment) were reported in the NAS– and NAS+ groups.

As shown in Table 1, a main effect of group, $F(2, 57)=10.5$, $p<.001$, on third trimester tobacco use was found such that both prenatal methadone-exposed groups, NAS– and NAS+, had significantly higher tobacco use than the COMP group ($p<.01$ for both groups), but did not differ from each other ($p>.05$). Additionally, a χ^2 analysis revealed that a significantly smaller proportion of the COMP group smoked cigarettes during the third trimester of pregnancy than the NAS– or NAS+ group ($p<.01$). There were no other significant differences between groups on any maternal demographic or self-reported substance use variables during pregnancy (see Table 1). [Approximate point of insertion for Table 1]

Infant Characteristics

As shown in Table 2, the NAS– group ($p < .01$), but not NAS+ group ($p > .05$), had significantly lower birth weights than the COMP group, $F(2, 57) = 4.4$, $p < .05$. When third trimester maternal tobacco use was added as a covariate to this analysis, there was no longer an effect of group status on birthweight. Groups did not differ on any other birth parameters. The NAS+ group spent on average significantly more days (~26 days) in the hospital than COMP (~2 days) and NAS– (~7 days) groups, $F(2, 57) = 64.1$, $p < .001$. [Approximate point of insertion for Table 2]

NNNS Summary Scores

NNNS summary scores and statistics for all groups are listed in Table 3. [Approximate point of insertion for Table 3] There were significant differences found for the regulation, quality of movement, and stress/abstinence summary scores. The COMP group differed from the NAS+ on the regulation summary score, $F(2, 56) = 7.9$, $p < .01$, and the quality of movement summary score, $F(2, 56) = 7.7$, $p < .01$. Infants in the COMP group had significantly better regulation ($p < .01$) and quality of movement ($p < .01$) than the NAS+ infants. NAS– group had intermediate scores on these two summary scales but showed no statistical difference from either the NAS+ or COMP group.

As would be expected, the COMP group had a significantly lower mean value on the stress/abstinence summary score than the NAS+ ($p < .05$) and NAS– groups ($p < .05$), $F(2, 55) = 4.8$, $p < .05$. No significant differences were found between group means for the following summary scores: habituation, attention, arousal, handling, excitability, lethargy, non-optimal reflexes, asymmetrical reflects, hypertonicity, and hypotonicity.

Influence of Maternal and Infant Characteristics on NNNs Summary Scores

To determine if any maternal and infant characteristics that differed between groups (e.g. maternal tobacco use, infant birth weight) influenced the relevant NNNs summary scores (regulation, quality of movement or stress/abstinence), ANCOVA analyses were conducted. Because length of stay was extended due to need for infant pharmacological treatment, length of stay was not added to these analyses to avoid multicollinearity. Because of limited power, more than one covariate could not be added to a model at a time. The addition of covariates did not improve any statistical models.

Discussion

The purpose of this ecologically valid study was to investigate the impact of prenatal methadone exposure with NAS and subsequent pharmacological treatment on infant neurobehavioral development at six weeks of age. Infants with prenatal methadone exposure were contrasted with a non-exposed group of demographically similar infants in whom prenatal exposure to tobacco, alcohol, marijuana, and prescription psychiatric medication was not exclusionary.

Preliminary statistical analyses revealed significant differences between the comparison and prenatal methadone-exposed groups on several maternal demographic and infant birth

parameter variables. The NAS+ and NAS- groups had higher proportions of women who smoked during pregnancy. Mothers in these groups also smoked more cigarettes per day in the third trimester of pregnancy than comparison mothers. Beyond maternal use of cigarettes, the groups did not differ from each other on maternal demographic and substance use/abuse variables. The use of a demographically similar comparison group of mothers rather than a non-substance using, middle-to-high socioeconomic status control group, is a strength of this study and accounted for pregnancy risk variables associated with economic disadvantage.

Methadone-exposed infants who did not require pharmacological treatment for NAS (NAS-) weighed significantly less than infants in the comparison group (COMP). This finding was no longer significant when third trimester cigarette use was statistically controlled. The effects of prenatal tobacco exposure on neonatal birthweight is supported by a well-established literature (see Rogers, 2009 for review). Pharmacologically-treated prenatal methadone-exposed (NAS+) infants spent, on average, two weeks longer in the hospital than the non-pharmacologically-treated (NAS-) infants and three weeks longer than comparison infants (COMP). This finding was expected as NAS+ infants were required to stay in the hospital until they were successfully weaned from opioid medications. Statistical models analyzing differences in lengths of stay between groups were not improved when maternal tobacco use or infant birth weight were added as covariates.

