

BODY TEMPERATURES AND OXYGEN CONSUMPTION DIFFER BETWEEN ACTIVE SLEEP EPISODES OF COMPLETE AND INCOMPLETE SLEEP CYCLES IN NEONATES

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In non-thermoneutral environments, sleep and thermoregulatory processes interact. Thus, in animals, disruption of the sleep cycle by wakefulness (W) maintains body homeothermia whereas occurrence of fast wave sleep implies its suspension. These transitions have never been studied in neonates, whose thermoregulatory processes operate fully during both active (AS, first sleep stage after W) and quiet sleep (QS). The analysis of the awakening process is of great importance since a relative inability to arouse has been found in infants at risk for Sudden Infant Death Syndrome. Cool exposure reduces QS at benefits of W and AS. The present study was undertaken to compare the AS episodes that precede a QS episode (AS 1: complete sleep cycles) with those which are followed by wakefulness (AS 0: complete sleep cycles). With regard to the sleep-thermoregulation interaction, it is held that body temperatures govern the sleep cycle, i.e. that their levels and/or response patterns differ according to the AS outcome. *Protocol:* 37 preterm neonates (37 ± 2 weeks post-conceptional age) were explored in an incubator during 2 consecutive morning naps (thermoneutrality and cool condition, 1.5°C below the thermoneutral air temperature). Sleep, oxygen consumption ($\dot{V}\text{O}_2$), esophageal temperature (T_{es}) and mean skin temperature were continuously recorded. *Results:* Cool exposure significantly shortened QS total duration and increased the frequency of incomplete sleep cycles ($+0.010 \pm 0.035 \text{ min}^{-1}$) at the expense of complete sleep cycles ($-0.004 \pm 0.015 \text{ min}^{-1}$). The duration of the AS 0 and AS 1 episodes was not modified by the thermal condition. $\dot{V}\text{O}_2$ and body temperatures differed according to the outcome of the AS episode. Whatever the thermal condition, in AS 1 episodes, $\dot{V}\text{O}_2$ was high at the beginning and decreased throughout the episode, as it continued during the succeeding QS episode. In contrast, AS 0 was characterized by lower values of body temperatures and $\dot{V}\text{O}_2$ at the episode onset. T_{es} increased throughout the AS 0 episode at thermoneutrality but not in the cool condition. *Conclusion:* Low levels of T_{es} and $\dot{V}\text{O}_2$ at the onset of the AS episode enhance sleep cycle interruption by W, during which T_{es} and $\dot{V}\text{O}_2$ are the highest while the thermal response to cool exposure is the most efficient. In contrast, QS is the least efficient stage as far as thermoregulation is concerned, but is privileged when energy conservation is needed. During cool exposure, the thermoregulatory function of the neonate overcomes the needs of energy conservation, even though the thermal stress is of low magnitude. This is achieved by preferential outcome of AS episode towards wakefulness. Outcome of active sleep episodes and corresponding body temperatures and oxygen consumption in neonates sleeping in thermoneutral or cool environment. Outcome of active sleep episodes in neonates sleeping in thermoneutral or cool environment: measurements of body temperatures and oxygen consumption. Interruption of neonates' sleep cycle induced to cool exposure. Active sleep (AS) episodes were separated into AS 1 and AS 0 depending on whether the sleep cycle was complete or not (AS followed by a quiet sleep (QS) episode or by wakefulness, respectively, with or without transitional intermediate sleep).

EFFECTS OF ENVIRONMENTAL TEMPERATURE AND SLEEP ON VENTILATORY RESPONSE TO HYPEROXIA IN PREMATURE NEONATES

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In neonates, previous studies have shown that the respiratory chemoreceptor sensitivity is modified by air temperature or by sleep stages. However the combined influence of both sleep and thermoregulation on the chemical control of breathing through chemoreceptors has never been studied. To assess whether there is an interaction between sleep, body temperature regulation and chemoreceptor functioning, 11 healthy premature neonates (post conceptional age 35.9 ± 2.1 weeks; $2,000 \pm 1,000$ g) underwent sleep hyperoxic challenge tests in neutral, cool and warm thermal conditions. It is held that this interaction could trigger chemoreceptor dysfunction which could be implicated in various neonatal respiratory disorders.

The neonates were successively exposed to thermoneutral air temperature (T_N), warm ($T_N + 2^\circ\text{C}$) and cool ($T_N - 2^\circ\text{C}$) environments during 3 morning naps. Abdominal, cheek skin temperatures and rectal temperature were continuously recorded. 71 hyperoxic challenge tests (100% O_2) of 30 sec duration each were done during quiet sleep (QS) and active sleep (AS) with a fitting face mask connected to a pneumotachograph. Before the test, 5 min was allowed for sleep stabilization.

Respiratory flows were determined and tidal volume, inspired and expired times, respiratory frequency and ventilation were calculated breath-by-breath. Data sampled during the hyperoxic challenge were compared with baseline data (30 sec before the test) during which the neonate breathed room air. The respiratory time was defined as the time elapsed between the test onset and the first significant decrease in ventilation which is considered as an adequate response to the hyperoxic challenge.

In cool environment, the drop in ventilation was higher during AS ($-36.5 \pm 10\%$) than in QS ($-18.6 \pm 11\%$; $p=0.03$) whereas this was not significant at thermoneutrality or during warm exposure. Thus, sleep and thermoregulatory processes interact with the chemoreceptor function that disagrees with the finding of Rigatto et al.* (1982). This difference could be explained by the fact that these authors have done the hyperoxic tests in thermoneutral environment, only.

