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Analysis of Cardiorespiratory Interactions in Infants at Increased Risk for Sudden Infant Death Syndrome

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Abstract

Sudden infant death syndrome (SIDS) is the sudden death of an infant under 12 months of age, which remains unexplained after complete autopsy and investigation. Prior research has suggested that SIDS is related to multiple risk factors involving sleep position, age, and preexisting biological vulnerabilities. These risk factors may contribute to a failure of an infant's autonomic nervous system (ANS) to maintain homeostatic control upon a direct physiological challenge, resulting in a sudden death. A practical way to study correlations between altered ANS functioning and homeostatic control in the developing infant is through the analysis of interactions between the cardiovascular and respiratory systems upon prenatal exposure to adverse substances. This study investigates the cardiorespiratory physiology of infants born in Cape Town, South Africa who were prenatally exposed to alcohol and tobacco smoke (with respect to age and sleep state). These substances have been shown to interact with, and likely impair functioning, of brainstem regions involved in homeostatic control. My primary hypothesis is that infants will exhibit changes in cardiorespiratory physiology and interactions upon prenatal exposure to alcohol and tobacco smoke, thus reflecting some ANS impairment. The overall findings in this study provide evidence of alterations in cardiorespiratory physiology in infants at risk for SIDS that converge with those from the epidemiological literature.

Executive Summary

Sudden infant death syndrome (SIDS) is the sudden, unexpected death of a seemingly healthy infant under the age of 12 months that cannot be explained after an extensive post-mortem investigation [2]. It remains one of the leading causes of death during the first year of life. Although an exact etiology for the event has not yet been discovered, significant progress has been made in effectively identifying risk factors. SIDS rates have decreased coincidentally with the increase in public awareness of these risk factors.

A widely accepted model, the Triple Risk Hypothesis proposed by Filiano et al. [8], states that the probability of SIDS is greatest when the infant 1) has an underlying biological vulnerability, 2) is in an unstable developmental period, and 3) has been exposed to exogenous stressors [2,9]. To explore the risk factor of exogenous stressors, I analyzed possible relationships between cardiorespiratory physiology and high prenatal exposure to alcohol and tobacco smoke in full-term infants. I chose to assess the cardiorespiratory physiology of these high-risk infants because of the ongoing speculation that sudden death without a known cause likely stems from the loss of cardiovascular and/or respiratory function, thus leading to fatal occurrences such as breathing cessation [27]. I hypothesized that the cardiorespiratory physiological characteristics of infants prenatally exposed to alcohol and tobacco smoke would be significantly different than those of unexposed infants, thus reflecting some autonomic impairment upon exposure to the exogenous stressor.

To assess for relationships between infant cardiorespiratory physiology and prenatal exposure to alcohol and tobacco smoke, I selected subjects who had been either unexposed or highly exposed (average of ≥4 drinks and ≥6 cigarettes per day for the duration of pregnancy) prenatally and who were assessed at either the newborn or 1-month period. I used both a linear

method to extrapolate values for their heart rates and breathing rates and a nonlinear method for values and characterizing cardiorespiratory interactions (i.e., cardiorespiratory synchronization and duration). I then conducted statistical analyses of these physiological values to assess the influence not only of prenatal exposures, but also of age and sleep state. These processes will be further described in the Methods section of this report.

My linear analyses revealed that most of the statistically significant differences between the subjects occurred with age. That is, cardiorespiratory physiology was different during the newborn period than it was during the 1 month period. Conversely, my nonlinear analyses did not reveal many statistically significant differences with age. Rather, most differences occurred between exposures. The linear results provide evidence for some ANS impairment involving heart and breathing rates themselves when approaching the 1-month period, which is in agreement with previous findings [20,22,26]. The nonlinear results suggest that cardiorespiratory physiology also changes upon the introduction of exogenous stressors, but these changes are not evident when analyzing the cardiovascular and respiratory systems as separate entities. That is, linear analysis insufficient for detecting changes in cardiorespiratory interactions, thus emphasizing the importance of the combined use of both methods for full characterization.

The results from this study contribute to the growing body of evidence of the effects of prenatal alcohol and tobacco smoke exposure on the autonomic and cardiorespiratory systems. These effects are pertinent to ongoing SIDS research, as alcohol and tobacco smoke exposure remain two of the most prevalent preventable correlates of infant mortality in the United States, with associated increases in SIDS risk two- to five-fold [6]. My results serve as a comparative baseline for future studies in this field, especially those involving cardiorespiratory physiology upon initiation of a direct physiological challenge such as an adverse prenatal exposure.

Acknowledgements

To my advisor, Dr. William Fifer: Thank you for giving me the opportunity to conduct SIDS research, guiding me through this process, and reminding me that it doesn't hurt to take a break, eat, and sleep every once in a while.

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Introduction

Sudden infant death syndrome (SIDS) is defined as the sudden death of a seemingly healthy infant under the age of 12 months that cannot be explained after an extensive investigation, including autopsy, death scene investigation, and review of clinical history [2]. In the United States, SIDS remains the leading cause of death among this age group and the third leading cause of all infant mortality (0.53 deaths per 1000 live births) [4]. My research group in Dr. William Fifer's lab has taken an interest in the physiology of infants in Cape Town, South Africa, as it is one of several places with elevated SIDS rates (3.41 deaths per 1000 live births) [4]. Thus, studying the physiology of infants in these increased-risk regions can provide us with additional information about possible biological contributors to SIDS.

The exact etiology of SIDS events remains unknown, so prevention efforts have been limited to spreading public awareness of risk factors. Filiano et al. [29] proposed a set of risk factors for SIDS, known as the Triple Risk Hypothesis, that have been promoted by the Safe Sleep campaign [19]. The SIDS rate has deceased coincidentally with the implementation of the Safe Sleep campaign and public awareness of Filiano et al.'s model; however, effects of prevention efforts have plateaued since 2006 [17,19]. The Triple Risk Hypothesis states that the probability of SIDS is greatest when the infant 1) has an underlying biological vulnerability such as prematurity, 2) is in an unstable developmental period (<6 months of age, with 2-4 months posing the highest risk), and 3) is exposed to exogenous stressors such as prone sleeping position, bed sharing, soft bedding, and in utero or environmental exposure to tobacco smoke, alcohol, and/or other drugs of abuse [2,9]. These risk factors are thought to decrease an infant's ability to respond to internal and external stressors known to increase heart rate (HR), heart rate variability (HRV), blood pressure (BP), and breathing rate (f) [27]. A schematic of Filiano et al.'s Triple Risk Hypothesis is displayed in Figure 1.

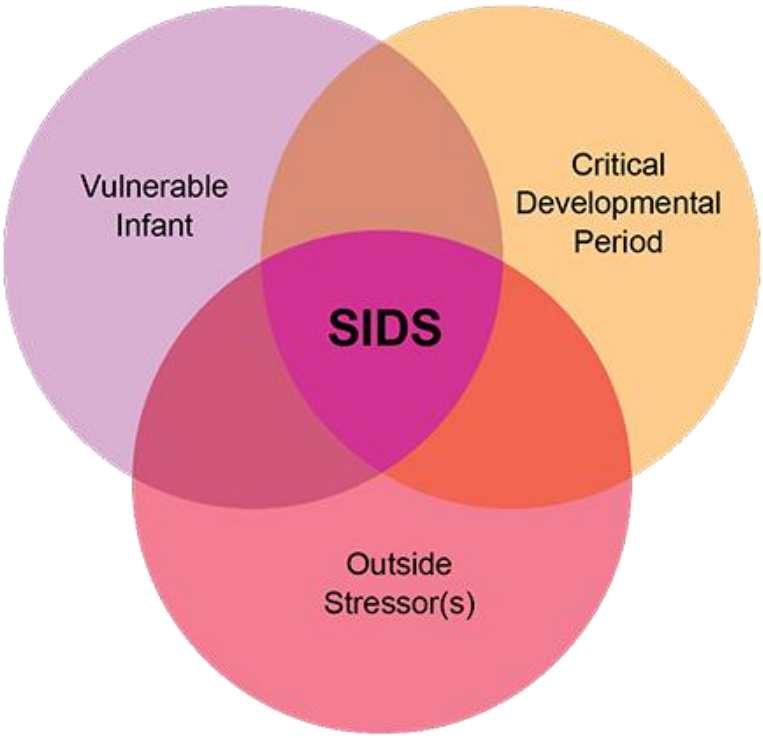

Figure 1. Triple Risk Model for SIDS proposed by Filiano et al. [8].

This study explores one of the three risk factors proposed by Filiano et al.: outside stressors (Figure 1). I specifically examine relationships between high prenatal exposure to alcohol and tobacco smoke on the cardiorespiratory physiology of full-term infants. Dr. Fifer's group suspects that exaggerated or diminished baseline variables (e.g., HR, HRV, f) and cardiorespiratory interactions reflect autonomic nervous system (ANS) impairments.

Autonomic Regulation of the Cardiovascular and Respiratory Systems

The nervous system is a network of neurons (or nerve cells) comprised of the central nervous system (or CNS, composed of the brain and spinal cord) and peripheral nervous system (or PNS, composed of everything outside of the brain and spinal cord). The PNS is further divided into sensory (afferent) division and motor (efferent) systems. Afferent signals originate

in the periphery and eventually reach the CNS, while efferent signals originate in the CNS to elicit involuntary and voluntary functions on effector organs such as the heart and lungs [32].

The autonomic nervous system (ANS), which controls the involuntary processes of the efferent division of the PNS, is composed of the sympathetic and parasympathetic systems [32]. The sympathetic system typically releases norepinephrine (NE) in response to intrinsic and/or extrinsic stressors via afferent autonomic pathways, which transmit responses to visceral organs via efferent autonomy pathways. Conversely, the parasympathetic system typically releases acetylcholine (ACh) to restore the body to restful conditions after stressful events [32].

Functions of the cardiovascular and respiratory systems are regulated by the ANS. Of particular interest to this study are the nerve fibers originating in medulla oblongata, a region in the brainstem that works together with the other two parts of the brainstem (midbrain and pons) and the hypothalamus to regulate heart rate, cardiac muscle contraction, blood pressure, respiratory rate, and other involuntary processes [32]. The majority of the nerve fibers in the sympathetic system that reach the heart and lungs increase Heart rate and respiratory rate, while those in the parasympathetic system tend to decrease these rates [32]. Heart rate is also influenced by respiration by a phenomenon known as respiratory sinus arrhythmia (RSA) [25]. In instances of RSA, respiratory rhythm results in variations in heart rate, causing heart rate to increase upon inhalation and decrease upon exhalation [39]. The net effect of these processes is a balance of the antagonistic effects of the sympathetic and parasympathetic systems [32].

Prenatal Adverse Exposures and Brainstem Functioning

Prenatal tobacco smoke and alcohol exposure remains one of the most prevalent preventable correlates of infant mortality in the United States, with an associated increase in SIDS risk two- to five-fold [6]. Additionally, a recent study revealed that of women whose

infants succumbed to SIDS, 36.8% of them reported smoking during pregnancy [3]. While a causal relationship between prenatal exposures to alcohol and tobacco smoke and SIDS has not been reported, there is a considerable amount of evidence of the correlations of these exposures with alterations on fetal autonomic development [2,3,6,7,9,16].

