Effects of maternal tobacco smoking, sleeping position, and sleep state on arousal in healthy term infants

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ORIGINAL ARTICLE

Objectives: To investigate whether a history of maternal tobacco smoking affected the maturation of arousal responses and whether sleeping position and infant age alters these relations.

Design: Healthy term infants (13 born to mothers who did not smoke and 11 to mothers who smoked during pregnancy) were studied using daytime polysomnography on three occasions: (a) two to three weeks after birth, (b) two to three months after birth, and (c) five to six months after birth. Multiple measurements of arousal threshold in response to air jet stimulation were made in both active sleep (AS) and quiet sleep (QS) when infants slept both prone and supine.

Results: Maternal smoking significantly elevated arousal threshold in QS when infants slept supine at 2–3 months of age (p<0.05). Infants of smoking mothers also had fewer spontaneous arousals from QS at 2–3 months in both prone (p<0.05) and supine (p<0.001) sleeping positions. In infants of non-smoking mothers, arousal thresholds were elevated in the prone position in AS at 2–3 months (p<0.01) and QS at 2–3 weeks (p<0.05) and 2–3 months (p<0.001).

Conclusions: Maternal tobacco smoking significantly impairs both stimulus induced and spontaneous arousal from QS when infants sleep in the supine position, at the age when the incidence of sudden infant death syndrome is highest.
Maternal smoking and sudden infant death syndrome

Table 1 Basic details of study groups

<table>
<thead>
<tr>
<th></th>
<th>Non-smoking</th>
<th>Smoking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of infants</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>Gestation (weeks)</td>
<td>40 (0.3) [38–41]</td>
<td>40 (0.4) [38–42]</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3498 (102) [2765–4015]</td>
<td>3595 (129) [3015–4190]</td>
</tr>
<tr>
<td>Apгар at 1 min</td>
<td>9 (5–10)</td>
<td>9 (5–10)</td>
</tr>
<tr>
<td>Apгар at 5 min</td>
<td>9 (7–10)</td>
<td>9 (6–10)</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>28 (2) [18–39]</td>
<td>32 (2) [26–42]</td>
</tr>
<tr>
<td>Age at 2–3 week study (days)</td>
<td>14 (1) [8–19]</td>
<td>15 (1) [10–21]</td>
</tr>
<tr>
<td>Weight at 2–3 week study (g)</td>
<td>3709 (129) [2750–4390]</td>
<td>3868 (140) [3155–4536]</td>
</tr>
<tr>
<td>Age at 2–3 month study (days)</td>
<td>74 (2) [63–83]</td>
<td>75 (2) [68–84]</td>
</tr>
<tr>
<td>Weight at 2–3 month study (g)</td>
<td>5453 (213) [4423–6480]</td>
<td>5805 (227) [4536–7000]</td>
</tr>
<tr>
<td>Age at 5–6 month study (days)</td>
<td>189 (5) [175–213]</td>
<td>176 (5) [155–200]</td>
</tr>
<tr>
<td>Weight at 5–6 month study (g)</td>
<td>7865 (359) [6200–9335]</td>
<td>7935 (223) [6804–8800]</td>
</tr>
</tbody>
</table>

Values are mean (SEM) (range).

excreted as cotinine (and 3-hydroxycotinine) in the urine, we analysed these metabolites as an indicator of maternal smoking.\textsuperscript{13} Urinary cotinine was analysed using a chemiluminescent enzyme immunoassay kit (Diagnostic Products Corporation, Los Angeles, California, USA) for the Immulite automated analyser. Results were correlated with a high performance liquid chromatography method\textsuperscript{13} by Passing Bablok regression ($r = 0.925$).

Recording methods

All infants were studied with daytime polysomnography recordings between 1000 and 1600 hours. Electrodes for recording physiological variables were attached to the baby while it fed, and, when drowsy, the infant was placed in a bassinet or cot at (5–6 months) under dim lighting and constant room temperature (22–23°C). The study did not begin until the infant was in a stable sleep state. Infants generally had both a morning and afternoon sleep interrupted by a midday feed, when sleep position was changed. Each infant slept in both prone and supine positions at each study. The initial sleep position was randomised for the morning of the first study, and the opposite position used for the morning of the second study. Positions were reversed again for the final study—that is, if the baby slept prone first at the first study, then it slept supine first at the second study, and the reverse used again at the third study. None of the infants studied slept prone routinely.