There were no significant mean differences between groups for most of the NNNS summary scales; however, ANOVA analyses revealed that non-exposed, comparison infants (COMP) had significantly better regulation and quality of movement than methadone-exposed infants pharmacologically-treated for NAS (NAS+). Methadone-exposed infants requiring pharmacological treatment for NAS (NAS+) did not differ from methadone-exposed infants who did not require pharmacotherapy for NAS (NAS-) on these measures. The NAS- group did not differ from the COMP group on any of these measures either. Importantly, whether reaching criterion for pharmacological treatment or not, prenatal methadone-exposed infants did not differ in stress/abstinence signs at six-weeks of age. These findings suggests that regardless of need for pharmacological treatment, prenatal methadone exposure may be associated with lingering neurobehavioral effects related to the stress/abstinence symptoms at six-weeks of age.

The NNNS regulation, quality of movement, and stress-abstinence findings replicate previous work that found relationships between prenatal opioid exposure, treatment for NAS, and non-optimal neurobehavioral outcomes on the NNNS summary scores at or before six weeks of life (Coyle et al., 2012; Jones et al., 2010; Velez et al., 2009). Jones et al. (2010) found that infants with prenatal methadone exposure who were pharmacologically treated for NAS had differences in NNNS summary scale scores, including quality of movement, as compared to exposed infants who did not require pharmacological intervention during the first two weeks of life. Coyle et al. (2012) reported that opioid-exposed infants showed improvements in self-regulation, attention and quality of movement and decreases in arousal, excitability, depression and hypertonia during the first month. Like our study, Velez et al. (2009) specifically examined prenatal methadone exposure, NAS, and pharmacological treatment for NAS; however, their neonates were assessed at three days of

age. The authors for this study found that infants requiring pharmacological treatment were more aroused, excitable, hypertonic, and took longer to habituate to stimuli than infants who did not require treatment. The current study complements these reports because infants were examined at a later age (six-weeks) and a non-exposed but at-risk comparison group was used. Because prenatal methadone-exposed and comparison infant group averages did not differ on most of the NNNS summary scores at six-weeks of age, neurobehavioral functioning of exposed infants may have improved over time. That said, stress/abstinence symptoms were still present in methadone-exposed infants regardless of need for pharmacological treatment during the neonatal period.

These findings replicate previous research that used a similar scale, the NBAS, to examine neurobehavioral development in infants with prenatal opioid exposure. Previous work consistently found that infants with prenatal opioid exposure had poorer state control and more irritability (Chasnoff et al., 1982; Strauss, et al., 1976; Strauss et al., 1975) which are components of the regulation construct of the NNNS. The quality of movement finding is consistent with the historic BSID literature that reported poorer scores on the Motor Development Index for infants with prenatal opioid exposure (Kaltenbach, 1996). Results from the current study build on past research by dividing the prenatal opioid exposure group into groups of infants who required and did not require pharmacological treatment. This study adds to the existing literature by describing unique neurobehavioral profiles of infants who have the added risk of pre-and postnatal opioid exposure in the context of a low socioeconomic environment. The unique neurobehavioral profile of these infants is different than infants who share the disadvantaged context but do not have opioid exposure throughout the prenatal period.

The findings of this study have important developmental implications. Previous work has demonstrated that early neurobehavioral differences measured by the NNNS as a result of prenatal exposures have been linked to later developmental delays during early childhood (Liu et al., 2010). The long-term implications of prenatal methadone exposure with or without NAS pharmacological treatment may persist throughout childhood and emphasize the need for early identification of developmental disorder markers. Such early identification can ensure proper intervention for maternal-child dyads in this high-risk population.

While ecological validity is a strength of this study, there are several limitations. Because access to names and contact information for all socioeconomically disadvantaged pregnant women in the region was not available, random sampling was not possible. The women who volunteered to participate were self-selecting and may have been fundamentally different than women who were not interested in participating. The findings of this study may not be generalizable to all women in methadone maintenance treatment and infants with prenatal methadone exposure.