Since large increase in the gain of these structures can induce breathing variability, the control of the thermal environment is of utmost importance in sleeping neonates exposed to events during which chemoreceptor control mechanisms operate.

* Rigatto H. et al. (1982) Early Human Development. 7(1):1-10

SPECTRAL ANALYSIS OF HEART RATE VARIABILITY DURING SLEEP IN CHILDREN WITH PARTIAL EPILEPSY

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Objective. Alterations of the autonomic control of cardiac activity in epileptic patients have been reported by several studies in the past and both ictal and interictal modifications of heart rate regulation have been described. Also, alterations of the autonomic control of cardiac activity can play an important role in the sudden unexplained death in patients with epilepsy (SUDEP). However, the eventual presence of specific changes in HRV during sleep, not correlated with seizures, has not been assessed in children with epilepsy; for this reason, we evaluated the features of the cardiac autonomic function during sleep without ictal epileptiform EEG activity in a group of children with partial epilepsy.

Methods. Eleven patients (5 males and 6 females; mean age 11.5 years, S.D. 3.65 years) affected by partial epilepsy were admitted to this study; 11 normal subjects (5 males and 6 females; mean age 12.9 years, S.D. 2.72 years) served as a control group. All subjects slept in the laboratory for two consecutive nights. The data were analyzed during the second night. Sleep was polygraphically recorded (including one ECG channel) and signals were digitally stored. A series of 5-minute ECG epochs were chosen from each sleep stage, during periods without evident ictal epileptiform activity in the EEG. ECG signals were analyzed for automatic detection of R waves and, subsequently, a series of time- and frequency-domain measures were calculated.

Results. Epileptic subjects tended to show an overall lower HRV in both time- and frequency-domain parameters, mostly during REM sleep and, to a lesser extent, during sleep stage 2. Among the different bands, this decrease was most evident for HF absolute power. For this reason, the LF/HF ratio was always higher in epileptic patients than in normal controls and the difference was statistically significant during SWS and REM sleep.

Conclusions. Our results seem to indicate that during sleep, a particular condition of basal modification in autonomic activity occurs (mostly during REM sleep) in partial epilepsy patients; this might represent an important factor contributing to the complex mechanism of SUDEP which takes place most often during sleep and supports the need of studying HRV specifically during this state in subjects with seizures.

ALTERED NITRIC OXIDE ACTIVITY IN ARTERIOLES OF EPISODIC HYPOXIA RATS

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Recurrent EH is a feature of sleep apnea that may result in systemic hypertension. Chronic EH (8 hours days for 35 days) in rats causes elevation of diurnal resting, mean arterial blood pressure (MAP). Using in-vivo video microscopy, we examined arteriolar reactivity in cremaster muscles of male rats after chronic EH. Arterioles of EH (n=6) and controls (n=6) were exposed to varying doses of norepinephrine, acetylcholine (ACH) and endothelin-1 (END). Separately, EH (n=5) and controls (n=5) were given one dose of NO synthase inhibitor, L-NAME. eNOS mRNA levels from the kidneys were examined. EH rats show a 16 mmHg increase in MAP over 35 days; controls none. Responses to NE and END were similar. ACH vaso-dilatation in EH rats was attenuated compared to controls. The degree of vaso-constriction in response to blockade of the NO system by L-NAME was significantly less (83% of baseline diameter) in arterioles of EH rats compared to that of controls (61% baseline diameter), implying lower basal resting NO release in the EH rats. Kidney mRNA eNOS levels were not different. These data suggest that chronic elevation of BP associated with EH involves increased peripheral resistance from decreased basal release or production of NO.

GESTATIONAL AND POSTNATAL EFFECTS OF PRONE SLEEPING

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Arousal from sleep is believed to be an important survival mechanism that may be impaired in victims of Sudden Infant Death Syndrome (SIDS). Prone sleeping has been demonstrated to be the major risk factor for SIDS in world-wide studies. It has previously been reported that arousability is impaired in prone sleeping healthy term infants. Similar studies have not been carried out in preterm infants, a group known to be at increased risk for SIDS. The aim of this study was to longitudinally compare arousal responses of term and preterm infants sleeping both prone and supine.

The control group for this study was 24 healthy term infants born at 38-42 wk (mean 40 ± 0.4 wk) gestation with normal birth weights 2765-4190g (mean 3540 ± 78 g). 14 preterm infants born at 30-35 wks (mean 32 ± 0.4 wks) gestation with birth weights 920-2337g (mean 1638 ± 102 g) were also studied. Preterm infants had received <2 days of mechanical ventilation, had normal cranial ultrasounds and their medical history was uneventful. All infants were studied using daytime polysomnography on 3 occasions: 2-3 weeks post-term, 2-3 months post-term, and 5-6 months post-term. Multiple measurements of arousal threshold (cm H₂O) in response to air-jet stimulation applied alternately to the nares were made in both active sleep (AS) and quiet sleep (QS) when infants slept both prone and supine. Arousal thresholds were compared between sleep states and sleeping positions with 2-way repeated measures ANOVA. Differences between term and preterm infants and between ages studied were tested with 2-way ANOVA. Data are presented as mean \pm sem and were considered statistically significant at the $p<0.05$ level.

