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# Prenatal programming of infant neurobehavior in a healthy population

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## Abstract

**Background—**Identifying the prenatal origins of mental conditions is of increasing interest, yet most studies have focused on high-risk populations and cannot disentangle prenatal and postnatal programming effects. Thus, we examined whether profiles of neurobehavior indicative of future risk could be identified in healthy 1–3 day old infants, and examined associations with perinatal risk factors.

**Methods—**Participants included 627 healthy mothers and term infants from a population-based US cohort. Neurobehavior was assessed within 24–72 hours after delivery with the NICU Network Neurobehavioral Scales (NNNS). A model-based clustering algorithm was used to derive neurobehavioral profiles from NNNS scores. Maternal health histories, pregnancy conditions and behaviors, labor/delivery factors and infant attributes were examined in relation to the neurobehavioral profiles.

**Results—**Seven discrete neurobehavioral profiles were identified, including one average functioning profile, and two inversely patterned below and above average profiles. Higher pregnancy weight gain (OR=1.44, 95%CI: 1.10, 1.88) and birthweight percentiles (OR=1.46, 95%CI: 1.10, 1.95) were associated with greater odds of below average newborn neurobehavior. Above average neurobehavior was associated with experiencing longer gestations (OR=1.29, 95%CI: 1.02, 1.64) and higher 5-minute APGAR scores (OR=2.43, 95%CI: 1.07–5.52). Maternal

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pregnancy alcohol use (OR=0.54, 95%CI: 0.33, 0.89), and fetal distress (OR=0.10, 95%CI: 0.01, 0.72) were associated with lower likelihood of having average neurobehavior.

**Conclusion—**Distinct profiles of neurobehavior can be derived in a healthy population of newborns, with different sets of perinatal factors predicting different patterns of neurobehavior. These findings suggest a potential *in utero* origin for mental health risk.

## INTRODUCTION

Identification of the early life origins of mental health conditions, including childhood cognitive and behavioral problems, is of increasing interest in order to better understand disease etiology and identify novel avenues for intervention[1]. The examination of newborn neurobehavior, considered a potential sentinel of future behavioral and cognitive functioning[2–4], provides a unique opportunity to identify prenatal influences on mental functioning before postnatal factors alter risk trajectories.

The Neonatal Intensive Care Unit Network Neurobehavioral Scale (NNNS) is a validated assessment that comprehensively measures neurobehavior among healthy and high risk infants[5] and prospectively predicts neurological problems, behavior and cognitive function in childhood[2–4]. Previous work has found NNNS performance to be associated with a range of adverse intrauterine exposures[4,6–13] and also placental epigenetic alterations that signifies exposure to an adverse fetal environment[14–16] among both healthy and higher risk infants. Moreover, one study of healthy infants established NNNS norms and observed several associations with typical markers of perinatal risk[17]. These studies suggest that the NNNS is sensitive to perturbations in the perinatal period and is a useful tool to explore developmental origins of disease hypotheses.

The NNNS involves an assessment of neurobehavior across 13 domains. The natural heterogeneity in neurobehavior can make it difficult to discriminate between healthy and at-risk infants, as well as understand overall level of functioning. The domains, while discrete, are not independent but represent elements of the character and control of neurobehavioral function and regulation. Moreover, they are likely to be coordinately regulated and/or coordinately disrupted. Thus, one way to organize NNNS information and capitalize on its wealth of information is to group infants according to scores across multiple subscales and derive discrete profiles of functioning. This approach would reveal groups who share similar patterns of neurobehavior across NNNS domains, and enhance discrimination of high-risk infants from the rest of the population. Two recent studies that used this strategy found similarly patterned high-risk and lower risk groups, although the number of profiles varied across samples[2,18].

