Arousal Responses to Somatosensory and Mild Hypoxic Stimuli are Depressed During Quiet Sleep in Healthy Term Infants

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Study Objectives: To compare arousal responses to somatosensory and hypoxic stimuli in sleeping human infants and to determine whether sleep state and postnatal age exerted similar changes in these arousal responses.

Design: We delivered somatosensory (nasal air-jet) stimulation and mild hypoxia (15% oxygen) to 10 healthy term infants aged 2 to 4 weeks, 2 to 3 months, and 5 to 6 months during identified sleep states. Hypoxic challenges were terminated at arousal, when the oxygen saturation fell below 85%, or at 5 minutes (failure to arouse).

Results: Infants failed to arouse to a greater percentage of hypoxia tests during quiet sleep (QS) than during active sleep (AS) at 2 to 3 months and 5 to 6 months of age (P<0.01). Infants failed to arouse to a greater percentage of hypoxic challenges during QS at 2 to 3 months and 5 to 6 months than at 2 to 4 weeks of age. Arousal latency to hypoxia was significantly longer in QS than in AS at each study age; however, arousal latency was not affected by postnatal age. Arousal thresholds to somatosensory stimulation were significantly greater in QS than in AS, except at 2 to 4 weeks of age. In AS, arousability to the air-jet was greater at 2 to 3 months compared to 2 to 4 weeks of age (P<0.05); in QS it was lower at 5 to 6 months compared to 2 to 4 weeks of age (P<0.05). Arousal latency to hypoxia and arousal thresholds to air-jet stimulation were not correlated within infants.

Conclusion: We conclude that arousal responses of infants to somatosensory and respiratory stimuli are similarly affected by sleep state and postnatal age. Infants are less arousable to both stimulus modalities in QS than in AS, and less arousable at 5 to 6 months of age than at 2 to 4 weeks in QS.

Key Words: Sudden infant death syndrome, hypoxia, arousability, sleep

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INTRODUCTION

AROUSAL FROM SLEEP SERVES AS A VITAL PROTECTIVE MECHANISM AGAINST CARDIORESPIRATORY FAILURE. In infants, failure to arouse (FTA) from sleep has been postulated to be involved in the sequence of events leading to sudden infant death syndrome (SIDS). Consequently, arousal responses to a range of stimuli have been investigated in human infants and in a number of animal models, including neonatal sheep, adult dogs, and neonatal piglets.

In animal models, both mechanical and cardiorespiratory stimuli have been used to induce arousal from sleep, including nasal and tracheal occlusion, laryngeal stimulation, hypotension, hypertension, hypoxia, hypercapnia, and hypoxia. These studies have consistently demonstrated that arousability is depressed in active sleep compared to quiet sleep. In contrast to findings in animals, arousal responses of human infants to mechanical and somatosensory stimuli are depressed in QS compared to AS. In infants, somatosensory stimulation has been widely used to avoid ethical and technical concerns associated with the administration of cardiorespiratory stimuli. Of the studies that have investigated the arousing effects of cardiorespiratory stimuli in infants, most have focused on responses during QS alone with few studies testing arousability in both sleep states.

Because of uncertainties about the effects of different forms of arousing stimuli in different models, our aim was to compare, in the same infants, arousal responses to both somatosensory (nasal air-jet) and respiratory (hypoxia) stimuli using established criteria for subcortical arousal. Because little information is available on the effects of sleep state and postnatal age on infant arousal responses to respiratory stimuli, we have also quantified arousal responses to hypoxia in both AS and QS in the same infants during their first 6 months after birth. We hypothesized that arousal responses to both somatosensory and respiratory stimuli would be similarly affected by sleep state and postnatal age.

METHODS

Ethical approval for this project was obtained from the Monash Medical Centre Human Ethics Committee. Written informed parental consent was obtained prior to each investigation.

