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Prenatal smoking and drinking are associated with altered newborn autonomic functions

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Abstract

Background: Prenatal smoking and drinking are associated with SIDS and neurodevelopmental disorders. Infants with these outcomes also have altered autonomic nervous system (ANS) regulation. We examined the effects of prenatal smoking and drinking on newborn ANS function.

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Contributors Statements

Dr Sania conceptualized and conducted statistical analysis, drafted the initial manuscript, and reviewed and revised the manuscript. Dr. Myers was a principal investigator of the study; he conceptualized and designed the study, supervised data collection and data processing, drafted the manuscript, reviewed, and revised it. Drs. Elliott, Fifer, and Odendaal were principal investigators of the study, they acquired funding, contributed to data analyses and interpretation of the results, and writing of the manuscript. Drs. Pini, Lucchini, and Shuffrey contributed to the interpretation of results and drafting the manuscript. Nugent, Barbosa and Angal participated in study supervision, data collection and processing, drafting and critical revision of the manuscript. Shreya participated in data analysis and drafting of the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest.

Category of study: Population Study

Consent Statement: Written informed consent was obtained from all participants. Ethical approval was obtained from Stellenbosch University, Avera Health, Sanford Health, the Indian Health Service and from participating Tribal Nations.

Methods: Pregnant women were enrolled in Northern Plains, USA (NP) and Cape Town (CT), South Africa. Daily drinking and weekly smoking data were collected prenatally. Physiological measures were obtained during sleep 12–96 hours post-delivery.

Results: 2,913 infants from NP and 4,072 from CT were included. In active sleep, newborns of mothers who smoked throughout pregnancy, compared to non-smokers, had higher breathing rates (2.2 breaths/min; 95% CI: 0.95, 3.49). Quit-early smoking was associated with reductions in beat-to-beat heart rate variability (HRV) in active (−0.08 sec) and quiet sleep (−0.11 sec) in CT. In girls, moderate-high continuous smoking was associated with increased systolic (3.0 mmHg, CI: 0.70, 5.24), and diastolic blood pressure (2.9 mmHg, CI: 0.72, 5.02). In quiet sleep, low-continuous drinking was associated with slower heart rate (−4.5 beat/min). In boys, low-continuous drinking was associated with reduced ratio of low-to-high frequency HRV (−0.11, CI: −0.21, −0.02).

Conclusions: These findings highlight potential ANS pathways through which prenatal drinking and smoking may contribute to neurodevelopment outcomes.

Introduction

Although many women modify their drinking and smoking behavior following pregnancy recognition[1,2], heightened risk of adverse outcomes associated with prenatal alcohol (PAE) and tobacco (PTE) exposures remains a public health concern, especially in low-resource settings and in populations with high prevalence of alcohol and tobacco use [3,4]. There is well documented evidence of role of prenatal drinking and smoking on increased fetal loss, stillbirth, preterm birth, intrauterine growth restriction, sudden infant death syndrome (SIDS) and other neurodevelopmental disorders [5–7]. Recent studies also linked PAE and PTE to dysregulation of the fetal and infant autonomic nervous system (ANS)[8,9] and the newborn central nervous system[10].

Alcohol and nicotine can directly affect the developing fetal brain leading to autonomic dysfunction and poor neurodevelopment[11,12]. Alcohol passed through the placenta is known to cause oxidative stress, mitochondrial damage, and alterations in the developing brain including malformations in the corpus callosum and cerebellum[13–15]. Nicotine alters fetal brain development by effects on cell proliferation and differentiation, specific neurotransmitters receptors, and disruption of neural activity in the brain[16]. Tobacco smoking and alcohol can also impact ANS development indirectly through shortening gestation and intrauterine growth restriction [17,18].

Impairment in the ANS regulation (cardiac, respiratory, and vascular) has been associated with SIDS, and adverse neurodevelopmental outcomes such as autism and ADHD[5,19,20]. Because of the increased incidence of SIDS among infants exposed to prenatal drinking and smoking [21], autonomic alterations associated with these exposures are important to understand. In addition to previous reports on alterations in SIDS victims' brainstem regions involved in autonomic regulation[22], members of the Prenatal and Alcohol in SIDS and Stillbirth (PASS) research team have reported on smoking and drinking associated alterations in the brainstem locations involved in cardiorespiratory functions and arousal in SIDS infants[23]. Other studies have shown that infants who subsequently die of SIDS have altered patterns of ANS activity during sleep including higher heart rate (HR), reduced

HR variability (HRV), and abnormalities in the beat-to-beat dynamics[24–30]. Inadequate responses to hypotension while asleep may also play a role in the fatal SIDS event[31,32].

Previous studies have o reported significant associations of prenatal drinking and smoking with reduced HRV in the fetus, and diminished HR responses to head-up tilting in newborns[8,33,34]. These studies have some methodological limitations including small sample sizes and limited exposure and covariate data. In the present study, we examined the role of prenatal drinking and smoking on parameters of ANS control in newborn infants in two large ethnically diverse cohorts of mother-infant dyads from the USA and South Africa. We hypothesized that prenatal drinking and smoking would affect newborn autonomic function and these effects will be contingent on timing, pattern and quantity of exposure. However, given the paucity of data with detailed measures of exposure as in the current study. we had no specific directional hypotheses.

Methods

Study population and data collection

Data for this study come from the Prenatal and Alcohol in SIDS and Stillbirth (PASS) Safe Passage Study. The Safe Passage Study was a prospective cohort study designed to evaluate the role of prenatal drinking and smoking on adverse pregnancy outcomes including stillbirth, SIDS, and fetal alcohol spectrum disorders (FASD). Participants were enrolled from antenatal clinics in Northern Plains, USA (South and North Dakota) and Cape Town, South Africa between August 2007 and January 2015. Pregnant women aged 16 years or older, at or after 6 weeks of gestation, carrying one or two fetuses were eligible to participate. Data on socioeconomic status, demographic, obstetric and medical history, periconceptional drinking and smoking were collected at the enrollment interview. Details on the study design and characteristics of the study population are published elsewhere[35].