While the latter studies demonstrated that profiles can be distilled from NNNS summary scores, two questions remain. First, Liu[2] identified a set of profiles among a sample of high-risk infants that were prenatally exposed to cocaine and other substances. It is not known whether similar profiles would be identifiable in healthy infants. Second, both Liu[2] and Sucharew[18] assessed neurobehavior among 1 month old infants. This lag in time between birth and NNNS assessment may have introduced postnatal programming effects as neurobehavioral programming extends well into the postnatal period[19,20]. Therefore, it

unclear whether the NNNS profiles derived in these studies was indicative of prenatal exposures, the postnatal environment or a mix of the two. Thus, working from a developmental origins of health and disease framework[21], we extend this prior work by deriving profiles of newborn neurobehavior using NNNS information collected at birth in a healthy population. We hypothesized that a set of discrete profiles characterized by gradations in poor, average, and above average neurobehavioral functioning would be observed. We also examined a wide range of prenatal maternal morbidities, birth, and infant attributes as predictive of profile membership. By replicating NNNS profiles in a different population, and at the earliest time points possible, our study illustrates the utility of the NNNS assessment for reliably discriminating meaningful variations in infant neurobehavior. This study also highlights novel sets of perinatal risk factors amenable to intervention that help explain differences in infant neurobehavior.

## METHODS

### Study Population

Participants were part of the Rhode Island Child Health Study, which enrolled mother and infant pairs following delivery at the Women and Infants Hospital of Rhode Island (Providence, RI, USA) from 2009–2013. Term infants (>37 weeks gestation) born small for gestational age (lowest $10^{th}$ percentile), or large for gestational age (highest $10^{th}$ percentile), based on birthweight and gestational age and calculated from the Fenton growth chart[22], were selected; infants appropriately sized for gestational age (AGA) matched on sex, gestational age (±3 days), and maternal age (±3 years) were also enrolled. Only singleton, viable infants were included in the study. Other exclusion criteria was maternal age (<18 or >40 years excluded), a life-threatening medical complication of the mother, and congenital or chromosomal abnormality of the infant. 804 infants were enrolled and 627 infants were assessed with the NNNS (78%). Low levels of missing NNNS summary scores was observed, with the exception of Habituation (n=343, 45% missing) as this subscale requires that the infant be in a sleep state to administer. There were no differences between participants with (n=627) and without (n=177) NNNS information according to any demographic perinatal factor considered in this study (data not shown), although infants missing NNNS information had mothers who were on average 1 year older than those with nonmissing NNNS information ($t$=−2.51, p=0.01). Study protocols were approved by Institutional Review Boards at Women and Infants' Hospital and Dartmouth College.

### Measures

**NICU Network Neurobehavioral Scale—**Infant neurobehavior was assessed with the NNNS by one certified psychometrician blinded to the prenatal history. Assessments were conducted after the first 24 hours of life but before hospital discharge[5,23]. Thirteen summary scores are derived from the exam and are listed in the Supplementary Table.

**Perinatal Predictors—**We examined maternal health, pregnancy conditions, health behaviors, labor/delivery factors, and infant attributes as predictors of neurobehavior. We examined these factors as they are known perinatal risks and are identifiable and amenable

to intervention in clinical settings. Predictor information was collected via medical record abstraction and self-report questionnaire.

*Maternal health history* was assessed as medical record indicated lifetime diagnoses of asthma, diabetes (any type), and pre-pregnancy obesity (body mass index ≥ 30, calculated from self-reported height and weight). *Pregnancy conditions* included the presence or absence of gestational diabetes, preeclampsia, depression, and anxiety/obsessive compulsive disorder/panic attacks as recorded in the medical record. Pregnancy weight gain was self-reported (standardized to have mean=0, standard deviation=1). Maternal health and pregnancy conditions were examined if the prevalence was >5%. *Pregnancy health behaviors* included any self-reported smoking (yes/no), alcohol use (yes/no), and hours of moderate/vigorous physical activity per week. Illicit drug use was not examined due to low prevalence (<1%). *Labor and delivery factors* as listed in the medical record included cesarean delivery, labor induction, medication use during labor, fetal distress, and breech birth (all yes/no). *Infant attributes* as listed in the medical record included birthweight percentile (standardized to have mean=0, standard deviation=1), gestational age at birth (weeks), and Apgar score (1 and 5 minutes after birth).

*Covariates* included self-reported maternal age, race (white/not white), education attainment (dichotomized as high school or less versus more than high school), and infant sex as listed in the medical record. Birthweight percentile was treated as a covariate, and also examined as an independent predictor of neurobehavior in separate models.

