Maternal cigarette-smoking during pregnancy adversely impacts neonatal neurological integrity with far ranging developmental consequences. Neurobehavioral assessment scales have shown increased behavioral excitability in newborn infants with prenatal cigarette-exposure (PCE), suggesting dose-response signs of stress/abstinence. In contrast to increased excitability, depressive effects of PCE have been found on fetal heart rate variability (HRV) and power spectra of heart rate rhythms. This study further examined the utility of analyses of HRV to detect effects of PCE on neurobehavioral integrity in newborn infants. Participants were 51 term infants who varied by maternal report in the amount of cigarette-use per day during pregnancy: 10 infants with Mild Exposure (ME, <10 cigarettes/day), 15 infants with Moderate/Heavy exposure (MHE, >10 cigarettes/day) and 26 Non-Exposed (NE) comparison infants. Infant heart rate was time-sampled every 5 seconds for 15 minutes and subjected to standard, previously used methods of spectrum analysis. Analyses of Co-Variance with the amount of maternal marijuana and alcohol use during pregnancy statistically controlled found significant dual dimensions of effects of PCE on several measures of HRV and power spectra. Infants with ME showed lower heart rate variability and power of spectral peaks than NE infants; infants with MHE showed greater heart rate variability and power of spectral peaks than NE infants. Whereas lower HRV and spectral peaks of ME infants may reflect depressive effects of mild hypoxic-ischemia, the higher HRV and spectral peaks of MHE infants may reflect excitatory effects of withdrawal that supersede effects of hypoxia.

**Abbreviations**

HRV: Heart Rate Variability; PCE: Prenatal Cigarette Exposure; CPM: Cycles Per Min; NE: No Exposure; ME: Mild Exposure; MHE: Moderate/Heavy Exposure; VTS: Variance of the Time Series; BSP: Basic Spectral Peak; DSP: Dominant Spectral Peak; SSDV: Sum of the Spectral Density Values.

**Introduction**

Maternal cigarette-smoking during pregnancy continues to be a significant concern for the health, behavior and development of children. Recent estimates of the number of women who smoke cigarettes during pregnancy range from an average of 8.4% to 20% of all women in the United States with rates ranging as high as 18% to 27% in some demographic groups [1]. In addition to containing the neurotoxins nicotine and carbon monoxide and 4000 other chemicals [2], that have genotoxic effects that alter DNA [3], and contribute to epigenetic effects [4], metabolites of cigarette smoke cross the placenta, act as vasoconstrictors, reduce uterine blood flow to the fetal brain, and create an hypoxic-ischemic condition that alters fetal brain development [5]. The effects of prenatal cigarette-exposure (PCE) on subsequent growth and development are well established 

Several studies indicate that the analysis of neonatal heart rate variability (HRV) also provides a sensitive and direct measure of the adverse effects of PCE on autonomic regulation. Regression analyses detected significant dose-response relationships between greater PCE and lower HRV in newborn infants after effects of maternal use of alcohol, caffeine and demographic

**Keywords**

- Maternal cigarette smoking
- Prenatal cigarette exposure
- Heart rate variability
- Nicotine withdrawal
- Spectrum analysis
- Newborn assessment

**Citations**

variables were statistically controlled [10]. In that study, infants with moderate/heavy (>10 cigarettes/day) PCE also showed a higher number of changes in behavioral state than infants with both mild (<10 cigarettes/day) and no PCE. No differences in behavioral activity were found between infants with mild and no PCE. Analyses of fetal heart rate (FHR) have similarly shown lower HRV in infants with both mild (1-10 cigarettes/day) and moderate (11-20 cigarettes/day) PCE than comparison fetuses with no PCE [11,12]. Spectrum analysis of fetal HRV showed that fetuses with PCE had significantly lower power in their heart rate rhythms than fetuses with no PCE [12]. Analogous effects of PCE have also been found in several linear and nonlinear measures of FHR, including decreased algorithmic complexity in HRV [13]. The studies indicate that PCE reduces HRV and disrupts its rhythmic organization in utero before the infant transitions to the postnatal environment.