Although there are over 4800 chemicals present in cigarette smoke, nicotine, a poisonous alkaloid, is the major pathogenic component [36]. Nicotine's lipid solubility and low molecular weight allow it to diffuse through the human placenta and subsequently enter fetal circulation at concentrations approximately 15% greater than the concentration in maternal circulation [36]. Nicotine molecules are then able to cross the blood-brain-barrier and act as agonists to nicotinic acetylcholine receptors (nAChRs), which are 16-subunit ligand-gated ion channels that are activated by the binding of acetylcholine [32,33]. These nAChRs are located in the medulla oblongata [6,25,29,33,34]. A study by Schechtman et al. found arousal mechanisms to be reduced at both the cortical and subcortical levels in SIDS victims prior to their deaths [20]. Epidemiological literature has produced a generally accepted hypothesis: that increased SIDS risk with prenatal exposure to alcohol and tobacco smoke is associated with nicotinic interactions with regulatory cardiorespiratory systems [9].

The group of serotonin (5-HT) receptors in the medulla oblongata, which are involved in cardiovascular and respiratory functions, is a major example of one such regulatory cardiorespiratory system (Figure 2).

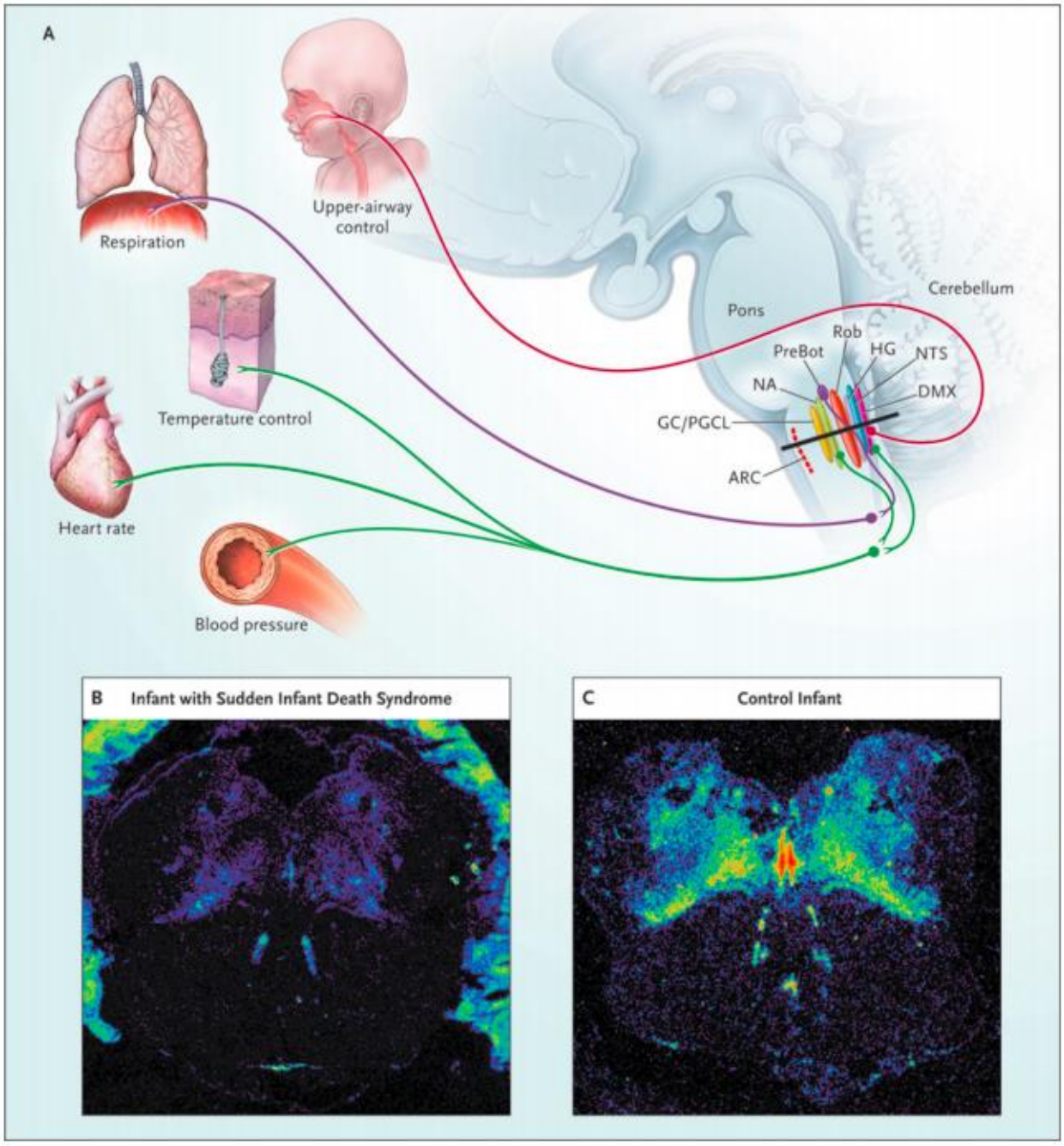

Figure 2. A) Schematic of the serotonergic (5-HT) system, with origination in the medulla oblongata within the brainstem (denoted by the black line), and its effector tissues in the airway, lungs, skin, heart, arteries, and veins. B) Tissue autoradiography of 5-HT type 1A (5-HT_{1A}) receptor binding (green) in a transverse section of the medulla oblongata of a SIDS infant in comparison to C) that of a control infant. Adapted from Kinney et al. [42].

Kinney et al. have provided evidence that nicotinic exposures have various effects on infant 5-HT receptors in the medulla oblongata, such as in the findings of the changes in the expression of 5-HT type 1A (5-HT_{1A}) receptors in the developing fetal medulla (Figure 2) [6,33,35]. Abnormalities such as these changes in 5-HT_{1A} expression may reduce normal respiration and arousal mechanisms during sleep [33,37]. Kinney et al.'s findings 5-HT abnormalities both in SIDS [41] and nicotine-exposed infants [6], along with Duncan et al.'s report of diminished cardiorespiratory functioning and arousal mechanisms—most of which are regulated in the brainstem—in infants with prenatal nicotinic exposure [33], support this hypothesis. These 5-HT abnormalities may contribute to Harper et al.'s proposed failure mechanism after a life-threatening event, which states that neural integration in the cardiac and respiratory centers (i.e., the brainstem and hypothalamus) in SIDS infants are somehow impaired and thus make them less capable oaf arousal or overcoming direct physiological challenges [9,27].

SIDS and Cardiorespiratory Physiology

Researchers have speculated that sudden death without a known cause likely stems from the loss of cardiovascular and/or respiratory function, thus leading to fatal occurrences such as breathing cessation [27]. Kinney et al.'s findings of medullary 5-HT abnormalities [6,35], the identification of abnormalities in sleep-wake arousal and cardiorespiratory function [20,26], evidence of tachycardia or bradycardia [27], and other physiological studies that have found neuronal and/or receptor abnormalities in the CNS [16,35,37] of infants who subsequently died of SIDS have provided evidence for an underlying biological basis for the event. This body of evidence has contributed to the recent focus on cardiorespiratory physiology in SIDS research.

Components of cardiac and respiratory physiology differ with sleep states, ages, and pathological conditions [39]. These changes include the nature of cardiorespiratory interactions,

which can be characterized through the analysis of cardiorespiratory coupling (CRC). Analyses of CRC model the cardiac and respiratory systems after self-sustained chaotic oscillators [27]. Instances of CRC occur when one of these systems adjust its rhythm upon weak interactions with the other, demonstrating that the two systems are not independent of each other [27]. This typically occurs when the systems initially have different rhythmic oscillatory frequencies, then eventually center their oscillations around a common frequency [31]. CRC is quantified m:n ratios, with m corresponding to the number cardiac R-peaks and n corresponding to the number of inspiratory onsets.

CRC helps to characterize the degree of rhythmic stability within the cardiac and respiratory systems during a given time period [10,12,25]. The determination of an optimal level of CRC has not been well-defined, as too high of an m:n ratio may reflect inefficient energy utilization, and too low of an m:n ratio may reflect diminished ability to respond to intrinsic and extrinsic stressors. However, SIDS risk has been attributed to diminished CRC, as rhythmic cardiorespiratory interactions suggest an adequately functioning ANS [25]. I take conclusions from the literature on CRC into account in the present study, as I am more focused on comparing CRC in specific age-, sleep state-, and exposure-divided groups within my cohort. This study involves the quantification of CRC in infants both exposed and unexposed prenatally to alcohol and tobacco smoke. My primary hypothesis is that prenatal exposure to alcohol and tobacco smoke will have a positive association with impaired cardiorespiratory physiology, thus reflecting autonomic impairments that resemble those that have been found in SIDS victims. This hypothesis would be supported by evidence of statistically significant changes ($p<0.05$) in physiological variables such as heart and breathing rate, as well as in degrees of CRC.

Methods

Subjects

Under the direction of the PASS Network (see Appendix for description), nurses at Tygerberg Hospital and affiliated clinics in Bellville, Cape Town, South Africa, enrolled subjects in the Safe Passage Study. Each subject that I selected from the cohort in the Safe Passage Study was a full-term infant (≥37 weeks post-conceptional age) with a birthweight ≥2500g and was of South African mixed ancestry.

Nurses conducted modification of the Timeline Follow-Back Interview (TLFB), which involved mothers' self-reported estimates of their tobacco and alcohol consumption patterns during pregnancy [38,40]. Based on the results of the TLFB, I divided subjects into two groups, belonging to either the classification of no prenatal exposure to alcohol or tobacco smoke or of high prenatal exposure to these substances. High prenatal exposure was defined as both ≥4 drinks and ≥6 cigarettes per day for the entire duration of pregnancy. I divided the exposed and unexposed groups by age (either newborn or 1 month of age), resulting in 4 subgroups: unexposed at birth (UB), unexposed at one month of age (U1M), highly exposed at birth (EB), and highly exposed at one month of age (E1M). I further divided the four groups by sleep state (either AS or QS) for a total of 8 subgroups.

In the TLFBs of the subjects in this study, no prenatal exposures to drugs of abuse were reported. There was no evidence of diabetes, hypertension, or pre-eclampsia during pregnancy in maternal medical records. None of the participating infants required any form of resuscitation at birth or admission to the Neonatal Intensive Care Unit, and none were twins. Because there was a predominantly equal distribution of male and female subjects in this cohort, sex differences were not analyzed as a possible confound. In addition, the relatively small number of subjects limits the effect of sex differences on the results.

Data Collection

The infant subjects were discharged from Tygerberg Hospital less than 24 hours after delivery, and their mothers returned with them within 48-96 hours for assessment. Nurses followed a standard physiological protocol that recorded infant heart rate, respiration, heart rate variability, blood pressure, and cortical brain activity during sleep within the first four days of life (i.e., the newborn period). Nurses again collected cardiorespiratory data in the same manner at a follow-up appointment approximately one month later (28 ± 7 days). Although it is known that the 2-4 month developmental period poses the highest risk for SIDS [9], the PASS Network chose the one month period for assessment due to 1) budgetary limits and 2) an increased likelihood for subjects to sleep during the time of data acquisition. Therefore, the one month period served as the more practical time period for follow-up assessment, as it had a much lower risk for data loss or low-quality signals.