## Data Analysis

A Gaussian-distributed recursively partitioned mixture model (RPMM)[16,24] was used to cluster infants into discrete profiles based the NNNS summary scores. This methodology is similar conceptually to the latent profile analysis (LPA) used to derive NNNS profiles in previous work[2,18] but differs in its computational efficiency. Both approaches can discern the optimal number of clusters in a dataset. Where LPA requires the *a priori* specification of the hypothesized number of clusters, and verification of the optimal number of clusters via model fitting and comparing goodness of fit statistics, RPMM derives the number of clusters in the dataset without such *a priori* specification and sequential model fitting. We used RPMM to identify discrete groups of infants according to their 13 NNNS summary scores. Once RPMM returned a set of profiles, we then verified that each profile was distinct by testing the differences in summary scores across profiles via ANOVA tests and also by visually inspecting profile plots. Next, average, below average (poor) and above average (positive) neurobehavioral profiles were identified: profiles with scores across NNNS domains greater than 0.5 standard deviations from the mean were considered as above or below average performance; scores less than 0.5 standard deviations from the mean were considered average. We implemented Tukey post-hoc tests to assess whether summary scores were significantly different between the below average and above average profiles, the below average and average profiles, and above average and average profiles. Next, to identify the perinatal predictors of profile membership, we fit multivariable logistic regression models with each profile as a binary outcome (1=profile of interest, 0=all other infants). Finally, to address multiple comparisons, we implemented the Benjamini-Hochberg

procedure to control for false discoveries by estimating q-values and the expected proportion of false discovery for associations with p-values<0.05[25]. Profiles were derived for the 627 infants with NNNS data. Multiple imputation procedures (PROC MI and MIANALYZE; SAS Institute Inc.) were used to impute missing values on perinatal predictors and covariates and pool estimates from five imputed datasets. Analyses were conducted with R (http://cran.r-project.org; RPMM package) and SAS (Version 9.3).

## RESULTS

### Participant Characteristics

Participant characteristics and amount of missing data prior to imputation per covariate are listed in Table 1 (n=627). Mothers were on average 29 years old, 72% white, 28% had a high school education or less, and were generally healthy. Twenty six percent were obese prior to pregnancy, women gained approximately 14.4 kg during pregnancy (range −7.2 to 44.6), and engaged in less than one hour of physical activity per week. One third used alcohol during pregnancy, and less than 5% smoked. Half of deliveries were cesarean, 28% were induced, and 23% had no medication during labor. Infants were born on average at 39 weeks gestation and mean birthweight percentile was 53%. Low levels of fetal distress and breech births occurred. Infants had high Apgar scores and half were male. Overall, there was a low level of missing perinatal and covariate information in this study with the exception of pregnancy weight gain (19.8%). Sensitivity analyses indicate no differences between those with and without pregnancy weight gain information according to maternal race ($\chi^2$=0.11, p=0.74), education ($\chi^2$=0.68, p=0.41), age (t=−1.51, p=0.13), gestational age at delivery (t=−0.30, p=0.76), or infant birth weight (t=0.70, p=0.48).

### NNNS Profiles

The RPMM analysis returned 7 neurobehavioral profiles. The mean NNNS summary scores were significantly different across each profile (Table 2) indicating the profiles were distinct. The supplementary figure displays the NNNS summary scores (standardized to be on the same scale) by profile. Profiles characterized by extreme NNNS scores were identified as were several less extreme profiles. Profiles 2, 4 and 7 were of particular interest as profiles 4 and 7 reflected infants with above average (profile 4) and below average (profile 7) neurobehavior (most scores > 0.5 standard deviations from the mean) whereas profile 2 reflected average neurobehavior (all summary scores were within 0.5 standard deviations from the mean; n=108, 17.1%). The above average profile (profile 4) infants (n=102, 16.2%) exhibited neurobehavior characterized by positive functioning and had the highest levels of attention, self-regulation, quality of movement, and habituation compared to all other profiles; they required the least amount of handling to maintain alertness during the exam, as well as had the lowest levels of excitability, hypertonicity, hypotonicity, and stress; they had low levels of arousal, lethargy, and average reflexes. Conversely, the below average functioning (profile 7) infants (n=63, 10%) had neurobehavior characterized by poorer functioning and had the lowest attention, self-regulation, and quality of movement; they also required the greatest amount of handling to maintain alertness during the exam, and had the highest levels of arousal, excitability, and stress; lethargy, hypertonicity, hypotonicity, reflexes, and habituation scores were average for this group. Results of the Tukey tests

indicated that the below average and above average profiles, and also the below average and average profiles, were significantly different across most domains (all $p<0.05$), except habituation, hypotonicity, asymmetric and non-optimal reflexes. Above average and average profiles were also significantly different across most domains (all $p<0.05$), except for habituation, lethargy, and the reflex measures. Visual inspection of the profile plots (Supplemental figure) confirmed these findings.