Exposures Assessment and characterization

Data on alcohol exposure were collected using a validated modification of the timeline follow back (TLFB) method[36], where, at each study visit, participants reported detailed information on daily drinking ±15 days of their last menstrual period at enrollment visit and thirty days prior to their last known drinking day at each study visit. Participants also reported on the number of days they smoked per week and number of cigarettes smoked per day at enrollment and during the prenatal visits. Depending on the number and timing of the study visits for each participant, some data on daily drinking and smoking were missing. Missing data were imputed using a non-parametric algorithm called K nearest neighbor algorithm (k-NN)[37]. Using clustering methods, based on the amount and timing of drinking and smoking, the participants were divided into 4 smoking and 10 drinking groups[38]. Because several of the drinking groups had a small number of subjects they were collapsed into 4 groups for the current analyses. Women were categorized as moderate-to-high continuous smoking, low-continuous smoking, quit-early smoking, and non-smoking; and moderate-to-high continuous drinking, low-continuous drinking, quit-early drinking, and non-drinking groups (See supplementary methods on exposure imputation and exposure clustering).

ANS data measurement and data processing

Details on ANS measurement, processing and normative values in our study population have been published previously[39]. Newborns were tested 12–96 hours after delivery. Electrocardiograms (ECG) and respiration rates were collected using custom hardware and software, and blood pressure (BP) was collected using a clinical monitor. The standard protocol involved recording ECG, respiration rate, and BP signals during a 10-minute baseline period and in response to three rapid (~3–5 seconds) 45° head-up tilts while the infant was in the prone position. The resting state measures allowed the characterization of autonomic traits, whereas changes in autonomic state in response to tilt assessed the infant's capacity to respond to an environmental challenge. As cardiorespiratory variables differ between sleep states, the records were subdivided into epochs coded as either active or quiet sleep states[40,41].

Baseline parameters: Baseline physiological variables were computed for 10 one-minute epochs prior to two baseline BP measurements. For each minute, the median the median R-wave to R-wave interval (RRi) was computed, and from the median RRi, heart rate (HR) was computed. The standard deviations of RRi (SD-RRi) and the square root of the mean of the squared successive differences in RRis (rMSSD) were computed as were low-frequency HRV (LF; HRV between 0.04 and 0.40 Hz) and high-frequency HRV (HF; HRV between 0.50 and 1.50 Hz) power from Fourier spectral analyses. The LF/HF ratio was also computed. For each minute, the median breath-to-breath interval, and breathing rate were computed. The long-term variability parameters (SD, LF power) evaluate a combination of sympathetic and parasympathetic nervous systems contributions to HRV. In contrast, measures of beat-to-beat variability (RMSSD, HF) are influenced largely by the parasympathetic nervous system, tied to rapid ANS reactivity. The LF/HF ratio has been interpreted as a measure of sympatho-vagal balance, with higher values indicating sympathetic predominance and lower values indicating parasympathetic predominance[42].

Parameters in responses to head-up tilting—For HR, acute responses to tilts were computed as maximum HR-minimum HR during the first 15 seconds in the head-up position. The median maximum-minimum HR over the 3 tilts were analyzed. The pre-tilt values for systolic and diastolic BP were recorded 90 seconds before tilts and head-up BP values were recorded during the period just before returning to the horizontal position, 90 seconds after head-up tilting. Median changes in BP over the three tilts were analyzed.

Statistical analysis

Linear regression models were used to estimate associations of each prenatal drinking groups (reference: non-drinkers) and smoking groups (reference: non-smokers) with ANS parameters with separate models for each of the ANS outcomes. All models included infant sex, hours of life at assessment and gestational age (GA) at birth as covariates. Models included maternal education, household crowding index, prenatal depression scores measured with the Edinburgh scale, and study site as potential confounders. Missing indicators were included in the multivariate models when covariate data were missing. Outcome variables that were not normally distributed were log transformed (SD-RRi, RMSSD). Analyses combining participants across sites were performed first, and then,

separately for each of the two sites. Additional sex stratified analyses were also performed. Because there were site differences in key characteristics of the participants, study site was included as a covariate in site combined models. Additional analyses stratified by site were also obtained. The overlap between exposure clusters (4 smoking groups and 4 drinking groups) was insufficient to examine the effects of interaction between drinking and smoking on ANS outcomes. Because we have related outcomes and covariates, adjustment for multiple comparisons (such as the Bonferroni method) was not appropriate[43–45]. We have reported all analyses and estimates (and confidence intervals) with equal emphasis and detail in the manuscript. All analyses were performed in SAS software version 9.4 (SAS Institute, Cary NC).

Ethics Statement:

Written informed consent was obtained from all participants. Ethical approval was obtained from Stellenbosch University, Avera Health, Sanford Health, the Indian Health Service and from participating Tribal Nations.

Results

The final analyses included 6,985 singletons born at term, of them 2,913 (42%) were from Northern Plains and 4,072 (58%) were from South Africa. We excluded infants with missing exposure data, preterm and post-term infants, and infants whose mothers used psychiatric medication during pregnancy (Supplemental Figure S1). South African women had poorer socioeconomic status, as reflected in lower educational attainment, higher unemployment rates, higher crowding index, and higher proportion living without a partner. Although mean gestational ages at birth were similar in both sites, South African newborns were about 400 g lighter on average (Table 1). ANS parameters were obtained later in South Africa (60. 3 hours, SD 51.3) compared to the Northern Plains site (29.9 hours, SD 47.3). This difference was due the hospital policy in South Africa of discharging infants within 24 hours of delivery, so they needed to be brought in for assessment later.

