The Influence of *In Utero* Exposure to Smoking on Sleep Patterns in Preterm Neonates

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Background: *In utero* exposure to smoking is known to adversely affect brain regions involved in behavioral state organization and could therefore interact with the neurophysiological development of neonates.

Study Objectives: The present study investigated the effects of prenatal smoking exposure on sleep patterns in the preterm neonate.

Design: Overnight sleep patterns were polysomnographically assessed at thermoneutrality. Sleep continuity and structure were scored for the respective frequencies, durations and percentages of active, quiet, and indeterminate sleep and wakefulness after sleep onset. The number and duration of body movements were also analyzed.

Setting: The neonatal intensive care unit at Amiens University Medical Center (France).

Participants: Healthy preterm neonates (postconceptional age: 33.9 ± 6.0 weeks) were enrolled according to whether their mothers had not smoked at all during pregnancy (control group, n = 19), smoked less during pregnancy (low-smoking group, S_{low}, n = 10), or smoked more (heavy-smoking group, S_{heavy}, n = 10) than 10 cigarettes per day throughout pregnancy.

Measurements and Results: Neonates born to heavy-smoking mothers had a significantly lower mean birth weight than controls (–21%) and displayed disrupted sleep structure and continuity: they slept less overall (with a higher proportion of active sleep and a lower proportion of quiet sleep) and had more wakefulness after sleep onset. Compared with controls, neonates from both smoking groups displayed more body movements and, as a result, more disturbed sleep.

Conclusions: High prenatal smoking exposure modifies sleep patterns in preterm neonates by disrupting sleep organization and increasing nocturnal body movements. These findings raise the question of the repercussions of these sleep disturbances (at what is a critical stage in brain development) on the child's physiological and neurobehavioral outcomes.

Keywords: Preterm neonates; Prenatal smoking exposure; Sleep structure; Body movements.

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NEONATES SPEND THE MAJOR PART OF THEIR EARLY LIFE ASLEEP. THE EARLY DEVELOPMENTAL PERIOD FEATURES MAJOR CHANGES IN SLEEP PATTERNS, reflecting the degree of brain maturation and plasticity. Sleep-wake state integrity is also of paramount importance in the neurophysiological development (e.g., body growth and vital functions) of neonates in general[1,2] and premature neonates in particular.

Several studies have reported that maternal cigarette smoking during pregnancy may have harmful effects on fetal development.[3,4] Cigarettes are known to deliver large amounts of chemical toxins to the fetus via the maternal bloodstream and increase the norepinephrine and dopamine concentrations in the central catecholaminergic systems of the developing brain.[5] This can have potentially negative effects (such as cell damage and disrupted development) on the central nervous system.[6] The brain regions involved in these smoking-related changes participate in neurobehavioral functions such as sleep-wake state organization and somatic motor control.[7,8]

In human adults, it has been previously reported that smokers have trouble falling asleep and suffer from poor sleep maintenance (and thus greater intra-sleep wakefulness).[9] Moreover, it has often been reported that both acute[10] and chronic[11] exposure to nicotine have adverse effects on sleep structure, even though the effects of nicotine depend on the dose and the administration route. Prenatal smoking exposure is also highly related to sudden infant death syndrome (SIDS). To improve the understanding of SIDS mechanisms, a number of studies have examined the effects of *in utero* exposure to smoking on autonomic functions during sleep in newborn infants. Although these studies pointed out significant effects of maternal smoking on arousal,[12-14] autonomic nervous system activity[15] or apnea,[16] they did not find any influence of prenatal smoking exposure on sleep structure. However, in two relatively old studies, Longo[17] and Fried et al[18] observed hyperactivity or hypertonicity in neonates following prenatal exposure to tobacco. Likewise, frequent physical and behavioral effects of prenatal exposure to nicotine have been observed in various animal species.[19,20] Taken as a whole, these studies show that *in utero* exposure to nicotine is associated with increased motor activity. Franck et al[21] also found that prenatal nicotine exposure profoundly altered the sleep-wake cycle of rat pups: total sleep time (TST) decreased as a result of increased awakening and decreased REM sleep.

The above observations emphasize the fact that despite the crucial role of sleep in neurophysiological development, there is a lack of data on the effects of prenatal smoking exposure on sleep patterns in human neonates. The available literature is heterogeneous and partly conflicting. The goal of the present study was to assess the influence of *in utero* exposure to smoking on sleep patterns in the developing preterm neonate. Specifically, we examined whether prenatal smoking exposure altered sleep structure and stability and motor activity.

