Effects of Sleep State and Postnatal Age on Arousal Responses Induced by Mild Hypoxia in Infants

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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 the sudden infant death syndrome (SIDS). In order to understand factors affecting arousability in infants, studies have been performed at postnatal ages relevant to SIDS. To avoid ethical and technical concerns associated with the administration of cardiorespiratory stimuli, arousal responses of sleeping infants have been assessed using somatosensory stimuli and body tilting. These studies have consistently found that infants are more arousable in active sleep (AS) than in quiet sleep (QS).

It has been proposed that mild hypoxia is not an effective stimulus for arousal in sleeping infants because the majority of normal infants fail to arouse in response to hypoxic challenges. However, most previous studies in infants have been conducted only in QS, with only 1 investigation being performed in both AS and QS. Consequently, little information is available on hypoxic arousal responses in AS, which, based on studies using somatosensory stimuli, is a state of increased arousability. In addition, arousal responses of infants to hypoxia have not been compared against the background of spontaneous arousals occurring during sleep, which occur frequently in young infants, particularly in AS.

Previous studies have usually defined arousal as vigorous body movements, eyes opening, awakening, crying, or a combination thereof. However, it has now been demonstrated that full cortical arousal from sleep, in both human adults and infants, is preceded by a stereotypical sequence of subcortical events. In sleeping infants, these subcortical processes may serve as protective mechanisms that restore airway patency, allow the infant to reposition his or her head while maintaining sleep efficiency, or both.

Because more knowledge is needed regarding arousability of infants in both sleep states in response to respiratory stimuli, the aims of this study were to compare arousal responses to mild hypoxia in both AS and QS and to contrast these induced responses with those of spontaneously occurring arousal, using indexes of subcortical arousal. In addition, we aimed to determine the effects of postnatal age on arousal responses. It was hypothesized (a) that hypoxia-induced arousal responses would be depressed in QS compared to AS and would occur more frequently and with shorter onset latencies than spontaneously occurring arousal (under normoxic conditions) in both sleep states, and (b) that these effects would be most marked at 2 to 3 months of age when the incidence of SIDS is highest.

METHODS

Subjects

Ethical approval was obtained from the Monash Medical Centre Human Research and Ethics Committee. Written informed parental consent was obtained prior to each study. Infants were recruited from Newborn Services, Jessie MacPherson Private Hospital, and the maternity wards at Monash Medical Centre, Clayton, Victoria, Australia.

The study group comprised 5 healthy term infants (4 girls, 1 boy) born at 38 to 41 weeks gestation (40 ± 0.5 weeks) with normal birth weights (mean 3615 ± 200 g, range 3110-4167 g), 5 healthy preterm infants (3 girls, 2 boys) born at 30 to 34 weeks gestation (mean 32 ± 0.7 weeks) with normal birth weights for gestational age (mean 2061 ± 239 g, range 1497-2933 g), and 1 small-for-gestational age infant (boy) born at 29 weeks gestation weighing 869 g. Infants were studied at 2 to 5 weeks, 2 to 3 months, and 5 to 6 months postterm corrected age. One term infant and 2 healthy preterm infants were only studied at 2 to 5 weeks. None of the mothers smoked while pregnant or following birth.

Participants:
Five healthy term and 6 healthy preterm infants were each studied at 2 to 5 weeks, 2 to 3 months, and 5 to 6 months postterm. All infants underwent daytime polysomnography during which nasal airflow was monitored using a purpose-built pneumotachograph. All infants were studied under both normoxic (21% O2) and hypoxic (15% O2, balance N2) conditions (presentation order randomized) in each sleep state at each study age. Tests were terminated at arousal, O2 saturation falling below 85%, or 5 minutes (failure to arouse).

Measurements:
Probability of failure to arouse and mean arousal latency were compared between each experimental condition, with each infant serving as its own control.

Results:
Infants aroused more frequently under hypoxic conditions than under normoxic conditions. Overall, arousal latencies were shorter during hypoxia compared to normoxia in both sleep states at each age. Arousal latencies were longer in QS compared to AS in both hypoxic and normoxic conditions.

Conclusion:
In sleeping infants, mild hypoxia serves as a stimulus for arousal in both AS and QS. Of particular significance is our finding that arousal from AS is readily elicited by mild hypoxia.