The prevalence of smoking was higher in South Africa (61%) than Northern Plains (24%). In South Africa, 23% pregnant women continued smoking in moderate to high amount and 35% continued with a low amount throughout the pregnancy while only 17% woman in Northern Plains continued. Women in the moderate-to-high continuous, mild continuous and quit early groups smoked 48.31 (SD 21.71), 15.72 (SD 10.28), and 8.81 (SD 9.92) cigarettes/ week, on average in first trimester, respectively (Figure 1 and Supplemental Table 1), with a declining number of cigarettes smoked in subsequent trimesters in all groups. Approximately half of the pregnant women in both sites reported drinking during pregnancy. The majority (43%) of NP participants quit drinking in the first trimester, only 8% drank throughout pregnancy, while 29% participants from SA continued to drink throughout (Supplemental Supplemental Table S3). Women in the high moderate-to-continuous, mild continuous and quit early groups drank 40. 9 (SD 60.1), 2.4 (SD 3.8), and 8.9 (SD 7.4) drinks in first trimester, respectively (Figure 1 and Supplemental Table 3). Women in the moderate-to-high continuous drinking group reported 2 (interquartile range, IQR 0–5), 1

(IQR 0–2), and 0 (IQR 0–1) binge episodes in trimester 1, 2 and 3 respectively. Women in low continuous and quit early group rarely binged.

Associations of smoking with baseline ANS parameters (Table 2, Supplemental Tables S5, S6)

In the site combined data analyses, compared to non-smokers, moderate-to-high-continuous smoking was associated with an increase in breathing rate in both sleep states ($p<0.001$, and $p=0.07$ respectively, Table 2). Similar results were seen for active sleep at each site (SA: 2.00 breaths/min, 95% CI: 0.43, 3.56, NP: 2.38 95% CI: −0.15, 4.91, Tables S-5, S-6). However, this association was primarily driven by results for girls (Figure 2, panel A, B, C).

There were no significant associations of smoking with HR parameters in the analyses with sites combined. In SA, compared to infants of non-smoker mothers, SD-RRi in active sleep was 0.02 s (95% CI: 0.002, 0.04) greater in the moderate-to-high-continuous smoking group (Supplemental Table S5), while rMSSD in active sleep (−0.08 sec, 95% CI: −0.14, −0.03) and quiet sleep (−0.11, 95% CI: −0.22, −0.001) was decreased in the quit-early smoking group (Tables S-5, S-6 respectively). In NP, SD-RRi in quiet sleep was increased in the quit-early group ($p=0.02$, Supplemental Table S6).

There were no significant associations of smoking with diastolic BP in combined analyses. In sex stratified analyses, moderate-to-high continuous smoking was associated with higher systolic (2.97 mmHg, CI: 0.70, 5.24, $p=0.01$) and diastolic BP (2.87 mmHg, CI: 0.72, 5.02, $p=0.009$) among girls only (Figure 2, panel D, G.)

Associations of alcohol with Baseline ANS parameters (Table 3, Supplemental Tables S7, S8)

In site combined analyses, compared to infants of non-drinker mothers, infants in the low-continuous drinking group had 4.53 beats/min lower HR in quiet sleep (95% CI: −7.75, −1.32, Table 3). Similar results were seen in SA for the low-continuous and moderate-to-high continuous drinking group (Supplemental Table S7). Compared to no drinking, low-continuous drinking was associated with a reduced ratio of LF/HF in active sleep in combined analyses (−0.08 95% CI −0.15, −0.02, Table 3), as well as in site stratified analyses (Tables S-7, S-8). Further stratification by sex revealed that this association was significant among boys only (Figure 2, Panel J, K, L).

In combined analyses, diastolic BP during quiet sleep was lower in low-continuous (−3.29 mmHg, 95% CI: −6.59, −0.006) and quit-early (−1.36 mmHg, 95% CI: 2.78, 0.05) drinking groups (Table 3) compared to non-drinkers. Low-continuous drinking was associated with reduced diastolic BP in the NP ($p=0.04$, Supplemental Table S8) but not in SA.

Associations of smoking on tilt responses (Table 4, Supplemental Tables S9, S10)

The decrease in active sleep breathing rates following head-up tilt was less pronounced in low-continuous smoking group (0.81 breaths/min, 95% CI: 0.07, 1.54, Table 4) compared to non-smokers. In stratified analyses, this association was significant in SA (Supplemental Table S9) but not in the NP (Supplemental Table S10).

In site combined analyses, the acute HR responses to tilt were increased during active sleep in the low-continuous (1.03 beats/min, 95% CI: 0.23, 1.83) and the moderate-to-high-continuous (1.22 beats/min, 95% CI: 0.23, 2.21) smoking groups compared to non-smokers (Table 4). The increases in the acute HR response in the moderate-to-high-continuous smoking group was significant in SA only (p=0.02, Supplemental Table S9). The decrease in RMSSD following head up tilt seen in the non-smoking (reference group) during active sleep was not seen in the low-continuous smoking group (0.35 sec, 95% CI: 0.03, 0.67, Table 4). A less marked reduction of RMSSD values in quiet sleep during head-up tilt was found in quit-early smoking group (p=0.05, Table 4) in site combined analyses.

In combined sites analyses, there were smaller decreases in systolic (3.05 mmHg, 95% CI: 1.22, 4.89) and diastolic BP (1.95 mmHg, 95% CI: 0.31, 3.58) following head up tilts during active sleep in the quit-early smoking group compared to non-smoker group (Table 4). These differences remained significant in NP (Supplemental Table S10). Decreases in diastolic BP to tilt in active sleep were also diminished in the low-continuous smoking group compared to non-smoking group, in site combined analyses (0.82 mmHg, 95% CI: −0.03, 1.69, Table 4) and in NP (2.57, 95% CI: 0.51, 4.63, Supplemental Table S10).

Associations of alcohol on tilt responses (Table 5, Supplemental Tables S11, S12)

The decrease in breathing rate in active sleep following head up tilting was less pronounced in the low-continuous drinking group (1.81 breaths/min, 95% CI: 0.32, 3.29, Table 5) compared to non-drinking group. In stratified analyses this association remained significant in NP but not in SA. In the NP, decreases in breathing in quiet sleep to head up tilt were diminished among low-continuous drinking (4.55 breaths/min, 95% CI: 0.19, 8.91, Supplemental Table S12),

Compared to non-drinking group, the decrease in RMSSD during quiet sleep was diminished in the quit-early drinking group (0.44 sec, 95% CI: 0.03, 0.84, Table 5) but not in site stratified analyses (Tables S-11 S-12).

