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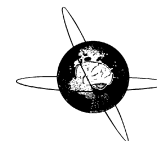
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The dynamics of cardiac autonomic control in sleeping preterm neonates exposed *in utero* to smoking

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HIGHLIGHTS

- Prenatally smoke-exposed neonates have low vagal and elevated sympathetic activities during sleep.
- Maternal smoking during pregnancy disrupts heart rate control dynamics in the neonate.
- The observed effects can be attributed to exclusively prenatal smoking exposure.

ABSTRACT

Objective: We aimed to determine whether *in utero* exposure to smoking may influence the activity and dynamics of cardiac autonomic control in preterm infants. We hypothesized that cardiac autonomic control is altered in preterm infants exposed prenatally to smoking and that these effects may vary as a function of the sleep state. **Methods:** We studied healthy, preterm neonates born to mothers who had smoked throughout pregnancy but not since birth ($n = 16$). *In utero*-exposed neonates were matched with control preterm neonates born to non-smoking mothers ($n = 18$). Cardiac autonomic control was monitored as a function of the sleep state by assessing heart rate variability with both linear and non-linear methods. **Results:** Preterm neonates with *in utero* exposure to smoking displayed alterations (relative to control neonates) in short-term cardiac autonomic control in all sleep states. These alterations included low vagal activity, elevated sympathetic activity, and low complexity and adaptability in heart rate control dynamics. **Conclusions:** Our results constitute direct evidence that cardiac autonomic activity and control are altered in sleeping preterm infants exposed to smoking *in utero*. **Significance:** These alterations may place the affected infants at a higher risk of neurological and cardiovascular complications, which could conceivably persist throughout childhood and adulthood.

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1. Introduction

Tobacco smoking during pregnancy has still a high prevalence worldwide, and is the most important potentially preventable cause of a range of adverse gestational and developmental outcomes (Salihu and Wilson, 2007). There is unequivocal evidence of a close relationship between maternal smoking during pregnancy on one hand and higher incidences of preterm birth, infant

morbidity and infant mortality on the other (Green et al., 2005). Worldwide, nearly 10% of all deliveries are preterm (Beck et al., 2010). Preterm birth is the leading cause of neonatal death (27%) (Lawn et al., 2010) and is a risk factor for elevated blood pressure (Hack et al., 2005) and hypertension (Eriksson et al., 2001) later in life.

Furthermore, a number of studies have found that smoking exposure at critical stages of fetal and infant development alters autonomic blood pressure control mechanisms (Browne et al., 2000; Cohen et al., 2008; Viskari-Lähdeoja et al., 2008), and that these effects persist for up to 1 year after birth (Cohen et al., 2010). Although a few studies have examined the influence of smoking exposure on heart rate variability (HRV), most failed to

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find a difference between control and smoke-exposed infants (Browne et al., 2000; Galland et al., 2000; Viskari-Lähdeoja et al., 2008). Other studies have yielded conflicting results, with either lower parasympathetic tone (Franco et al., 2000) or lower sympathetic tone (Thiriez et al., 2009) in infants born to smoking mothers. This discrepancy may be due to shortcomings in study design. The influence of sleep states has not always been considered (Browne et al., 2000; Thiriez et al., 2009; Viskari-Lähdeoja et al., 2008), even though active sleep (AS) and quiet sleep (QS) differ in terms of autonomic function (Frasch et al., 2007). Also, it has not been possible to determine whether the observed effects were due to prenatal exposure or postnatal exposure (Browne et al., 2000; Franco et al., 2000; Galland et al., 2000; Viskari-Lähdeoja et al., 2008).

There is now evidence to support the hypothesis championed by Goldberger et al. (1990), whereby the HR in the developing human infant is subject to non-linear and possibly chaotic-like changes (Sugihara et al., 1996). A conventional spectral analysis of HRV (as used in the above-cited studies) can provide information on cyclic variations but not on the dynamic properties of the fluctuations. Non-linear methods are typically designed to assess quality, scaling and correlation properties rather than the magnitude of variability assessed by conventional HRV methods. To the best of our knowledge, non-linear methods of HRV analysis have never been used to study the effects of smoking exposure. Importantly, non-linear methods are known to be suitable for analyzing non-stationary time series and may provide additional power in characterizing complex systems such as cardiac autonomic control in infants (Mäkikallio et al., 2002; Morren et al., 2005).