In term infants arousal threshold was elevated in QS compared with AS when infants slept both prone and supine at all three ages. In the preterm infant group, arousal threshold in QS was significantly elevated compared to AS at 2-3 wks and 2-3 mo in both positions, there was no difference at 5-6 mo. In term infants arousal thresholds were significantly higher in both AS and QS when infants slept prone at 2-3 wk and 2-3 mo, but not at 5-6 mo. In the preterm group, the prone position only impaired arousal at 2-3 mo, and this was in both AS and QS. Postnatal age did not alter AS arousal thresholds in either group of infants in the prone position. When infants slept supine AS thresholds were lower at 2-3 mo than 2-3 wks in both groups. Postnatal age had no effect on arousal thresholds in QS in either position in preterm infants. However, arousal threshold increased significantly with postnatal age in QS, in both positions in term infants. When arousal responses were compared between term and preterm infants at matched ages preterm infants were significantly easier to arouse at 5-6 mo in both sleeping positions.

We have demonstrated that the prone sleeping position significantly impairs arousal in both AS and QS. The finding that in preterm infants this impairment was only at 2-3 mo, the age when the risk of SIDS is highest is of significance. Importantly at 5-6 mo sleeping position did not affect arousability in either group of infants. This study provides an insight into why the risk for SIDS is increased when infants sleep prone.

This project was supported by SIDSaustralia and SIDassist.

A DEFINITION OF INFANT AROUSAL FROM SLEEP

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Currently there is no universally accepted definition of arousal in young infants. This has made comparison of studies investigating arousal responses difficult to compare. The aim of this study was, therefore, to develop a definition of arousal, both spontaneous and stimulus-induced in the human infant which would provide consistency across sleep states, postnatal ages and could be used for both term and preterm infants.

24 healthy term infants and 24 healthy preterm infants were studied using daytime polysomnography. Recordings of EEG, EOG, submental EMG, ECG, instantaneous HR, and thoracic and abdominal breathing movements were made. Term infants were studied on 3 occasions: (a) 2-3 weeks after birth; (b) 2-3 months post-term and (c) 5-6 months post-term. Preterm infants had an additional study at 36-38 wks gestational age.

A pulsatile air-jet (frequency 3Hz) delivered to the nostrils of the infant was used as an external arousal stimulus in both AS and QS. The probability of spontaneous arousal was calculated as the frequency of arousal which coincided with calibration of each stimulus. In determining the criteria for an arousal response we used changes in the physiological variables previously reported to be important in the overall arousal response. A change in ventilation pattern of more than 2 breaths, a body movement away from our stimulus, an associated increase in sub-mental EMG and an increase in heart rate of greater than 10% above baseline. All these changes needed to occur within 7 s of the stimulus onset. The 10s immediately preceding the stimulus presentation provided the baseline level used to assess the change in each variable.

Frequencies of responses in cardiovascular and behavioral variables for both stimulus-induced and spontaneous arousals were compared between sleep states, infant groups and across ages, using Chi Square analysis, a p value of <0.05 was considered significant.

A change in HR was consistently the least frequently occurring response across sleep states and ages in both groups of infants. Consequently, arousal was defined as a change in 3 or more of the 4 variables. Using this definition, changes in the four arousal criteria at the time of arousal were not different between AS and QS in the term infants at any of the ages studied. In the preterm infants only HR changes were significantly less frequent in QS compared to AS at 2-3 weeks post-term ($p<0.001$). Frequencies of responses in each of the four variables were also not different between the two groups of infants at matched post-conceptual ages. Additionally, there was no difference in the frequency of changes with increasing postnatal age in term infants and in preterm infants the only difference was in HR in QS.

In this study we have developed and tested a definition of arousal that can be used in young infants and which is consistent across sleep states, postnatal age and gestational age. The definition is simple to use and does not require sophisticated analysis of EEG.

This project was supported by SIDSaustralia, Sudden Infant Death Research Foundation (South Australia) and SIDassist

ACUPUNCTURE TO THE SACRAL SEGMENT PROMOTES SLEEP STATES AND RELAXES THE URINARY BLADDER

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It is known clinically that acupunctural stimulation to the sacral segment suppresses hyperactivity of urinary bladder, resulting in prevention of enuresis. To elucidate the mechanisms of this suppression, we examined effects of the acupuncture on the mobility of urinary bladder in urethane anesthetized rats simultaneously with those on electroencephalogram (EEG).

Rats were anesthetized with urethane (1.0 g/kg i.p.). Polyethylene catheter was inserted to the urinary bladder to record the pressure and to infuse saline. Stainless steel bolts for recording EEG were screwed to the skull overlying the frontal and parietal cortices. An acupunctural needle was set at the periosteum of the 3rd segment of sacrum, and the needle was rotated manually at a speed of about 3 turns/sec for 1 minute as acupunctural stimulation. The stimulation was applied mainly when the bladder contracted spontaneously after infusion of saline (0.4 – 0.8 ml). During experiment under urethane anesthesia, high amplitude slow-wave EEG and slow-wave EEG with slightly faster and lower amplitude appeared alternatively; each condition lasted several seconds or more. Spontaneous bladder contraction was observed mainly in the latter condition.

The acupunctural stimulation completely suppressed the contraction for 2 to 23 minutes in 41 of 106 cases. In 28 of 41 successive cases, EEG pattern shifted to high amplitude slow waves. The EEG change was observed only when the stimulation suppressed the bladder contraction. The same EEG change could be observed by the acupunctural stimulation applied when the bladder was empty with no contraction.

The results suggest that the acupunctural stimulation to the 3rd sacral segment affects sleep promoting systems as well as micturition systems.

THE FINGERS HEAT UP AS WE FALL ASLEEP

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Introduction: Originally it was believed that the evening decline in core body temperature was the best predictor of sleep initiation (1). However, more recently the increase of distal skin temperature (hands and feet) has been shown to be more related to sleep latency(2). Further exploration of the physiology associated with sleep initiation would seem to be warranted, especially for its possible relevance to a better understanding of sleep difficulties such as sleep onset insomnia. Most studies have examined distal skin temperatures with relatively long time intervals of measurement only over the typical bed retiring period of time. Therefore, the present study investigated the rapid changes of finger temperature from lights out to sleep onset at various circadian phases.