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Study Objectives: It has been suggested that mild hypoxia may not be a potent stimulus for arousal during sleep in infants because infants frequently fail to arouse from quiet sleep (QS). Our aim was to characterize arousal responses of sleeping infants in both active sleep (AS) and QS under normoxic and mildly hypoxic (15% O2) conditions over the first 6 months of life.

Participants: Five healthy term and 6 healthy preterm infants were each studied at 2 to 5 weeks, 2 to 3 months, and 5 to 6 months postterm. All infants underwent daytime polysomnography during which nasal airflow was monitored using a purpose-built pneumotachograph. All infants were studied under both normoxic (21% O2) and hypoxic (15% O2, balance N2) conditions (presentation order randomized) in each sleep state at each study age. Tests were terminated at arousal, O2 saturation falling below 85%, or 5 minutes (failure to arouse).

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Study Protocol

Daytime polysomnography was performed between 10:00 AM and 4:00 PM at the Adamson Sleep Centre of the Melbourne Children’s Sleep Unit, Monash Medical Centre. Electrodes for recording polysomnographic variables were attached during a morning feeding, after which infants were placed supine in a bassinet. Electrode impedance was measured (Model EZM4B, Grass Instrument Co., Quincy, Mass, USA) prior to recording, and electrodes were reapplied, if necessary, to maintain impedance levels below 10 KΩ. Studies were performed during both morning and afternoon sleep periods, following the infant’s regular feeding schedule. Environmental temperature remained constant (21°C-24°C), while noise and light levels were kept to a minimum.

Polygraphic recordings were made (Grass Instrument Company, Quincy, Mass, USA) of electroencephalogram, electrooculogram, submental electromyogram, electrocardiogram, instantaneous heart rate (HR), thoracic and abdominal breathing movements (Resp-ez Piezoelectric sensor, EPM Systems, Midlothian, Va, USA) and SpO2 (Biox 3700e Pulse Oximeter, Ohmeda, Louisville, Colo, USA). Sleep state was assessed as QS, AS, or indeterminate sleep using electroencephalogram, behavior, HR, and breathing-pattern criteria; data from indeterminate sleep were excluded from the analyses. Infant sleep diaries were completed for the 2 days preceding each study to ensure that sleep patterns obtained in the laboratory were similar to those in the home.

Stimulus and Protocol

Nasal airflow was measured using a miniaturized pneumotachograph attached to a silicone rubber nose mask. A continuous bias flow of medical-grade gas was passed across the pneumotachograph at 5 L per minute, causing a pressure of 0.25 cmH2O within the mask. Carbon dioxide (CO2) was monitored in expired gas sampled at 100 mL per minute (PETCO2). A differential transducer attached to the pneumotachograph was electronically balanced to account for the bias flow and capnograph gas withdrawal.

A custom-drilled 3-way tap (Swagelock, Ohio, USA) was used to change the bias-flow gas supply from air (21% O2) to 15% O2 (balance N2). Pressure relief valves (Norgren, Melbourne, VIC, Australia) were placed within the normoxic and hypoxic gas lines to ensure that pressure within the circuit did not exceed 45 cmH2O. There was no significant difference in temperature between the normoxic and hypoxic gas supplies.

Normoxic and hypoxic tests were randomly presented in either the morning or afternoon sleep period to eliminate any time-of-day effect. A 2-minute control period (with mask / pneumotachograph attached) obtained at the commencement of each sleep period provided baseline values of cardiorespiratory variables and respiratory gas values. A further 1-minute control period was then obtained prior to each normoxic and hypoxic test. For the normoxic tests, the infant simply continued to breathe 21% O2 for 5 minutes in the absence of arousal (FTA) or until a spontaneous arousal occurred. For the hypoxic challenges, the inspired gas was changed from 21% O2 (air) to 15% O2. Tests were terminated at either arousal, at 5 minutes in the absence of arousal (FTA), or if the SpO2 fell below 85%. Replicate tests (n = 3) were obtained in each sleep state for both the normoxic and hypoxic tests. Recovery periods of at least 2 minutes were provided between successive hypoxic challenges. Tests were not performed during periods of periodic breathing. Tests inducing periodic breathing were continued until 1 of the termination criteria was met.