The purpose of the present study was to further explore the utility of spectrum analysis of HRV as an assessment of the effects of prenatal CE on neurobehavioral development in the newborn infant, after the infant has transitioned to the postnatal environment. In essence, spectrum analysis uncovers the frequencies and power (strength) of the many oscillating sensory, physiological and behavioral systems that contribute to the rhythmic variability of heart rate over time. For example, a lower powered cycle in HRV at the frequency of respiratory-sinus arrhythmia (20 cycles per minute) has been detected in many conditions where the health, autonomic regulation and/or development of the infant have been compromised [14]. Lower-powered cycles or spectral peaks are also evident at the frequency of the Basic Rest-Activity Cycle at 1.5 cycles per hour in infants with fetal growth retardation [15], and measures indicative of poor autonomic regulation [16]. Spectrum analysis of the HRV of fetuses with PCE in the study reported above detected a significantly lower-powered cycle at .3 cpm, the frequency at which spontaneous cyclic motility of the fetus contributes to fetal HRV [17]. The cycle in spontaneous motility develops through mid-gestation and has also been used to reflect the health of the developing fetus. For example, lower-powered rhythms averaging .3 cpm have been found in cyclic motility in fetuses of diabetic mothers [17]. Whereas the above study using spectral analysis of fetal HRV examined effects of PCE on HRV and power spectra in utero, the current study explored how different levels of PCE may differentially impact HRV and the power of its spectral rhythms when the infant may be experiencing withdrawal from nicotine. Whereas the typical lower HRV and lower-powered spectral peaks were expected to be found in infants with mild PCE, we hypothesized that the greater excitability and behavioral activity characteristic of infants with moderate/heavy PCE would be evident in their HRV and power spectra.

MATERIALS AND METHODS

Participants

Infants were selected from newborn infants residing in the Term Nursery of a large city hospital based on the medical records of both mothers and infants. After obtaining informed consent, mothers were interviewed on the day of infant assessment using a standard questionnaire used in previous studies of prenatal exposures in order to obtain a history of the amount of cigarette-, alcohol-, and other maternal drug-use during pregnancy [18]. Infants were excluded from the sample if mothers reported using anti-depressant medications, cocaine or any illicit drug, other than marijuana, during pregnancy. The study included 25 infants with PCE and 26 Non-Exposed (NE) comparison infants. Following methods used in previous studies of effects of PCE on neurobehavioral development [6,10], the 25 infants with PCE group were further divided into two groups based on reported maternal cigarette-use during the first trimester of pregnancy: Mild Exposure (ME, n = 10) and Moderate/Heavy exposure (MHE, n = 15). The amount of maternal cigarette-use during the first trimester of pregnancy was chosen because this is the period during which cyclic motility develops [17], and PCE has been demonstrated to have its greatest effects on this neurobehavioral outcome measure [12]. All infants were full term and full birth weight (>2500 g: M = 3353.84, SD = 458.82), except for two MHE infants who had birth weights of 2352 and 2416 g. Infants showed no abnormal signs on routine physical and neurological examinations (Table 1).

Infant and maternal characteristics for the three groups are listed in Table 1. Results of One-way Analyses of Variance indicated that NE, ME and MHE groups did not reliably differ in maternal age or years of education or infant gestational age, birth weight, birth length, head circumference, Apgar scores, or age (in hr) at testing (all p's > .25). Chi Square tests showed that groups also did not reliably differ in the distribution of infant sex (p > .31) or maternal ethnicity (p > .38). As long found in studies of prenatal cigarette-exposure [19,20], cigarette-using mothers more often reported drinking alcohol (PCE = 16; NE = 8), X2(1) = 7.23, p < .01, and smoking marijuana (PCE = 6; NE = 0), X2(1) = 5.73, p < .02, and smoking marijuana (PCE = 6; NE = 0), X2(1) = 7.23, p < .01, than NE comparison mothers. No reliable differences between the two PCE groups were found in the number of mothers using alcohol and/or marijuana during pregnancy (p > .25).