Table 1—Clinical Parameters for the 3 Groups of Neonates

	Control group n = 19	S_{low} group n = 10	S_{heavy} group n = 10
Cigarettes / day	0	4.9 (1–9) ***	16.2 (10–25) ***, †††
Gestational age, weeks	31.7 (29.1–34.4)	31.2 (28.6–34.1)	31.2 (27.8–33.3)
Birth weight, g	1675 (1080–2180)	1488 (850–2190)	1325 (949–2074) *
Postnatal age, days	19.8 (1–48)	20.2 (0–61)	29.7 (0–61)
Postconceptional age, weeks	34.5 (30.3–36.9)	34.1 (29.1–38.7)	35.7 (32.0–37.7)
Weight at the time of the study, g	1975 (1020–2525)	1807 (963–2810)	1997 (1010–2635)
Mechanical ventilation, days	3.4 (0–6)	3.8 (0–6)	5.1 (0–8)
Arterial oxygen saturation, %	97.6 (96.4–98.3)	97.9 (96.5–99.0)	97.4 (96.3–97.9)
Respiratory rate, breaths/min	52.1 (44.2–61.4)	52.0 (39.7–63.4)	54.6 (45.5–61.1)
Heart rate, beats/min	146 (137–161)	150 (135–166)	146 (133–167)

Values are means and ranges. *$P < 0.05$; ***$P < 0.001$ versus control group. †††$P < 0.001$ versus S_{low} group.

MATERIAL AND METHODS

Infants

Forty healthy preterm neonates (means ± SD, gestational age: 31.3 ± 1.7 weeks, postconceptional age: 33.9 ± 6.0 weeks) were recruited from the neonatal intensive care unit (NICU) at Amiens University Medical Center (France) after their parents had been informed of the protocol and had given their written consent. The protocol was approved by the Picardy Regional Institutional Review Board. All neonates were free of neurological, respiratory, and cardiac disorders and had not received caffeine treatment or oxygen therapy. Some infants received mechanical ventilation via endotracheal tube, but no studied infant had been on ventilatory support for at least 7 days before the study. Recordings were performed after determining that there had been no changes in health status in previous days. None of the infants had suffered from sleep deprivation in the 48 hours prior to the sleep recording session.

In order to determine the level of *in utero* exposure to smoking, mothers were asked to evaluate the daily number of cigarettes that they had smoked during pregnancy. Even if various studies have pointed out that such self-reports are acceptably reliable,[22,23] the results remain dependent on the subjective report of the mother. The mothers' medical records were reviewed, paying special attention to any history of smoking during pregnancy for comparison with the self-report. A short questionnaire was used to acquire additional information; neonates whose mothers reported substance abuse or passive smoking were excluded from the study. On the basis of the questionnaire responses, 3 homogeneous groups of neonates (Table 1) were constituted, according to whether their mothers had not smoked during pregnancy (control group), smoked <10 cigarettes per day (low-smoking group, S_{low}), or smoked >10 cigarettes per day (heavy-smoking group, S_{heavy}) throughout pregnancy. To preserve the homogeneity among the 3 groups of neonates, the data of one infant in the S_{heavy} group were discarded from analyses based on a very low birth weight history and a significantly older postnatal age at the time of the recording (87 days).

As breastfeeding during the early postnatal period has been found to reduce some of the adverse effects of prenatal smoking exposure on sleep,[24] and as differences in the method of feeding or in feeding formulas have been found to modify sleep patterns,[25] neonates of the control, S_{low} and S_{heavy} groups were all recruited from the NICU among mothers who did not breast-feed their infants. Infants were not exposed to environmental tobacco smoke after birth.

Polysomnographic Recordings

Overnight polysomnographic recordings were made between 19:00 on day 1 and 08:00 on day 2, and began just after a feed. The infants slept in a separate room, i.e., isolated from routine nursing activities, noise, and light changes. Neonates were nursed in a closed, convectively heated incubator (Médipréma ISIS, Chambray-les-Tours, France) at thermoneutrality, in order to avoid any thermal effect on sleep patterns.[26] The room's air temperature was kept constant at 22°C (relative air humidity: 30% to 40%, air velocity ≤0.04 m/sec, natural convection). Neonates wore only a small diaper and were lying in the supine position on a mattress.