Data recordings for all physiological variables were approximately 1 hour long. There recordings occurred approximately 30 minutes after eating, with infants placed in the prone position, as it was presumed to be the more challenged position. The first 10 minutes was considered the first baseline interval (pre-tilt), which was the only time interval used in assessing fetal physiology in this study. The next ten minutes corresponded to the interval after an induced cardiorespiratory challenge (a 45-degree head-up tilt during sleep). This process was repeated twice using the same time intervals for a second and third baseline-tilt sequence. Cardiovascular data were collected via electrocardiograms (ECGs) at a sampling rate of 500 Hz, with leads placed on the left abdomen, left scapula, and right scapula. Respiratory tracings were simultaneously collected using a respiratory inductance belt (Ambulatory Monitoring, Inc.), which was placed around each infant's chest. Signals were digitized at a sampling rate of 20 Hz [1].

Filtration and Preparation of Physiological Signals

Prior to analysis, the raw cardiorespiratory data were filtered to minimize the noise due mostly to excessive infant movement (done with the help of Nicoló Pini, M.S.). I used the Graphical Marking and Data Acquisition (GMARK) program to mark cardiac R-peaks in each subject's ECG and inspiratory onsets in each subject's respiratory tracing in order to use these two datasets for analysis (sample recordings displayed in Figure 3, left). The algorithm that GMARK programming is based on [14] allows it to automatically detect cardiac R-peaks and inspiratory onsets; however, I inspected these automated marks and manually corrected them when necessary.

In my analysis, it was necessary to correct for sleep state, as the two predominant sleep states within the first 6 months of life in healthy term infants, active sleep (AS) and quiet sleep (QS), have been associated with different physiological characteristics [15]. In newborns, QS epochs are associated with rhythmic inter-breath intervals (i.e., low respiratory variability), absence of markers for rapid eye movements (non-REM sleep) in their electroencephalograms (EEGs). AS epochs are associated with irregular inter-breath intervals (high respiratory variability), increased heart rate and heart rate variability, and rapid eye movements (REM sleep) [5,15,27]. QS signals also have lower amplitudes in their EEG signals than those in AS [5].

Sleep state epochs (i.e., periods of distinct sleep states) were determined in Matlab using an algorithm based on the contrasting characteristics between AS and QS stated above. This method has been validated by comparison to the accuracy of outcomes of standard polysomnographic and behavioral sleep state coding, which characterizes QS by small and large muscle movements and AS by twitches, facial expressions, and sucking motions [5,27].

I assessed the filtered data for outliers on the level of individual time segments. Outliers were identified as those above the high-cutoff value ($1.5x75^{th}$ percentile interquartile range) and

below the low-cutoff value (1.5x25th percentile interquartile range). Prior to exclusion, I assessed the specific degrees of prenatal exposure (amounts of cigarettes smoked and drinks consumed by their mothers) experienced by subjects with outlier data. This was done to identify possible associations between outlier physiological data and high degrees of prenatal exposure (i.e., excessive amounts of cigarettes or drinks per day). I then excluded the outlier time segments from the calculation of the average of all segments corresponding to that particular subject. Even after outlier exclusion, some subjects' averages were still outside its corresponding group's high- or low-cutoff values. These are displayed as open circles in the box plots in Figures 4-7.

Time Domain Analysis

Time domain analysis is a linear method of measuring and subsequently comparing baseline physiological parameters. After filtration and preparation, I separated the original 600-second filtered recordings into individual 60-second segments. I then processed these 60-second segments in Matlab in order to extrapolate the mean values of the following variables: heart rate (HR), R-R intervals (RRi), root mean squares of successive differences (RMSSD-RRi), standard deviations of R-R intervals (SD-RRi), breathing rate (f), inter-breath intervals (BBi), and standard deviations of inter-breath intervals (SD-BBi). I then averaged these variables, obtained from the group of 60-second segments corresponding to a particular subject, for each subject in order to ensure properly weighted contributions of each into the mean computations for each of their respective groups.

The variability induced by respiratory sinus arrhythmia is an important factor to consider in assessing SIDS risk, as indicators of autonomic dysregulation, such as tachycardia, decreased arousals, high sympathetic activation, and decreased HRV have been found in SIDS infants prior to the event [9,25]. HRV is a measure of the difference in time intervals between subsequent

cardiac R-peaks and is commonly used in clinical settings to assess cardiovascular functioning [32]. It is generally reflective of parasympathetic activation and the ANS's ability to adapt to direct physiological challenges [25]. An "optimal" HRV level has been difficult to define, as both upper and lower bounds of HRV are associated with impaired regulatory capacity: too low of a level is likely to reflect decreased homeostatic adaptability or chronic stress, while too high of a level is likely reflect ANS instability and inefficient energy utilization [25].

The root mean squares of successive differences in R-R intervals (RMSSD-RRi) is the time domain measure similar to HRV in spectral or frequency analysis. These RMSSD-RRi values—statistical measures of the differences between consecutive cardiac R-peaks—were obtained for each 60-second segment of infant ECG and respiration dataset and analyzed as correlates to high-frequency heart rate variability (HF-HRV). While RMSSD-RRi values were used to quantify beat-to-beat variability, measurements of the standard deviation of cardiac R-R intervals (SD-RRi) were used to quantify overall HRV. For each subgroup containing average data from each subject, I performed equal variance, two-tailed t-tests for statistical significance. I compared these values across different subgroups (e.g., unexposed infants in active sleep vs. in quiet sleep, unexposed vs. exposed infants in active sleep) as well as to values reported by the PASS Network [1].

Synchronization Analysis

Time domain analysis relies on the assumption that the systems being measured are linear. The baseline physiological values obtained from the time domain analysis served as basic predictors of the nature of cardiorespiratory interactions and therefore acted as supplements to the synchronization analysis, a method which accounts for the nonlinearity in various oscillatory systems. Phase synchronization analyses assume that the systems of interest are self-sustained

and either interacting with or oscillating independently of each other [31]. The phenomenon has been found in several physiological systems, including in the autonomic nervous system [39]. Phase synchronization between the cardiac and respiratory systems occurs when a fixed number of heart beats occur per every inspiratory onset. In these instances, the two dynamic, non-identical systems become "phase locked", during which their respective frequencies are adjusted as a result of their weak interactions. This is modeled by Equation 1,

$$mf_m - nf_n = \text{constant} \quad (1)$$

where m is the number of heart beats, n is the number of inspiratory onsets, f_m is the frequency of heart beats, and f_n is the frequency of inspiratory onsets. Phase synchronization between heart beats and inspiratory onsets serves as direct evidence of cardiorespiratory coupling [39].

After proper filtration and peak marking as described for the time domain analysis, I separated the original 600-second filtered recordings into individual 180-second segments. I used the same marks of cardiac R-peaks and inspiratory onsets as used in the time domain analysis (done with GMARK). The amount of time between each event marker (i.e., an interval between consecutive R-peaks or consecutive inspiratory onsets) defined one complete cycle.

I then used a Matlab algorithm (developed by Nicoló Pini, M.S.) to calculate the temporal distances between the marks for inspiratory onsets and both preceding and successive cardiac R-peaks to obtain absolute and relative distances between the peaks with respect to time ($\Psi(t)$). These distances were visualized and compared as plots of distance on the x-axis and respiratory cycle on the y-axis, both with respect to time. These plots are referred to as synchrograms (Figure 3, top right).

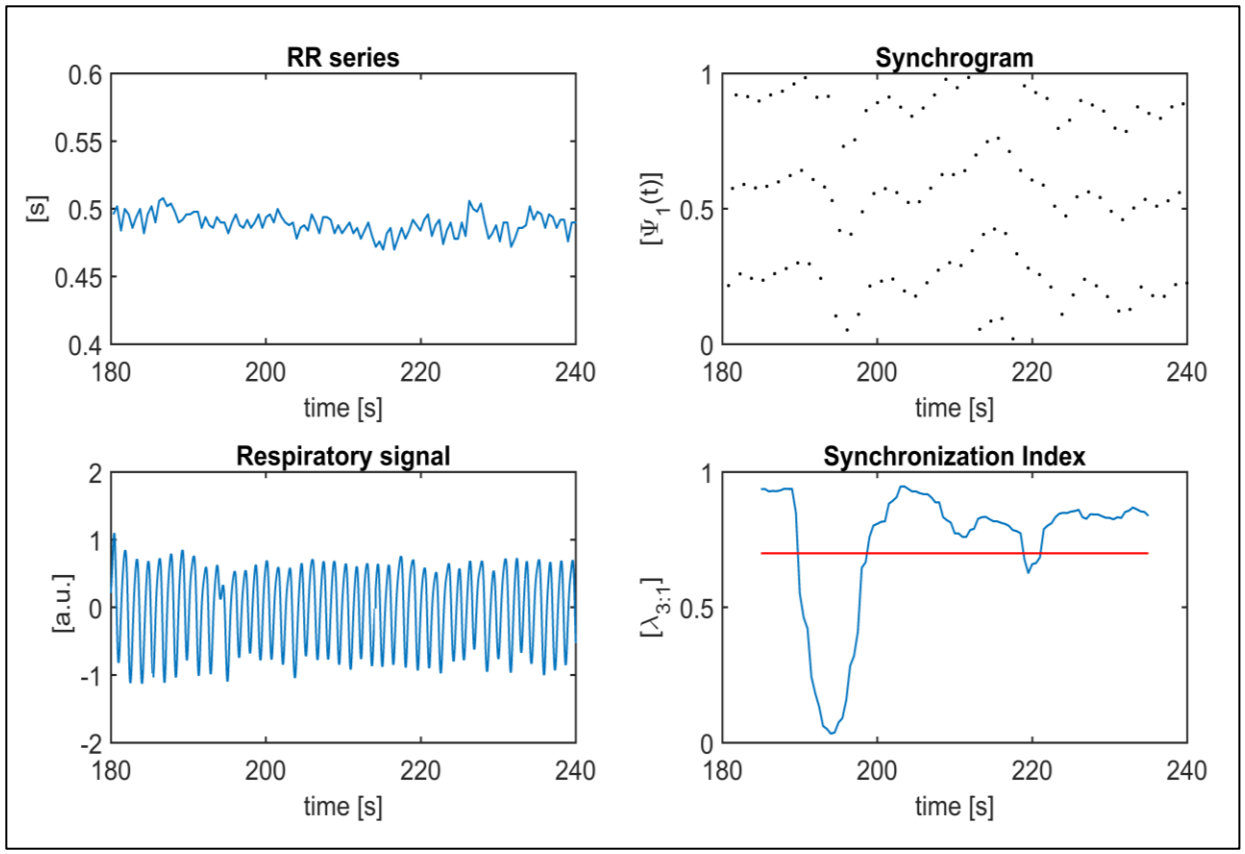

Figure 3. Sample linear and nonlinear data of unexposed newborns in QS. Top left: R-R intervals at 500 Hz. Bottom left: respiratory tracing at 20 Hz. Top right: synchrogram of distances between cardiac R-peaks (x-coordinates) and respiratory cycles (y-coordinates) with respect to time. Parallel horizontal lines indicate time intervals during which cardiorespiratory coupling is exhibited. All other values indicate intervals of varying levels of de-synchronization (i.e., phases are spread randomly over the interval [0,2π]). Bottom right: synchronization index (approximately 0.7) based on the instances of synchronization from the synchrogram (0 = no synchronization, 1 = maximum synchronization).