Method: Fourteen healthy, good sleeping subjects (11 males, 3 females, mean age = 28.14 yrs) participated in a modified 48-hour wakeful bedrest constant routine while PSG was monitored continuously and rectal temperature (RT) and skin temperature (ST) of the palmar distal surface of the right index finger was recorded at 30-second intervals. Across this 48-hour period multiple sleep latency tests (MSLTs) were administered every half hour. The sleep latency was the interval between lights out and the first of three consecutive 30-second epochs of sleep after which time the subject was aroused from sleep.

Results: Baseline finger ST was recorded half-hourly before the start of each MSLT. There was a significant circadian rhythm of baseline ST with average peak of 33.5 deg C at 0300 hrs and nadir of 31.5 deg C at 1600 hrs. The core RT circadian rhythm nadir and peak respectively followed these ST phases by 2-3 hours. During the MSLT trials ST showed consistent changes. During physical adjustment to supine position at the beginning of each trial there was a drop of ST of about 0.6-0.8 deg C over a period of one minute. This was followed by a steady rise of ST at a rate of about 0.6 deg C per minute until the trial was terminated at the third consecutive epoch of sleep or, in the case of longer sleep latencies, until ST leveled out at an asymptotic value. The asymptotic values had a circadian rhythm in phase with the baseline ST rhythm but 1-4 deg C higher depending on the initial starting baseline values and because ST never exceeded about 35 deg C.

Conclusions: The circadian rhythm of finger temperature is almost the reciprocal of core temperature, probably serving the function of helping to drive the circadian rhythm of core temperature through heat dissipation from vasodilation or conservation from vasoconstriction. The initial drop of ST with settling down may result from increased sympathetic vasoconstriction associated with the brief muscular effort required to adjust to the sleeping position. The subsequent, more pronounced increase of ST would be a result of decreased sympathetic tone preceding sleep onset and be a part of what has been described as the "sleepening" process. It would be of interest to compare the magnitude of this normal response with that in sleep onset insomniacs who may instead show a sympathetic arousal as indicated by a further drop of ST when presented with an opportunity to fall asleep.

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MULTIPLE SITES OF ENDOGENOUS EXCITATION OF THE RESPIRATORY SYSTEM IN REM SLEEP

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Background. Physiological regulation in REM sleep is characteristically non-homeostatic. Control occurs with internal feedforward systems rather than reflexively through feedback. The logic, origin and sites of action of this feedforward control are generally not known. In the respiratory system, feedforward control is evident as an endogenous excitatory drive, which can account for the rapid and irregular breathing and the lowered threshold to CO₂ of REM sleep. In the present study, hoping to determine more about this endogenous drive, we dissociated the neurons of the respiratory system by mechanically ventilating cats to apnea, in which state respiratory neurons and muscles are excitable, even active, but are not reciprocally bound into a rhythmic network, and compared the activity of medullary respiratory neurons to the activity of the diaphragm. Simultaneous excitation of neurons and the diaphragm in REM sleep would suggest that the endogenous drive has a single source and/or that the effect is hierarchical (i.e., from premotor to motor neurons). Independent excitation would indicate that there are multiple sources and sites of action of the endogenous drive.

Methods. Adult, intact and unanesthetized cats were implanted with electrodes for recording the EEG, pontogeniculo-occipital waves, and diaphragmatic electromyograms. A fistula was created to allow intubation of the trachea. During recording sessions, the animal breathed and was ventilated through the tracheal fistula, and a microelectrode was passed into the medullary ventral respiratory group. Action potentials of single respiratory neurons were recorded along with sleep parameters, diaphragmatic activity and ventilation. The animal was ventilated to apnea and CO₂ was injected into the air delivered by the ventilator to maintain a level that was approximately 85% of the end tidal level in NREM sleep.

Results and Conclusion. All possible patterns of excitation of the diaphragm and of a respiratory neuron were observed in REM sleep: i. simultaneous excitation of the neuron and diaphragm, as occurs during spontaneous breathing, ii. excitation of only the neuron, and iii. excitation of only the diaphragm. We conclude that there are multiple sources of endogenous drive to the respiratory system in REM sleep and/or that gating distributes the drive independently to the oscillator, and pre-motor and motor neurons.

Support: NIH HL21257 & HL62589, GAANN P200A80102

HYPOCAPNIA INHIBITS REM SLEEP IN CATS

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At altitude, ventilation is increased, and sleep is disrupted. The increased ventilation in response to hypoxia at altitude causes hypocapnia. To determine whether hypocapnia could contribute to altitude-induced sleep disturbances, we studied the effect of different levels of CO₂ and O₂ on sleep. Adult cats were prepared for recordings of sleep and respiratory (airflow, tidal CO₂, intratracheal pressure and diaphragmatic EMG) parameters. Three protocols in five intact, unanesthetized cats were used. Recording periods were three hours long. In the first protocol (hypocapnic normoxia) the animals were intubated through a tracheal fistula, connected to a ventilator and ventilated to apnea while CO₂ was held constant at either 65%, 75%, 85% or 95% of eupneic levels. In the second protocol (hypocapnic hypoxia) animals were intubated and allowed to breathe hypoxic gas mixtures (10-15% O₂ in N₂) and CO₂ was allowed to fall. In the third protocol (isocapnic hypoxia) animals were intubated and allowed to breathe hypoxic gas mixtures and CO₂ was maintained at the eupneic level. Control animals breathed room-air through a tracheal tube. The results obtained from observations of more than 300 REM periods revealed that, independent of the fraction of inspired oxygen, REM sleep was least at lower levels of CO₂. In hypocapnic normoxia and hypocapnic hypoxia REM sleep episodes and time decreased progressively as CO₂ decreased. In isocapnic hypoxia REM sleep episodes and time did not differ significantly from control animals. NREM sleep was not significantly affected by hypocapnia in any protocol. These results indicate that REM sleep disturbances at high altitude may be caused by hypocapnia.