### Predictors of NNNS Profiles

Table 3 lists the adjusted associations between perinatal risk factors and membership to each neurobehavioral profile compared to all other infants. Different sets of perinatal factors predicted membership to each profile. Infants of mothers who consumed alcohol while pregnant, or that experienced fetal distress were less likely to belong to the average neurobehavioral group (profile 2). Also, infants of mothers who experienced greater weight gain during pregnancy, or infants with higher birthweight percentiles were more likely to belong to the below average or poor neurobehavioral group (profile 7). By contrast, infants born at older gestational ages or who had higher 5 minute APGAR scores were more likely to belong to the above average or positive neurobehavior group (profile 4). The magnitude of these associations was moderate to large. For example, a one standard deviation increase in maternal pregnancy weight gain was associated with 44% greater likelihood of poor infant neurobehavior (profile 7). Prenatal alcohol use was associated with 46% lower likelihood of average neurobehavior (profile 2). A one unit increase in 5 minute Apgar score was associated with a 2.43 greater odds (95%CI: 1.07, 5.52) of belonging to the above average neurobehavior group (profile 4).

While NNNS summary scores were on average less extreme for profiles 1, 3, 5, and 6, profile membership was associated with various factors (Table 3). Profile 3 infants were more likely to have mothers with a history of asthma, less likely to have used alcohol while pregnant, or more likely to engage in physical activity during pregnancy. Also, profile 3 infants had smaller birthweight percentiles and were born at earlier gestational ages. Mothers of profile 5 infants were more likely to have used alcohol or smoked during pregnancy, and these infants were more likely to have experienced fetal distress. The magnitude of these associations was moderate to large. For example, infants born to mothers with a history of asthma had 2.42 times the odds (95%CI: 1.31, 4.26) of belonging to profile 3 than other groups. No associations were observed with any perinatal factor and profile 1 or 6.

The q-values for the associations between perinatal factors and NNNS profiles were 0.05. This indicates that 5% of the 14 associations with p-values<0.05 were false discoveries. As such, we expect less than one of the tests to be a false discovery (i.e., 0.05*14=0.7 false discovery, or less than 1 one false discovery).

## DISCUSSION

The results of this study indicate that distinct profiles of infant neurobehavior can be reliably derived from NNNS summary scores in a healthy population within days after birth. These findings are particularly noteworthy as we replicated some of the same profiles identified in

previous work[2,18] in a healthier, younger sample. The low false discovery rate indicates that these findings were not due to chance. Moreover, as we examined newborns within 24–72 hours following delivery, neurobehavior was not likely confounded by a mixture of prenatal and postnatal influences. Thus, our findings suggest that programming of neurobehavior begins *in utero*, and that neonate neurobehavior is sensitive to perinatal risks that are amenable to intervention in existing clinical settings.

Different sets of perinatal risks associated with the average and poor neurobehavioral profiles. Our findings indicated that healthy mothers with uncomplicated pregnancies who abstain from prenatal alcohol use may be more likely to have infants with average neurobehavioral functioning. NNNS scores for this group were largely consistent with 50$^{th}$ percentile norms identified by Fink et al.[17] and was similarly patterned to low-risk profiles identified in other samples[2,18] indicating that an average/low-risk group can be reliably identified from NNNS scores.

We identified a below average or poor neurobehavioral profile that was characterized by low levels of self-regulation and attention and high levels of arousal and excitability. Summary scores for these domains were at or above the 90$^{th}$ (arousal, excitability) and 10$^{th}$ (self-regulation) percentile norms indicating poor performance in these areas[17], and was similarly patterned to high-risk NNNS profiles found in other samples[2,18]. Pregnancy weight gain was a robust predictor of membership in this profile. There could be many potential mechanisms linking pregnancy weight gain to neurobehavior that we were unable to examine in this study, such as maternal dietary factors[26,27] and prenatal glucocorticoid exposure[28]. We encourage future work to explore the potential mechanisms linking pregnancy weight gain to poor neurobehavior in infancy.