Electrophysiological recordings included right and the left centro-occipital leads electroencephalographic leads (EEG), an electrocardiogram (ECG) and respiratory movement monitoring (assessed by transthoracic impedance using the ECG patch electrodes). Eye movements were monitored by a piezoelectric quartz transducer attached to an eyelid. Body movements were recorded by accelerometers attached to a wrist and the opposite ankle. Transcutaneous arterial oxygen saturation values were recorded with a pulse oximeter (Oximax MAX-N, Tyco Healthcare group LP, Nellcor Puritan Bennett Division, CA). All recordings were continuously monitored on a polysomnogram (Alice 4, Respironics, Nantes, France).

Sleep states were scored visually in 30-sec periods, according to Curzi-Dascalova and Mirmiran.[27] In addition, infants were video-recorded throughout the night. Although video monitoring was not used to score sleep states, it helped to exclude nursing interventions and feeding episodes from the analysis. The scorers were blinded to the infant's designated study group and the study hypothesis. Sleep states were differentiated on the basis of concordance between EEG and REM signals. As necessary, breathing parameters were superimposed on these measurements to help define states. Wakefulness was defined as a state in which the infant's eyes were open (whether scanning

Table 2—Effects of *In Utero* Exposure to Smoking on Sleep Stability Parameters

	Control group	S_{low} group	S_{heavy} group	Group effect (*F;* P values)
SPT, min	677 ± 50	667 ± 37	701 ± 67	NS
TST, min	622 ± 65	587 ± 35	510 ± 58	$F_{2,36}$ = 12.4; P < 0.001
WASO, % SPT	8 ± 7	12 ± 4	27 ± 4	$F_{2,36}$ = 41.4; P < 0.001
f WASO/h	0.5 ± 0.2	0.5 ± 0.2	0.7 ± 0.2	$F_{2,36}$ = 3.6; P = 0.037
f SSC/h	2.4 ± 0.7	2.7 ± 0.7	2.8 ± 0.8	NS
AS, min	416 ± 44	367 ± 57	399 ± 90	NS
QS, min	150 ± 65	156 ± 37	79 ± 31	$F_{2,36}$ = 7.4; P = 0.002
IS, min	56 ± 52	64 ± 32	32 ± 27	NS
Longest AS episode, min	34.1 ± 12.2	32.2 ± 10.6	38.7 ± 13.8	NS
Longest QS episode, min	20.5 ± 7.5	23.4 ± 9.1	14.6 ± 4.3	$F_{2,36}$ = 3.8; P = 0.032

Values are given as means ± SD. NS: non-significant; SPT: sleep period time; TST: total sleep time; WASO: wakefulness after sleep onset; f WASO: frequency of wakefulness after sleep onset episodes; f SSC: frequency of sleep-wake state changes; AS, IS, and QS: active, indeterminate, and quiet sleep.

the environment or not) and when frequent body movements occurred. Crying or fussing was sometimes observed. Active sleep (AS) was defined as continuous EEG activity with REM, while quiet sleep (QS) was defined as discontinuous EEG activity without REM. Periods that did not meet the criteria for either AS or QS (i.e., discontinuous EEG with REM or [most commonly] continuous EEG without REM) were defined as indeterminate sleep (IS).

Data Analysis

The temporal organization of sleep states falls into 2 categories:[28] sleep structure and sleep stability, i.e., the ability to maintain sleep. The following criteria were taken into account: sleep period time (SPT), beginning at the first sleep onset and ending at the last awakening; the percentage and frequency of wakefulness after sleep onset (WASO), expressed as a proportion of the SPT; total sleep time (TST), defined as the difference between SPT and WASO durations; and the percentage and frequency of the different sleep states, expressed as a proportion of TST. The duration of the longest QS episode, which may be related to the development and maturation of the central nervous system of the neonate,[29] was also analyzed.

Special attention was paid to the number and duration of body movements. All active periods separated by <1 sec were regarded as a single movement. Conversely, motor activity lasting <1 sec but within a quiet period was not considered to be a movement. Nocturnal awakening episodes with frequent body movements were not taken into account in the analysis. The temporal structure of movements was evaluated by measuring the frequency and the total duration of body movements (TDM), expressed relative to the TST and to each sleep state.