Compared to non-drinking group, decreases in systolic BP in active sleep following head up tilts were less pronounced in the moderate-to-high-continuous drinking group in site combined analyses (1.22 mmHg, 95% CI: 0.11, 2.33, Table 5), which remained significant in SA (Supplemental Table S11), but not in the NP (Supplemental Table S12). In NP, decreases in diastolic BP in quiet sleep were diminished among moderate-to-high-continuous drinking group (4.36 mmHg, 95% CI: 1.41, 7.30, Supplemental Table S12).

Discussion

In this study, data from two large socio-culturally and ethnically diverse cohorts were used to evaluate the effects of prenatal drinking and smoking on ANS parameters extracted from physiological signals acquired from term newborns at birth. We found significant associations of prenatal smoking with newborn breathing and rMSSD during baseline, as well as greater acute changes in HR, and diminished changes in systolic and diastolic BP in response to head up tilts. There was a significant association between smoking and blood pressure among girls only. Prenatal drinking was associated with reductions in BP,

reductions in the ratio of low to high frequency HRV, and diminished breathing rate and systolic BP responses to tilt. The associations of prenatal smoking and drinking were more pronounced in South African newborns, where women, on average, drank and smoked at higher levels, and were more likely to continue to drink and smoke throughout the pregnancy than women in the Northern Plains.

Moderate to high continuous smoking was associated with higher breathing rates. This extends the literature linking prenatal smoking to alterations in regulation of infant respiration, specifically regarding the literature reporting increases in the frequency and length of obstructive apneas[46]. Evidence from animal studies indicates maternal smoking leads to nicotine-induced cholinergic sensitivity of respiratory neurons of neonatal mice[47], a mechanism that could predispose newborns to SIDS. Prenatal smoking has also been shown to adversely impact airway development, leading to abnormalities associated with an increased tendency to wheeze and higher susceptibility to respiratory infections in early childhood[48].

Our results support the conclusion that prenatal smoking alters key aspects of cardiovascular regulation in newborn infants. The finding that prenatal smoking is associated with increases in baseline BP is consistent with a prior report by Beratis et al [49,50]. With regard to the responses to tilt, in a prior study in the NP, smaller increases in HR following head up tilting were found in tobacco exposed infants[8]. Consistent with this, in our study, infants of non-smokers had significant increases in HR following tilt whereas infants exposed to smoking did not (see Table 4). However, in contrast Browne et al. found infants of smoking mothers had a larger decrease in blood pressure to tilt[44], though the number of subjects assessed in the newborn period was small (26 non-smokers, 24 smokers).

Ours is the first study to report a significant reduction in the ratio of low-to-high frequency HRV among infants whose mothers drank throughout the pregnancy in moderate-to-high amounts. This ratio is interpreted as a measure of sympathovagal balance, although this remains controversial[51] has been utilized as potential marker for social arousal and engagement[52]. The changes found suggest that prenatal drinking is associated with a greater relative parasympathetic control of HR. In contrast, an increase in the ratio of low-to-high frequency HRV associated with prenatal smoking was reported in other smaller studies[53,54]. Greater effects of smoking on the ratio of low-to-high HRV among boys is potentially related to their greater vulnerability, given that male infants who are more likely to die of SIDS[55]. Similar sex differences in autonomic regulation are also reported in animal studies[56]. Taken together, these results indicate that the effects of smoking and drinking are not mediated through a common neurophysiological or psychological mechanism.

We also found moderate-to-high continuous smoking was associated with increases in systolic and diastolic BP among newborn girls. Similar effects were reported in prior studies among adolescent girls [57,58]. Unlike previous studies in newborns and fetuses[8,9], we found no statistically significant associations of prenatal drinking on HR and HRV in active sleep. However, the average amount of alcohol consumed in our study populations was considerably lower than in previous studies. We also did not observe any significant

associations of smoking on newborn baseline HR despite significant associations with fetal HR among the same cohort[9].

The findings related to first trimester exposures followed by cessation in the quit early groups are potentially caused by the impact of withdrawal from smoking and drinking on maternal autonomic function and psychology, and, in turn, effects of these changes on fetal and infant physiology. Smoking cessation during pregnancy is known to elevate maternal blood pressure[59,60]. In addition, women quitting smoking and drinking may experience depressed mood, anxiety and stress symptoms[61,62]. Although we adjusted for maternal depression in the analyses, residual confounding is plausible. Another limitation of our study is measurement error in the exposure assessment. Since smoking and drinking were based on self-reports, it is likely that there was underreporting by some pregnant women exposed to anti-smoking and anti-drinking information[63] and this could have contributed to less robust findings. In addition, data on medications related to smoking cessation or heart arrhythmias were not collected, therefore we could not account for these potential contributions in the analyses. Given the low proportion of women quitting smoking in both sites and low socioeconomic condition of the participants, it was unlikely that many would have access to such interventions.

Strengths of our study include extensive prospectively collected alcohol and smoking data with rigorous quality controls, which allowed us to evaluate the role of both quantity and timing of the exposure. To date, ours is the largest epidemiological study to examine the role of PAE and PTE on newborn ANS in a population-based study. We measured multiple aspects of autonomic activities including HRV, BP and breathing rates, which allowed evaluation of their coordinated activities. We also had detailed information on several potential confounders that were not accounted for in previous studies. While studies conducted in laboratory settings have precise measurements of smoking and alcohol exposures and increased confounder control, such findings are not directly generalizable to human population health. Our findings are generalizable to pregnant women and their infants of similar racial, ethnic and socioeconomic backgrounds.