We identified an above average or positive neurobehavioral group that was inversely patterned from poor functioning profile and was characterized by high levels of attention, self-regulation and low excitability. Scores for self-regulation and excitability were at or above the 90$^{th}$ and 10$^{th}$ percentile norms respectively indicating good performance in these domains[17], and this profile was patterned similarly to healthiest neurobehavioral profile identified by Liu[2]. Longer gestations and higher 5 minute APGAR scores were predictors of this profile. As this sample was limited to term infants, these associations could reflect gradations in neurological and neurobehavioral maturity within the term period. It follows that with longer gestations, and potentially enhanced neurodevelopmental maturity, these infants would also have higher 5 minute APGAR scores as we observed. These findings underscore the sensitivity of the NNNS to discriminate a high functioning group within a healthy sample.

Though less extreme in attention and self-regulatory domains, profile 3 infants had the highest degree of lethargy, hypotonicity, lowest arousal and the poorest reflexes. Scores were at or above the 90$^{th}$ percentile for lethargy, non-optimal reflexes and the 10$^{th}$ percentile for arousal, hypotonia norms indicating poor performance in these areas[17]. Profile 3 was associated with several perinatal factors including maternal asthma morbidity, and lower birthweight and younger gestational age. Deficits in motor, tone, and arousal domains may reflect the neurobehavioral and neurological immaturity associated with being born smaller

and prior to 40 weeks gestation. Moreover, asthma was a robust predictor of membership to this profile. Asthma during pregnancy may influence infant motor and reflex domains potentially via physiologic processes related to the disease, medication use to manage the condition, or both. Future work should replicate these findings and also explore how maternal asthma morbidity could affect infant neurobehavior.

Profiles 5, a less extreme neurobehavioral group, was associated with some established perinatal risks including smoking and alcohol use during pregnancy and fetal distress. These findings indicate that variability in less-extreme neurobehavior can be explained in part by these known risks.

This study has some limitations. While the method we used to derive profiles of neurobehavior across NNNS summary uses all available data from participants, meaning all participants contributed some information to the derivation of profiles, some summary score information was not collected because infants have to be in a certain state to be assessed[5]. This is particularly true for Habituation as infants have to be asleep for this domain to be assessed (Habituation was missing for 45%). Exclusion of the Habituation subscale from profile derivation due to infant wakefulness may mean that information from the highest and lowest functioning children may have been excluded. For example, infants who are wakeful could either be satiated, well-rested, and healthy or they could be distressed, sickly, or uncomfortable. Making exclusions based on wakefulness could mean that the full distribution of potential Habituation scores has been truncated, which in turn may limit our ability to discriminate important differences in infant's ability to habituate and accumulate to stimuli. Other limitations of this study include use of medical record diagnoses of pregnancy conditions. Such records could be incomplete or contribute to misclassification. Also, self-reported information on pregnancy weight gain and health behaviors were used which likely underreport risk. Additionally, we lacked information on medication use during pregnancy, which could influence neonate neurobehavior. While we found no association with medication use during labor, we encourage future work to examine medication use throughout pregnancy as potentially influencing infant neurobehavior. Finally, we were unable to identify the physiologic mechanisms linking perinatal risks to infant outcomes. Future work should examine molecular pathways (e.g., epigenetic mechanisms) that may help explain how these perinatal factors contribute to programming infant neurobehavior.

This study also has several strengths. We used a validated measure of infant neurobehavior that has prospectively predicted behavioral, cognitive and neurological health in toddlers and school-aged children[2–4]. Also, the NNNS was assessed at birth, thereby preventing confounding by postnatal environmental influences. Additionally, we examined healthy infants rendering our findings more generalizable to the general population. Finally, we examined a wide range of perinatal factors as predictors of neurobehavior, which can help inform both obstetric and pediatric clinical practice.

## CONCLUSION

In this study, we capitalized on the wealth of information returned by the NNNS to identify distinct profiles of infant neurobehavior that signify different levels of risk. We identified

some of the same neurobehavioral profiles as in past work but among a different population of younger and healthier infants using a different statistical method. This indicates that the NNNS can reliably discriminate meaningful variations in infant neurobehavior, and that statistical clustering techniques can effectively identify high and low risk groups. Moreover, we found that different perinatal factors associated with different profiles, which may in turn contribute to later neurologic, behavioral and cognitive functioning[2–4]. The public health impact of these findings is significant as these perinatal risks are readily identifiable and amenable to intervention in existing clinical settings. Programs designed to promote factors like healthy weight gain and effective management of chronic conditions during pregnancy may not only improve maternal health, but may also safeguard infant neurodevelopment as well.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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**Table 1**