I used these distances to identify instances in which there were set numbers of cardiac R-peaks (m) per inspiratory onset (n). Using Nicoló Pini's algorithm, I extrapolated m:n ratios (m=1-6, n=1) with corresponding time durations of these ratios' occurrences. Epochs of CRC were identified when marks of overlapping cardiac R-peaks and inspiratory onsets formed parallel horizontal lines (Figure 3, top right). This pattern indicated either a predominantly constant distance between peaks or a fixed fluctuation around a constant for a given set of breathing cycles. The latter accounted for the inherent noise present in physiological systems

[27]. Phases were proportional to the m:n ratios throughout the subjects' ECGs and respiratory tracings.

Using the m:n ratios from synchrograms, I obtained an overall synchronization index ($\lambda_{m:n}$) for all the subjects in a given subgroup (Figure 3, bottom right). Synchronization indices ranged from 0 to 1, with 0 reflecting no synchronization and 1 reflecting maximum synchronization within the given dataset.

I determined total synchronization (%) for each 180-second segment by taking the sum of all m:n ratios within each's subject's dataset. As in the time domain analysis, I took averages of the resulting values for each subject to ensure equal weight in the means of the total synchronizations for each group. The same equal variance, two-tailed t-tests were done, and values were compared across groups.

Results

Baseline Cardiorespiratory Physiology

Variable	Original N	Low Cut-Off	High Cut-Off	Final N	Outliers	Mean ± SD	5th	95th
HR (bpm, AS)	39	93	162	39	0.00%	128±12	107	143
HR (bpm, QS)	35	101	145	34	2.86%	123±9	107	141
SD-RRi (sec, AS)	39	-0.008	0.063	39	0.00%	0.029±0.012	0.012	0.052
SD-RRi (sec, QS)	35	-0.010	0.057	35	0.00%	0.026±0.011	0.008	0.047
rMSSD-RRi (sec, AS)	38	-0.005	0.029	35	7.89%	0.013±0.006	0.004	0.028
rMSSD-RRi (sec, QS)	34	-0.012	0.045	34	0.00%	0.019±0.011	0.006	0.044
f (breaths/min, AS)	40	26.8	82.8	40	0.00%	56.6±8.0	44.5	71.0
f (breaths/min, QS)	33	22.3	74.3	32	3.03%	48.2±7.6	35.7	62.0

A. Unexposed newborns (UB)

Variable	Original N	Low Cut-Off	High Cut-Off	Final N	Outliers	Mean ± SD	5th	95th
HR (bpm, AS)	38	118	181	37	2.63%	149±11	127	166
HR (bpm, QS)	45	118	162	45	0.00%	140±9	126	157
SD-RRi (sec, AS)	38	0.002	0.045	37	2.63%	0.025±0.007	0.013	0.040
SD-RRi (sec, QS)	44	-0.008	0.042	44	0.00%	0.018±0.007	0.007	0.029
rMSSD-RRi (sec, AS)	37	-0.004	0.023	34	8.11%	0.010±0.004	0.004	0.018
rMSSD-RRi (sec, QS)	45	-0.001	0.023	45	0.00%	0.011±0.004	0.005	0.019
f (breaths/min, AS)	36	32.8	76.9	35	2.78%	56.1±8.5	40.2	70.8
f (breaths/min, QS)	43	25.8	63.3	42	2.33%	45.7±6.9	34.7	58.4

B. Unexposed infants at 1 month of age (U1M)

Variable	Original N	Low Cut-Off	High Cut-Off	Final N	Outliers	Mean ± SD	5th	95th
HR (bpm, AS)	37	100	145	35	5.41%	123±8	108	137
HR (bpm, QS)	37	87	165	37	0.00%	126±13	104	145
SD-RRi (sec, AS)	37	-0.009	0.074	37	0.00%	0.033±0.013	0.015	0.059
SD-RRi (sec, QS)	37	-0.002	0.048	36	2.70%	0.024±0.009	0.007	0.045
rMSSD-RRi (sec, AS)	37	-0.004	0.037	36	2.70%	0.017±0.007	0.008	0.031
rMSSD-RRi (sec, QS)	37	-0.009	0.044	37	0.00%	0.017±0.008	0.006	0.032
f (breaths/min, AS)	37	34.4	80.4	36	2.70%	57.2±8.0	42.4	72.5
f (breaths/min, QS)	35	24.1	69.2	34	2.86%	47.0±8.1	32.8	61.9

C. Exposed newborns (EB)

Variable	Original N	Low Cut-Off	High Cut-Off	Final N	Outliers	Mean ± SD	5th	95th
HR (bpm, AS)	39	122	164	37	5.13%	145±8	133	162
HR (bpm, QS)	34	108	171	34	0.00%	138±10	117	152
SD-RRi (sec, AS)	38	-0.002	0.048	36	5.26%	0.025±0.010	0.010	0.044
SD-RRi (sec, QS)	33	-0.004	0.034	33	0.00%	0.016±0.007	0.008	0.030
rMSSD-RRi (sec, AS)	37	-0.006	0.028	35	5.41%	0.012±0.005	0.006	0.023
rMSSD-RRi (sec, QS)	34	-0.003	0.025	32	5.88%	0.011±0.005	0.004	0.021
f (breaths/min, AS)	38	31.8	79.3	38	0.00%	56.5±6.6	45.9	68.2
f (breaths/min, QS)	33	22.4	66.9	31	6.06%	46.0±9.1	34.2	64.7

D. Exposed infants at 1 month of age (E1M)

Table 2. Baseline values for infants at both ages (newborn and 1 month) and at both exposures (prenatally unexposed and exposed to alcohol and tobacco smoke) obtained from time domain analysis, with outlier rejection.

Statistical values for HR, SD-RRi, RMSSD-RRi, and breathing rate during the newborn and 1-month period for each of the 8 subgroups are reported in Table 2 above.

In my cohort, I found only a minimal number of whole-subject outliers, with the highest percentage at 8.11% in the U1M group for RMSSD-RRi. Outlier rejection occurred mostly within the data of the individual 60-seconds segments. As stated in the methods section, I analyzed outliers, with the expectation that outlier data in one physiological variable would coincide with outlier data in another (e.g., association of elevated HR values with greater than 10 cigarettes and 10 drinks per day). However, I did not find any significant variable-exposure relationships in doing so.

Mean HR was higher in AS than in QS for the unexposed groups at both the newborn and 1 month periods. I observed a similar trend in these groups' SD-RRi values and breathing rates. Conversely, RMSSD-RRi values were slightly lower in AS than in QS. There were net decreases in mean HR, SD-RRi, and RMSSD-RRi at the 1 month period with respect to the newborn period, and breathing rate averages only slightly decreased with age.

Like the unexposed groups, the exposed groups also contained only a minimal number of outliers in their mean physiological parameters, with the highest percentage at 6.06% in the E1M group for breathing rate. Mean HR was higher in AS than in QS during the newborn period but lower at the 1 month period for upon exposure to alcohol and tobacco smoke. Mean SD-RRi values were higher in AS than in QS at both ages, and RMSSD-RRi values at both ages were predominantly similar. Mean breathing rate was greater in AS than in QS at both ages. There were net decreases in mean SD-RRi and RMSSD-RRi and a net increase in HR at the 1 month period with respect to the newborn period, and breathing rate averages only slightly decreased with age. Comparisons across sleep states, ages, and exposures are reported in Figures 4-6.

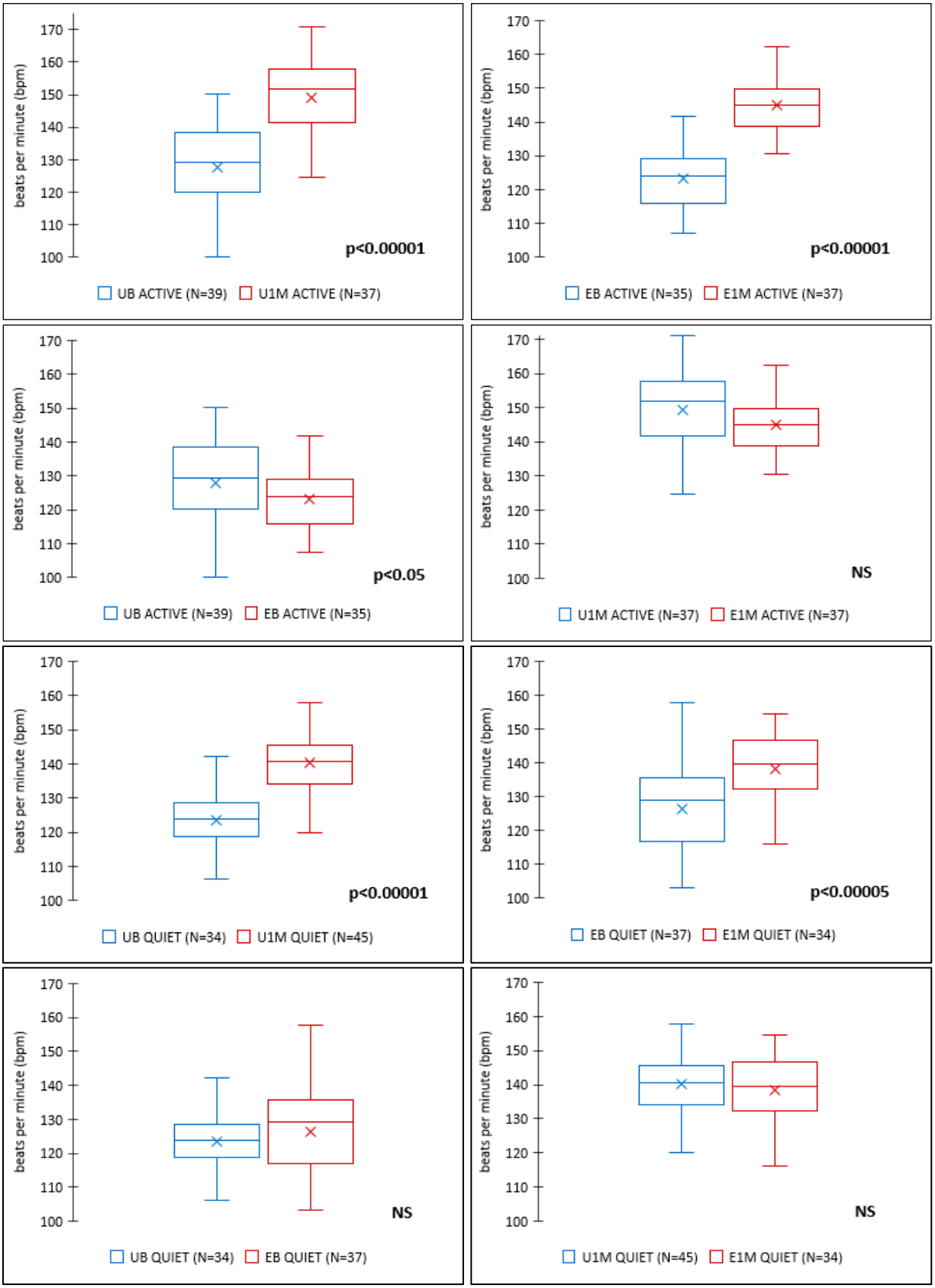

Figure 4. Comparisons of mean baseline heart rates across different groups.