Conclusion

We observed that prenatal smoking and drinking alters autonomic function in term newborns. Specifically, effects of smoking on breathing rate, beat-to-beat HRV and effects of drinking on low-to-high frequency HRV highlight the pathways through which prenatal drinking and smoking could be linked to risk of SIDS and neurodevelopmental disorders like ADHD[24–26,64]. While the changes in physiological measurement we presented might not meet thresholds for what may be termed clinical significance, they do indicate changes in ANS regulation profiles. Given that SIDS occurs when multiple factors intersect (vulnerable infant, critical period and exogenous trigger) even small shifts in physiology can move infants into high-risk categories. Similar shifts in physiology have been reported in other at risk populations such as preterm infants[65]. The current study provides new evidence to support the conclusion that prenatal exposure to smoking and alcohol can alter newborn autonomic function. Given the high prevalence of prenatal smoking and drinking and their known associations with postnatal outcomes such as SIDS, and poor neurodevelopment,

early examination of ANS parameters can serve as a marker for identification of newborns at risk of adverse outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Impact:

- In this prospective cohort study of 6,985 mother-infant dyads prenatal drinking and smoking were associated with multiple ANS parameters.
- Smoking was associated with increased neonatal breathing rates among all infants, and heart rate variability (HRV) and blood pressure (BP) among girls.
- Drinking was associated with reductions in HR and BP among all newborns, and reductions in the ratio of low to-high frequency HRV among boys.
- These findings suggest that prenatal smoking and drinking alter newborn ANS which may presage future neurodevelopmental disorders.

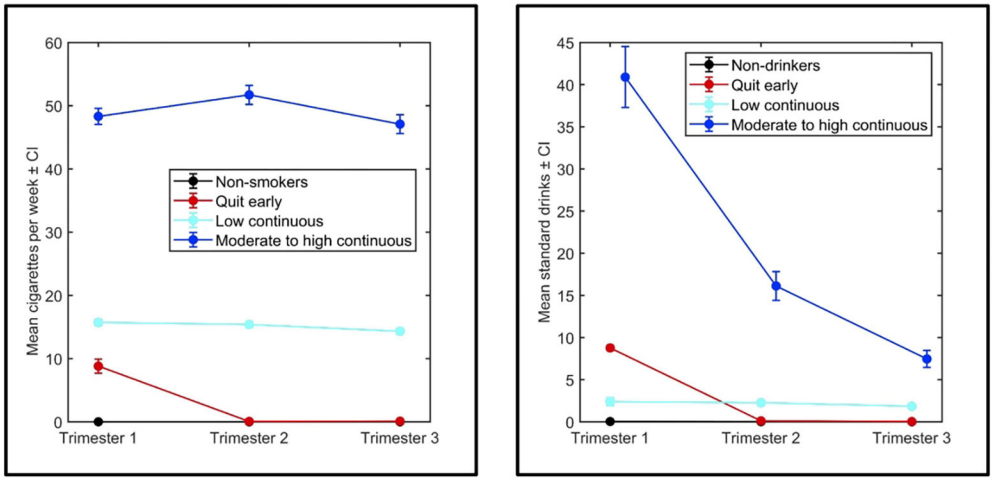

Figure 1:
Magnitude of smoking and drinking in each trimester by clusters

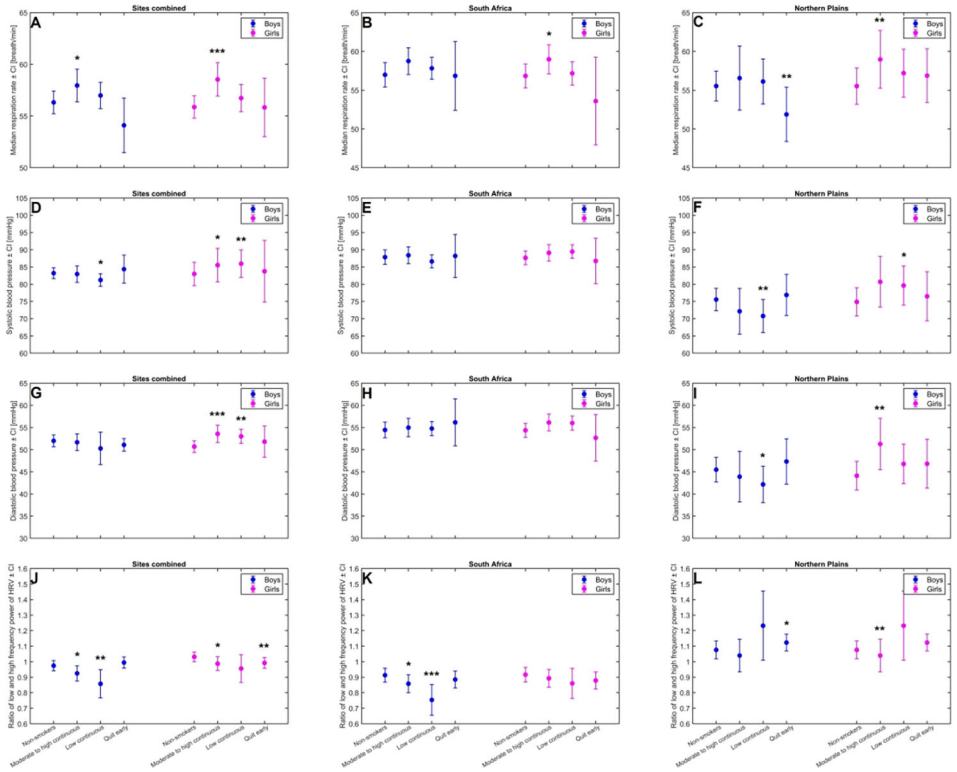

Figure 2: Marginal means in each exposure categories, stratified by study site and infant sex.

Panel A, B and C shows median respiration rate for smoking exposure groups for both sites, South Africa, and Northern Plains, respectively.

Panel D, E, F shows Systolic BP for smoking exposure groups for both sites, South Africa and Northern Plains, respectively.

Panel G, H, I show diastolic BP for smoking exposure groups for both sites, South Africa and Northern Plains, respectively.

Panel J, K, L shows ratio of low-to-high frequency heart rate variability for alcohol exposure groups for both sites, South Africa, and Northern Plains, respectively.