Support: NIH HL21257 & HL62589, GAANN P200A80102

SLEEP INFLUENCES ON HUMAN CUTANEOUS BLOOD FLOW (CBF)
STUDIED BY LASER DOPPLER FLOWMETRY (LDF)

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NREM sleep induces a gradual reduction of sympathetic activity that reaches the lowest level during slow waves sleep, whereas REM sleep is characterized by a wide sympathetic variations. CBF is regulated by sympathetic adrenergic innervation that mediate vasoconstriction responses. There are some differences between cranial and limb skin sympathetic innervation.

Pletismographic studies evidence during sleep stages difference in CBF related to sympathetic activity in segmental sites.

OBJECTIVE: to study CBF variations during the different sleep stages in cranial and segmental sites of human healthy subjects. In particular to evaluate following parameters: 1) mean CBF, 2) vasomotion 3) spontaneous phasic variations, 4) vasodilatory response to local thermic stimulation, 5) correspondence between CBF vasoconstriction and sympathetic skin response (SSR) 6) differences between cranial and segmental districts of the above mentioned parameters.

METHODS: polisomnographic study of 10 subjects (age 24-36) for one adaptation night and for a second recording night, monitoring following parameters: 1) electroencephalography (C3, CZ, O2), 2) electroculography (ROC - LOC), 3) milioideo electromiography, 4) forehead and fingertip CBF by LDF 5) forehead and hand SSR 6) skin temperature 7) EKG 8) continuous digital blood pressure (Portapress), 9) oronasal flow and toraco-abdominal movements.

RESULTS: After following asleep appear spontaneous phasic vasoconstriction responses (VR) in limb sites not correlated with breathing, body or limbs movements, blood pressure variations. VR frequency decreases gradually during NREM sleep from stage 1-2 to slow waves stages. The highest VR frequency, in all subjects, is recorded during REM sleep. VR were not observed in cranial sites.

THE EFFECT OF SLEEP STATE ON VENTILATORY RESPONSES TO MILD HYPOXIA IN 2-3 MONTH OLD INFANTS

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An impaired ability to arouse and/or augment breathing in response to a respiratory challenge may be a cause of Sudden Infant Death Syndrome. Previously, ventilatory responses to hypoxia have been investigated in quiet sleep (QS) with little attention given to arousal. Our aim was to compare ventilatory responses to hypoxia in active sleep (AS) and QS in tests inducing arousal and compare them with tests that failed to arouse.

9 term infants, aged 2-3 months, were studied using daytime polysomnography during which ventilatory and arousal responses to hypoxia were characterised. Control data were obtained over one minute prior to 15% O₂ inhalation. Tests were terminated at either arousal, after 5 minutes, or if SpO₂ dropped to 85%. Breath-by-breath measurements of respiratory rate, tidal volume (\dot{V}_T /kg) and minute ventilation (\dot{V}'_i /kg) were obtained. Summaries of respiratory and blood gas variables were obtained for each 30 second epoch of 15% O₂ administration and expressed as a percentage change relative to control levels.

We performed 21 tests in AS (8 infants) and 28 in QS (9 infants). All AS tests were terminated by arousal and provided ventilatory data suitable for analysis. Tests that induced periodic breathing or were terminated due to SpO₂ reaching 85% were excluded from further analysis. 21 of the QS tests (in 7 infants) were suitable for breath-by-breath analysis. In 8 of these tests (6 infants) the infants did not arouse, while the remaining 13 tests (5 infants) induced arousal. Four infants both aroused and failed to arouse within repetitive QS tests. Repetitive tests were averaged within infants in each arousal or non-arousal condition.

In non-arousal tests during QS infants demonstrated an increase in \dot{V}'_i within 90s of hypoxia onset that decreased towards control levels by 5 minutes. In tests inducing arousal during QS, the initial hyperpnoea was greater than in QS non-arousal and AS tests, however, it too was unsustainable. Tests during AS demonstrated the lowest rate of initial hyperpnoea with \dot{V}'_i below control levels at arousal.

As arousal latency was significantly longer in QS than AS ($p < 0.001$), \dot{V}'_i was compared between AS and QS with respect to the point of arousal. While a marked increase in \dot{V}'_i prior to arousal was found in QS, there tended to be a slight decrease in \dot{V}'_i in AS tests prior to arousal.

This study demonstrates that infants are able to mount a greater ventilatory response to mild hypoxia in QS than AS, and that arousal may serve as a more potent protective mechanism in AS than in QS.

EFFECTS OF SLEEP STATE AND AGE ON AROUSAL RESPONSES TO MILD HYPOXIA IN INFANTS

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A major hypothesis to explain Sudden Infant Death Syndrome (SIDS) is that infants fail to arouse when confronted with a life-threatening event. While arousal responses of infants to hypoxia have been investigated in quiet sleep (QS), there is limited knowledge of their responses in active sleep (AS) and the effect of postnatal age on arousability. Therefore, the aim of this study was to compare the effects of sleep state longitudinally in infants up to 6 months of age.