According to current literature, there is no direct evidence to show that *in utero* exposure to smoking alters cardiac autonomic control in neonates, and the underlying pathophysiological mechanisms remain poorly understood. The objective of the present study was to determine whether *in utero* exposure to smoking in preterm infants may influence the activity and dynamics of cardiac autonomic control. We hypothesized that cardiac autonomic control (as measured in an HRV analysis) is altered in preterm infants exposed prenatally to smoking and that these effects may vary as a function of the sleep state. The HRV's characteristics were examined with linear and non-linear methods, in order to extract more detailed quantitative and qualitative information.

2. Methods

2.1. Patients

Enrolment of preterm neonates, including eligibility requirements and informed consent, have been described in detail previously (Stéphane-Blanchard et al., 2013). None of the neonates had disorders or treatments (for at least the 7 days preceding the study) known to influence cardiac autonomic control. To control whether infants displayed an acid/base disturbance caused by a respiratory and/or metabolic problem, base excess was measured during the first 7 days of life. The local institutional review board approved the study, which complies with the Declaration of Helsinki.

Shortly after each infant's arrival in the NICU, a structured questionnaire on prenatal history and exposure to tobacco smoke was administered. Medical records were reviewed for any mention of smoking during pregnancy and were compared with the mother's statement. Neonates whose mothers reported (i) illicit substance abuse or (ii) passive smoking at home or at work during their pregnancy were excluded from the study. Only neonates born to women who reported smoking more than 1 cigarette per day throughout the entire pregnancy were included in the study. The

included, exposed infants were matched for gestational age at birth and postmenstrual age at the time of the study with control infants born to non-smoking mothers.

2.2. Study protocol

The study protocol has been described in detail previously (Stéphane-Blanchard et al., 2013). Neonates were recorded polygraphically (two electro-encephalograms, eye movements, an electrocardiogram, respiratory signal, body movements, oxygen saturation) at thermoneutrality, in the supine position during a morning nap.

Sleep states were scored as recommended by the Pediatric Task Force (Grigg-Damberger et al., 2003). All artifacts or other events that might have influenced the infant's HR were identified manually and excluded from the analysis.

2.3. HRV analysis

Recording and calculation of linear HRV parameters have been described elsewhere (Stéphane-Blanchard et al., 2013). Briefly, electrocardiogram signals were sampled at 2000 Hz. R wave detection, calculation of RR intervals and HRV analysis were performed with Kubios HRV[®] software (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland).

Standardized time- (mean HR, SDNN, r-MSSD and pNN25) and frequency-domain (power spectra in the very low (VLF), low (LF) and high (HF) frequency bands, LF/HF ratio) HRV parameters were extracted in order to characterize both overall, short- and long-term cyclic components responsible for HR variability.

2.3.1. Detrended fluctuation analysis (DFA)

DFA quantifies the intrinsic, fractal-like correlation properties of dynamic systems (Peng et al., 1995). A scaling exponent α represents the time series' correlation properties (see Appendix A). If $\alpha < 0.5$, the signal is anti-correlated (i.e. there are negative correlations in the signal); if $\alpha = 0.5$, the signal is uncorrelated (white noise); lastly, if $\alpha > 0.5$, there are positive correlations in the signal. In the present study, we sought to characterize the scaling behavior of the fluctuation function on short and long timescales, in order to establish whether there were short- or long-range correlations. Therefore, two scaling exponents were estimated (with a linear fit) over a specific scaling range for each segment: α_1 in the range $4 \leq n \leq 16$ and α_2 in the range $16 \leq n \leq 64$, which respectively characterize correlation behavior on short and intermediate timescales.

2.3.2. Approximate entropy (ApEn) and sample entropy (SampEn)

ApEn quantifies the unpredictability of fluctuations in a time series (see Appendix A) and yields the logarithmic probability that patterns of observations will repeat themselves within determined tolerance limits on next incremental comparisons. A low value of ApEn corresponds to lower complexity and a more predictable time series. SampEn is similar to ApEn but does not take account of self-matches and thus reduces the superimposed bias. SampEn is also less dependent on the length of the time series (Richman and Moorman, 2000). Both ApEn and SampEn are estimates of the negative natural logarithm of the conditional probability that a data of length N having repeated itself within a tolerance r for m points will also repeat itself for $m + 1$ points. SampEn was designed to reduce bias in ApEn and agrees more closely with the theory on data with known probabilistic content.