Subjects

Ten healthy term infants (5 girls/5 boys) were recruited from the Monash Medical Centre, Melbourne, Australia. Gestational ages at birth were 38 to 41 weeks (39.8 ± 0.4 weeks, mean ± SEM) and birth weights ranged from 2890 to 4725 grams (3516 ± 178 g). Apgar scores were 8 to 9 (median, 9) at 1 minute and 9 to 10 (median, 9) at 5 minutes. Polysomnographic studies were performed in each infant at 2 to 4 weeks, 2 to 3 months, and 5 to 6 months after birth, but 1 infant was unavailable for the 2 to 3 month study. Maternal ages were 20 to 40 years (34.0 ± 1.4 years), and none of the mothers smoked while pregnant or after delivery. Participation in the study was entirely voluntary with no monetary incentive provided.

Arousal Criteria

For both stimulus protocols, subcortical arousal was determined according to 4 criteria previously described with the presence of at
least 3 required to designate an arousal. These criteria included a change in both amplitude and frequency of the ventilatory pattern for more than 2 breaths, an observed behavioural response (usually turning the head away from the stimulus), a heart-rate acceleration of more than 10% above baseline, and an increase in phasic submentalis electromyogram activity. All of these changes must have occurred within 7 seconds of the onset of the air-jet stimulus or simultaneously during hypoxia. This period was chosen to allow time for the heart rate to reach a maximum level. 17 The 10 seconds of recording immediately preceding the air-jet stimulus and hypoxic arousals were used to obtain baseline measures to assess the degree of change in each physiologic variable. Previous validation analyses of subcortical arousal responses to air-jet stimulation identified an interrater reliability of 99.2% (248 of 250 arousal responses). 23

Arousal Stimuli

The somatosensory stimulus was a pulsatile nasal air-jet stimulus (3 Hz for 5 seconds), applied using an established procedure. 17,25-27 The stimulus was delivered alternately to the nostrils during both AS and QS, allowing arousal thresholds to be calculated. 17,25 In brief, if the infant failed to arouse, the air pressure was increased when the stimulus was again presented to that nostril; when arousal occurred, the pressure was decreased. Arousal threshold was calculated as the mean stimulus driving pressure between each arousal and nonarousal response. 17 Prior to each stimulus presentation, the pressure was calibrated on the chart record (although not presented to the infant). In order to determine the probability of a spontaneous arousal from sleep, the number of arousals that occurred during stimulus calibration was measured.

For hypoxia tests, nasal airflow was measured using a small pneumotachograph attached to a nose mask, modified from that employed by Cohen and colleagues. 46,47 Small, medium, and large nose masks were constructed based on nose molds of infants at each study age. These silicone rubber masks permitted flexibility in the pneumotachograph assembly while ensuring an adequate seal. No additional mask sealant was required. Leaks were detected as a baseline shift in respiratory flow, a sudden reduction in expired carbon dioxide levels, or both. The entire mask-pneumotachograph assembly weighed 48 grams with low dead space (4.0-6.5 mL) and resistance (0.44 cm H2O at 10 L/minute).

A continuous bias flow of medical-grade gas was passed across the pneumotachograph at 5 liters per minute, causing a pressure of 0.25 cm H2O within the mask. A differential pressure transducer (Model PT5A, Grass Instrument Co., Quincy, MA, USA) was used to measure airflow via 2 ports positioned 5 mm on either side of a fine wire-mesh screen within the pneumotachograph. An additional port located 1 cm from the proximal end of the pneumotachograph was used to monitor expired carbon dioxide at a sampling rate of 100 mL per minute. The transducer was electronically balanced to account for the bias flow and capnograph drift.

When using the air-jet stimulation, there was no difference in arousal thresholds between left and right nostrils, and so the data were pooled. The maximal driving pressure of the air jet was 900 cm H2O; therefore, an arousal threshold of 900 cm H2O was recorded if an infant failed to arouse following 2 or more successive presentations at this maximal stimulus intensity. 26 Hypoxia tests that were terminated due to the SpO2 being less than 85% were excluded from analyses. Parental infant sleep diaries for the 2 days preceding each study to ensure that the sleep obtained in the laboratory was similar to typical sleep patterns in the home.

Data Analysis

Arousalability to the nasal air jet was measured as arousal thresholds, whereas arousability to hypoxia was measured as both the probability of arousal (FTA) and arousal latency. For hypoxic challenges, the probability of FTA to hypoxia was assessed as both a) the percentage of infants who failed to arouse to 1 or more hypoxia tests and b) the percentage of tests to which infants failed to arouse.