Procedure

Infant assessment: Infants were studied between scheduled feedings when they were 15 to 62 hr. old (M = 29.11, SD = 11.28). The infant was placed on its side in a temperature-controlled isoolate in a darkened, quiet location within the nursery. Electrodes were placed on the infant's abdominal and pectoral regions and were attached to a Vagal Tone Monitor III (VTM III) via a Corometrics Neonatal Monitor. The VTM III determined and stored a moving-average of heart rate that was time-sampled at 5-second intervals. Continuous data were obtained for 15 minutes after the infant had remained undisturbed for 10 minutes, thus resulting in 180 time-sampled observations of neonatal heart rate.

Spectral analysis of neonatal heart rate: The 180 measures of heart rate were subjected to standard spectrum analytic techniques used in previous studies of autonomic nervous system regulation in the fetus and newborn infant including fetuses exposed to maternal cigarette-use during pregnancy [12,15,16]. Linear, quadratic, and cubic trends in the data were first removed to improve the stationarity of the time series. The residual variance of the time series was then spectrum analyzed using a Blackman-Tukey window. Spectral peaks were considered significant if the power of those peaks exceeded that of white
Table 1: Maternal and Infant Demographics (3 groups).

<table>
<thead>
<tr>
<th>Maternal characteristics</th>
<th>No-Exposure</th>
<th>Mild Exposure</th>
<th>Mod/Heavy Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>28.35</td>
<td>29.1</td>
<td>29.53</td>
</tr>
<tr>
<td>Education (yr)</td>
<td>13.96</td>
<td>14.25</td>
<td>12.13</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>39.48</td>
<td>40.26</td>
<td>39.56</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3449.96</td>
<td>3503.7</td>
<td>3087.33</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>50.67</td>
<td>51.13</td>
<td>49.78</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>33.95</td>
<td>34.38</td>
<td>33.4</td>
</tr>
<tr>
<td>Apgar 1</td>
<td>8.38</td>
<td>8.1</td>
<td>8.27</td>
</tr>
<tr>
<td>Apgar 5</td>
<td>9</td>
<td>8.9</td>
<td>9</td>
</tr>
<tr>
<td>Age at testing (hr)</td>
<td>29</td>
<td>26.65</td>
<td>30.93</td>
</tr>
</tbody>
</table>

Abbreviations: YR: year; WK: week; G: grams; cm: centimeters; hr: hours

Table 2: Outcome Measures in Cigarette-Exposure Groups.

<table>
<thead>
<tr>
<th>Outcome Variables</th>
<th>Non-Exposed</th>
<th>Mild-Cigarette Exposed</th>
<th>Mod/Heavy Cigarette-Exposed</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRV</td>
<td>43.43</td>
<td>16.32</td>
<td>43.41</td>
</tr>
<tr>
<td>VTS</td>
<td>27.44</td>
<td>14.51</td>
<td>33.57</td>
</tr>
<tr>
<td>Basic Spectral Peak</td>
<td>26.26</td>
<td>13.66</td>
<td>43.42</td>
</tr>
<tr>
<td>Dominant Spectral Peak</td>
<td>26.55</td>
<td>13.46</td>
<td>44.09</td>
</tr>
<tr>
<td>SSDV</td>
<td>392.70</td>
<td>207.58</td>
<td>478.20</td>
</tr>
<tr>
<td>Heart Rate Mean</td>
<td>126.2</td>
<td>129.8</td>
<td>126.6</td>
</tr>
<tr>
<td>Number of Peaks</td>
<td>2.9</td>
<td>2.2</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Abbreviation: HRV: heart rate variability; VTS: variance of the time series

Figure 1 Model power spectrum of frequency by amplitude with designated Kolmogorov-Smirnov 95% confidence interval.
noise as determined by a Kolmogorov-Smirnov nonparametric test at a 95% confidence level. This analysis detected oscillations in heart rate at frequencies ranging from .1 to 6 cycles per minute (cpm) with a resolution of .1 cpm.

The statistical variance of the 180 measures of heart rate was used to assess each infant’s overall Heart Rate Variance (HRV). Variance of the Time Series (VTS), the residual variance following the regression analyses, was also determined for each infant. Measures obtained from the power spectra included the power of the a) Basic Spectral Peak (BSP) – the first significant spectral peak, b) Dominant Spectral Peak (DSP) – the spectral peak with the highest power and c) Sum of the Spectral Density Values (SSDV) – a value describing the overall cumulative power of the spectrum. Figure 1 shows a typical power spectrum with spectral peaks and Kolmogorov-Smirnov confidence interval. In this figure, the Basic Spectral Peak and Dominant Spectral Peak occurred at the same frequency, .3 cpm. Following standard recommendations, log transformations of the measures based on heart rate variance were conducted to normalize their distributions for statistical comparisons [21].