Table 1

Maternal and neonatal characteristics.

	Control group n = 18	Smoking group n = 16
<i>Maternal data</i>		
Cigarettes per day	0	14 (2–25)***
Maternal age (years)	25 (19–37)	27 (18–34)
<i>Neonatal data</i>		
Gestational age (weeks)	31.4 (28.4–33.7)	31.4 (28–34.1)
Birth weight (g)	1427 (810–1940)	1326 (790–2105)
5 min APGAR score	8.9 (7–10)	8.8 (6–10)
Base excess (mmol/L)	–8.2 (–17.1 to 4.7)	–5.9 (–20.0 to 0.7)
Postnatal age (days)	33.3 (18–59)	33.5 (17–57)
Postmenstrual age (weeks)	36.1 (34.8–37.3)	36.2 (34.4–38.8)
Weight at the time of the study (g)	2082 (1675–2325)	2023 (1220–2830)
Male:female gender ratio	7:11	6:10
Duration of caffeine citrate therapy (days)	11 (0–23)	13 (0–24)
Duration of mechanical ventilation (days)	3.5 (0–6)	4.0 (0–7)

Values are quoted as the mean (range).

*** $P < 0.001$.**Table 2**

Sleep, ventilatory and temperature data.

	Control group	Smoking group
Total sleep time (min)	134.7 ± 13.3	137.2 ± 7.9
Wakefulness after sleep onset (min)	17.1 ± 11.1	13.1 ± 7.9
AS (%)	58.4 ± 7.9	65.6 ± 10.3*
QS (%)	27.7 ± 6.0	22.7 ± 6.1*
Incubator temperature (°C)	32.4 ± 0.9	32.8 ± 0.6
Arterial oxygen saturation (%)	97.7 ± 1.2	97.3 ± 1.4
Respiratory rate (breaths min ⁻¹)	52.1 ± 9.4	53.3 ± 11.8

Values are quoted as the mean ± standard deviation. * $P < 0.05$. There were no significant intergroup differences in the incubator temperature, respiratory rate and arterial oxygen saturation in either active sleep (AS) or quiet sleep (QS).

2.4. Statistical analysis

Statistics were computed using Statview software (SAS Institute, Inc., Cary, NC). Calculation of the sample size was calculated according to Bland (1995) for comparison of two means. Results indicated recruitment of 17 subjects in each group in order to detect a difference of 0.4 in the DFA scaling exponent α_1 between infants born to smoking or non-smoking mothers, with 90% power at the 5% level. All HRV parameters that did not follow a normal distribution in a Kolmogorov–Smirnov test (spectral parameters) were transformed using a Box–Cox transformation. To stabilize the variance, values expressed as percentage (i.e. pNN25) were arcsined-transformed. Two-way analyses of variance for repeated measures were used to test for differences in HRV parameters between groups (the between-subject factor) and between sleep states (the within-subject factor). When F values were significant, two-tailed unpaired t tests were computed separately for AS and QS. The threshold for statistical significance was set to $P < 0.05$. Data are quoted as the mean ± SD.

3. Results

3.1. Patients

The infants' and mothers' characteristics are summarized in Table 1. The final study population (gestational age: 31.4 ± 1.5 weeks; birth weight: 1379 ± 349 g; postmenstrual age: 36.2 ± 0.9 weeks; weight at the time of the study: 2083 ± 277 g) consisted of 18 control infants (the control group) and 16 prenatally smoke-exposed infants (the smoking group). The duration of caffeine citrate therapy and mechanical ventilation did not differ

significantly when comparing the smoking and control groups. Although none of the infants were exposed to environmental tobacco smoke after birth, 7 of the 16 smoking mothers breastfed (as did all the non-smoking mothers). However, we did not observe any differences in linear or non-linear HRV parameters according to whether the infants in the smoking group had been breastfed or not.

The smoking and control groups did not differ significantly in terms of the total sleep time or the duration of wakefulness after sleep onset (Table 2). Relative to the control group, the proportion of AS was significantly higher in the smoking group ($P = 0.0302$) and the proportion of QS was significantly lower ($P = 0.0212$). There were no significant intergroup differences in the incubator temperature, respiratory rate and arterial oxygen saturation in either AS or QS.