*p<0.1, **p <0.05, ***p <0.01 indicates significance of the difference in marginal mean in comparison to the reference group, non-smoker, or non-drinker

Table 1:

Characteristics of the study population (n=6915) by site

		Cape Town, South Africa N= 4072	Northern Plains, USA N=2913
		N[1] (%) or mean (SD)	N[1] (%) or mean (SD)
Maternal characteristics			
Maternal age, years		24.7 (5.8)	26.8 (5.3)
Marital status			
	Married/cohabiting	2195 (53.9)	2290 (78.6)
	Unmarried	1877 (46.1)	623 (21.4)
Education			
	Primary school education	313 (7.7)	46 (1.6)
	Some high school education	2738 (67.2)	480 (16.5)
	High school completed	876 (21.5)	531 (18.2)
	Higher than high school	142 (3.5)	1855 (63.7)
Employment status			
	Employed	1183 (29.1)	1914 (65.1)
	Unemployed	2889 (70.9)	999 (34.3)
BMI, kg/m^2			
	<18.5	287 (7.1)	61 (2.1)
	18.5–25	2010 (49.4)	1045 (35.9)
	25– 30	957 (23.5)	877 (30.1)
	30+	818 (20.1)	930 (31.9)
Parity			
	Nulliparous	1597 (38.8)	935 (32.1)
	Parity 1–2	1265 (31.1)	937 (32.2)
	Parity >3	739 (18.2)	546 (18.7)
Mode of delivery			
	Normal and assisted delivery	3650 (89.70)	2234 (76.72)
	Cesarean section	419 (10.30)	678 (23.28)
Crowding index		1.6 (0.9)	0.7 (0.6)
Depression, Edinburgh Depression Score		12.77 (5.92)	5.46 (4.34)
Infant characteristics			
Birth weight, g		3107.7 (447.3)	3520. 9 (457.3)
Gestational age, weeks		39.4 (1.2)	39.4 (1.1)
Sex			
	Female	1985 (48.8)	1469 (50.4)
	Male	2087 (51.2)	1444 (49.6)
Hours of life at measurement		60.26 (51.27)	29.93 (47.56)

Table 2:

Association of prenatal smoking with ANS measures at baseline in combined site analyses (Infants whose mothers did not smoke during pregnancy formed the reference group. Results with p-values <0.01, <0.05, <0.10 are bolded)

	Non-smoker Mean (95%CI)	**Quit early Mean difference (95%CI) P value**	**Low continuous Mean difference (95%CI) P value**	**Moderate to high continuous Mean difference (95%CI) P value**
HR (bpm)	126.13 (125.41, 126.84)	1.07 (−0.72, 2.87) (0.24)	0.096 (−0.88, 1.07) (0.84)	−0.019 (−1.21, 1.16) (0.97)
log_{10}SD-RRi (s)	−1.61 (−1.62, −1.59)	−0.005 (−0.04, 0.02) (0.72)	0.002 (−0.014, 0.02) (0.20)	**0.02 (−0.001, 0.04) (0.06)**
log_{10}RMSSD (s)	−1.95 (−1.96, −1.94)	−0.027 (−0.060, 0.0005) (0.10)	−0.0002 (0.02, 0.01) (0.98)	0.015 (−0.007, 0.04) (0.17)
Breaths/min	56.05 (55.29, 56.82)	−1.09 (−3.03, 0.84) (0.26)	0.79 (−0.25, 1.85) (0.13)	**2.22 (0.95, 3.49) (0.0006)**
Systolic blood pressure (mmHg)	83.12 (81.96, 84.28)	0.88 (−2.15, 3.91) (0.56)	0.44 (−1.11, 1.98) (0.57)	1.24 (−0.65, 3.14) (0.19)
Diastolic blood pressure (mmHg)	50.82 (49.86, 51.77)	1.44 (−1.03, 3.92) (0.25)	0.87 (−0.39, 2.14) (0.17)	**1.53 (−0.02, 3.09) (0.053)**
Low frequency power of HRV	−5.88 (−5.90, −5.86)	0.0003 (−0.059, 0.059) (0.992)	0.002 (−0.029, 0.034) (0.881)	**0.03 (−0.003, 0.07) (0.075)**
High frequency power of HRV	−6.83 (−6.85, −6.80)	**−0.077 (−0.14, −0.011) (0.02)**	0.009 (−0.026, 0.045) (0.59)	**0.04 (−0.0013, 0.085) (0.057)**
Ratio of low and high frequency power of HRV	0.94 (0.92, 0.97)	**0.05 (−0.007, 0.11) (0.08)**	0.006 (−0.025, 0.038) (0.709)	0.013 (−0.025, 0.051) (0.504)
Quiet sleep				
HR (bpm)	120.53 (119.39, 121.67)	−0.49 (−3.15, 2.17) (0.71)	0.58 (−1.04, 2.19) (0.48)	1.03 (−1.04, 3.11) (0.32)
log_{10}SD-RRi (s)	−1.74 (−1.76, −1.72)	**0.05 (−0.002, 0.10) (0.06)**	−0.01 (−0.05, 0.02) (0.43)	0.02 (−0.02, 0.06) (037)
log_{10}RMSSD (s)	−1.93 (−1.95, −1.90)	0.009 (−0.04, 0.07) (0.73)	−0.017 (−0.05, 0.016) (0.31)	0.007 (−0.036, 0.051) (0.74)
Breaths/min	45.68 (44.65, 46.72)	−1.78 (−4.19, 0.63) (0.14)	0.16 (−1.31, 1.63) (0.82)	**1.70 (−0.18, 3.58) (0.07)**
Systolic blood pressure (mmHg)	82.75 (81.13, 84.38)	−0.29 (−4.01, 3.41) (0.87)	0.72 (−1.52, 2.97) (0.52)	0.06 (−2.75, 2.87) (0.96)
Diastolic blood pressure (mmHg)	50.17 (48.97, 51.37)	−0.70 (−3.45, 2.03) (0.61)	−0.21 (−1.87, 1.45) (0.80)	−1.32 (−3.40, 0.74) (0.21)
Low frequency power of HRV	−6.27 (−6.32, −6.21)	0.12 (0.00074, 0.24) (0.04)	−0.029 (−0.103, 0.043) (0.424)	0.056 (−0.036, 0.15) (0.234)
High frequency power of HRV	−6.84 (−6.89, −6.80)	−0.04 (−0.149, 0.067) (0.45)	−0.037 (−0.103, 0.028) (0.26)	0.028 (−0.055, 0.113) (0.506)
Ratio of low and high frequency power of HRV	0.58 (0.53, 0.63)	**0.117 (−0.003, 0.238) (0.056)**	0.015 (−0.057, 0.087) (0.68)	0.039 (−0.054, 0.134) (0.40)