RESULTS

Spectrum analyses detected reliable oscillations in heart rate variability for all but three infants – one in each of the three groups (all p's < .05). The number of significant spectral peaks (cycles) in the power spectra ranged from 1 to 3. No differences were found among groups in the number of reliable spectral peaks evident in the power spectrum, \( F(2, 46) = 7.9, p < .01 \). HRV. The BSP, or first significant peak, occurred at .3 cpm for 31 infants, .6 cpm for 12 infants, and .9 cpm for 2 infants. These rhythms in heart rate correspond to the previously found cycle in motility at .3 cpm or its multiples (harmonics). Chi Square analyses showed no reliable differences in the distributions of the frequency of these peaks across the three groups (p's > .42). Three infants in the sample (one in each of the three groups) also had significant peaks at 1.46 cpm and 1.79 cpm. Thus, these analyses first indicate that newborn infants show rhythms in HRV that correspond to the previously found cycle in motility at .3 cpm.

To examine effects of PCE on measures of HRV and the power spectrum, comparisons among the three groups were conducted via One- way Analyses of Covariance using Type III Sums of Squares to partial out the independent effects of alcohol- and marijuana-use. Significant effects were found for the level of prenatal cigarette-exposure for all measures of heart rate variability and measures of the power spectrum: Heart Rate Variability, \( F(2, 46) = 5.77, p < .006 \); Variance of the Time Series, \( F(2, 46) = 4.55, p < .02 \); power of the Basic Spectral Peak, \( F(2, 43) = 4.54, p < .02 \); power of the Dominant Spectral Peak, \( F(2, 43) = 4.33, p < .02 \) and Sum of the Spectral Density Values, \( F(2, 46) = 4.53, p < .02 \). No differences were found among groups in their mean resting heart rates, \( F(2, 46) = .365, p > .70 \), nor were any significant effects found for the covariates of prenatal alcohol- (all p's > .68) or marijuana-exposure (all p's > .41) on any of these outcome measures.

Differences among the three PCE groups were determined by LSD post-hoc comparisons. A similar pattern of differences among groups was found for all measures of HRV and the power spectrum shown in Table 2. ME infants had significantly lower heart rate variances as measured by both HRV and VTS, had significantly lower power of spectral peaks at both the BSP and DSP and had a significantly lower SSDV than infants with both NE and MHE (all p's < .05). In contrast, MHE infants had significantly greater heart rate variances as measured by both HRV and VTS, had significantly higher power of spectral peaks at both the BSP and DSP and had a significantly higher SSDV than infants with both NE and ME (all p's < .05), except for finding no reliable difference from NE and MHE infants in HRV (p > .19). That is, whereas infants with mild cigarette exposure during pregnancy had significantly lower values on all variance and spectral features than non-exposed infants, infants with moderate to heavy exposures had significantly higher values on those same measures than non-exposed infants. Figure 2 shows a modal power spectrum of an infant in each of the three PCE groups, delineating the differences in power at the same .3 cpm frequency. Figure 3 shows three-dimensional landscapes of the power spectra for all the infants in the NE Figure 3a, ME, Figure 3b and MHE Figure 3c groups, respectively. The generally lower power of spectral peaks in the ME infants is evidenced by the “flatter” spectral landscape, compared to the spectral landscape of NE infants. The greater power of spectral peaks in MHE infants is evidenced in the “more jagged” landscape with sharper, higher peaks.