3.2. Time-domain HRV parameters

The mean HR and time-domain HRV parameters in the control and smoking groups in AS and QS are shown in Table 3. The sleep state had a significant influence on HR (which was higher during AS than during QS) and SDNN (which was lower during AS than during QS). There was no significant effect of prenatal exposure to smoking on HR or SDNN. In contrast, r-MSSD and pNN25 were significantly lower in the smoking group than in the control group during both AS ($P = 0.0039$ and 0.0126 ; respectively) and QS ($P = 0.0023$ and 0.0221 ; respectively).

3.3. Frequency-domain HRV parameters

The mean values of frequency-domain HRV parameters in the control and smoking groups in AS and QS are shown in Table 4. The sleep state had a significant influence on the absolute values of VLF, LFn.u., the LF/HF ratio (which were higher during AS than during QS) and HFn.u. (which was lower during AS than during QS). Absolute values of HF were significantly lower in the smoking group than in the control group during both AS ($P = 0.0017$) and QS ($P = 0.0038$). Relative to the control group, LFn.u. was significantly higher in the smoking group (AS: $P = 0.0011$ and QS: $P = 0.0218$), whereas HFn.u. was significantly lower (AS: $P = 0.0011$ and QS: $P = 0.0218$). As a consequence, the LF/HF ratio was significantly higher in the smoking group than in the control group during both AS ($P = 0.0018$) and QS ($P = 0.0061$).

Table 3

Mean values of HR and HRV time-domain parameters in the control and smoking groups during AS and QS.

	Control group		Smoking group		Group effect	Sleep-state effect
	AS	QS	AS	QS		
HR (bpm)	147.1 ± 8.3	142 ± 9.2	150.5 ± 6.9	145.2 ± 11.2	NS	<i>P</i> = 0.001
SDNN (ms)	20.2 ± 5.5	17.1 ± 8	20.9 ± 6.4	13.5 ± 5.8	NS	<i>P</i> = 0.0032
r-MSSD (ms)	13 ± 6.1	11.7 ± 5.6	7.7 ± 3.2	6.2 ± 2.8	<i>P</i> = 0.0009	NS
pNN25 (%)	10 ± 11.1	6.1 ± 7.1	2.4 ± 3.6	1.3 ± 2.7	<i>P</i> = 0.0056	NS

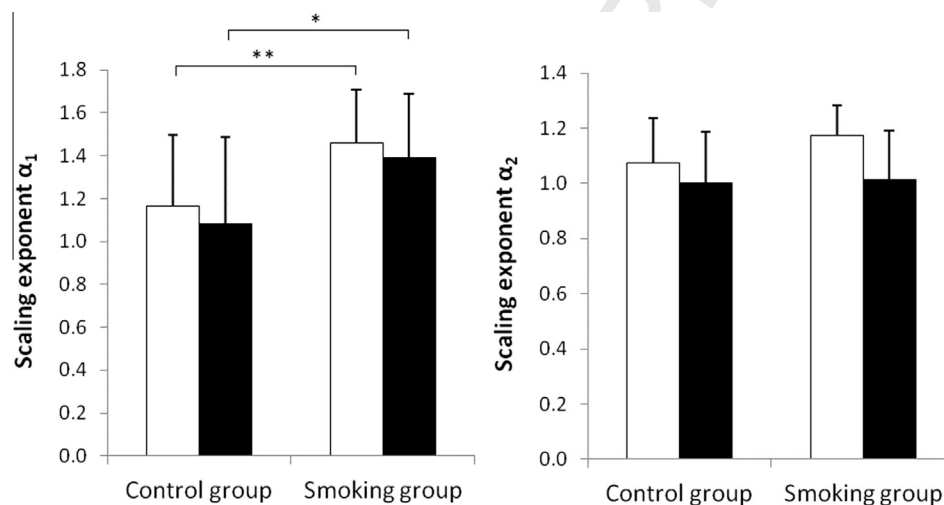
Values are quoted as the mean ± SD. NS: non-significant in a two-way analysis of variance.

Table 4

Mean values of HRV frequency-domain parameters in the control and smoking groups during AS and QS.