There was a significant decrease in HR upon exposure for newborns in AS ($p<0.05$), which was not evident at 1 month of age. No significant changes were found upon exposure for infants at either age during QS. The most consistent trend observed was the significant increase in mean HR from the newborn to 1 month period (~12-20 bpm), regardless of sleep state or the degree of alcohol and tobacco smoke exposure ($p<0.0005$ for each comparison). These changes are consistent with the significant increases reported by the PASS Network [1]. Additionally, this increase in HR is consistent with previous reports of HR developmental trends in both normal and at-risk infants [1,20,22,26].

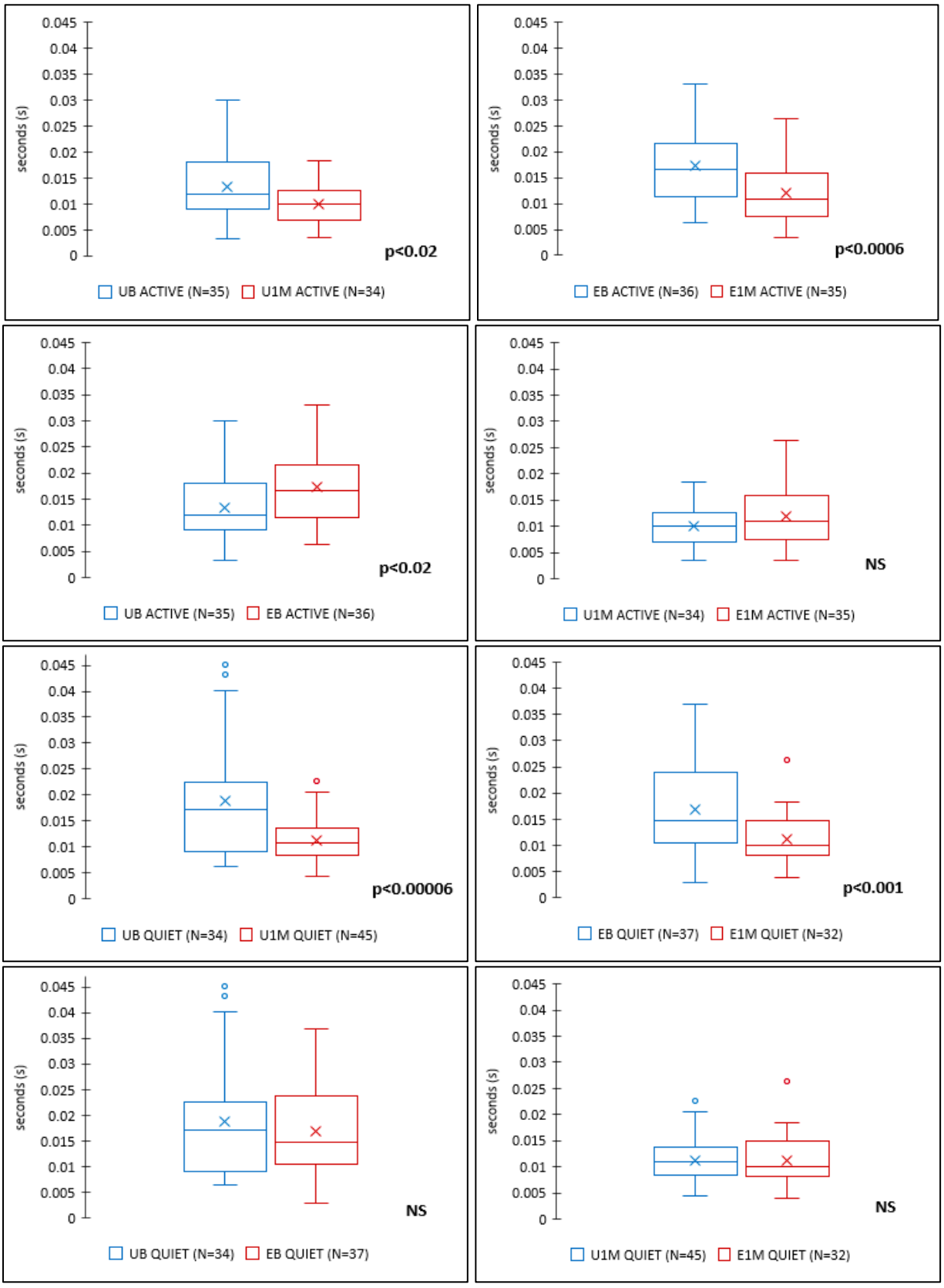

Figure 5. Comparisons of root-mean-square of successive differences between cardiac R-R intervals (RMSSD-RRi) across different groups.

There were significant decreases in RMSSD-RRi values from the newborn to 1 month period across both sleep states and in both unexposed and exposed groups. The least significant decrease was found in unexposed infants in AS ($p<0.02$), despite the much greater increase observed in the HR values within this group (Figure 4). This also occurred for the exposed infants in QS, but to a lesser degree ($p<0.001$). The only significant change in RMSSD-RRi upon exposure is observed in its increase in newborns in AS, which is consistent with the corresponding decrease in HR exhibited in Figure 4 for this group. Infants at 1 month of age in AS, and at both newborn and 1 month in QS, did not exhibit any significant changes with prenatal exposure.

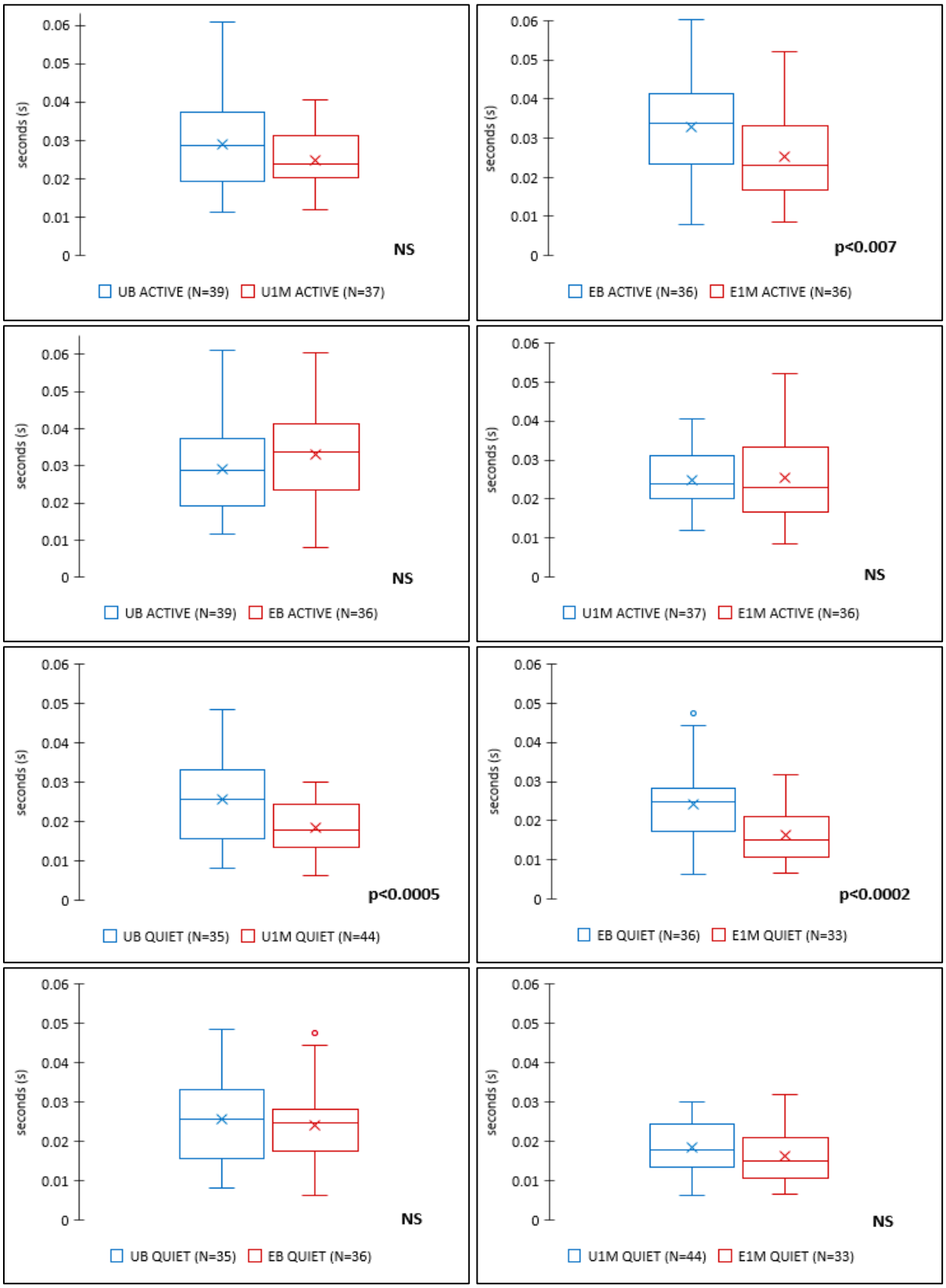

Figure 6. Comparisons of standard deviations between cardiac R-R intervals (SD-RRi) across different groups.

The trends for the newborn to 1 month group comparisons reported in Figures 4 and 5 were similar to those of the SD-RRi values in Figure 6, with exception to the unexposed groups in AS. Both the exposed and unexposed groups in QS showed significant decreases in SD-RRi ($p<0.0002$). A significant decrease from the newborn to 1-month period ($p<0.007$) is observed in the exposed groups in AS, but these values are, on average, slightly higher than those in QS. This pattern is not evident in the unexposed groups in AS. There were no significant changes in SD-RRi in any of the groups upon exposure.

There were no statistically significant differences between respiration rate or inter-breath intervals in any of the exposed or unexposed groups, at both ages and in both sleep states. For this reason, respiratory rate, standard deviations of inter-breath intervals (SD-BBi), and RMSSD-BBi values were not included in this time domain analysis report.

Cardiorespiratory Coupling

The synchronization analysis revealed several significant differences between the unexposed and exposed groups that were not present in the linear time domain analysis. The box plots in Figure 7 do not indicate the same significant changes in cardiorespiratory interactions from the newborn to 1 month period as were reported in the HR, RMSSD-RRi, and SD-RRi values in the time domain analysis previously described (Figures 4-6). This suggests that linear assumptions may be more useful for evaluating the influence of vagal tone on cardiorespiratory physiology, rather than the interactions themselves. Statistical values for the total synchronization (%) and total duration of synchronization (seconds) are reported in Table 3 below.