Table 3:

Association of prenatal alcohol with ANS measures at baseline in combined site analyses. (Infants whose mothers did not drink during pregnancy formed the reference group. Results with p-values <0.01, <0.05, <0.10 are bolded)

	NonMean (95%CI)	**Quit early Mean difference (95%CI) P value**	**Low continuous Mean difference (95%CI) P value**	**Moderate to high continuous Mean difference (95%CI) P value**
Active sleep				
HR (bpm)	126.36 (125.67, 127.05)	0.46 (−0.37, 1.30) (0.27)	−0.76 (−2.74, 1.22) (0.45)	0.56 (−0.54, 1.67) (0.31)
log_{10}SD-RRi (s)	−1.61 (−1.61, −1.59)	−0.005 (−0.02, 0.009) (0.51)	0.004 (−0.03, 0.04) (0.80)	−0.003 (−0.02, 0.015) (0.73)
log_{10}RMSSD (s)	−1.96 (−1.97, −1.94)	−0.005 (−0.02, 0.01) (0.49)	0.022 (−0.01, 0.58) (0.23)	0.006 (−0.01, 0.03) (0.53)
Breaths/min	56.05 (55.28, 56.82)	−0.14 (−1.03, 0.75) (0.76)	−1.61 (−3.72, 0.50) (0.13)	−0.45 (−1.64, 0.73) (0.45)
Systolic blood pressure (mmHg)	84.22 (83.09, 85.35)	−0.21 (−1.55, 1.13) (0.31)	−1.19 (−4.43, 2.05) (0.47)	−0.43 (−2.19, 1.31) (0.62)
Diastolic blood pressure (mmHg)	52.04 (51.12, 52.97)	0.22 (−0.87, 1.33) (0.68)	−0.79 (−3.45, 1.87) (0.56)	−0.48 (−1.92, 0.95) (0.50)
Low frequency power of HRV	−5.86 (−5.88, −5.84)	−0.015 (−0.043, 0.011) (0.26)	−0.017 (−0.082, 0.048) (0.609)	−0.013 (−0.05, 0.022) (0.45)
High frequency power of HRV	−6.86 (−6.89, −6.84)	−0.007 (−0.038, 0.022) (0.62)	**0.089 (0.017, 0.16) (0.015)**	**0.036 (−0.004, 0.077) (0.079)**
Ratio of low and high frequency power of HRV	1.002 (0.98, 1.02)	−0.014 (−0.04, 0.01) (0.31)	**−0.08 (−0.15, −0.02) (0.008)**	**−0.04 (−0.07, −0.006) (0.02)**
Quiet sleep				
HR (bpm)	122.28 (121.16, 123.41)	0.07 (−1.28, 1.42) (0.10)	**−4.53 (−7.75, −1.32) (0.006)**	−1.41 (−3.30, 0.46) (0.13)
log_{10}SD-RRi (s)	−1.72 (−1.74, −1.69)	−0.001 (−0.03, 0.02) (0.92)	−0.02 (−0.08, 0.05) (0.61)	0.002 (−0.03, 0.04) (0.92)
log_{10}RMSSD (s)	−1.94 (−1.96, −1.91)	−0.006 (−0.04, 0.02) (0.64)	0.006 (−0.06, 0.08) (0.85)	0.017 (−0.022, 0.06) (0.39)
Breaths/min	45.84 (44.82, 46.86)	−0.51 (−1.73, 0.71) (0.41)	−0.54 (−3.45, 2.36) (0.71)	0.48 (−1.22, 2.20) (0.57)
Systolic blood pressure (mmHg)	84.02 (82.48, 85.55)	−1.22 (−3.14, 0.70) (0.21)	−3.46 (−7.92, 0.98) (0.12)	0.13 (−2.38, 2.65) (0.10)
Diastolic blood pressure (mmHg)	43.61 (40.65, 46.56)	**−1.36 (−2.78. 0.05) (0.06)**	**−3.29 (−6.59, 0.006) (0.05)**	0.99 (−0.86. 2.84) (0.29)
Low frequency power of HRV	−6.23 (−6.28, −6.18)	0.015 (−0.04, 0.07) (0.62)	−0.02 (−0.17, 0.11) (0.72)	0.003 (−0.08, 0.08) (0.93)
High frequency power of HRV	−6.86 (−6.90, −6.81)	−0.02 (−0.08, 0.02) (0.32)	−0.014 (−0.14, 0.11) (0.82)	0.04 (−0.03, 0.11) (0.28)
Ratio of low and high frequency power of HRV	0.61 (0.56, 0.67)	0.03 (−0.02, 0.09) (0.22)	0.02 (−0.12, 0.16) (0.75)	−0.02 (−0.11, 0.05) (0.53)

Table 4:

Association of prenatal smoking with mean changes in cardiorespiratory variables in response to 45° head-up tilts in site combined analyses. (Infants whose mothers did not smoke during pregnancy formed the reference group. Results with p-values <0.01, <0.05, <0.10 are bolded)