Definition of Subcortical Arousal

For both normoxic and hypoxic protocols, subcortical arousal from sleep was determined according to 4 criteria previously described, with the presence of at least 3 required to designate an arousal response. These criteria were a change in both amplitude and frequency of the ventilatory pattern for more than 2 breaths; an observed behavioral response (usually turning the head away from the stimulus); a HR acceleration of greater than 10% above baseline; and an increase in submental electromyogram activity. All of these changes must have occurred simultaneously to be considered an arousal. The 10-second period of recording immediately preceding the arousal was used to obtain baseline data to assess the degree of change in each physiologic variable.

Data Analysis

Comparisons were made between normoxic and hypoxic arousal responses in the following analyses, with each infant serving as his or her own control. Hypoxic tests that were terminated due to SpO2 < 85% were excluded from analyses of FTA and arousal latency.

Normality was assessed in all data using the Shapiro-Wilks’ statistic. Stratified Cochran’s c2 statistics were used to compare the probability of arousal within the normoxic and hypoxic conditions at each study age. Paired Student t tests were used to compare (a) total sleep time (TST) in the laboratory during normoxia and hypoxia; (b) sleep duration in the home versus laboratory; (c) SpO2 and PETCO2 levels at control versus point of arousal or FTA in both normoxic and hypoxic conditions; and (d) arousal latency during normoxic and hypoxic conditions. One-way analysis of variance for repeated measures with Student-Newman-Keuls posthoc analysis was used to investigate the effect of postnatal age on sleep duration in the laboratory. Arousal latencies in normoxic and hypoxic conditions were calculated using a value of 300 seconds in infants who failed to arouse. All results are presented as mean ± SEM, and significance was accepted at P < .05.
RESULTS

Over the 3 ages studied, 134 tests were conducted under normoxic conditions (over all ages combined), 69 in QS, and 65 in AS; replicate tests were performed in 71% of sleep periods (Table 1). None of the tests were terminated due to the SpO2 falling below 85%, and none induced periodic breathing. A total of 181 hypoxia tests were successfully conducted (97 in QS and 84 in AS), with replicate tests being performed in 61% of sleep periods (Table 1). Eighteen hypoxia tests were excluded from further analysis due to the SpO2 falling below 85%. Seventeen hypoxic challenges (in 13 infants) that induced periodic breathing were included in the calculation of arousal latencies.

Respiratory Gas Data

The SaO2 and PETCO2 at the point of arousal or at the end of the 5-minute challenge were compared with baseline values obtained during the 1-minute control periods preceding each test (Tables 2 and 3, respectively). In normoxic tests, there was no significant change in PETCO2 throughout the test period (Table 2). Small but significant differences were found between PETCO2 levels at the point of arousal or FTA and control levels, with an increase in QS at 2 to 3 months and a decrease in AS at 5 to 6 months (Table 3). During hypoxic tests, significant O2 desaturation was observed in both sleep states at all 3 ages, and a small but significant (P < .05) decrease in PETCO2 was observed during QS at 2 to 3 months of age.

Probability of FTA During Normoxia and Hypoxia

The probability of FTA under normoxic and hypoxic conditions was calculated as both (a) the percentage of infants who failed to arouse to 1 or more test and (b) the percentage of tests that failed to induce arousal (Table 4).

Using stratified Cochran’s χ2 statistic on both the number of infants and number of tests that failed to induce arousal, arousal occurred significantly more often during hypoxic tests than during normoxic tests (P < .05). When individual χ2 tests were performed within each sleep state at each age, this significant difference was only present duringQS at 2 to 5 weeks and 2 to 3 months (percentage of tests). However, a consistent trend remained for infants to arouse more often in response to hypoxia in comparison to the probability of spontaneously occurring arousal under normoxic conditions.

Arousal Latency During Normoxia and Hypoxia

The times from stimulus onset to arousal during normoxia and hypoxia were calculated. The FTA tests were included as 300 seconds in arousal latency calculations (Figure 1). Arousal latency was shorter in hypoxia compared with normoxia in both AS and QS at 2 to 3 months of age, and there was a trend for arousal latency to be shorter in response to hypoxia than normoxia in each sleep state at each of the ages, with the exception of QS at 5 to 6 months of age.

Effects of Sleep State on Arousal Latency

Mean arousal latency was longer in QS compared with AS during normoxic tests at all 3 ages studied; however, this only reached statistical significance at 2 to 3 months of age (P < .05) (Figure 2). During hypoxic tests, arousal latency was significantly longer in QS compared with AS at all 3 ages studied (P < .05).