Variable	Original N	Low Cut-Off	High Cut-Off	Final N	Outliers	Mean ± SD	5th	95th
Total Synch. (%, AS)	39	-0.117	0.226	39	0.00%	0.0657±0.0642	0.003	0.180
Total Synch. (%, QS)	35	-0.304	0.551	35	0.00%	0.1307±0.1183	0.000	0.321
Total Synch. Duration (sec, AS)	39	-6.590	16.139	39	0.00%	5.2009±3.5573	0.534	12.944
Total Synch. Duration, (sec, QS)	35	-12.835	27.072	35	0.00%	7.3049±5.8185	0.000	17.748

A. Unexposed newborns (UB)

Variable	Original N	Low Cut-Off	High Cut-Off	Final N	Outliers	Mean ± SD	5th	95th
Total Synch. (%, AS)	35	-0.090	0.158	34	2.86%	0.0543±0.0512	0.000	0.164
Total Synch. (%, QS)	44	-0.255	0.574	44	0.00%	0.1600±0.1333	0.001	0.438
Total Synch. Duration (sec, AS)	34	-7.031	13.139	32	5.88%	3.4475±2.6304	0.000	8.259
Total Synch. Duration, (sec, QS)	44	-10.163	25.459	44	0.00%	6.9809±4.8029	0.000	15.469

B. Unexposed infants at 1 month of age (U1M)

Variable	Original N	Low Cut-Off	High Cut-Off	Final N	Outliers	Mean ± SD	5th	95th
Total Synch. (%, AS)	37	-0.062	0.104	37	0.00%	0.0296±0.0247	0.001	0.079
Total Synch. (%, QS)	35	-0.142	0.449	33	5.71%	0.1660±0.1125	0.021	0.387
Total Synch. Duration (sec, AS)	34	-9.493	15.821	34	0.00%	3.9306±3.0161	0.178	8.602
Total Synch. Duration, (sec, QS)	37	-4.447	17.944	37	0.00%	7.1992±3.3703	1.751	12.518

C. Exposed newborns (EB)

Variable	Original N	Low Cut-Off	High Cut-Off	Final N	Outliers	Mean ± SD	5th	95th
Total Synch. (%, AS)	36	-0.049	0.081	35	2.78%	0.0210±0.0265	0.000	0.081
Total Synch. (%, QS)	34	-0.389	0.837	34	0.00%	0.2541±0.1735	0.013	0.560
Total Synch. Duration (sec, AS)	35	-5.608	9.347	33	5.71%	2.2763±2.2289	0.000	6.201
Total Synch. Duration (sec, QS)	34	-6.963	22.716	34	0.00%	8.0830±4.9211	1.154	16.977

D. Exposed infants at 1 month of age (E1M)

Table 3. Total synchronization (%) and duration of synchronization (seconds) for m:n ratios of R-peaks to inspiratory onsets by age, sleep state, and exposure, with outlier rejection.

Although outlier rejection at the level of individual 180-second segments was synchronization analysis produced whole-subject outliers than the time domain analysis in all groups. The greatest percentages were in total synchronization duration in both the U1M and E1M group in AS (5.88% and 5.71%, respectively) and in total synchronization in the EB group in QS (5.71%). Extreme outlier data were analyzed in the same way as in the time domain analysis, but no significant physiological variable-exposure associations were found.

A consistent increase is observed in both the mean total synchronization and mean duration in QS with respect to AS, regardless of age or exposure. The greatest differences in both the total synchronization and total duration are observed in the E1M group (0.2304% and 5.8067 second increases in QS). The least significant differences between the two sleep states are observed in the UB group (0.0650% and 2.1040 second increases in QS). Although there are differences in the mean total synchronization and duration with age, they are not nearly as pronounced as the differences in the cardiorespiratory physiology variables computed and compared in the time domain analysis.

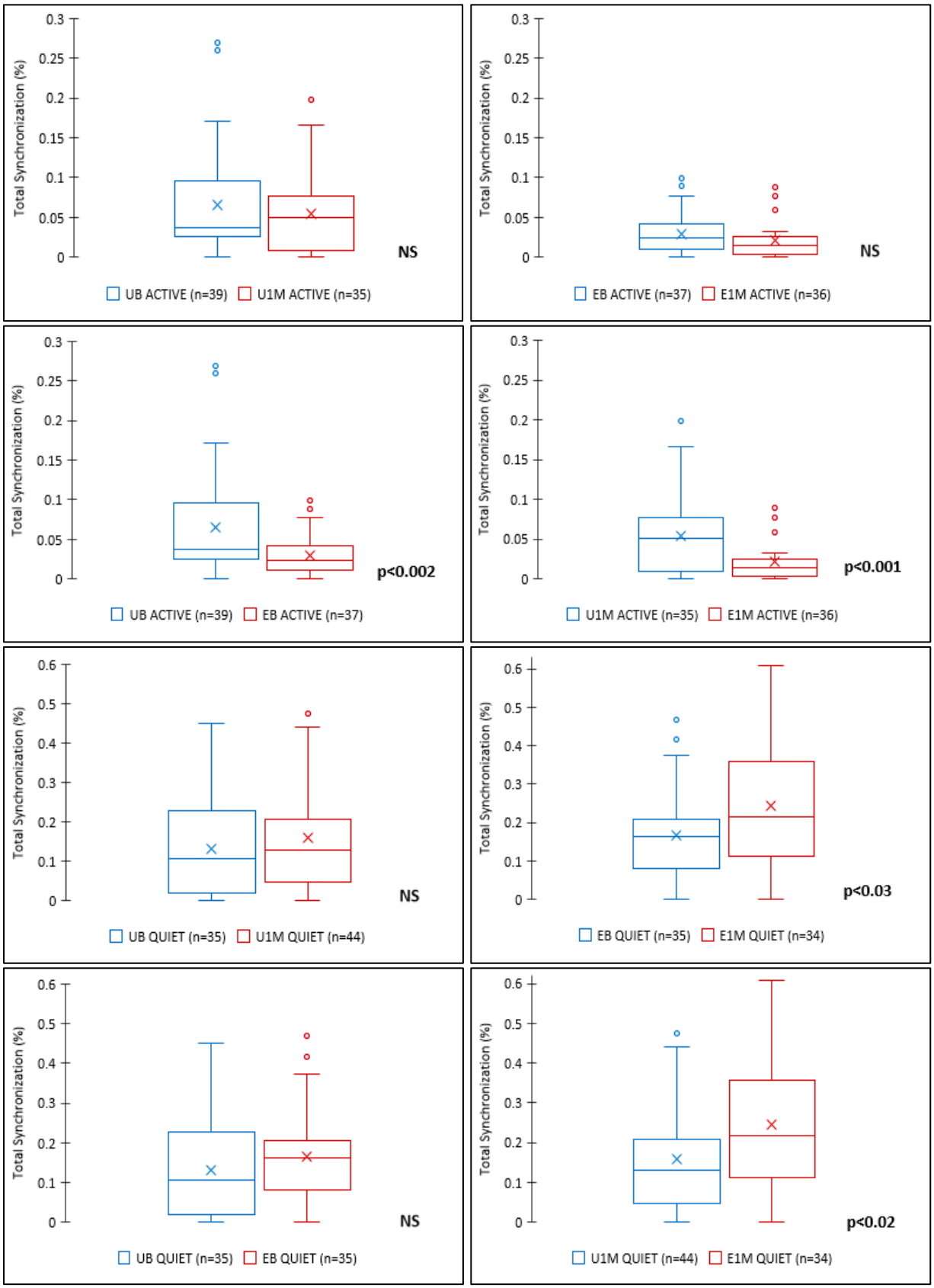

Figure 7. Total synchronization (%) for various m:n ratios.

There is a net increase in total synchronization and duration in QS with respect to AS. In QS, the total synchronization values are nearly double of those observed in AS. This is observed in the expanded y-axes in Figure 7 (y_{max} = 0.3 in AS, y_{max} = 0.6 in QS). There are statistically significant changes in the total synchronization with prenatal exposure to alcohol and tobacco smoke, with decreases in AS at both ages and increases in QS only at 1 month of age. This increase in QS with exposure is not evident in the UB and U1M comparison. It is also important to note the net decreases in the ranges of the values themselves, especially with prenatal exposure in AS at both ages. These changes are not as evident in QS.

Interactions Among Groups

Variable	Unexposed				Exposed			
	Sleep State Differences		Age Differences		Sleep State Differences		Age Differences	
	Newborn	1 month	Active Sleep	Quiet Sleep	Newborn	1 month	Active Sleep	Quiet Sleep
HR (bpm)	0.0812	*0.0001	*0.0000	*0.0000	0.1991	*0.0031	*0.0000	*0.0000
SD-RRi (sec)	0.2385	*0.0001	0.08646	*0.0004	*0.0015	*0.0001	*0.0070	*0.0002
rMSSD-RRi (sec)	*0.0126	0.19354	*0.01596	*0.0001	0.8265	0.5325	*0.0006	*0.0010
Total Synch. (%)	*0.0029	*0.0004	0.40376	0.3107	*0.0000	*0.0000	0.1594	*0.0275

Table 4. P-values for sleep state and age differences without changes in exposure. Asterisks (*) denote statistically significant differences ($p<0.05$), rounded to four decimal places.

Differences in the physiological variables in Tables 2 and 3 and Figures 4-7 are reported as p-values in Tables 4 and 5. Sleep state differences for HR were present only in infants at 1 month of age. These were both decreases in QS with respect to AS, with a greater decrease in the unexposed group (U1M) than in the exposed group (E1M). SD-RRi values were also lower in QS at 1 month of age for both unexposed and exposed groups, as well as in exposed newborns (EB). Similar to the HR comparisons, the greatest decrease in SD-RRi was present in the U1M group. The only significant sleep state difference in the RMSSD-RRi computations was for unexposed newborns (UB), which exhibited an increase in QS with respect to AS. Breathing

rates were significantly lower in QS than they were in AS, regardless of age or exposure. The greatest difference in the sleep state comparison of breathing rate was in the E1M group. Total synchronization percentages were significantly greater in QS than they were in AS for both ages and exposures, with the greatest increase in the E1M group.

The analysis of age differences revealed significant increases in HR at 1 month of age, regardless of sleep state or exposure. The greatest increase in HR was in the unexposed group in QS. The SD-RRi values of both unexposed and exposed infants was greater at 1 month of age than at the newborn period in QS. Conversely, SD-RRi was lower at 1 month in QS than in AS in exposed infants. There were significant decreases in RMSSD-RRi from the newborn to 1 month period, regardless of exposure or sleep state. The greatest decrease was observed in the unexposed group in QS. Total synchronization was greater in QS for the U1M group in comparison to the UB group, but this difference was not nearly as statistically significant as those computed when considering sleep state differences.

Variable	Exposure Differences	
	Newborn	1 month
HR (bpm, AS)	0.0564	0.0857
HR (bpm, QS)	0.2437	0.3934
SD-RRi (sec, AS)	0.1732	0.8306
SD-RRi (sec, QS)	0.5081	0.1850
rMSSD-RRi (sec, AS)	* 0.0145	0.0982
rMSSD-RRi (sec, QS)	0.3966	0.9529
Total Synch. (%, AS)	* 0.0096	* 0.0009
Total Synch. (%, QS)	0.2041	* 0.0165

Table 5. P-values for differences in cardiorespiratory physiology upon prenatal exposure to alcohol and tobacco smoke, without changes in sleep state or age. Asterisks (*) denote statistically significant differences ($p<0.05$).