Statistical Analysis

Statistics were computed using Statview software (version 5.0, SAS Institute Inc., Cary, NC). Normal data distribution was checked using a Kolmogorov-Smirnov test. One-way analyses of variance (ANOVA), with group as the between-subject fac-

tor were used to test differences in sleep structure and stability parameters. Body movement parameters were analyzed using 2-way ANOVAs, with sleep state as the within-subject factor and group as the between-subject factor. Subscripts beneath *F* values represent the degrees of freedom. When *F* values were significant, paired or unpaired *t*-tests were computed. Values expressed as percentages were arcsine transformed in order to stabilize the variance.[30] The significance threshold P was set to < 0.05. Data are given as means ± 1 SD.

RESULTS

Infants

Table 1 shows that the greater *in utero* exposure to smoking was associated with lower birth weight. Compared with non-exposed (control) neonates, prenatal smoking exposure decreased birth weight by 11% in the S_{low} group and 21% in the S_{heavy} group, although the difference was only significant in the latter (P = 0.020). By the time of the study, intergroup differences in body weight were no longer observed. This could be due (at least in part) to the fact that polysomnographic recordings in neonates in the S_{heavy} group were performed at a greater postnatal age, even though the difference in this latter parameter was not statistically significant.

The time spent under mechanical ventilation did not differ significantly between the control, S_{low} and S_{heavy} groups. Prenatal smoking exposure did not modify the mean blood oxygen saturation, or heart or respiratory rates during the nocturnal polysomnographic recordings. Heart and respiratory rates were higher in AS than in QS in all groups of neonates (heart rate: 152 ± 11 and 145 ± 9 beats/min, respectively, P < 0.001; respiratory rate: 58.3 ± 9.7 and 49.1 ± 7.2 breaths/min, respectively, P = 0.002).

Effects of Prenatal Smoking Exposure on Sleep Organization

The mean duration of recording was identical in the 3 groups (11.4 ± 1.1 h). All the neonates had well-defined sleep-wake cycles. *In utero* exposure to smoking modified both sleep stabil-

Table 3—Effects of *In Utero* Exposure to Smoking on Sleep Body Movement Parameters

	Control group	S_{low} group	S_{heavy} group	Group effect (*F*; P values)
Total duration of body movement, %				
AS	7.9 ± 2.3	11.7 ± 3.6	13.3 ± 5.8	$F_{2,36}$ = 7.7; P = 0.002
IS	4.9 ± 2.1	8.8 ± 3.5	10.6 ± 5.5	$F_{2,36}$ = 9.6; P < 0.001
QS	3.3 ± 1.9	6.8 ± 3.6	8.6 ± 5.7	$F_{2,36}$ = 7.8; P = 0.001
All sleep states	4.9 ± 2.1	8.8 ± 3.5	10.6 ± 5.4	$F_{2,36}$ = 9.5; P < 0.001
Body movement frequency, min^{-1}				
AS	0.81 ± 0.31	1.09 ± 0.40	1.22 ± 0.34	$F_{2,36}$ = 5.2; P = 0.010
IS	0.76 ± 0.30	1.06 ± 0.41	1.17 ± 0.34	$F_{2,36}$ = 5.5; P = 0.008
QS	0.71 ± 0.31	1.00 ± 0.39	1.10 ± 0.32	$F_{2,36}$ = 5.3; P = 0.010
All sleep states	0.77 ± 0.31	1.05 ± 0.40	1.16 ± 0.34	$F_{2,36}$ = 5.1; P = 0.011

Values are given as means ± SD. AS: active sleep; IS: indeterminate sleep; QS: quiet sleep.

ity (Table 2) and structure (Figure 1). These effects were only observed in neonates born to heavy-smoking mothers (S_{heavy} group).

The 3 groups did not differ in terms of SPT. Although *in utero* exposure to smoking did not modify the overall frequency of sleep-wake state changes, neonates in the S_{heavy} group showed an increase in the frequency of WASO episodes (P = 0.019 *vs.* controls) and in the amount of WASO relative to SPT (P < 0.001 *vs.* controls). As a result, TST was lower in the S_{heavy} group than in the control group (–18%, P < 0.001).