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Normality was assessed in all data using the Shapiro-Wilks statistic with significance taken at P < 0.05. Mantel-Haenszel \( \chi^2 \) statistics were used to compare the probability of spontaneous arousal to air-jet-induced arousal and the probability of arousal within each sleep state at each age to both forms of stimulation. Paired t-tests were used to compare a) arousal thresholds between nostrils, b) sleep duration in the home versus laboratory, c) SpO2 and end-tidal carbon-dioxide tension levels at control versus point of arousal or FTA, and d) the effect of sleep state on arousability to both stimuli at each study age. One-way ANOVA for

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RESULTS

A total of 157 hypoxia tests were successfully conducted, 94 in QS and 63 in AS, with replicate tests obtained in 66% of sleep epochs. Ten hypoxia tests in both AS and QS over the 3 ages were excluded from further analysis because tests were terminated when the SpO2 fell below 85% (Table 1). Thirteen tests (10 infants) that induced periodic breathing were included in the arousal latency calculations but not respiratory analysis (Table 1).

A total of 507 air-jet stimuli were presented in AS and 583 in QS (over all ages), providing 236 arousal threshold determinations in AS and 225 in QS. Data regarding the number of air-jet presentations and arousal thresholds in each sleep state at each age are presented in Table 1. The frequency of spontaneous arousals was significantly higher in AS than QS at each study age (P<0.01); however, in both sleep states, infants aroused significantly more often to the air jet when compared to spontaneous arousal (P<0.05).

Sleep Data

There was no significant difference in daytime sleep duration in the laboratory when compared with the infants’ typical sleeping patterns at home (parental sleep diaries) at any age. Total sleep time in the laboratory was significantly longer at 2 to 4 weeks of age (143 ± 9 min) than at 2 to 3 months (107 ± 10 min) and 5 to 6 months (97 ± 9 min) (P<0.05). The amount of AS decreased with age, whereas the amount of QS remained unchanged (Table 2).

Respiratory Gas Data (Normoxia versus Hypoxia)

Following 15% oxygen inhalation, significant oxygen desaturation was observed, relative to baseline levels, at the point of arousal or at 5 minutes (FTA) in both sleep states at each age (Table 3). A small but significant (P<0.01) decrease in end-tidal carbon-dioxide tension levels was observed during QS at 2 to 3 months of age, but we do not consider this to be clinically significant (Table 3).

In response to the hypoxic challenges, all infants aroused in AS at each study age; however, responses were variable in QS with infants either arousing or failing to arouse to repeated tests. Ten percent of infants did not arouse to 1 or more hypoxic challenges in QS at 2 to 4 weeks, 33% at 2 to 3 months, and 22% at 5 to 6 months of age. When expressed as a percentage of tests that failed to induce arousal, there was no significant difference in the probability of FTA between AS and QS at 2 to 4 weeks of age. However, infants failed to arouse to a significantly higher percentage of hypoxia tests in QS than in AS at both 2 to 3 months and 5 to 6 months of age (P<0.01) (Figure 1). The probability of infants failing to arouse to hypoxia was greater at 2 to 3 months and 5 to 6 months of age compared to 2 to 4 weeks (P<0.05) (Figure 1). In response to nasal air-jet stimulation, none of the infants failed to arouse in either sleep state at any age.
Effects of Sleep State and Age on Arousal Latency to Hypoxia

Arousal latency to hypoxia was determined when a) FTA tests were excluded and b) when FTA tests were included as 300 seconds in arousal-latency calculations. When FTA tests were excluded, arousal latency tended to be longer in QS than in AS, although a significant sleep-state–related difference was found at only 2 to 4 weeks of age (Table 2). When FTA tests were included in the analyses, arousal latency was significantly longer in QS than in AS at each study age (Figure 2). Postnatal age had no significant effect on arousal latency to hypoxia in either sleep state when FTA tests were both included (Figure 3) and excluded from the analyses.