	Control group		Smoking group		Group effect	Sleep-state effect
	AS	QS	AS	QS		
VLF (ms ²)	180.5 ± 115.9	174.4 ± 193.3	312.7 ± 296.4	79.9 ± 101	NS	<i>P</i> = 0.0428
LF (ms ²)	178.5 ± 140.6	143.4 ± 203.5	178 ± 92.2	113.8 ± 220.6	NS	NS
HF (ms ²)	52 ± 36.9	50 ± 44.9	19.1 ± 11.6	11.7 ± 9	<i>P</i> = 0.0006	NS
LFn.u.	75.4 ± 13.2	63.8 ± 26.1	88.6 ± 7.1	81.8 ± 10.9	<i>P</i> = 0.0026	<i>P</i> = 0.002
HFn.u.	24.6 ± 13.2	36.2 ± 26.1	11.4 ± 7.1	18.2 ± 10.9	<i>P</i> = 0.0028	<i>P</i> = 0.0026
LF/HF	5.6 ± 5.4	3.5 ± 3.1	13 ± 7.2	9.2 ± 7.4	<i>P</i> = 0.0009	<i>P</i> = 0.0052

Values are quoted as the mean ± SD. NS: non-significant in a two-way analysis of variance.

**Fig. 1.** Mean ± SD values of the scaling exponents α_1 and α_2 (as calculated in a DFA) during AS (empty bars) and QS (black bars) in the control and smoking groups. **P* < 0.05; ***P* < 0.01.

3.4. DFA, ApEn and SampEn

The sleep state had a significant influence on the scaling exponent α_2 , which was higher during AS than during QS (*P* = 0.0036). The opposite pattern was observed for ApEn (*P* = 0.0022) and SampEn (*P* = 0.0009). Relative to the control group, the scaling exponent α_1 was significantly higher in the smoking group during both AS (*P* = 0.0067) and QS (*P* = 0.024). There was no significant intergroup difference in the scaling exponent α_2 (Fig. 1). ApEn and SampEn were significantly lower in the smoking group than in the control group during AS only (*P* = 0.0017 and 0.0014; respectively) (Fig. 2).

4. Discussion

Our results showed that maternal smoking during pregnancy is associated with alterations in short-term cardiac autonomic con-

trol during both AS and QS. Importantly, the preterm neonates had not been exposed to smoking after birth. These changes were characterized by low vagal activity, elevated sympathetic activity and disrupted HR control dynamics (i.e. less complexity and adaptability).

Our present data on time- and frequency-domain HRV parameters consistently demonstrated the presence of low short-term variability (fast HR fluctuations, as highlighted here by r-MSSD, pNN25 and the power spectrum in the HF band) in preterm neonates born to smoking mothers. These alterations may reflect poor cardiac vagal control. Our findings agree partly with previous data on 10-week-old term infants with smoking exposure in whom changes in autonomic control were characterized by low HF power during REM sleep (Franco et al., 2000). We also found that prenatal smoking exposure was associated with an increase in sympathetic activity (as highlighted here by the power spectrum in the LF band). These slow HR oscillations are commonly attributed to baroreceptor-mediated vasomotor control. Thus, our results are

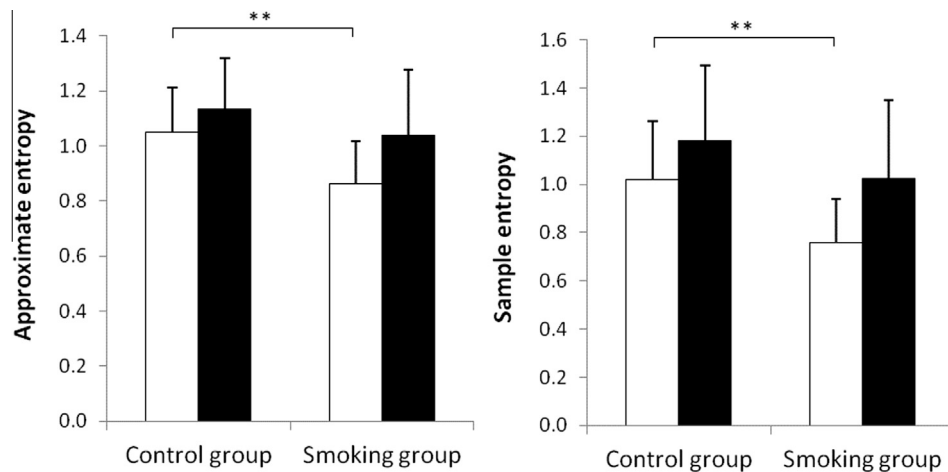


Fig. 2. Mean $\pm$ SD values of ApEn and SampEn during AS (empty bars) and QS (black bars) in the control and smoking groups. ** $P < 0.01$.