The mean RMSSD-RRi value for exposed infants had greater statistically significant increases than that of unexposed infants in the newborn period in AS. Total synchronization percentages during both the newborn and 1 month periods in AS were greater in the unexposed infants, with a more statistically significant increase in the 1 month group. Conversely, total

synchronization was significantly greater in the exposed subjects than in the unexposed subjects in QS at 1 month. This difference was not found in the newborn subjects in QS upon prenatal exposure. No statistically significant differences were found in HR, SD-RRi, or breathing rate for either of the sleep states or ages.

I made 42 comparisons of different variables, making my results subject to false positives (i.e., results by chance). Therefore, I conducted a false discovery rate test for all p-values in Tables 4 and 5. All adjusted p-values had Benjamini-Hochberg significance at a false discovery rate of 5%, thus extending the validity of my results.

Discussion

Baseline Cardiorespiratory Physiology

The baseline cardiorespiratory physiological values and trends in the present study for the unexposed group are similar to those recently reported by the PASS Network [1]. For both groups, mean HR was higher in AS than in QS, and the range of values was approximately the same in both datasets. Similar trends were observed in the SD-RRi and breathing rate (f) values; however, their decreases from AS to QS were not as statistically significant in this study's cohort as they were in the larger study conducted by PASS Network [1]. This cohort exhibited slightly greater RMSSD values than subjects in the PASS study in both sleep states. This is important to note, as baseline cardiorespiratory physiological variables that are associated with ANS functioning have been found to differ in SIDS victims when compared to age-matched controls [20]. Specifically, they have been found to have higher heart rates in both AS and QS as well as altered HRV, with the latter being slightly more evident in QS [20].

The most statistically significant difference in the 1-month analysis was in the greater RMSSD-RRi values in this study vs. those in the PASS Network's study [7] in both sleep states

(0.017 vs. 0.009, respectively). Respiratory rate (f) of exposed newborns as well as all baseline parameters for exposed infants at the 1 month period have not been reported in the literature and therefore could not be compared in the same manner.

The observed increase in HR from the newborn to 1-month period is typically associated with either increased sympathetic activation or decreased parasympathetic activation [2,9]. This result was expected based on information gathered from the literature, as autonomic control should theoretically decrease as newborns approach the developmental period during which they are at highest risk for SIDS [2,9]. This observation may reflect increased instability of the ANS as described by Harper et al., specifically on the premise that most of the development of the parasympathetic nervous system occurs around 1 month of age [9]. By this reasoning, it is possible that infants have diminished regulatory capacity at 1 month of age, in comparison to the other ages in the 0-6 month risk period. Diminished regulatory capacity may help to explain an infant's decreased capability to adapt to intrinsic or extrinsic stressors.

The smaller but still statistically significant decrease in HR upon prenatal exposure in AS in newborns still works to identify a possible association between prenatal exposures and autonomic impairments. Most notable is the observation of the opposite effect in this comparison (i.e., a decreased HR instead of the significant increases from the newborn to 1 month period).

The HR values reported in this study support Filiano et al.'s Triple Risk Hypothesis for SIDS [29]. The 1 month period was characterized by increased HR in both sleep states, while prenatal exposure resulted in decreased HR in AS. These opposing outcomes suggest that there may be some optimal HR in the developing infant and that physiological stressors may cause variables such as HR to deviate from it. Too high of a HR may reflect inefficient energy expenditure, while too low of a HR may reflect decreased arousability.

It is widely accepted that cardiac R-R intervals, and thus HR, should carry some degree of variation (i.e., HRV) in order to maintain homeostasis when encountering challenges that cause cardiorespiratory physiology to deviate from this baseline state. RMSSD-RRi values have been used as measures of short term beat-to-beat HRV [25]. Direct measures of HRV such as RMSSD-RRi provide more quantitative information about ANS adaptability during neurodevelopment.

HRV, and thus RMSSD-RRi, generally reflect the degree of parasympathetic activation in the cardiorespiratory system [25]. Therefore, it was expected that changes in one direction in RMSSD-RRi would exhibit changes in the opposite direction across groups to those found in HR (e.g., a high HR would correspond to a low RMSSD-RRi for that subject). A baseline level of HRV is characteristic of a properly functioning ANS in healthy mammals, as regulatory systems are geared toward restoring homeostasis after environmental or psychological stimuli and therefore should not be static [22,25].

HRV has been found to be diminished at 1 month of age, but then follows an increasing trend approaching 6 months of age [22]. This suggests that rapid maturation of the parasympathetic nervous system occurs around 1 month of age, so it would be inherently less stable and more subject to dominant control by the sympathetic nervous system. This conclusion was supported by the trend observed in the increased HRs from the newborn to 1-month period across both sleep states and in both unexposed and exposed groups. This is consistent with the hypothesis of altered autonomic control as infants approach the critical period from 2-4 months of age [29]. These findings are also consistent with a study by Harper et al., which reported diminished HRV in SIDS victims and may suggest that these events are a combination between decreased parasympathetic modulation and increased sympathetic activation during the 0-6

month developmental period, resulting in an imbalance between the sympathetic and parasympathetic nervous systems [20].

Harper et al.'s theory was partially supported by the comparison of UB and EB groups in AS, which showed a significant increase in RMSSD-RRi and a corresponding decrease in HR with prenatal exposure (Figure 5). While this change in RMSSD-RRi provided evidence of a marked physiological change upon exposure, it only occurred in newborns in AS. This observation did not align with expectations, as there were no marked physiological changes in the infants at 1 month of age despite the presumed increased risk. The lack of significant changes in RMSSD-RRi in QS supports the complementary hypothesis that infants have greater homeostatic ability during this sleep state in comparison to AS.

The standard deviation of cardiac R-R intervals (SD-RRi), or the overall deviation from consistent cardiac periodicity, are direct measures of long-term HRV [25]. In a prior study by the PASS Network, this factor was shown to be a better marker for PAE [7]. The significant decreases in SD-RRi from the newborn to 1-month period (Figure 7), regardless of exposure, reflect a possible decreased in overall HRV approaching the critical period. These findings further support the previous reports of the significant effect of age on HRV, as especially evident in QS [1,27]. Conversely, SD-RRi in AS has been reported to be less significant [1].

The significant decrease in HRV in the exposed groups with respect to the unexposed groups from the newborn to 1-month period in AS supports the proposed decrease in ANS regulatory capacity upon prenatal exposure to smoking and alcohol. This is especially pertinent to functions involving responses to physiological challenges such as decreases in blood pressure or increases in HR.

Overall, these findings provide further evidence of major physiological changes during the 0-1 month period as well as support of the association of suboptimal HRV with SIDS risk [9]. Despite fewer significant changes in cardiorespiratory physiology between exposed and unexposed infants at both ages, many of the values presented in the time domain analysis suggest that infants are at higher risk for SIDS during the 1 month period. However, further studies are needed to assess vagal tone and regulation in response to physiological stressors, as the reported values are only baseline measurements that can serve as predictors of ANS behavior upon the introduction of these stressors.

Cardiorespiratory Coupling

The nonlinear method of synchronization analysis is required for the realistic analyses of physiological systems [10-13]. This method works in combination with linear time domain analysis to more fully assess two vital characteristics of the ANS and, more specifically, medullary function—parasympathetic modulation and cardiorespiratory interactions.

The net increases in total synchronization in QS are consistent with a previous state-related analysis [27] as well as a CRC feasibility study [21]. In the latter, Fifer et al. reported the greater stability of CRC dynamics in QS, with phase synchronization centered around a constant frequency. Conversely, this characteristic was reported to exhibit less consistency in AS [21]. Additionally, the corresponding decreases in respiratory rate in QS with respect to AS support the prior association of greater degrees of cardiorespiratory synchronization with lower respiratory rate [39].

The synchronization percentages in the present study support the mentioned findings and further suggest that vital ANS functions such as gas exchange and arousal (e.g., the ability to

respond to physiological stressors) are closer to optimal levels in QS than in AS. A possible explanation for the overall lower total synchronization percentages in AS is the supported theory that respiration is inherently more "noisy" in AS and is therefore more likely to exhibit less synchronization. A previous sleep state EEG analysis has reported this phenomenon in both infants and adults [5].

The determination of an optimal level of synchronization and coupling has not been well-defined. However, it was expected that exposed infants would exhibit diminished CRC. The observations in QS are contrary to this expectation; but these differences are less probable than those computed in AS by a factor of ~10 ($p<0.03$ vs. $p<0.002$, respectively). Based on these finding, it is still possible to infer that over-synchronization can also be representative of an ANS impairment. It has been proposed that this cardiac and respiratory cycle phase locking competes with respiratory sinus arrhythmia during cardiorespiratory interactions [30]. This rests on the premise that the cardiac and respiratory systems can become too entangled and subsequently have a decreased capability of adapting to physiological challenges; that is, there may be a possible necessity for the two systems to "detangle" from each other. This is analogous to the well-studied mechanism of HRV that occurs in R-R intervals in response to physiological stressors [25]. An additional hypothesis is that the fluctuation between low and high synchronization in AS and QS serve as intrinsic stressors that may serve to "train" the ANS to respond to physiological challenges; and perhaps the data obtained from the present study provide a time frame for this developmental process [27].

Interactions Among Groups

Overall, it seems that the largest changes occurred with age in the time domain analysis, but with sleep state in the synchronization analysis. The significant differences in the age, sleep state, and exposure comparisons served as a means for assessing interactions among these variables. The decrease in HR in QS with respect to AS at 1 month of age, regardless of exposure, suggest greater parasympathetic tone in QS, which is in agreement with previous findings [23,24,26]. The accompanying decreases in SD-RRi in QS for both unexposed and exposed subjects are consistent with the theory that long-term HRV is a correlate for sympathetic tone. These findings suggest greater parasympathetic regulation at 1 month of age. RMSSD-RRi values were expected to increase in QS, which would be consistent with the increased parasympathetic activity. However, the only difference was in the UB group. Although this RMSSD-RRi observation is not as pertinent to the 1-month analysis, it still exhibits a greater value in QS than in AS. Additional evidence for parasympathetic activity in QS is shown in the significantly decreased breathing rates within this sleep state, regardless of age or exposure. The greater synchronization percentages in QS at both ages and exposures suggests that there is some interaction between parasympathetic activity and cardiorespiratory synchronization.

The increases in HR from the newborn to 1 month period support the theory of decreased parasympathetic tone when approaching the critical period. The greatest increase in HR from the newborn to 1 month periods in QS in the unexposed group, which does not contradict my previous sleep state findings, as the net values of HR are still lower in QS than in AS. With this consideration, the increases in SD-RRi values at 1 month in QS (for both exposures) are expected based on their reciprocity to HR. Together with the HR trends, the SD-RRi values suggest increased sympathetic activity during the 1 month period in QS, as the developing infant

approaches the critical period. The opposite effect on SD-RRi that is observed in the exposed infants at 1 month of age (lower value in QS than in AS) provides additional evidence of a marked physiological change upon prenatal exposure. Perhaps it is the case that ANS functioning is dominated by sympathetic activity in unexposed infants, but shifts to parasympathetic activity upon exposure to alcohol and tobacco smoke.