Recordings were made on a Grass Polygraph model 78A 16 channel recorder (Grass Instrument Company, Quincy, Massachusetts, USA) of electroencephalogram, electro-oculogram, submental electromyogram, electrocardiogram, instantaneous heart rate, thoracic and abdominal breathing movements (Resp-ez Piezo-electric sensor (EPM Systems, Middlothan, Virginia, USA), and blood oxygen saturation (SpO$_2$; Biox 3700e Pulse Oximeter; Ohmeda, Louisville, Colorado, USA). Sleep state was assessed as QS, AS, or indeterminant sleep using electroencephalographic, behavioural, heart rate, and breathing pattern criteria.\textsuperscript{16}

Stimulus and arousal criteria

A pulsatile air jet (frequency 3 Hz for five seconds) delivered to the nostrils of the infant was used to induce arousal in both AS and QS, and arousal thresholds were calculated from the delivery pressure as described previously.\textsuperscript{13} Briefly, the stimulus was presented alternately to the left and right nostrils; if the infant failed to be aroused, the air jet pressure was increased and the stimulus again presented to that nostril. Whenever an arousal response occurred, the pressure was then decreased. The changes in pressure between each presentation ranged from 25 to 200 cm H$_2$O, but were usually 100 cm H$_2$O. The maximum pressure setting was 950 cm H$_2$O. Arousal threshold was calculated as the mean pressure between each arousal and non-arousal response. In the prone position in QS, stimuli at the maximum pressure sometimes failed to elicit an arousal. In these cases, when two successive stimuli presented to the same nostril failed to elicit a response, an arousal threshold of 950 cm H$_2$O was recorded. In determining whether a presentation elicited an arousal response, the four criteria previously described\textsuperscript{13} were used: a change in ventilation pattern of more than two breaths, an observed behavioural response, a heart rate acceleration more than 10% above baseline, and an increase in submental electromyographic activity. All of these changes had to occur within seven seconds of the stimulus onset, which allowed heart rate to reach a maximum. The 10 seconds of recording immediately preceding the stimulus presentation provided baseline data used to assess the change in each variable following the stimulus. A change in at least three of the four criteria was required for it to be designated an arousal response.

To assess the possible effect of changes in physiological variables on arousal, abdominal skin temperature, rectal temperature, heart rate, and oxygen saturation were recorded before each stimulus presentation. Mean respiratory rates were calculated over three minutes at the beginning of the first AS and first QS period for each sleeping position during a time when there were no stimulus presentations.

Data analysis

Data were first tested using the Kolmogorov-Smirnov normality test and the Levene Median test for equal variance. Patient variables and arousal thresholds for left and right nostrils were compared using a one way analysis of variance (SigmaStat version 2; SPSS Inc). No difference between arousal thresholds was found between nostrils in either sleeping position or between smoking and non-smoking groups. Accordingly, the data for each nostril were pooled for all subsequent analyses of threshold. Mean arousal thresholds for AS and QS were calculated for each infant and compared within individual studies and between studies using a two way analysis of variance for repeated measures. Missing values (two for the non-smoking group and one for the smoking group in study 1; two for the non-smoking group in study 2; and three for both the non-smoking and smoking groups in study 3) were calculated following the protocol outlined by Snedecor and Cochran.\textsuperscript{17} Comparisons of arousability between the smoking and non-smoking groups at matched conceptual ages were made with two way analysis of variance. The probability of spontaneous arousal was calculated as the number of spontaneous arousals occurring during each stimulus calibration (sham stimulus), expressed as a percentage of the total number of calibrations. Comparisons of spontaneous arousal probabilities between sleep states, infant groups, and sleep positions and compared with test arousal.
proportions were made using χ² analysis. Sleep durations recorded by parents in the sleep diaries were compared with those obtained during the sleep studies with Student's paired t test. Sleep cycle length was determined as the time between the onset of successive epochs of AS; only complete epochs of AS and QS were included in this analysis. Mean sleep epoch length and sleep cycle were compared between groups and studies using two way analysis of variance. All values are expressed as mean (SEM), and p < 0.05 was considered significant.

RESULTS

Maternal smoking
In the non-smoking group (n = 13), all mothers reported that they did not smoke and that no one else in the household smoked during or after the pregnancy. This was confirmed by infant urinary cotinine levels measured at the 2–3 months study which were all < 10 ng/ml. Eight of these infants were breastfed. In the maternal smoking group (n = 11), mothers reported routinely smoking 3–20 cigarettes/day (mean (SEM) 15 (6)/day). Seven of the infants were breastfed. Two mothers reported that they stopped smoking when pregnancy was confirmed at six to eight weeks gestation, but resumed smoking 10–15 cigarettes/day as soon as the infants were born. One of these infants, who was bottle fed, had < 10 ng/ml cotinine in the urine. In the remaining infants (n = 10), urinary cotinine levels ranged between 10.6 ng/ml (8.2 µg/mmol) and 286 ng/ml (409 µg/mmol), mean 82.5 ng/ml (110 µg/mmol). There was no correlation between maternal smoking and infant urinary cotinine levels; two mothers who reported smoking the least number of cigarettes had infants with the highest cotinine levels (both infants were breast fed).