10 healthy term infants born at 38-41 wks gestation (birthweight 3516±178g) were studied at 2-4 wks, 2-3 mo and 5-6 mo post term. Apgar scores at 1 and 5 minutes were 8-9 and 9-10, respectively. Infants underwent daytime polysomnography during which airflow was monitored using a purpose-built pneumotachograph. One minute control periods were obtained prior to each test, following which 15% O₂ was administered. Tests were terminated at either arousal, SpO₂ reaching 85% or at 5 minutes. Replicate tests were obtained.

Tests terminated due to SpO₂ reaching 85% were excluded from analyses. The probability of arousal in each sleep state was determined using chi-square analysis. Mean arousal latency was then calculated for AS and QS. Paired t-tests were used to investigate sleep state effects at each study age while one-way repeated measures ANOVA with Bonferroni post-hoc analysis was used to investigate age effects in each sleep state. Significance was taken at $p < 0.05$.

A total of 157 tests were successfully conducted, 94 in QS and 63 in AS. Ten of these tests were excluded from analyses due to SpO₂ reaching 85%. Replicate tests showed no evidence of habituation to hypoxia. All tests in AS induced arousal, whereas in QS 88% of tests resulted in arousal at 2-3 wks, 58% at 2-3 mo and 61% at 5-6 mo. Significant differences between sleep states ($p < 0.01$) in probability of arousal were present at 2-3 mo and 5-6 mo. Arousal latency was significantly longer in QS than AS at each age, however no age-related effects were found.

Our study has demonstrated that the probability of arousal to mild hypoxia is significantly lower in QS than AS at 2-3 months and 5-6 months post-term age. Furthermore, in infants less than 6 months, arousal latency is significantly longer in QS than in AS and is not affected by postnatal age.

Supported by the Sudden Infant Death Research Foundation (South Australia)

MICROINJECTION OF OREXIN A AND B INTO TRIGEMINAL MOTOR NUCLEUS INCREASES MASSETER MUSCLE TONE

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Orexins (hypocretin) are excitatory neuropeptides that are involved in a wide-range of physiological processes, including sleep-wake homeostasis, and regulation of motor control. Deficiency in orexin neurons is implicated in narcolepsy, which is characterized by poor sleep homeostasis and intrusions of REM sleep-like muscle atonia during wakefulness (cataplexy) (1). Recent evidence suggests that orexin-synthesizing neurons of the lateral hypothalamus are relatively active during wakefulness and inactive during sleep (2). Orexin neurons send axonal projections throughout the brain and spinal cord, including both trigeminal and hypoglossal motor nuclei (3), which contain motoneurons that express orexin receptors (4). Based on orexin projections to motor nuclei and the presence of orexin receptors on motoneurons, it is hypothesized that orexins are directly involved in the regulation of motoneuronal excitability across the sleep-wake cycle. To assess the role of orexins on muscle tone regulation, we tested the hypothesis that microinjection of orexin A and B into the trigeminal motor nucleus would excite masseter muscles, which are innervated by trigeminal motoneurons. In 6 decerebrate, unanaesthetized cats, we unilaterally microinjected 0.5 ml of 0.1 mM orexin A or B into the trigeminal motor nucleus while monitoring EMG activity of left and right masseter muscles. Injection of orexin A into the trigeminal motor nucleus increased (Wilcoxon's matched-pairs sign-ranked test: $df = 5$; $T = 2$; $p = 0.028$) ipsilateral masseter muscle tone (integrated EMG activity) by 154.3 ± 209.3 % (range: 4.1–482.8 %) compared with baseline control. The latency and duration of the response were: 7.0 ± 6.6 s (range: 1–19 s) and 17.3 ± 23.1 min (range: 1.5–52.0 min), respectively. Injection of orexin B into the trigeminal motor nucleus increased ($df = 5$; $T = 2$; $p = 0.028$) ipsilateral masseter muscle tone by 105.7 ± 153.0 % (range: 4.1–402.4 %) compared with baseline control. The latency and duration of the response were: 5.3 ± 3.1 s (range: 2–10 s) and 2.5 ± 3.5 min (range: 0.58–9.7 min), respectively. There was no significant difference between the effect of microinjection of orexin A or B on masseter muscle tone increase ($df = 5$; $T = 2$; $p = 0.5$), latency to response ($df = 5$; $T = 2$; $p = 0.916$), or response duration ($df = 5$; $T = 2$; $p = 0.173$). Unilateral injection of 0.5 ml of artificial cerebral spinal fluid into the trigeminal nucleus ($n = 5$) had no effect on the EMG activity of left or right masseter muscles. We conclude that orexin A and B postsynaptically excite trigeminal motoneurons, and are directly involved in the regulation of muscle tone. Loss of orexin function may therefore disturb normal motor processes, and thereby contribute to the motor system pathology of narcolepsy/cataplexy.