in agreement with a number of studies showing that infants exposed to smoking *in utero* have an impaired autonomic system; in turn, this can result in abnormal blood pressure control in the first few weeks of postnatal life (Browne et al., 2000; Cohen et al., 2008; Viskari-Lähdeoja et al., 2008).

Cardiac autonomic control dynamics were also assessed using DFA, which quantifies the intrinsic, fractal-like correlation properties of RR interval series. The normal HR time series is “fractal-like” and appears to (i) display the fractal property of self-similarity over various time scales and (ii) show $1/f$ fluctuation. A breakdown in this scale-invariant, fractal organization could lead to either uncorrelated randomness or highly predictable behavior, both of which may result in a less adaptable system (Goldberger, 1996; Mäkilä et al., 2002). In the present study, we found that short-term scaling exponent α_1 was higher in smoke-exposed preterm neonates than in non-exposed controls. This finding has many implications. When considering the interbeat interval time series, the mean $\pm$ SD value of α_1 was 1.12 ± 0.37 (i.e. close to $1/f$ noise) for the control subjects and 1.43 ± 0.27 (i.e. close to Brownian noise) for the exposed subjects. The presence of $1/f^2$ behavior in the HR time series in neonates in the smoking group suggests that the power in the HF band was lower than in the LF band. The fact that prenatal smoking exposure alters α_1 (but not α_2) also suggests the presence of short-term effects (related to parasympathetic activity) on heartbeat dynamics. Lastly, the difference between $1/f$ scale-invariant behavior in controls and more highly correlated behavior in smoke-exposed neonates indicates low HRV complexity in the latter, which might be physiologically harmful.

This premise is supported by our data on ApEn and SampEn, which are important measures of the disorder in the HR signal. These methods provide a generalized measure of regularity. A deterministic signal with high regularity has a higher probability of remaining close for longer vectors of the series and hence has very small ApEn and SampEn values. In contrast, a random signal has very low regularity and yields high ApEn and SampEn values (Richman and Moorman, 2000). Hence, the values of ApEn and SampEn will be abnormally low in individuals with cardiac disorders because they indicate low beat-to-beat variability (Goldberger, 1996). In the present study, we found that the mean values of ApEn and SampEn were significantly lower in the exposed group (0.95 ± 0.19 and 0.89 ± 0.25 , respectively) than in the control group (1.09 ± 0.17 and 1.10 ± 0.28 , respectively) during AS but not during QS. This may again suggest that cardiac auto-

nomic control is less complex and less adaptable in prenatally smoke-exposed neonates.

The present study had some limitations. Firstly, the study population was relatively small; other intergroup differences might have emerged from a larger sample. Secondly, the evaluation of prenatal exposure to cigarette smoke was based on maternal self-reports and medical records. This may have led to underreporting of smoking during pregnancy. However, many studies have shown that self-reports are acceptably reliable (George et al., 2006; McDonald et al., 2005). Furthermore, we had already validated the questionnaire used in the present study (Stéphan-Blanchard et al., 2011). Thirdly, it is well known that smoking during pregnancy is strongly correlated with preterm delivery (Green et al., 2005). However, we did not explore a combination of these two risk factors in the present study. Thus, we do not know whether preterm infants at the same corrected age would have differed from term infants because of fetal insult or because early adaptation to extra-uterine life may have altered their developmental trajectory. Indeed, preterm birth is known to accentuate sympathetic drive (Fyfe et al., 2014) and alter the HR's scaling properties (Bickel et al., 1998). Hence, we cannot presently account for this putative source of bias. One could imagine that preterm birth and smoking exposure may have exacerbated cardiac autonomic control dysfunction concomitantly and interdependently. Finally, we found a significant difference between control and smoke-exposed infants in sleep structure. Since cardiac autonomic control differs according to sleep states (Frasch et al., 2007), some differences in HRV results attributed to prenatal exposure to smoking may have been influenced by more time spent in AS in the smoking group. However, no statistical interaction between the groups and the sleep states was found, and differences in linear and non-linear HRV parameters between the groups, excepting for the entropy calculations, were always significant and similar in both AS and QS.