The amount of QS (in min) was lower in the S_{heavy} group (P = 0.003 *vs.* controls; P < 0.001 *vs.* S_{low}). Moreover, neonates in the S_{heavy} group showed shorter duration of the longest QS episode than controls (P = 0.030) and neonates in the S_{low} group (P = 0.013). This was not found for the longest AS episode. As a result, the relative duration of AS was higher in the S_{heavy} group than in either the control group (+15%, P = 0.012) or the S_{low} group (+24%, P = 0.004) and was reflected by a decrease in the percentage of QS (–34% *vs.* controls, P = 0.024; –41% *vs.* S_{low}, P = 0.001). There were no differences among the 3 groups in terms of the percentage of IS.

Effects of Prenatal Smoking Exposure on Motor Activity

Variables describing body movements for the 3 groups of neonates in the different sleep stages are shown in Table 3. In all groups of neonates, the TDM (expressed as a percentage of time in each sleep stage) was higher in AS than in IS, and higher in IS than in QS (10.3% ± 4.4%, 7.3% ± 4.3% and 5.6% ± 4.2%, respectively; $F_{2,36}$ = 917.3; P < 0.001; each comparison was statistically significant). A similar result was found for the frequency of body movement (0.99 ± 0.38, 0.94 ± 0.38 and 0.89 ± 0.37/min, respectively; $F_{2,36}$ = 77.7; P < 0.001).

In utero exposure to smoking increased significantly the TDM: the control group spent less time moving than the S_{low} group (P < 0.001) or the S_{heavy} group (P < 0.001). Similar results were found for the frequency of occurrence of body movements: the control group's sleep was less disturbed by body movements than that of the S_{low} group (P = 0.043) or the S_{heavy} group (P = 0.004). No difference between neonates from low-smoking mothers and neonates from heavy-smoking mothers was observed in this respect.
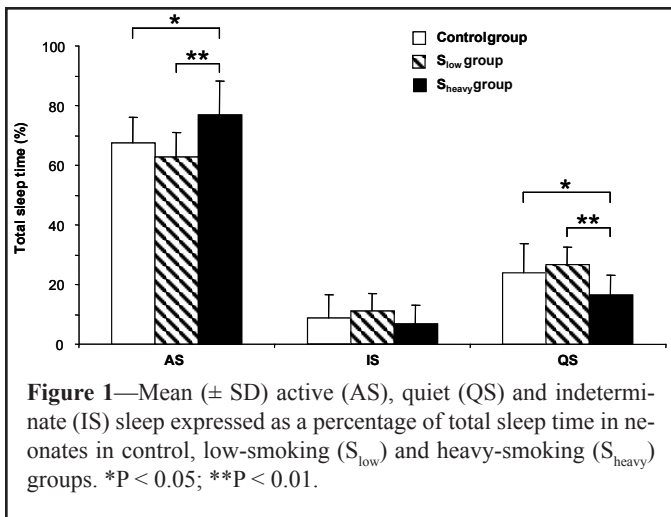
DISCUSSION

The present study deals with the influence of *in utero* exposure to smoking on sleep patterns in preterm neonates. We chose to investigate sleep structure and stability and body activity during sleep because these parameters reflect brain maturation and provide information on the neuronal control of physiological and behavioral functions in neonates. In the present study, the control group of neonates yielded similar sleep structure and body activity data to those previously recorded in our laboratory[31] or by other investigators[32] in neonates of similar postconceptional age.

To the best of our knowledge, this study is the first to show that high levels of prenatal smoking exposure strongly modify sleep patterns in preterm neonates. The present data demonstrate altered sleep structure (decreased TST and QS proportion, increased AS proportion) and higher sleep fragmentation (greater frequency of WASO and sustained body movements) in preterm neonates born to heavy-smoking mothers. The results need to be interpreted with caution, as neonates in the S_{heavy} group were studied at a greater postnatal age (nearly 10 days) than the others, even though this difference was not statistically significant. As a consequence, some differences in sleep and body activity parameters attributed to prenatal exposure to heavy smoking may have been influenced by either brain maturation or more time spent in the NICU in the S_{heavy} group.