Effects of Sleep State and Age on Arousal Thresholds to Air-Jet Stimulation

Arousal thresholds to the air jet were significantly greater in QS compared to AS at each study age, with the exception of 2 to 4 weeks of age (Figure 2). In response to nasal air-jet stimulation, arousal thresholds were lower in AS at 2 to 3 months compared with 2 to 4 weeks ($P<0.05$) and higher in QS at 5 to 6 months than at 2 to 4 weeks of age ($P<0.05$) (Figure 3).

Correlation of Arousal Latency (Hypoxia) and Arousal Threshold (Air Jet)

There were no significant correlations within infants between arousal latencies to hypoxia and arousal thresholds to nasal air-jet stimulation (Study 1 AS $r_s = 0.4$, $N=9$; Study 1 QS $r_s = -0.5$, $N=8$; Study 2 AS $r_s = 0.4$, $N=8$; Study 2 QS $r_s = 0.5$, $N=8$; Study 3 AS $r_s = -0.3$, $N=6$; Study 3 QS $r_s = -0.4$, $N=9$).

DISCUSSION

This study demonstrates for the first time that arousal responses of healthy term infants to both a somatosensory and a respiratory stimulus are depressed in QS compared to AS. We also demonstrated that arousability to both stimuli was similarly affected by postnatal age in infants aged between 2 weeks and 6 months.

Effect of Sleep State on Infant Arousal Responses

In response to nasal air-jet stimulation, we found that arousal responses were depressed in QS compared to AS at all ages studied, except at 2 to 4 weeks. This finding supports our previous studies in term infants under various environmental and clinical conditions$^{7,26,27}$ and that was also demonstrated using different somatosensory$^{10,13,21}$ and mechanical$^9$ stimuli. Although we failed to demonstrate a significant sleep-state–related difference at 2 to 4 weeks of age, the trend was present and may have been significant had we been able to study more infants.

We demonstrated a similar sleep-state–related difference in arousability to hypoxia, in terms of both probability of arousal (FTA) and arousal latency. The majority of previous studies of arousal responses to hypoxia have been investigated in QS alone,$^{5,8,11,14-16}$ with only 1 study examining both sleep states.$^4$ Furthermore, arousability has been quantified only in terms of the percentage of infants, tests that failed to arouse, or both.$^4,8,11,14,15$ Our inclusion of arousal latency in response to hypoxia provides a continuous measure of arousal that is analogous to our measure of arousal threshold in response to air-jet stimulation. Our study highlights the importance of taking sleep state into consideration because all hypoxic challenges presented during AS induced arousal at each study age. In contrast, a significant proportion of tests in QS failed to induce arousal. A sleep-state–related difference in the probability of arousal to hypoxia was present at 2 to 3 months and 5 to 6 months. In addition, arousal latency to hypoxia was significantly longer in QS compared to AS at each study age. For this latter analysis, we included FTA tests as 300 seconds in the arousal-latency calculations; however, this would still provide an underestimate of the true arousal latency in those infants who failed to arouse after 5 minutes of exposure to hypoxia. Even when these FTA tests were excluded from arousal-latency calculations, arousal latency tended to be longer in QS than in AS. In a previous study, we showed that SpO₂ at arousal was not different between sleep states, indicating that infants desaturate more quickly in AS.$^{50}$ Although arousal responses to both stimuli were similarly affected by sleep state, we were unable to demonstrate a significant correlation between arousal latency to hypoxia and arousal threshold to nasal air-jet stimulation in either sleep state at any age.

Our finding that QS is a state of diminished arousability compared to AS in response to mild hypoxia is supported by previous studies using a mild asphyxia stimulus (5% carbon dioxide / 13% oxygen / balance nitrous).$^{18,24}$ In the only other study to investigate hypoxic arousal responses of infants in both sleep states, no such sleep-state–related differences in arousability were found.$^4$ However, that study was conducted in a substantially smaller group of infants, and arousal was quantified only as the percentage of infants who failed to arouse.