The decreases in RMSSD-RRi from the newborn to 1 month period, with the greatest decrease in the unexposed group in QS, support this hypothesis. It provides further evidence of sympathetic activity in the absence of exposure. The increase in total synchronization in QS from the newborn to 1 month period suggests that there is an interaction between age, sympathetic activity, and cardiorespiratory synchronization. This is contrary to the conclusion drawn in the sleep state analysis. It seems that parasympathetic activity exhibits an association with synchronization across different sleep states, but that sympathetic activity exhibits this association across different ages.

Most of the differences between exposed and unexposed subjects occurred with age. The only significant change obtained from the time domain analysis upon prenatal exposure was the lower mean RMSSD-RRi in newborns in AS, which suggests greater sympathetic activation. Total synchronization is decreased in newborns in AS, which serves as further evidence of a marked physiological change upon prenatal exposure. Together, these results suggest that prenatal exposure to alcohol and tobacco smoke may promote greater sympathetic activity and decrease CRC in AS. However, in QS, the exposed infants had greater total synchronization at 1 month. This suggests that there exists an optimal level of synchronization in the developing infant that is altered upon prenatal exposures.

Conclusion

I conducted these analyses of cardiorespiratory interactions in order to contribute to the establishment of biomarkers for SIDS risk and prenatal exposures, with the goal of more informed early intervention. This is especially important in areas with elevated SIDS rates such as the Western Cape in South Africa and the Northern Plains in the United States. Future studies should take into account maternal susceptibility to tobacco and alcohol consumption during pregnancy in these regions and the influence, if any, of impoverished prenatal conditions, lower levels of maternal education and age, and cultural norms [7].

The use of a noninvasive, nonlinear method in the present study successfully provided information on characteristic CRC patterns in developing high-risk infants. The patterns of diminished RMSSD and SD-RRi and increased HR observed in the linear analysis during the 1 month period were similar to prior studies [1,22,27], which provides further evidence of increased vulnerability during this period of rapid ANS development. Comparison to these prior studies also reveals the significantly higher HR at 1 month than at 2 months of age, suggesting that the 1 month period is a transient period of decreased stability, parasympathetic modulation, and ability to respond to direct physiological challenges. The nonlinear analysis did not reveal significant differences with age, but rather with exposure. Decreased synchronization, and thus CRC, were most evident in exposed infants at both ages in AS. These observations support the original hypothesis that exposed infants would exhibit decreased CRC, reflecting some ANS impairment.

Although this study provides information about the beginning of infant development, more data points are needed to better characterize cardiorespiratory physiology and markers of the progression of ANS development during the high-risk period for SIDS (0-6 months). A more conclusive approach would be to analyze time domain and CRC data collected from SIDS

victims in order to compare them to the data of infants considered to be at risk. However, the small number of subjects that ultimately succumbed to SIDS in the PASS Network's cohort would give this hypothetical study less statistical power.

Further analyses must be conducted in order to provide additional information on CRC patterns in developing infants. My results serve as a comparative baseline for those studies, especially those involving the assessment of similar variables upon initiation of a direct physiological challenge. This would more adequately model how the cardiorespiratory physiology of an infant at risk for SIDS reacts to stressors prior to the event, especially with consideration of the failure mechanism proposed by Harper et al. [9].

Limitations

The parameters for the highly exposed subjects were reliant on maternal recollection of smoking, alcohol use, and exposure to other drugs of abuse. Therefore, I expect that there was some degree of under-reporting throughout the study. Additionally, although there was a lower cutoff to the amount of maternal alcohol and tobacco smoke consumption for classification into the exposed group, I did not establish an upper limit. Therefore, some (but very few) mothers reported consuming ≥8 drinks and ≥10 cigarettes per day for the entire duration of pregnancy. As stated in the Methods section (p. 10-11), I ensured that the physiological variables corresponding to these exceptionally high exposures were not outliers with respect to the subjects with exposures closer to the lower cutoff ≥4 drinks and ≥6 cigarettes per day.

Another limitation is that a small number of subjects provided data in more than one group (i.e., data from one infant at both ages, both sleep states, or both exposures). I suspect that although this serves as a potential confound, it would not significantly alter the results since

these infants were only a small subset of the cohort. A repeated measures analysis, which was not conducted in this study, would correct for any slight differences contributed by these subjects.

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Appendix

About the PASS Network

I conducted this study using data collected from the Safe Passage Study by the Prenatal Alcohol in SIDS and Stillbirth (PASS) Network, in collaborations with the Department of Developmental Neuroscience and the Sackler Institute for Developmental Psychobiology at the New York State Psychiatric Institute/Columbia University Medical Center. The goal of the Safe Passage Study is to investigate the risk of prenatal alcohol exposure (PAE) for SIDS, stillbirth, and fetal alcohol spectrum disorders (FASD). This is an international, prospective study that has amassed a cohort of infant physiological data (n=11692) from affiliated clinical sites in North and South Dakota in the United States, and in Cape Town in South Africa– both of which have SIDS rates that are amongst the highest in the world (3.46 and 3.41 per 1000 live births, respectively) [4]. Possible explanations to these increased statistics are mostly related to environmental factors such as socioeconomic status, health literacy, and poor post-natal environments. Additionally, there is a greater number of mothers who consume alcohol and tobacco smoke during pregnancy, have lower levels of education, and are of younger age [7]. The PASS Network seeks to take this wide range of factors into consideration in order to better understand their relationships to infant health and development, through studies with highly generalizable results.

The PASS Network's goal is to acquire as much prospective data as possible for use in the investigation of adverse prenatal exposures, both alone and in consideration of other maternal, environmental, and genetic factors, on fetal ANS function, SIDS and stillbirth risk, and later developmental outcomes.

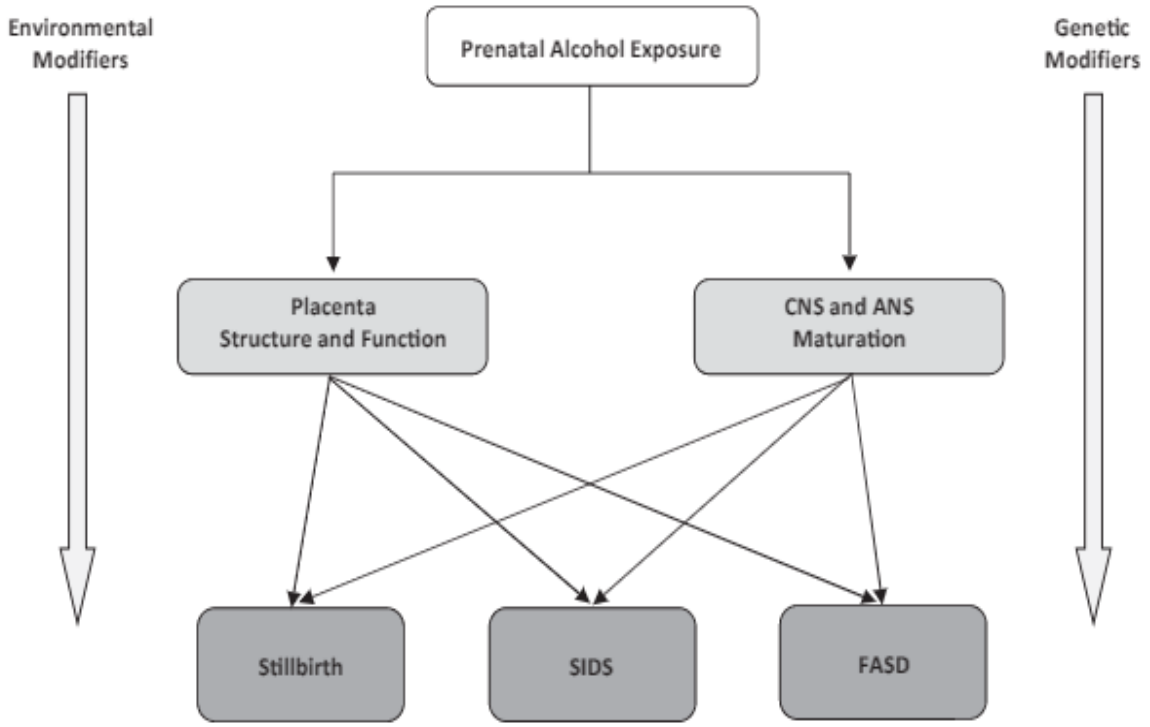

Figure 8. The PASS Network's proposed model of adverse outcomes due to PAE and environmental and genetic modifiers [4].

The primary hypothesis of the PASS Network is that prenatal adverse exposures increase risk for SIDS, stillbirth, and FASD by a mechanism similar to that in Figure 8. Initial analyses from the PASS Network demonstrated that whether or not an infant was pre- or post-natally exposured to alcohol and/or tobacco smoke, the infant still exhibits a significant physiological change as he or she approaches the unstable developmental period (2-4 months of age). Specifically, infants may have more difficulty in overcoming direct physiological challenges that tend to increase heart rate. Some of these outcomes are subject to sleep state, which, in the case of human fetuses and infants, are present in the two distinct states of active (AS) and quiet (QS) sleep [1,5]. These findings, along with those contributed from the current study, have the potential to more clearly identify predictors for vulnerability to SIDS, stillbirth, and FASD so that early intervention can be done for infants at risk.

IRB Approval

Institutional Review Board (IRB) approvals were obtained for the experiments conducted under the PASS Network's Safe Passage Study, including the participating clinical sites in North Dakota, South Dakota, and Cape Town, and data processing centers in New York City, NY. The demographics of the subjects in the Safe Passage Study include mixed racial, ethnic, and socioeconomic backgrounds at varying levels of prenatal exposure to alcohol and tobacco smoke within their respective regions.

List of Abbreviations

ACh: Acetylcholine
ANS: Autonomic nervous system
AS: Active sleep
BP: Blood pressure
CNS: Central nervous system
CRC: Cardiorespiratory coupling
EB ACTIVE: Exposed at birth in active sleep
EB QUIET: Exposed at birth in quiet sleep
ECG: Electrocardiogram
EEG: Electroencephalogram
E1M ACTIVE: Exposed at 1 month of age in active sleep
E1M QUIET: Exposed at 1 month of age in quiet sleep
f: Breathing rate
FASD: Fetal alcohol spectrum disorder
HR: Heart rate
HRV: Heart rate variability
nAChR: Nicotinic acetylcholine receptor
NE: Norepinephrine
PAE: Prenatal alcohol exposure
PNS: Peripheral nervous system
QS: Quiet sleep
RMSSD-RRi: Root-mean-square of successive differences between consecutive cardiac R-R intervals
RSA: Respiratory sinus arrhythmia
SD-RRi: Standard deviation of cardiac R-R intervals
SIDS: Sudden infant death syndrome
TLFB: Timeline follow-back interview
UB ACTIVE: Unexposed at birth in active sleep
UB QUIET: Unexposed at birth in quiet sleep
U1M ACTIVE: Unexposed at 1 month of age in active sleep
U1M QUIET: Unexposed at 1 month of age in quiet sleep
5-HT: Serotonin
5-HT$_{1A}$: 5-HT receptor type 1A