However, the difference in sleep patterns between the control and S_{heavy} groups can not easily be attributed to more stressful experiences in the first days of life (nursing interventions, environmental factors) since medical history (oxygen therapy, mechanical ventilation) and treatments (caffeine) did not differ among the 3 groups of neonates. Infants in the S_{heavy} group spent more time in the NICU than controls, but the effects of heavy prenatal smoking exposure on sleep patterns are the opposite of those observed with care activity stimulations. Such stimulations inevitably induce more short periods of sleep deprivation, leading to compensatory increases in sleep time and/or intensity during recovery. Moreover, neonates respond to selective or total sleep deprivation by compensatory increases in QS time only.[33,34]

Secondly, the difference in sleep patterns between the control and S_{heavy} groups would reflect the effect of advancing age

Figure 1—Mean (± SD) active (AS), quiet (QS) and indeterminate (IS) sleep expressed as a percentage of total sleep time in neonates in control, low-smoking (S_{low}) and heavy-smoking (S_{heavy}) groups. *P < 0.05; **P < 0.01.

rather than the effect of prenatal smoking exposure. It is commonly established[35] that the early development of sleep is characterized by a rapid increase in the proportion of QS while AS remains fairly constant. The development continues during the first year, with an increasing proportion of QS, mainly at the expense of AS. Moreover, nocturnal sleep continuity increases with advancing age, as a consequence of a concomitant increase in sleep state duration and decreased the frequency of nocturnal wakefulness. Once again, the present results on sleep patterns in neonates in the S_{heavy} group go in an opposite way than those of advancing age. Hence, the difference in sleep patterns between the control and S_{heavy} groups can not be attributed to their difference in postnatal age, and even suggests an underestimation of the effect of heavy prenatal smoking exposure on sleep patterns.

Our findings are consistent with literature reports of the teratogenic effect of nicotine on the developing central nervous system. Nicotine produces adverse signaling in brain regions such as the reticular activating system, which is involved in the control of activity, arousal, waking and REM sleep. Prenatal smoking exposure can induce up-regulation of nicotinic receptors in the brain, resulting in greater release of dopamine, norepinephrine, serotonin, and acetylcholine.[5] Nicotine's excitatory effects on the reticular activating system could thus disrupt normal sleep processes and result in greater activity, arousal, and REM sleep.

The neurotoxic effects of *in utero* exposure to smoking with respect to sleep-wake properties have not been thoroughly studied, and the available data are partly conflicting. Our results for nocturnal body movements are consistent with previous studies, which reported greater motor activity in both human neonates[17,18] and animal species[19,20] exposed *in utero* to nicotine. In contrast, our findings on sleep structure disagree with most other studies in human neonates, which have reported a lack of sleep structure differences between controls and infants exposed prenatally to maternal smoking.[12-16] However, as mentioned above, none of these studies were specifically designed to assess this relationship, and the disparity of the methodologies does not enable confirmation of the absence of influence of prenatal smoking exposure. Some studies assessed the influence of prenatal smoking exposure on infant arousability, so that the sleep structure would have been disturbed by the

arousal stimuli.[12-14] In other studies, the sleep analysis pooled data from neonates of 0.5 weeks postnatal age with those from 11-week-old children,[15,16] and it was not always possible even to distinguish whether the effects of smoking occurred prenatally or postnatally.[16] Nevertheless, an alteration in vigilance state organization has been found in newborn rats exposed prenatally to nicotine (2 or 4 mg/kg/day).[21] Although these authors reported a significant reduction in REM sleep following prenatal nicotine exposure (in contrast to our present findings), they also found less total sleep and more waking.

Altered sleep organization may have physiological significance. Initial evidence is provided by the disruption of sleep stability, with a decrease in TST and increases in the frequency of WASO and nocturnal body movements. Maternal smoking during pregnancy increases the risk of SIDS, and the relationship between SIDS and prenatal smoking exposure appears to be dose-dependent.[36] Strikingly, prenatal smoking exposure produced sleep pattern abnormalities that contrast with those found in infants at risk of SIDS since one suggested etiology for SIDS involves alterations in both stimulus-induced and spontaneous arousal from sleep in infants exposed prenatally to tobacco smoke.[37] This comparison requires caution since preterm infants studied in NICU may not have the same sleep stability (stressful environment), as infants born at term and living at home at the time of the study.[37] Indeed, routine care procedures could not be completely avoided; and handling, noise, and lightning in the NICU are well-known factors that increase arousals or sleep state transitions.[38] The results of the present study suggest, however, that if prenatal smoking exposure does increase the risk of SIDS, one possible pathophysiological mechanism may involve an absence of organized, central control (when required), rather than a decrease in the ability to arouse from sleep.