Participant characteristics (n=627)

| Predictors (n) | % or Mean (SD) | n (%) missing |
|---|---|---|
| *Demographic Factors* | | |
| Maternal age, years | 29.5 (5.5) | 5 (0.8) |
| Maternal education, high school or less | 27.5 | 9 (1.4) |
| Maternal race, white | 71.8 | 5 (0.8) |
| *Maternal Health History* | | |
| Asthma, ever diagnosed | 15.2 | 50 (8.0) |
| Diabetes, ever diagnosed | 5.5 | 48 (7.7) |
| Obesity, pre-pregnancy BMI ≥30 | 26.2 | 34 (5.4) |
| *Pregnancy Conditions* | | |
| Gestational diabetes, yes | 9.5 | 5 (0.8) |
| Preeclampsia, yes | 6.5 | 5 (0.8) |
| Depression, yes | 13.8 | 16 (2.6) |
| Anxiety/OCD/panic attack, yes | 11.6 | 5 (0.8) |
| Weight gain during pregnancy, kg | 14.4 (6.3) | 124 (19.8) |
| *Pregnancy Health Behaviors* | | |
| Smoking, yes | 4.7 | 8 (1.3) |
| Alcohol use, yes | 32.1 | 72 (11.5) |
| Physical activity, hours per week | 0.4 (0.9) | 63 (10.5) |
| *Labor and Delivery Factors* | | |
| Cesarean section, yes | 50.7 | 5 (0.8) |
| Labor induced, yes | 27.8 | 5 (0.8) |
| Medication use for labor, yes | 76.9 | 5 (0.8) |
| Fetal distress, yes | 8.7 | 5 (0.8) |
| Breech birth, yes | 4.9 | 5 (0.8) |
| *Infant Attributes* | | |
| Infant gender, male | 48.9 | 5 (0.8) |
| Birthweight percentile | 53.6 (34.1) | 8 (1.3) |
| Gestational weeks | 39.0 (1.0) | 5 (0.8) |
| Apgar score, minute 1 | 7.9 (1.2) | 13 (2.1) |
| Apgar score, minute 5 | 8.9 (0.4) | 18 (2.9) |

**Table 2**

NNNS descriptive statistics and ANOVA tests to assess differences in summary scores across the 7 profiles

| NNNS Summary Scale | Scores, Mean (SD) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Profile 1 n=66** | **Profile 2 n=107** | **Profile 3 n=80** | **Profile 4 n=101** | **Profile 5 n=102** | **Profile 6 n=108** | **Profile 7 n=63** | **p** |
| Attention | 4.16 (1.25) | 4.36 (1.20) | 3.79 (1.23) | 5.15 (1.16) | 4.56 (1.35) | 3.61 (1.01) | 3.29 (0.93) | <0.001 |
| Quality of movement | 4.39 (0.49) | 4.34 (0.57) | 4.22 (0.55) | 4.66 (0.46) | 3.86 (0.56) | 3.84 (0.67) | 3.80 (0.70) | <0.001 |
| Self-regulation | 5.34 (0.60) | 5.07 (0.37) | 5.00 (0.54) | 5.95 (0.47) | 4.41 (0.62) | 3.98 (0.67) | 3.80 (0.66) | <0.001 |
| Habituation | 7.31 (0.96) | 7.43 (0.37) | 6.70 (1.82) | 7.45 (0.95) | 7.40 (1.00) | 7.12 (1.36) | 7.00 (1.81) | 0.02 |
| Stress abstinence | 0.15 (0.96) | 0.14 (0.07) | 0.16 (0.06) | 0.11 (0.05) | 0.20 (0.05) | 0.21 (0.07) | 0.21 (0.08) | <0.001 |
| Arousal | 3.73 (0.46) | 4.11 (0.38) | 3.36 (0.48) | 3.44 (0.43) | 4.58 (0.64) | 4.87 (0.52) | 5.07 (0.41) | <0.001 |
| Handling | 0.30 (0.19) | 0.35 (0.18) | 0.26 (0.22) | 0.21 (0.14) | 0.41 (0.24) | 0.49 (0.26) | 0.49 (0.25) | <0.001 |
| Excitability | 2.74 (1.34) | 3.81 (1.03) | 2.21 (1.24) | 1.19 (0.86) | 6.30 (1.98) | 7.19 (1.91) | 7.94 (1.79) | <0.001 |
| Lethargy | 6.95 (2.48) | 5.43 (2.05) | 8.53 (2.67) | 5.50 (1.97) | 5.04 (2.55) | 6.50 (2.10) | 6.97 (1.98) | <0.001 |
| Hypertonicity | 1.03 (0.17) | 0 (0) | 0 (0) | 0 (0) | 1.27 (1.30) | 0.26 (0.74) | 0.68 (0.50) | <0.001 |
| Hypotonicity | 0.59 (0.82) | 0.39 (0.64) | 1.03 (1.16) | 0.33 (0.49) | 0.44 (0.54) | 0.49 (0.69) | 0.60 (0.71) | <0.001 |
| Non-optimal reflexes | 6.33 (1.84) | 5.27 (1.66) | 7.2 (1.75) | 5.71 (1.77) | 5.86 (1.94) | 5.90 (2.58) | 5.35 (2.54) | <0.001 |
| Asymmetrical reflexes | 1.52 (1.24) | 1.61 (1.29) | 1.94 (1.37) | 1.54 (1.44) | 1.60 (1.27) | 1.78 (1.40) | 1.19 (1.12) | 0.04 |