Furthermore, the mechanisms by which prenatal smoking exposure is associated with high sympathetic activity, low parasympathetic activity, and low complexity and low adaptability in the dynamics of cardiac autonomic control during the early postnatal period remain elusive. Some researchers have attributed these changes to the direct effects of nicotine, although this hypothesis runs counter to the pro-vagal nature of the nicotinic pathway (Floto and Smith, 2003). Acetylcholine is a critical factor in all stages of human brain development, and nicotine is a specific stimulant of nicotinic acetylcholine receptors (nAChRs). By provid-

ing excessive cholinergic stimulation during fetal life, nicotine from maternal smoking may impair the coordination of the many events in cell replication, differentiation and synaptic development that are required for correct assembly of the fetal nervous system (Slotkin, 1998). nAChRs have a major role in the function of the autonomic nervous system within the brainstem (Wang et al., 2003). The neural control of HR is determined by the activity of preganglionic, parasympathetic, cardiac vagal neurons located within the nucleus ambiguus in the medulla (Mendelowitz, 1999). Activation of nAChRs facilitates inhibitory neurotransmission to premotor cardiac vagal neurons (Wang et al., 2003). However, a prominent hypothesis in adults (Yun et al., 2005) suggests that the body's compensatory response to chronic nicotine exposure (which generally promotes vagal function) may paradoxically induce even greater sympathetic overactivity. There are well-known examples in which the exposure of biologic equilibria to intermittent, short-acting challenges produces paradoxical long-term responses by eliciting opposing, compensatory mechanisms. In the present context, the autonomic dysfunction related to chronic smoking exposure may operate through the biologic remodeling of nAChRs. Over-exposure of these receptors to stimulation has been shown to result in their down-regulation and desensitization to adaptive responses (Galzi and Changeux, 1995). The differential responses of the autonomic nervous system highlight the importance of taking chronobiologic factors into account when studying the pattern and maturation of this system in preterm neonates.

Indeed, the period of maturation from 32–33 weeks to after birth in preterm infants is characterized by a decrease in the sympathetic modulation of blood pressure and an increase in the parasympathetic modulation of HR (Yiallourou et al., 2013). In turn, the latter may result in a shift from an uncorrelated, random walk to strong negative autocorrelations among HR interbeat intervals (Bickel et al., 1998). Our findings indicate that even one month after birth (i.e. with no exposure to smoking), preterm infants still showed altered dynamics of cardiac autonomic control during sleep (low vagal activity, elevated sympathetic activity, low complexity and impaired adaptability in HR series). This suggests that smoking exposure *in utero* may delay the maturation of autonomic nervous system activity and control. This idea is supported by Thiriez et al.'s report (2009) on the absence of the expected relationship between advancing age and HRV parameters in infants born to smoking mothers. The present data add to a growing body of literature showing that prenatal exposure to smoking may have postnatally apparent effects lasting up to two years after birth (Cohen et al., 2010; De Rogalski Landrot et al., 2007). Prenatal smoking exposure is a risk factor for preterm birth, and both factors may contribute to the onset of cardiovascular dysfunction in adults born preterm (Cohen et al., 2008, 2010). Indeed, it is increasingly acknowledged that a harmful intrauterine environment is associated with a greater cardiovascular risk later in life. In light of this theory on the "fetal origins of adult disease", one can speculate that impaired autonomic control (and perhaps impaired maturation) in preterm infants born to smoking mothers may have long-term, masked effects that may have a role in the underlying pathogenesis of cardiovascular complications during adolescence and adulthood, such as elevated blood pressure (Hack et al., 2005) and hypertension (Eriksson et al., 2001).