Our study also highlights the importance of taking into consideration the species being investigated. In contrast to the findings in human infants, studies using animal models have demonstrated opposite patterns of sleep-state–related differences in arousability. Studies using neonatal sheep,$^{28-30}$ adult dogs,$^{40-43}$ and neonatal piglets$^{44,45}$ have shown that arousal responses to a number of cardiorespiratory stimuli are impaired in AS compared to QS. We initially thought that these differing patterns of sleep-state arousability between animal models and human infants may be due to the different stimulus modalities used to induce arousal. While most studies conducted on animals have employed cardiorespiratory stimuli, investigations of human infants have predominantly used somatosensory stimuli. However, we have found that infant arousal responses are similar to both forms of stimulation. Therefore, we postulate that the different effects of sleep state on arousability between humans and animal models may reflect species differences in sleep-state organization, maturation of sleep architecture, or both. In neonatal
sheep, for example, the duration of AS and QS periods (4 and 6 minutes, respectively) are substantially shorter than those obtained in this study (Table 2).

**Effect of Postnatal Age on Infant Arousal Responses**

We found that infants in AS were less arousable to air-jet stimulation at 2 to 4 weeks than at 2 to 3 months of age. However, previous studies by our group did not find these maturation changes to be significant. In contrast, arousal responses to hypoxic stimulation showed no maturation changes in AS when quantified either in terms of probability of arousal or arousal latency.

In QS, arousability was found to decrease with increasing postnatal age. In accordance with previous findings, we found that infants had higher arousal thresholds in response to air-jet stimulation at 5 to 6 months than at 2 to 4 weeks of age. Although we found no maturation change in the hypoxic arousal response in QS when quantified in terms of arousal latency, infants failed to arouse to a greater percentage of hypoxic challenges in QS at 2 to 3 months (42%) and 5 to 6 months (39%) than at 2 to 4 weeks of age (12%). This decrease in arousability with increasing postnatal age in QS has also been demonstrated in response to mild asphyxia.

**Methodologic Considerations**

The use of a mask-pneumotachograph system in this study may have provided some cutaneous stimulation; however, we did not test arousal responses until the infant had been in a stable sleep state for at least 2 minutes after mask application. In addition, in a subsequent study, we directly compared spontaneous arousal under normoxic conditions and hypoxic arousal under the same conditions and found that infants aroused more frequently and at shorter latencies in hypoxia than in normoxic conditions.

Ethical considerations limited our hypoxic stimulus to 15% oxygen inhalation with the termination of tests if the SpO2 fell below 85%. Data from these terminated tests (10/157) were excluded from further analysis. We do not believe that exclusion of these tests affected results because they occurred across both sleep states and at all 3 ages studied (Table 1). Our hypoxic stimulus was mild in comparison to animal models that have used inhalation of 0% oxygen, 28% or 10% oxygen. The opposing patterns of sleep-state arousability between human infants and animal models may be due in part to differences in stimulus intensity, but we consider this unlikely.

Previously, it has been reported that hypoxia is a poor arousing stimulus in QS in human infants. The greater incidence of arousal during QS in our study compared to previous investigations may be due to different definitions of arousal. In previous studies, full cortical arousal was defined as infants waking, crying, or both. Given that infant arousal responses to a variety of stimuli follow a stereotypic pattern of a spinal, respiratory, startle, and then a cortical response, we employed a multifactorial subcortical definition of arousal without awakening. These subcortical responses may serve as protective mechanisms during sleep, while maintaining sleep integrity, with full cortical arousal only being necessary if these subcortical mechanisms fail.

**CONCLUSIONS**

Arousal responses of sleeping infants aged between 2 weeks and 6 months to both somatosensory and respiratory stimuli are depressed in QS compared to AS. In addition, both forms of stimuli are similarly affected by postnatal age, with arousability being unchanged in AS and decreasing in QS. Although we did not demonstrate a strong correlation within infants between the 2 stimuli in arousability, the overall similarity of effects of sleep state and postnatal age on arousability suggests that somatosensory stimuli such as our air jet provide useful tools for assessing infant arousability.

Arousal serves as an important protective mechanism during sleep, particularly in AS when rapid hypoxemia may develop due to diminished ventilatory responsiveness and increased metabolic demands. Therefore, cardiorespiratory failure, when coupled with an inability to arouse to hypoxia during an asphyxial challenge may be involved in the pathogenesis of sudden infant death syndrome.

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**REFERENCES**

28. Fewell JE, Baker SH. Arousal from sleep during rapidly developing hypoxemia in...