Sleep Data

The TST in the laboratory did not differ between normoxic and hypoxic conditions at 2 to 5 weeks (normoxia TST: 58 ± 5 minutes; hypoxia TST: 67 ± 6 minutes; n = 11 infants), 2
to 3 months (normoxia TST: 53 ± 7 minutes; hypoxia TST: 67 ± 7 minutes; n = 8 infants), or 5 to 6 months (normoxia TST: 39 ± 5 minutes; hypoxia TST: 44 ± 4 minutes; n = 8 infants).

Infant sleep patterns in the laboratory were compared with those at home (matched for time of day) as recorded by parental sleep diaries. At 2 to 5 weeks of age, there was no significant difference in daytime sleep duration in the laboratory (126 ± 8 minutes) compared with the infants' typical sleeping patterns at home (119 ± 9 minutes). However, at both 2 to 3 months and 5 to 6 months of age, daytime sleep duration was significantly longer in the laboratory than at home (124 ± 10 minutes versus 55 ± 13 minutes and 84 ± 4 minutes versus 49 ± 12 minutes, respectively). The TST in the laboratory was significantly longer at both 2 to 5 weeks (138 ± 8 minutes) and 2 to 3 months (122 ± 9 minutes) than at 5 to 6 months (84 ± 4 minutes) (P < .01).

DISCUSSION

This study demonstrates for the first time that mild hypoxia induces subcortical arousal from both AS and QS in healthy infants during the first 6 months of life. Of particular significance is our finding that arousal from AS is readily elicited by mild hypoxia. The unique protocol of this study allowed comparison of hypoxic arousal responses with spontaneously occurring arousal under identical test conditions in normoxic conditions.

Only 1 previous study of human infants has examined hypoxic arousal in both sleep states,23 and the majority of studies have been conducted in QS only.16-22 We confirmed previous findings that, in QS, infants frequently fail to arouse to a mild hypoxic stimulus.15 However, the measurement of responses in both sleep states combined with the comparison of hypoxic responses with those under identical normoxic condition has allowed us to confirm that hypoxia is an effective stimulus for arousal in young infants.

Our study quantified infant arousal responses as both the probability of FTA and arousal latency. In other studies, the presentation of data on only the number of infants who aroused or the number of tests to which all infants aroused has provided limited, although valuable, data on the arousal process. In our studies, the inclusion of arousal latency has provided a continuous measure of arousability and allows comparison with other measures of arousal threshold index that have been previously published.8-11 No previous study of arousal responses of infants to either hypoxic or normoxic stimuli has measured arousal latency.

Arousalability During Normoxia and Hypoxia

We analyzed our data as both the percentage of infants and tests that failed to induce arousal to allow comparison with previous studies. In both forms of analysis, infants tended to arouse more frequently in hypoxia than in normoxia in both sleep states. This difference only reached statistical significance in QS at 2 to 5 weeks and 2 to 3 months when expressed as number of tests. In addition, arousal latency also tended to be shorter in hypoxic challenges when compared to normoxia, with these differences reaching statistical significance at 2 to 3 months of age in both AS and QS.

Effects of Sleep State on Arousalability During Normoxia and Hypoxia

Our finding of a greater arousal latency in QS than in AS in response to the hypoxic challenges is in accordance with previously published data using somatosensory stimuli from our group8,11 and from others15,34; a similar state-related difference was demonstrated using body tilting.7 In support of this state-related difference in arousability, spontaneous arousal from sleep occurs more frequently in AS than in QS.8,33 The only other study that has investigated hypoxic arousal responses in both sleep states in infants found no sleep-state-related difference in arousability.23 However, it was found that, in AS, the majority (75%) of 2 to 3-month-old infants (n = 4) aroused in response to hypoxia (15% O2 inhalation over a 3- to 5-minute period). The difference between those findings and others may have been due to differences in criteria for arousal and the use of overt awakening as the end point of each test.23