DISCUSSION

Maternal cigarette-smoking during pregnancy has long been shown to increase the risk for children to have a wide range of adverse developmental consequences, including Sudden Infant Death Syndrome, attention deficits, impulsivity and many other childhood behavior problems [4]. The adverse effects of PCE on neurologic development are evident in the newborn infant [20], and may be produced through at least two pathways [6]. One pathway includes the effects of the metabolites of cigarette smoke that act as vasoconstrictors, depriving the fetus of oxygen, thus resulting in a condition of mild, chronic hypoxia-ischemia. In a second pathway, nicotine up-regulates nicotinic cholinergic receptor-binding sites that not only change fetal synaptic activity in the brain but may also create passive nicotine addiction and withdrawal symptoms in the newborn infant [20]. The adverse effects of conditions involved in these two pathways have typically been shown in a wide range of measures of neurobehavioral integrity in otherwise full term, full birthweight infants. Standard neurobehavioral exams have shown that newborn infants with PCE show poorer habituation and state regulation, heightened irritability, greater numbers of tremors and startles and a dose-response relationship with neonatal signs of visual stress and degree of stress/abstinence [6,8]. Linear associations between the amount of PCE and lower HRV, as well as increased numbers of changes in behavioral state in infants with the heaviest exposures, have been found in full term, full birth weight newborns [10]. Lower HRV [11] and disrupted temporal organization of HRV, in dinging lower-powered spectral peaks, has also been found in fetuses with PCE who subsequently were born full term and full birth weight [12]. Underlying much of this work are the implicit and/or explicit assumptions that a linear, dose-response relationship exists between the amount of PCE and the degree of neurobehavioral adversity.
Figure 2: Power spectra from infants with mild PCE, no PCE (comparison) and moderate/heavy PCE on the same axis. Note the higher power of the power spectrum of the infant with moderate/heavy exposure and the low power of the power spectrum of the infant with mild exposure, relative to the power spectrum of the comparison, non-exposed infant.

Figure 3: Figure 3 shows three-dimensional landscapes of the power spectra for all the infants in the NE (3a), ME (3b) and MHE (3c) groups, respectively. Individual infants are represented along the z axis. The generally lower power of spectral peaks in the ME infants is evidenced by the “flatter” spectral landscape, compared to the spectral landscape of NE infants. The greater power of spectral peaks in MHE infants is evidenced in the “more jagged” landscape with sharper, higher peaks.
In contrast to this typical dose-response relationship, the present study provides intriguing evidence that there may be two separate and opposite effects of PCE on neurobehavioral development in newborn infants, as measured by HRV and its power spectra. Whereas infants with mild PCE (<10 cigarettes/day) showed lower heart rate variability and lower power of spectral peaks, infants with moderate/heavy exposure (>10 cigarettes/day) showed greater heart rate variability and greater power of spectral peaks than infants with no PCE. These dual dimensions were found with several measures of heart rate variability and the heart rate power spectrum. The overall statistical variance of 180 time-samples of heart rate described the changes in the infant’s unperturbed resting heart rate over time. Continued differences in the Variance of the Time Series indicate that the regression analyses did not differentially affect the distribution of HRV on which the spectral analyses were conducted. Finding differences in both measures of spectral peaks revealed that PCE groups differed in strength of the cycles throughout the power spectrum. No differences in the number of spectral peaks also indicate that the differences in power were not the result of the power being differentially distributed in a greater or lower number of spectral peaks. Last, the Sum of the Spectral Density Values similarly showed that the PCE groups differed in the overall power of the entire power spectrum, independent of the power or number of the spectral peaks. The dual dimensions were found on all of these measures, except for finding no significant difference between NE and MHE groups on overall HRV. Thus, while infant groups varying in the amount of PCE did not differ in their mean heart rates, they differed in how their heart rates varied around those means.

Differences found in the power of spectral peaks may result from at least two processes. First, the power of the spectral peak or cycle may reflect how well the rhythmic rise and fall over time in neonatal heart rate fits a sinusoidal-shaped wave at the frequency of .3 cpm. Much like a statistical linear correlation, the better a line fits the variability in data, the higher is the correlation coefficient. As such, the lower-powered and higher-powered spectral peaks at .3 cpm of infants with mild and moderate/ heavy PCE, respectively, suggests the infants’ heart rate cycles recurred in less precise and more precise rhythmic manners. Second, the lower- and higher-powered spectral peaks of infants with ME and MHE, respectively, may directly result from lesser and greater amounts of variability in heart rate. Importantly, these differences in heart rate variability may provide the basis for, or result from, lesser and greater changes in infant arousal, excitability and behavioral activity. For example, previous work has shown changes in behavioral state activity correspond to changes in the infant’s HRV [22,23] and, unlike infants with mild PCE, infants with moderate to high PCE (>10 cigarettes/day) have also been shown to have higher numbers of changes in behavioral state than non-exposed infants [10]. Because the variability in heart rate centered around the frequency of spontaneous cyclic motility (at .3 cpm), it is reasonable to suggest that the lesser and greater amounts of HRV found in infants with mild and moderate/heavy PCE, respectively, would be associated with lesser and greater amounts of spontaneous motor activity. These differences in motor activity and behavioral state could provide the basis for infants with mild PCE to be described as clinically “flat”, at low arousal and as showing fewer state changes. Infants with moderate/heavy PCE could be described as being more highly aroused and showing greater motor and behavioral state activity- or excitable.