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AUTONOMIC MARKERS OF MICROAROUSAL DURING NORMAL HUMAN SLEEP

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Studies in patients with obstructive sleep apnea or with periodic leg movements have suggested that autonomic responses such as increases in heart rate and blood pressure may provide a sensitive marker of brief arousal during sleep. In healthy subjects, Sforza et al. (2000) showed a relationship between heart rate (HR) variation and cortical (i.e., microarousal –MA– or transient activation phases) or subcortical (i.e., bursts of K-complexes or of delta waves) indices of arousal. In the present study we further characterise the autonomic markers of arousal by analysing the relationship between changes in HR and blood pressure (BP) and microarousal occurrence in healthy subjects. After a habituation night, 12 males healthy volunteers (aged 32.5 +/- 6.8 years) underwent one experimental session during which sleep EEG, ECG, and beat-to-beat blood pressure (Portapres) were simultaneously recorded. Recordings were visually scored at 30-sec intervals according to Rechtschaffen and Kales and MA were identified using ASDA scoring rules. Autonomic arousals were defined as either periods of at least six successive decreases in R-R values (HR arousals) or as six successive increases in mean BP (BP arousals). Results showed a higher variability of HR and BP during a MA than during a same time frame preceding the MA (in 82.8 % of MA an increase in R-R intervals variance was observed and in 80.9 % an increase in mean BP variance). During the entire sleep period, modest correlations were found between the number of autonomic arousal and MA occurrences ($r=0.33$ for HR arousal and $r=0.23$ for BP arousal); however, during REM sleep, a strongest correlation was observed between MA and BP arousal ($r=0.67$) but not between MA and HR arousal ($r=0.16$) whereas the converse was true for SWS ($r=0.76$ between HR arousal and MA; $r=-0.04$ for BP arousal and MA). These results suggest that autonomic and cortical arousals are differently regulated in REM and SWS.

THERMOREGULATORY ADAPTATIVE MECHANISMS IN NEONATES' SLEEP STAGE

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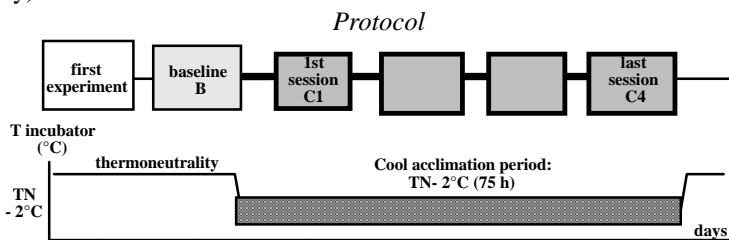
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Introduction Contrary to human adults and animals, body temperature regulation is fully efficient in Active (AS) and Quiet (QS) sleep in neonates. Thus, brief cool exposures increase the oxygen consumption (VO_2) through non shivering thermogenesis by stimulation of brown adipose tissue. During a prolonged cool condition, there are thermoregulatory adaptative processes leading to a protection of deep body temperature. In adults, this is explained by an increase of the sensitivity of the thermoregulatory system due to a change in the gain or a shift in the hypothalamic set temperature. In neonates, the changes of these adaptative mechanisms in the different sleep stages have never been studied and it would be unwise to extrapolate the results found in brief cool exposure to prolonged condition.

To assess whether adaptative thermoregulatory processes are operative in AS as well as in QS, 6 neonates (33 ± 4 weeks gestation, 19 ± 14 days old, 2.3 ± 0.2 kg) body mass were exposed during a prolonged cool exposure.

Material and methods Sleep, esophageal (T_{es}), skin (T_{sk}) temperatures and VO_2 were recorded during the baseline (B: $33.3 \pm 0.6^\circ\text{C}$), the first and last sessions of the cool acclimation period which lasted 75 hrs (air temperature: 2°C under the thermoneutrality).



Results The first cool exposure did not affect T_{es} whereas T_{sk} sharply decreased. VO_2 increased by 25% throughout the acclimation period. This increase was significant in both AS ($+1.1 \pm 0.5 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$; $p=0.005$) and QS ($+1.6 \pm 0.4 \text{ ml}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$; $p=0.005$) and was not significantly different between the sleep stages. The sensitivity of thermoregulatory system, described by the ratio VO_2/T_{es} and VO_2/T_{sk} , was significantly increased by cool acclimation in both AS and QS.

Conclusion The results pointed out that the adaptative thermoregulatory processes occurring during cool exposure are operative in AS and QS. These processes can be mediated through changes at the level of the central controller (changes in the gain and/or in the hypothalamic set point temperature) and/or by an increase in the sensitivity of the thermoregulatory effector (brown adipose tissue).

Keywords sleep, neonate, thermoregulation, cool acclimation

EFFECTS OF PROLONGED SLEEP LOSS ON GENE EXPRESSION

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Knowledge of the molecular correlates of sleep and wakefulness is essential if we are to understand the restorative processes occurring during sleep and the cellular consequences of sleep deprivation. To this end, we have recently performed a systematic screening of gene expression across behavioral states. We have compared mRNA levels of ~10,000 genes in the rat cerebral cortex after 3-8 hours of spontaneous sleep, sleep deprivation, or spontaneous waking (1-3). Only <0.1% of these genes was differentially expressed across behavioral states. A few of them, unknown, had higher expression in sleep relative to waking/sleep deprivation. By contrast, most genes were upregulated in waking/sleep deprivation relative to sleep. They included immediate early genes, mitochondrial genes, growth factors, and chaperones. In this study we want to extend this analysis to characterize the molecular consequences of long-term sleep deprivation. Thus, we have performed a systematic screening of cortical gene expression in rats sleep deprived for 4-10 days using the disk-over-water apparatus.

Nine pairs of long-term sleep deprived (TSD) rats and yoked control (TSC) rats were sacrificed when body temperature was at baseline level (n=4), 1°C below baseline (n=3), or 5°C below baseline (n=2). Body temperature in TSC rats was always at baseline level. Non-REM sleep and REM sleep were reduced by 80% and 96% in TSD rats, and by 28% and 37% in TSC rats, respectively. Total RNA was extracted from the cerebral cortex and used to screen ~10,000 genes as before (1-3). Almost all genes previously found (1-3) to be upregulated by waking and short-term sleep deprivation did not change their expression or were down-regulated in TSD rats. In fact, almost all genes whose expression was affected by prolonged sleep loss showed lower mRNA levels in TSD rats relative to TSC rats and to normally sleeping, spontaneously awake, or short-term sleep deprived rats. Only 2 genes, coding for the enzymes arylsulfotransferase (AST) and serum/glucocorticoid-regulated serine/threonine kinase, were upregulated in TSD rats. The induction of AST appeared to be related to the duration and/or intensity of sleep loss.