Comparisons of FTA between the results obtained in this study for QS and those reported from previous researchers are made difficult by differences in (a) administration of inspired gases (nasal mask versus head hoods), (b) duration and intensity of the hypoxic challenge; (c) method of reduction in FIO2 (single step or stepwise), and (d) ages of the infants studied. However, previous investigators have, like us, found that infants frequently fail to arouse to hypoxia in QS. Studies by Ariagno et al23 using a mask and an FIO2 of 0.15 showed that 20% (1/5) of term infants (aged 1 to 4 months) failed to arouse, while Hunt15 found similar rates of 24% (5/21) in 2-month-old infants. These levels are somewhat lower than in our study, in which 38% to 55% of infants failed to arouse. These differences may be due to the small number of infants (n = 5) used in the study by Ariagno et al23 or may result from the finding that infants may both arouse and fail to arouse to successive stimuli. Hence, without repeated tests, an overestimation of arousability may be obtained. Studies using a head hood and an FIO2 of 0.15 have found differing rates of FTA. Milerad et al11 reported FTA in 67% (12/18) of infants (4-14 weeks), while McCulloch et al15 reported FTA in 30% (6/20) of 2-month-old infants, and Dunne et al17 reported FTA in 33% (33/49) of 1 to 14-week-old infants. Studies using more or less severe hypoxic stimuli or using differing protocols have also demonstrated similar findings in relation to FTA. Infants also failed to arouse to a milder reduction in FIO2; to 0.17 for 10 minutes.16 In contrast, exposure to an FIO2 of 0.11 induced arousal in all infants in 1 study22 and 68% in another.15 Despite differences in frequencies of FTA to hypoxia, all of these studies are consistent in the finding that infants frequently fail to arouse in response to a hypoxic challenge. This in itself may not be a reflection of the inadequacy of the hypoxic challenge to elicit an arousal response in QS, but may be due to the ventilatory response to mild hypoxia being adequate to maintain oxygenation without necessitating arousal from sleep. In contrast, arousal in response to hypoxia may be a more vital response in AS, which is a state of greater O2 consumption.

In the present study, repeated tests (n = 3) were conducted in each sleep state, providing novel data that infants both arouse and fail to arouse to repeated tests in QS. When examined both in terms of arousal latency and FTA, our findings were not explained by habituation to the stimulus, as arousal latencies did not consistently increase with repeated tests, nor did infants become increasingly less arousable.

The majority of previous studies have only been concerned with overt behavioral awakening and have not assessed subcortical arousals as we did. Recent data obtained in infants, children, and adults have demonstrated that arousal is not an all-or-none process but is graded, involving a hierarchy of events.24-29 In infants, arousal can be initiated by a sigh coupled with a startle, followed by “thrashing” body movements and then full cortical arousal. This sequence has been consistently observed in both sleep states for both spontaneous and induced arousal.12,24,26 These subcortical arousal mechanisms may be important to prevent injury (ie, by removing an asphyxial stimulus or restoring airway patency) while maintaining sleep efficiency.12

There are a number of limitations to our study. Firstly, the subjects were a heterogeneous group of healthy infants that included 5 term infants, 5 premature infants, and 1 infant who was small for gestational age. However, infants were studied at matched postconceptional ages, and each was studied in both normoxic and hypoxic conditions, thus serving as his or her own control. In addition, there was no difference in the amount of time spent asleep in normoxic or hypoxic conditions. Furthermore, the sleep of the infants in the laboratory was also not adversely affected compared to that at home. Secondly, the mask pneumotachograph system employed in this study may have provided cutaneous stimulation, which could, in itself, alter arousability. However, by comparing arousal responses during hypoxia and normoxia with the mask attached, we believe we have eliminated this potential problem. To further restrict the effects of cutaneous stimulation on ventilatory and arousal responses, no tests were performed for at least 1 to 2 minutes.
after application of the mask. Thirdly, we calculated arousal latencies for FTA tests using a value of 300 seconds. We believe that the inclusion of these FTA tests was valid because the infants concerned were not consistent nonresponders to hypoxia, as they did arouse to some hypoxic tests. Exclusion of the FTA tests from arousal-lateness calculations would therefore have provided an underestimation of the true value. The inclusion of maximal stimulus thresholds (under FTA conditions) has been performed previously in investigations of arousal responses to hypertension, hypotension, sound, and nasal air-jet stimulation.5,6,10,35,36

In conclusion, we have demonstrated for the first time that mild hypoxia is an effective stimulus for subcortical arousal in human infants in both AS and QS during the first 6 months of postnatal life, with respect to both arousal latency and probability of arousal. Quantification of spontaneous arousals during normoxic conditions has provided convincing evidence of the effectiveness of chemosensory stimuli in inducing arousal, which previously has been equivocal using other published protocols that omit spontaneous arousal.

REFERENCES