5. Conclusions

In conclusion, the most striking finding in the present study was that preterm neonates born to smoking mothers display differences in cardiac autonomic activity and control when compared with control neonates. The main strength of the present study lies

in its demonstration that both linear and non-linear HRV parameters were affected by exclusively prenatal smoking exposure (i.e. there was no known postnatal exposure). Although more research is needed, it is clear that smoking during pregnancy places infants at a higher risk of developmental difficulties, which might conceivably persist throughout childhood and adulthood. Therefore, public health interventions aimed at reducing the risk of prematurity and cardiovascular dysfunction are of paramount importance. The present results support the implementation of prenatal interventions designed to reduce adverse pregnancy outcomes.

Uncited references

Acharya et al. (2004), Giddens and Kitney (1985), and Grigg-Damberger et al. (2007).

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Conflict of interest: All authors have no conflict of interest to declare.

Appendix A

Detrended fluctuation analysis

A correlation is extracted for different time scales, as follows. First, the RR interval time series is integrated:

$$y(k) = \sum_{j=1}^k (RR_j - \overline{RR}), \quad k = 1, \dots, N$$

where $\overline{RR}$ is the mean RR interval.

Next, the integrated series is divided into segments of equal length n . Within each segment, a least-squares line is fitted to the data. Let $y_n(k)$ denote these regression lines. Next, the integrated series $y(k)$ is detrended by subtracting the local trend within each segment. The root-mean-square fluctuation of this integrated, detrended time series is given by:

$$F(n) = \sqrt{\frac{1}{N} \sum_{k=1}^N (y(k) - y_n(k))^2}$$

This computation is repeated over different segment lengths to yield the index $F(n)$ as a function of segment length n . If the time series is self-similar, the fluctuation function $F(n)$ increases by a power-law: $F(n) \sim n^\alpha$. A linear relationship on a double-log graph indicates the presence of fractal scaling, and so the fluctuations can be characterized by scaling exponent α (the slope of the regression line relating $\log F(n)$ to $\log n$).

Approximate and sample entropy

The approximate entropy (ApEn) is computed as follows. First, a set of length m vectors u_j is formed:

$$u_j = (RR_j, RR_{j+1}, \dots, RR_{j+m-1}), \quad j = 1, 2, \dots, N - m + 1$$

where m is the embedding dimension and N is the number of measured RR intervals.

The distance between these vectors is defined as the maximum absolute difference between the corresponding elements, i.e.:

$$d(u_j, u_k) = \max\{|RR_{j+n} - RR_{k+n}| | n = 0, \dots, m-1\}$$

Next, for each u_j , the relative number of vectors u_k for which $d(u_j, u_k) \leq r$ is calculated. The index is denoted by $C_j^m(r)$ and can be written as:

$$C_j^m(r) = \frac{\text{number of } \{u_k | d(u_j, u_k) \leq r\}}{N - m + 1} \quad \forall k$$

Due to this normalization, the value of $C_j^m(r)$ is always smaller or equal to 1 but is always greater than $1/(N - m + 1)$, since u_j is also included in the count. Next, take the natural logarithm of each $C_j^m(r)$ and average over j to yield:

$$\Phi^m(r) = \frac{1}{N - m + 1} \sum_{j=1}^{N-m+1} \ln C_j^m(r)$$

Lastly, ApEn is obtained from:

$$\text{ApEn}(m, r, N) = \phi^m(r) - \phi^{m+1}(r)$$

Thus, the value of ApEn depends on three parameters: the length m of the vectors u_j , the tolerance value r , and the data length N . The input variables r and m must be set before ApEn is calculated. In the present study, the value of m was set to 2. The tolerance r has a strong effect on ApEn and so is typically set to be a fraction of the standard deviation of the data (SDNN). Here, r was set to 0.2 SDNN.

In Sample Entropy (SampEn), the self-comparison of u_j is eliminated by calculating $C_j^m(r)$ as:

$$C_j^m(r) = \frac{\text{number of } \{u_k | d(u_j, u_k) \leq r\}}{N - m} \quad \forall k \neq j$$

Now, the value of $C_j^m(r)$ will be between 0 and 1. Next, the values of $C_j^m(r)$ are averaged to yield:

$$C^m(r) = \frac{1}{N - m + 1} \sum_{j=1}^{N-m+1} C_j^m(r)$$

Lastly, SampEn is obtained from:

$$\text{SampEn}(m, r, N) = \ln(C^m(r)/C^{m+1}(r))$$

The values set for the embedding dimension m and for the tolerance parameter r are the same as those for the ApEn calculation.

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