The statistical analyses may be underpowered due to small group size; however, the sample size is comparable to several other recent studies on this topic (e.g. Coyle et al., 2012; Jones et al. 2010). Due to the small sample size, the complexity of statistical models was limited when examining potential covariates that contributed to infant outcomes. Social desirability bias may have influenced self-reports regarding alcohol and substance use and abuse during

pregnancy. Participants in all groups may have been using substances throughout their pregnancies. While having maternal pregnancy urine toxicology and breathalyzer screens would have been ideal, such screens were not part of the standard of care for women in the comparison group and were only randomly used in the methadone maintenance group as part of the clinics' standards of care. There was not enough urine toxicology screening data to run meaningful statistical analyses. There was an attempt to control for the possibility of prenatal substance use/abuse by recruiting a demographically similar comparison group with alcohol, tobacco, marijuana, and psychiatric medication use histories.

Infant exposure to prescribed medications was also a variable for which we could not control. Though the groups did not differ in maternal use of licit prescription medications, a number of variables could have contributed to infant outcomes including dose of the prescription, adherence to prescription regimen, and individual differences in medication metabolism. Though the majority of infants (65%) who required pharmacological intervention to treat NAS were only prescribed one medication, a little over a third of the infants in this group required 2nd or 3rd line drugs. Due to the sample size, analyses could not be conducted to determine if specific pharmacological treatments or combination of treatments (prenatal or postnatal) may have contributed to neurobehavioral outcomes.

Another limitation of this clinical study was that infant hospital length of stay was related to NAS pharmacological treatment status. Pharmacologically-treated prenatal methadone-exposed (NAS+) infants spent approximately two weeks longer in the hospital than non-pharmacologically-treated prenatal methadone-exposed (NAS-) infants. NAS+ infants were required to stay in the hospital until they were successfully weaned from opioid medications. NAS+ infants' poorer postnatal clinical outcomes may reflect exposure to more adverse prenatal conditions than NAS- infants which are related to maternal use of other recreational or prescribed substances (Choo et al., 2004; Pritham et al., 2012). However, maternal use of alcohol or tobacco did not differ between prenatal methadone-exposed groups based on the maternal interview (Table 1). Statistical models using length of stay as the dependent variable and group as the quasi-independent variable were not changed when maternal tobacco, alcohol or marijuana were added as covariates. Finally, though the main focus of this study was to examine prenatal variables contributing to NAS treatment, many postnatal maternal factors that may have impacted infant neurobehavioral development within the first six weeks of life were not considered.

This is the first study to date that has used a demographically similar comparison group of infants, rather than a control group, to examine prenatal methadone exposure, NAS and pharmacological treatment, and infant neurobehavioral profiles as measured by the NNNS after release from the hospital in a rural, Caucasian sample. This design helped to identify the unique post-NAS neurobehavioral profiles of newborns with prenatal methadone exposure who did and did not require treatment in this population. These findings will assist health care professionals in developing unique parenting intervention techniques for high-risk dyads in this methadone maintenance treatment population.

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Table 1**Maternal Demographics and Substance Use**

	COMP (n=21)		NAS- (n=16)		NAS+ (n=23)		<i>p</i>
	Mean	SD	Mean	SD	Mean	SD	
Maternal Age	24.7	2.9	27.3	3.6	26.0	3.8	<i>ns</i>
PPVT (IQ)	95.8	8.0	94.6	6.0	95.3	6.9	<i>ns</i>
Hollingshead SES	26.3	8.8	24.0	7.6	23.0	8.6	<i>ns</i>
Pregnancy Cigarette Use (% Yes) ^a	38		100		87		<.001
Pregnancy Cigarettes/Day ^a	3.0	4.3	10.9	6.1	9.7	6.9	<.001
Pre-pregnancy Alcohol Use (% Yes)	62		68		77		<i>ns</i>
Pre-pregnancy AA/DD (oz.)	2.0	2.3	3.9	4.7	3.2	2.7	<i>ns</i>
Pregnancy Marijuana Use (% Yes)	33		63		52		<i>ns</i>
Pregnancy Marijuana Use (times/week)	1.4	2.6	1.4	2.4	1.8	2.7	<i>ns</i>
Pregnancy Opioid Nonprescription Use (% Yes)	10		31		9		<i>ns</i>
Pregnancy SSRI Use (% Yes)	10.0		12.5		17.3		<i>ns</i>
Pregnancy Benzodiazapine Use (% Yes)	0.0		12.5		9.0		<i>ns</i>
Methadone dose at birth (mg)	–	–	101.9	51.0	101.5	64.5	.001
							<i>ns</i>