There were no significant differences between the smoking and non-smoking groups with respect to any of the measures presented in table 1.

Arousal threshold
Table 2 presents the total number of stimuli and responses elicited to both test and sham stimuli in each sleep state and sleep position for both smoking and non-smoking groups.

Effects of maternal smoking
When arousal thresholds were compared between groups, maternal smoking significantly elevated arousal threshold in QS when infants slept supine at 2–3 months (p < 0.05). In the prone position in AS, the non-smoking group had significantly higher arousal thresholds at 2–3 months (p < 0.01) (table 2). Figure 1 gives mean arousal thresholds for both smoking and non-smoking groups of infants sleeping both prone and supine.

Effects of sleep state
In the smoking group, arousal thresholds were significantly elevated in QS compared with AS at 2–3 months (p < 0.001) and 5–6 months (p < 0.001) in both sleeping positions. In the non-smoking group, arousal thresholds were also elevated in QS compared with AS at 2–3 months when infants slept prone and at 5–6 months in both sleeping positions.

Effects of sleeping position
Sleeping position had a significant effect on arousability in the non-smoking group of infants. Arousal thresholds were significantly elevated in the prone position in AS at 2–3 months (p < 0.01) and QS at 2–3 weeks (p < 0.05) and 2–3 months (p < 0.001) in both sleeping positions. Conversely, sleeping position had no effect on arousal responses in either AS or QS at any of the three ages studied in the smoking group.

Effects of postnatal age
Arousalability was also affected by postnatal age, but effects differed according to sleep state. In AS, arousal thresholds were unchanged with age in either the smoking or non-smoking group. In contrast, arousal thresholds increased with postnatal age in QS in both sleep positions in both groups. In both groups, when infants slept supine, arousal thresholds were significantly higher at 5–6 months than at 2–3 weeks (p < 0.01). In the prone position, arousal thresholds were significantly higher at 5–6 months than at 2–3 weeks in the smoking group (p < 0.01) and the non-smoking group (p < 0.05).

Table 2 Numbers of infants studied and responses elicited to both test and sham stimuli at each study for each sleep state and sleep position for non-smoking and smoking groups

<table>
<thead>
<tr>
<th>Age at study</th>
<th>Smoking group</th>
<th>Non-smoking group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AS</td>
<td>QS</td>
</tr>
<tr>
<td></td>
<td>Prone</td>
<td>Supine</td>
</tr>
<tr>
<td>2–3 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of infants</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Total stimulus presentations</td>
<td>389</td>
<td>315</td>
</tr>
<tr>
<td>Arousal threshold (cm H₂O)</td>
<td>208 (31)</td>
<td>157 (21)</td>
</tr>
<tr>
<td>% spontaneous arousal</td>
<td>4</td>
<td>7†††</td>
</tr>
<tr>
<td>2–3 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of infants</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Total stimulus presentations</td>
<td>317</td>
<td>249</td>
</tr>
<tr>
<td>Arousal threshold (cm H₂O)</td>
<td>154</td>
<td>106 (15)†††</td>
</tr>
<tr>
<td>% spontaneous arousal</td>
<td>4†††</td>
<td>51††</td>
</tr>
<tr>
<td>5–6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of infants</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Total stimulus presentations</td>
<td>77</td>
<td>52</td>
</tr>
<tr>
<td>Arousal threshold (cm H₂O)</td>
<td>265 (99)†</td>
<td>122 (29)†††</td>
</tr>
<tr>
<td>% spontaneous arousal</td>
<td>3</td>
<td>8†††</td>
</tr>
</tbody>
</table>

* p<0.05; ** p<0.01; *** p<0.001 prone v supine.
† p<0.05; †† p<0.01; ††† p<0.001 AS v QS.
‡ p<0.05; ‡‡ p<0.01; ‡‡‡ p<0.001 non-smoking v smoking groups.
AS, Active sleep; QS, quiet sleep.
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Effects of sleeping position

The probability of spontaneous arousal from sleep tended to be higher in the supine position than when infants slept prone; however, this difference only reached significance in AS at 2–3 weeks in the non-smoking group (p<0.05).

Physiological variables

There was no difference between groups in baseline HR or O₂ saturation in either sleep state or position at any of the ages studied. Maternal smoking increased respiratory rate by 11–12 breaths/min at 2–3 weeks in QS when infants slept both prone (p<0.01) and supine (p<0.05). Abdominal and rectal temperatures were lower by 0.3 and 0.4°C respectively in AS and QS in the smoking group compared with the non-smoking group in the supine position at 2–3 weeks (p<0.05). At 2–3 months, abdominal temperature was also lower by 0.4°C in QS in the smoking group (p<0.05).