Determining the causal mechanisms for finding two neurobehavioral dimensions, in contrast to most of the extant literature, is beyond the scope of this study. We can speculate, however, that the dual neurobehavioral dimensions found in this study may result from dual pathways through which maternal cigarette smoking during pregnancy affects early development. First, the lower HRV and lower-powered spectra of infants with mild PCE may be the direct product of the adverse effects of chronic mild hypoxia-ischemia on neurobehavioral integrity - the effects of which are often characterized by lethargy and depressed newborn activity [24,25]. Mild hypoxia may produce the same effects as found in many other studies in which a wide range of conditions that insult neurobehavioral integrity result in lower HRV and lower powered spectra [14,16]. In contrast, the greater HRV and powered spectra may reflect the effects of moderate/high PCE on nicotinic cholinergic receptor-binding sites and withdrawal from passive nicotine addiction. The strong effects of withdrawing from moderate/high PCE may supersede the depressant effects of mild hypoxia. The higher HRV and higher-powered spectral peaks may correspond to increased motor activity and excitability, as well as higher withdrawal scores on the Finnegan assessment [9]. Interestingly, finding dual dimensions resulting from direct and indirect pathways have similarly been found in the effects of prenatal cocaine exposure on neonatal neurobehavioral integrity [26]. The findings of this study were also based on methods that may be especially sensitive to the different effects of varying PCE levels on measures of neurobehavioral integrity described above. The present study based the level of PCE exposure on the number of cigarettes mothers smoked only during the first trimester of pregnancy. This period of exposure was chosen because cyclic motility - associated with the outcome measure being studied - develops during the first trimester up until mid-gestation. In this manner, the amount of PCE exposure was assessed for the period when the system being measured was developing. As would be expected, PCE during the first trimester has significantly stronger effect on HRV and power spectra at .3 cpm than PCE during the second or third trimesters [12]. These methods resulted in determining the effects of PCE on the activity of a specific oscillating system. By doing so, the assessment was sensitive to the dual dimensions of neurobehavioral function examined in this study.

The findings and speculations described above are of course subject to important limitations. First, this study relied on a retrospective self-report interview to determine the level of maternal cigarette-use in the first trimester of pregnancy. While the interview used in this study has previously been used to successfully differentiate other forms of prenatal drug exposure, including levels of maternal cigarette-smoking during pregnancy [18], future study would benefit from prospective measures of PCE and direct measures of cotinine or nicotine exposure. Future study would also benefit from the assessment of concomitant behaviors such as motor activity and changes in behavioral state and other measures often assessed in standard neurobehavioral examinations, as well as a measure of neonatal abstinence. 
syndrome specifically designed to assess nicotine withdrawal. Last, direct assessment of parasympathetic and sympathetic nervous system activity may help elucidate the bases of the inhibitory and excitatory dimensions found in this study.

CONCLUSION

In conclusion, in contrast to a typical dose-response relationship, this study found two separate and opposite dimensions of neurobehavioral effects of prenatal cigarette-exposure. Whereas lower levels of prenatal exposure were associated with lower HRV and power spectra that have previously found in a wide range of conditions in which central and autonomic integrity of newborn and young infants have been compromised, higher levels of PCE were associated with greater HRV and power spectra that may contribute to, or result from, higher levels of infant neurobehavioral activity that may indicate a pattern of nicotine withdrawal.

ACKNOWLEDGEMENTS

Thanks to Laura Stephens for help in data collection and management

REFERENCES