**Table 3**

Odds ratios (95% confidence intervals) for the association between perinatal factors and NNNS profiles[^]

| | Profile 1 | Profile 2 Average | Profile 3 | Profile 4 Above average | Profile 5 | Profile 6 | Profile 7 Below average |
|---|---|---|---|---|---|---|---|
| *Maternal Health History* | | | | | | | |
| Asthma, ever diagnosed | 0.74 (0.34, 1.61) | 1.13 (0.64, 1.99) | 2.42 (1.37, 4.27) | 1.01 (0.56, 1.82) | 0.63 (0.32, 1.23) | 0.72 (0.38, 1.33) | 0.94 (0.49, 1.99) |
| Diabetes, ever diagnosed | 0.58 (0.17, 2.06) | 1.55 (0.72, 3.40) | 1.60 (0.68, 3.77) | 0.49 (0.17, 1.43) | 1.09 (0.43, 2.73) | 1.34 (0.59, 3.05) | 0.39 (0.09, 1.78) |
| Pre-pregnancy obesity | 1.41 (0.81, 2.54) | 0.71 (0.43, 1.20) | 1.37 (0.80, 2.35) | 0.87 (0.52, 1.44) | 1.01 (0.61, 1.68) | 1.13 (0.70, 1.84) | 0.72 (0.38, 1.38) |
| *Pregnancy Conditions* | | | | | | | |
| Gestational diabetes | 1.15 (0.50, 2.66) | 1.32 (0.68, 2.54) | 0.87 (0.38, 2.02) | 1.01 (0.49, 2.09) | 1.00 (0.37, 2.02) | 1.13 (0.56, 2.27) | 0.43 (0.13, 1.42) |
| Preeclampsia | 0.46 (0.11, 1.99) | 1.01 (0.43, 2.39) | 1.33 (0.53, 3.35) | 0.43 (0.13, 1.42) | 1.26 (0.53, 3.00) | 0.96 (0.39, 2.37) | 2.05 (0.84, 5.00) |
| Depression | 1.26 (0.63, 2.53) | 1.00 (0.55, 1.82) | 0.94 (0.47, 1.87) | 0.92 (0.49, 1.71) | 0.77 (0.40, 1.51) | 0.97 (0.53, 1.77) | 1.38 (0.68, 2.79) |
| Anxiety, OCD, panic attack | 1.24 (0.61, 2.50) | 0.63 (0.32, 1.25) | 1.34 (0.70, 2.57) | 1.24 (0.69, 2.25) | 0.73 (0.36, 1.49) | 0.79 (0.40, 1.57) | 1.29 (0.64, 2.62) |
| Weight gain during pregnancy | 0.97 (0.74, 1.27) | 0.91 (0.73, 1.13) | 1.00 (0.78, 1.29) | 1.04 (0.84, 1.29) | 0.92 (0.74, 1.15) | 0.92 (0.74, 1.14) | 1.44 (1.10, 1.88) |
| *Pregnancy Health Behaviors* | | | | | | | |
| Smoking | 1.73 (0.54, 5.50) | 0.51 (0.12, 2.22) | 0.22 (0.02, 1.61) | 0.39 (0.09, 1.71) | 3.04 (1.20, 7.71) | 1.49 (0.52, 4.03) | 0.57 (0.08, 4.41) |
| Alcohol use | 1.48 (0.87, 2.55) | 0.54 (0.33, 0.89) | 0.53 (0.28, 0.99) | 0.81 (0.50, 1.31) | 1.68 (1.06, 2.65) | 1.32 (0.84, 2.06) | 1.09 (0.62, 1.93) |
| Physical activity | 1.06 (0.80, 1.41) | 0.88 (0.66, 1.16) | 1.31 (1.03, 1.66) | 1.05 (0.82, 1.33) | 0.81 (0.60, 1.11) | 1.07 (0.85, 1.36) | 0.77 (0.52, 1.13) |
| *Labor and Delivery Factors* | | | | | | | |
| Cesarean section | 0.81 (0.47, 1.39) | 1.04 (0.67, 1.61) | 0.67 (0.40, 1.11) | 0.93 (0.60, 1.47) | 1.10 (0.69, 1.73) | 1.19 (0.77, 1.85) | 1.36 (0.78, 2.40) |
| Labor induced | 1.00 (0.55, 1.81) | 1.07 (0.66, 1.72) | 1.55 (0.93, 2.61) | 0.84 (0.51, 1.39) | 0.80 (0.48, 1.34) | 1.00 (0.62, 1.61) | 0.87 (0.46, 1.64) |
| Medication use for labor | 0.65 (0.35, 1.21) | 1.25 (0.70, 2.32) | 0.94 (0.52, 1.73) | 1.55 (0.85, 2.85) | 0.95 (0.55, 1.64) | 0.62 (0.38, 1.03) | 2.07 (0.85, 5.00) |
| Fetal distress | 2.01 (0.88, 4.58) | 0.10 (0.01, 0.72) | 0.74 (0.28, 1.98) | 0.60 (0.23, 1.57) | 2.09 (1.00, 4.37) | 1.44 (0.68, 3.03) | 1.08 (0.37, 3.19) |