Sleep maintenance and stability are crucial for growth and brain development, especially in preterm neonates. The period from 34 to 40 weeks of postmenstrual age is a developmental window during which neurobehavioral systems (e.g., sleep-wake states and body movements) undergo a transition into an organized neonatal pattern, with well-defined sleep cycles.[39] Thus, the effects observed here testify to the difficulty of the exposed infants of organizing and maintaining their sleep, probably as a consequence of a comparative immaturity. The fact that the duration of the longest QS episode was significantly shorter in neonates of the S_{heavy} group argues in favor of this hypothesis because QS is a highly controlled sleep stage, which could be representative of the development of cortical control.[29] Moreover, an increase in nocturnal body movements and a decrease in TST may have deleterious effects (caused by long-term sleep deprivation) and could place the exposed infants at a higher risk of a negative developmental outcome.

The second aspect relates to the disruption of sleep structure in significantly smoke-exposed preterm neonates, who show greater percentages of WASO and AS and a corresponding reduction in QS, compared with controls. A markedly altered postnatal sleep pattern observed in neonates whose mothers smoked during pregnancy also suggests suboptimal central nervous system organization and could thus explain disrupted regulation of autonomic and vegetative functions, which are known to interact during sleep. It is commonly assumed that both cardiac and respiratory functions vary according to the

sleep state. In AS, heart rate variability and breathing are always irregular, and central apnea occurs very frequently.[40] Moreover, previous studies from our group have shown that chemical control of breathing is sleep state dependent.[41] The high amount of AS observed in this study could increase the risk of cardio-respiratory disorders during sleep, since exposed infants might present less stable breathing and a greater incidence of cardio-respiratory challenges (e.g., apnea). It has been suggested that modifications in sleep-wake ontogeny may trigger SIDS events by producing misalignments between vegetative functions that normally become integrated during the postnatal period, thus leading to the failure of homeostatic processes.[42]

Our findings indicate that even after 29.7 days without post-natal exposure to smoking or nicotine, and despite the fact that neonates in the S_{heavy} group were nearly 10 days older, infants exposed *in utero* to high levels of smoking still showed an altered organization of the various behavioral states. These data add to a growing body of literature that shows that prenatal exposure to neurodevelopmental teratogens may have long-lasting, postnatally apparent effects. Moreover, long-term cognitive disorders during childhood and adolescence have been demonstrated. For example, prenatal smoking exposure can lead to deficits in sustained attention and impulsivity in adolescence[43] and a higher risk of attention deficit hyperactivity disorder in childhood[44]—effects that could be mediated (at least in part) by sleep changes. Indeed, studies have demonstrated that sleep disturbances in preterm infants are related to the same later neurodevelopmental outcomes found after prenatal smoking exposure.[45,46] Thus, it is important to examine the longevity of the observed differences in brain activity. The developing brain is known for its plasticity and ability to reorganize in response to stimulation provided by the post-natal environment. Consequently, it is possible that intergroup differences may become smaller over the course of development. Longitudinal studies are necessary to assess the persistence of behavioral state effects caused by prenatal smoking exposure.

In conclusion, *in utero* exposure to heavy smoking was found to modify sleep patterns in preterm neonates. Our data evidenced a concomitant disruption in sleep organization and an increase in nocturnal body movements. Abnormal sleep processes may alter compensatory responses to autonomic cardiovascular/respiratory challenge and increase the likelihood of life-threatening events later in life. Although more research is needed, it is clear that smoking during pregnancy places infants at a higher risk of developmental difficulties which could persist throughout early and middle childhood. Therefore, examining the neurodevelopmental trajectories of neonates exposed to maternal smoking (and of those who were not) could lead to greater understanding of potential deficits in the exposed group, better prediction of outcomes and, potentially, more effective compensatory clinical interventions.

ABBREVIATIONS

AS: Active sleep
ECG: Electrocardiogram
EEG: Electroencephalogram
f: Frequency (/min)
IS: Indeterminate sleep
NICU: Neonatal intensive care unit

NS: Non-significant
QS: Quiet sleep
REM: Rapid eye movement
S_{low}: Neonates born to mothers in the low-smoking group
S_{heavy}: Neonates born to mothers in the heavy-smoking group
SIDS: Sudden infant death syndrome
SPT: Sleep period time (min)
TDM: Total duration of body movements (%)
TST: Total sleep time (min)
WASO: Wakefulness after sleep onset

DISCLOSURE STATEMENT

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