AST, in rodents, is responsible for the catabolism of catecholamines and its strong induction in TSD rats suggests that sleep may be important to interrupt the activity of catecholaminergic systems active during waking. These results also suggest that sustained sleep loss may be associated with decreased levels of many cortical transcripts. (Supported by Neurosciences Research Foundation). (1) Cirelli and Tononi, *Molec Brain Res* 56:293,1998; (2) *ibid*, *Brain Res* 885:303,2000; (3) *ibid*, *J Neurosci* 20:9187,2000.

CEREBRAL CIRCULATION IN SLEEP: RESPONSE TO HYPOXIA

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Rapid-eye-movement (REM) sleep is remarkable among sleep-wake states for its elevated cerebral blood flow (CBF). Recently it has been proposed that sensitivity of cerebral vessels to low PO_2 is a fundamental property that determines the level of CBF (Lenzi et al 1999). If this were to be true, oxygen levels should be a powerful determinant of CBF in all behavioural states. In two specific tests of the hypothesis we: (a) contrasted the response of the cerebral circulation during REM and non-REM sleep to transient, episodic arterial oxygen desaturations designed to mimic sleep apnea (*HYPOXIA IN SLEEP*); and (b) determined the changes of CBF associated with REM and non-REM occurring against a background of continuous hypoxia (*SLEEP IN HYPOXIA*).

Lambs ($n = 8$) were instrumented to record beat-beat cerebral blood flow (CBF) using a 2 mm diameter TRANSONICS™ transit time ultrasonic flow probe implanted around the superior sagittal sinus, and implanted with catheters to record cerebral perfusion pressure (CPP) and electrodes to define sleep-wake states. Arterial oxygen saturation (SpO_2) was recorded with a NELLCOR™ pulse oximeter. CBF was contrasted between REM and non-REM sleep occurring naturally during normoxia (FiO_2 0.21) and during hypoxia induced by reducing FiO_2 to 0.10 either (a) transiently (60 sec) within individual sleep epochs (*HYPOXIA IN SLEEP*); or (b) continuously (1 hr) across repeated sleep epochs (*SLEEP IN HYPOXIA*).

Under baseline (normoxia) conditions, CBF (ml/min) was significantly greater in REM than in non-REM (19 ± 1 vs. 15 ± 1 , $P < 0.01$, mean \pm SE, $n = 4$, t-test). During continuous hypoxia, significant increases of CBF from control values occurred in REM (24 ± 7 percent, $P < 0.05$) and non-REM (14 ± 5 percent, $P < 0.05$), and the greater CBF (ml/min) of REM (23 ± 2) compared with non-REM (17 ± 1) was preserved ($P < 0.02$). Similarly, there was preservation of the significantly greater CBF of REM compared with non-REM under conditions of transiently (60 sec) imposed hypoxia ($n = 6$).

As the major cerebral blood flow differences of sleep (REM > non-REM) are preserved in hypoxia, regardless of its duration, sensitivity of cerebral vessels to PO_2 appears to be a fundamental determinant of cerebral blood flow in sleep.

Lenzi P-L, Zoccoli G, Walker A & Franzini C. Cerebral blood Flow Regulation in REM Sleep: A Model for Flow-metabolism Coupling. Archives Italiennes de Biologie, 1999

BLOOD-BRAIN BARRIER PERMEABILITY TO GLUCOSE IN DIFFERENT BRAIN FUNCTIONAL CONDITIONS

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Blood-Brain Barrier (BBB) permeability modulation may subserve homeostasis of the brain's internal environment in front of local metabolic and hemodynamic changes. To clarify the issue we measured regional cerebral Blood Flow (BF) and glucose Permeability-Surface area product (PS) on adult Sprague-Dawley rats in different cerebral functional conditions, namely Quiet Wakefulness (QW, n=3) or Active Sleep (AS, n=2) after one week's recovery from surgery in unrestrained animals, and 1% Halothane, 70% Nitrous oxide anesthesia (HN, n=5). Briefly, $^3\text{H-D-Glucose}$ (30 mCi) and $^{14}\text{C-iodoantipyrine}$ (10 mCi) were bolus injected in the inferior vena cava, while blood was withdrawn from an arterial catheter for 12 s at a constant rate (1). The animal was then decapitated with an air-pressure operated blade, controlled from outside the box. Blood and brain tissue sample radioactivity measures (b scintillation spectrometer) allowed BF and PS computation in the medulla, pons, cerebellum, midbrain, diencephalon, hippocampus and cerebral hemispheres.

Mean PS value was significantly lower in the HN group than in either QW or AS (9.59 ± 3.64 vs. 15.69 ± 2.49 and 9.59 ± 3.64 vs. 14.49 ± 3.22 respectively, ml/min-100g, means \pm SD, $p < 0.001$, ANOVA with Dunnet's T3 correction). In each functional condition (HN, QW, SA) regional PS was highly and positively correlated with regional BF (Pearson, $p < 0.005$).

Since the fraction of perfused brain capillaries (hence capillary surface area S) is constant (2), our results can be attributed to changes of BBB glucose transporter kinetics reflecting cerebral energy demand and, within groups, by a positive correlation between mean regional BF and uniformity of individual capillary flow rates: a higher BF increase in the fraction of slowly perfused cerebral capillaries equals in fact functionally to an increase in S (3).

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