| | Profile 1 | Profile 2 Average | Profile 3 | Profile 4 Above average | Profile 5 | Profile 6 | Profile 7 Below average |
|---|---|---|---|---|---|---|---|
| Breech birth | 1.53 (0.57, 4.15) | 0.78 (0.29, 2.06) | 0.94 (0.32, 2.80) | 0.63 (0.22, 1.84) | 1.31 (0.52, 3.30) | 1.78 (0.80, 3.97) | 0.26 (0.03, 1.91) |
| *Infant Factors* | | | | | | | |
| Birthweight percentile[#] | 0.89 (0.68, 1.16) | 1.00 (0.80, 1.24) | 0.73 (0.56, 0.93) | 0.96 (0.77, 1.20) | 1.16 (0.92, 1.45) | 1.01 (0.81, 1.25) | 1.46 (1.10, 1.95) |
| Gestational weeks | 0.95 (0.72, 1.25) | 0.87 (0.69, 1.09) | 0.62 (0.48, 0.80) | 1.29 (1.02, 1.64) | 1.08 (0.86, 1.36) | 1.08 (0.87, 1.35) | 1.33 (0.98, 1.81) |
| Apgar score, minute 1 | 0.86 (0.70, 1.05) | 1.08 (0.88, 1.34) | 0.95 (0.78, 1.16) | 1.03 (0.84, 1.26) | 1.57 (1.14, 2.15) | 0.87 (0.74, 1.04) | 0.96 (0.75, 1.21) |
| Apgar score, minute 5 | 0.94 (0.46, 1.89) | 0.71 (0.42, 1.21) | 0.65 (0.36, 1.16) | 2.43 (1.07, 5.52) | 2.00 (0.92, 4.33) | 0.78 (0.46, 1.33) | 0.98 (0.47, 2.03) |

^ Odds ratios reflect the likelihood of infants being in a particular profile as compared to all other infants. Models are adjusted for maternal age, race, education, infant gender, and birthweight percentile.

[#] Birth weight percentile model is adjusted for maternal age, race, education and infant gender.

Birthweight percentile and weight gain during pregnancy have been standardized such that a one unit change corresponds to a one standard deviation change.