	Non-smoker Mean (95%CI)	**Quit early Mean difference (95%CI) (P value)**	**Low continuous Mean difference (95%CI) (P value)**	**Moderate to high continuous Mean difference (95%CI) (P value)**
Active sleep				
Δ sustained HR (bpm)	2.52 (2.08, 2.96)	0.56 (−1.64, 0.52) (0.31)	−035 (−0.95, 0.24) (0.24)	−0.29 (−1.03, 0.45) (0.44)
Δ acute HR (bpm)	22. 98 (22.39, 23.57)	−0.69 (−2.13, 0.75) (0.34)	**1.03 (0.23, 1.83) (0.01)**	**1.22 (0.23, 2.21) (0.02)**
Δ rMSSD-RRi (s)	−0.57 (−0.80, −0.33)	0.46 (−0.12, 1.04) (0.12)	**0.35 (0.03, 0.67) (0.03)**	−0.09 (−0.49, 0.30) (0.64)
Δ change in breathing (breaths/min)	−3.73 (−4.27, −3.19)	0.35 (−0.98, 1.68) (0.60)	**0.81 (0.07, 1.54) (0.03)**	−0.005 (−0.91, 0.90) (0.99)
Δ systolic blood pressure (mmHg)	−6.04 (−6.76, −5.30)	**3.05 (1.22, 4.89) (0.001)**	0.68 (−0.29, 1.65) (0.16)	−0.22 (−1.42, 0.98) (0.72)
Δ diastolic blood pressure (mmHg)	−6.49 (−7.31, −5.83)	**1.95 (0.31, 3.58) (0.01)**	0.82 (−0.03, 1.69) (0.06)	0.08 (−0.98, 1.14) (0.88)
Quiet sleep				
Δ sustained HR (bpm)	3.39 (2.82, 3.98)	0.89 (−0.60, 2.38) (0.24)	−0.38 (1.19, 0.0.41) (0.34)	0.34 (−0.65, 1.32) (0.50)
Δ acute HR (bpm)	22.39 (21.54, 23.24)	**−1.89 (−4.11, 0.32) (0.09)**	0.08 (−1.08, 1.24) (0.89)	0.40 (−1.03, 1.83) (0.85)
Δ rMSSD-RRi (s)	−1.06 (−1.41, −0.71)	**−0.92 (−1.82, −0.015) (0.05)**	0.12 (−0.36, 0.61) (0.61)	0.27 (−0.32, 0.86) (0.36)
Δ change in breathing (breaths/min)	−3.73 (−4.27, −3.19)	0.30 (−1.19, 1.79) (0.69)	−0.36 (−1.17, 0.46) (0.39)	−0.23 (−1.22, 0.77) (0.65)
Δ systolic blood pressure (mmHg)	−5.07 (−5.89, −4.25)	−0.19 (−2.38, 2.00) (0.86)	−0.02 (−1.12, 1.07) (0.97)	0.37 (−0.95, 1.69) (0.58)
Δ diastolic blood pressure (mmHg)	−5.83 (−6.53, −5.12)	−0.24 (−2.08, 1.61) (0.80)	0.34 (−0.57, 1.26) (0.46)	0.32 (−0.78, 1.43) (0.57)

Table 5:

Association of prenatal drinking with mean changes in cardiorespiratory variables in response to 45° head-up tilts in both study sites. (Infants whose mothers did not drink during pregnancy formed the reference group. Results with p-values <0.01, <0.05, <0.10 are bolded)

	Non-drinker Mean (95%CI)	**Quit early Mean difference (95%CI) (P value)**	**Low continuous Mean difference (95%CI) (P value)**	**Moderate to high continuous Mean difference (95%CI) (P value)**
Active sleep				
Δ sustained HR (bpm)	2.29 (1.86, 2.71)	−0.21 (−0.72, 0.28) (0.40)	−0.22 (−1.45, 1.00) (0.72)	0.17 (−0.51, 0.86) (0.62)
Δ acute HR (bpm)	23.08 (22.51, 23.64)	−0.23 (−0.91, 0.44) (0.50)	1.33 (−0.29, 2.95) (0.10)	0.09 (−0.83, 1.01) (0.85)
Δ rMSSD-RRi (ms)	−0.34 (−0.57, −0.12)	−0.11 (−0.38, 0.16) (0.42)	0.09 (−0.56, 0.75) (0.77)	−0.15 (−0.52, 0.22) (0.42)
Δ change in breathing (breaths/min)	−3.95 (−4.46, −3.43)	0.47 (−0.14, 1.09) (0.12)	1.81 (0.32, 3.29) (0.02)	−0.29 (−1.14, 0.55) (0.49)
Δ systolic blood pressure (mmHg)	−5.60 (−6.29, −4.90)	0.15 (0.69, 1.01) (0.31)	0.39 (−1.57, 2.37) (0.69)	**1.22 (0.11, 2.33) (0.03)**
Δ diastolic blood pressure (mmHg)	−6.09 (−6.71, −5.47)	0.32 (−0.43, 1.07) (0.40)	0.39 (−1.35, 2.13) (0.66)	0.55 (−0.43, 1.54) (0.26)
Quiet Sleep				
Δ sustained HR (bpm)	3.81 (3.24, 4.38)	−0.37 (−1.04, 0.31) (0.28)	−0.82 (−2.42, 0.78) (0.31)	0.38 (−0.51, 1.28) (0.39)
Δ acute HR (bpm)	22.17 (21.33, 23.01))	−0.68 (−1.66, 0.31) (0.17)	0.29 (−2.08, 2.66) (0.80)	−0.12 (−1.43, 1.17) (0.84)
Δ rMSSD-RRi (ms)	−1.09 (−1.44, −0.75)	**0.44 (0.03, 0.84) (0.04)**	−0.58 (−1.55 0.38) (0.23)	−0.21 (−0.75, 0.32) (0.43)
Δ change in breathing (breaths/min)	−2.39 (−2.97, −1.81)	−0.08 (−0.76, 0.61) (0.83)	−1.09 (−2.71, 0.52) (0.18)	−0.34 (−1.24, 0.56) (0.46)
Δ systolic blood pressure (mmHg)	−4.85 (−5.65, −4.04)	−0.34 (−1.32, 0.64) (0.50)	−0.35 (−2.53, 1.83) (0.75)	−0.05 (−1.27, 1.17) (0.93)
Δ diastolic blood pressure (mmHg)	−5.81 (−6.50, −5.13)	−0.03 (−0.85, 0.79) (0.94)	0.11 (−1.72, 1.94) (0.90)	0.27 (−0.75, 1.30) (0.59)