Sleep characteristics

There was no difference between the total amount of time infants slept in the laboratory and that recorded by parental sleep diaries at home over the 48 hours before the studies, averaged for the same time of day, in either group of infants. In addition, there was no difference between groups in the length of either AS or QS, or total time asleep, at any of the three ages studied.

DISCUSSION

This study shows that maternal tobacco smoking significantly impairs both stimulus induced and spontaneous arousal from QS when infants are sleeping in the supine position. Of significance to SIDS, this effect is strongest at the age when SIDS incidence is highest. Any impairment in arousability from sleep that compromised ventilation or cardiovascular responses to hypoxia or asphyxia could contribute to the final pathway to SIDS.

A consistent feature of SIDS epidemiology is the age distribution at which most SIDS cases occur. The incidence is low in infants less than 1 month of age (the time of our first study), peaks at 2–4 months (the time of our second study), and then declines after 5 months (the time of our third study).11 Although previous studies of the effects of maternal smoking on arousability have identified higher arousal thresholds to both auditory10 and hypoxic11 stimuli, these studies were not, however, carried out longitudinally in the same infants. In addition, the first study10 combined data from both term and preterm infants; however, we have shown that arousability is affected by prematurity.11 Results of the later study11 may have been confounded by illicit drug use during pregnancy in four out of 13 mothers in the smoking group.

Our study provides new evidence on the effects of maternal smoking and sleeping position. The finding that sleeping position affected arousability from sleep only in the non-smoking group may be of particular importance. Previous studies of arousability from sleep in infants exposed to maternal smoking have only been carried out in infants sleeping in the supine position; however, this difference only reached significance in AS at 2–3 weeks in the non-smoking group (p<0.05).

Spontaneous arousal

The probability of spontaneous arousal from sleep was significantly lower than the probability of arousal in response to air jet stimulation in both sleep states and sleep positions in all studies in both groups of infants (p<0.001).

Effects of maternal smoking

Maternal smoking had a significant effect on the probability of spontaneous arousal from sleep at 2–3 months in both prone (p<0.05) and supine (p<0.001) sleeping positions, compared with the non-smoking group (table 2).

Effects of sleep state

Although the probability of spontaneous arousal was higher in AS compared with QS in both positions in both groups of infants, this difference only achieved significance in the smoking group at 2–3 months in the prone position (p<0.001) and at all three ages studied in the supine position. In the non-smoking group, the probability of spontaneous arousal was significantly higher in AS compared with QS at 5–6 months in the prone position (p<0.01) and 2–3 weeks in the supine position (p<0.01).

Figure 1 Mean arousal thresholds for smoking and non-smoking groups sleeping both prone and supine in quiet (QS) and active (AS) sleep. (A) Prone AS; (B) supine AS; (C) prone QS; (D) supine QS.
Our findings are consistent with previous studies by our group as they confirm that sleep state has a considerable effect on auditory arousal thresholds in infants being elevated in QS compared with AS. Previous studies have not examined the effects of maternal smoking or sleeping position on responses in the two sleep states and our study provides new evidence that sleep state exerts this influence regardless of the effects of exposure to maternal smoking or sleeping position. Although both maternal smoking and the prone position have been reported to impair arousability, previous studies were only carried out during either AS, a state where arousal is readily initiated in infants, or QS, and individual responses in the two states were not compared. We have previously reported fetal brain may also be due to the direct toxic effects of nicotine and that the effects of maternal smoking on later neurological outcome may not all be secondary to hypoxia-ischaemia. Nicotine interacts directly with endogenous nicotinic acetylcholine receptors in the brain and can profoundly affect central nervous system function and development; it has been shown that [1H]nicotine binding sites are heavily concentrated in the tegmental nuclei of the brain, which are involved with cardiopulmonary integration, somatic motor control, and arousal. Infants in the two groups of our study did not, however, differ in birth weight or Apgar scores, indicating that infants had not suffered severe growth restriction or severe hypoxia in utero.

Our study could not differentiate between the effects of maternal smoking during pregnancy and those occurring postnatally. However, none of the infants in the non-smoking group were exposed to cigarette smoke either before or after birth and none of the fathers in this group smoked. In infants, urinary cotinine levels are affected by method of feeding. Other workers have reported that the prone sleeping position increased the time spent in QS. However, our present study showed no difference in the length of epochs of either AS or QS, or total time asleep between the infants in the smoking and non-smoking groups at any of the three ages studied. Hence our findings of an elevation in QS threshold in the smoking group when sleeping supine is probably not due to alterations in sleep characteristics. In conclusion, we have shown that maternal tobacco smoking impairs both spontaneous and stimulus induced arousal from QS of infants sleeping in the recommended supine sleeping position. Arousal from sleep was impaired by prone sleeping regardless of maternal smoking or sleep state. We suggest that impaired arousal responses in infants of smoking mothers may blunt the ability of an infant to respond to a life threatening stimulus.

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