SES, socioeconomic status;

AA/DD, Absolute alcohol/drinking day

Pregnancy = Third trimester of pregnancy

^aCOMP different than NAS- and NAS+ (p<.05)

Neonatal Characteristics

Table 2

	COMP (n=21)		NAS- (n=16)		NAS+ (n=23)		<i>F</i> / <i>x</i> ²	<i>p</i>
	Mean	SD	Mean	SD	Mean	SD		
Gestational Age (weeks)	39.4	1.3	39.4	1.5	39.0	1.5	0.6	<i>ns</i>
Birth Weight (kg) ^b	3.5	0.4	3.1	0.4	3.3	0.5	4.4	<.05
Birth Length (cm)	51.3	4.1	50.0	2.5	51.3	2.6	1.0	<i>ns</i>
Head Circumference (cm)	34.0	1.3	34.2	1.2	34.1	1.4	0.1	<i>ns</i>
Length of Stay (Days) ^c	2.1	0.7	7.1	6.9	26.0	10.3	64.1	<.001
Follow Up Age (days)	39.8	10.7	41.9	12.3	43.1	18.9	0.3	<i>ns</i>
Breastfeeding (% breastfed at least some of the time) ^d	–	–	50		43		0.5	<i>ns</i>
Sex (% female)	48		39		48		0.2	<i>ns</i>

^bCOMP different than NAS- (*p*<.05)^cNAS+ different than COMP and NAS- (*p*<.01)^dBreastfeeding data was only collected from the prenatal methadone-exposed groups

Table 3

NNNS Subscale Scores between Groups

	COMP (n=21)			NAS- (n=16)			NAS+ (n=23)			<i>F</i>	<i>p</i>
	Mean (SD)	Range		Mean(SD)	Range		Mean(SD)	Range			
Habituation	5.6 (1.6)	3.0 – 7.3		8.5 (0.7)	8.0–9.0		6.0 (1.9)	4.5–7.7		2.3	<i>ns</i>
Attention	5.0 (1.4)	3.0 – 7.2		4.5 (1.3)	2.3–6.1		4.5 (1.4)	2.6 – 6.5		0.6	<i>ns</i>
Arousal	4.0 (0.9)	2.4 – 5.5		4.3 (0.9)	2.9–6.2		4.4 (0.8)	2.9–5.7		1.3	<i>ns</i>
Regulation ^d	6.0 (0.9)	4.0–7.8		5.2 (1.0)	3.1–6.9		4.8 (1.0)	2.8–7.4		7.4	<.01
Handling	0.3 (0.3)	0.0 – 0.8		0.4 (0.3)	0.0–0.9		0.4 (0.3)	0.0–0.8		0.5	<i>ns</i>
Quality of Movement ^d	5.1 (0.7)	3.0–6.0		4.6 (0.8)	3.4–5.7		4.1 (0.9)	2.2–5.5		7.7	<.01
Excitability	2.7 (2.1)	0.0–6.0		3.8 (2.7)	0.0–8.0		4.1 (2.5)	0.0–8.0		1.9	<i>ns</i>
Lethargy	4.1 (2.4)	1.0–9.0		4.4 (2.4)	0.0–8.0		3.9 (2.5)	1.0–11.0		0.5	<i>ns</i>
Non-optimal reflexes	5.4 (2.2)	1.0–9.0		4.9 (2.8)	0.0–9.0		5.6 (2.2)	2.0–10.0		0.2	<i>ns</i>
Asymmetrical Reflexes	1.1 (0.9)	0.0 – 3.0		1.3 (0.9)	0.0–3.0		1.1 (1.1)	0.0–4.0		0.1	<i>ns</i>
Hypertonicity	0.5 (0.9)	0.0 – 4.0		0.9 (1.0)	0.0–3.0		0.7 (1.1)	0.0–3.0		0.6	<i>ns</i>
Hypotonicity	0.3 (0.6)	0.0–2.0		0.6 (0.7)	0.0–2.0		0.6 (0.9)	0.0–3.0		1.2	<i>ns</i>
Stress/Abstinence ^e	0.1 (0.04)	0.02 – 0.14		0.1 (0.1)	0.04–0.51		0.1 (0.1)	0.02–0.49		4.9	<.05

^dCOMP different than NAS+ ($p<.01$)^eCOMP different than NAS- and NAS+ ($p<.05$)