Sleep-Related Changes in the Regulation of Cerebral Blood Flow in Newborn Lambs

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Study Objectives: The interplay between cerebral perfusion pressure (CPP) and vascular resistance leads to fluctuations in cerebral blood flow (CBF). The relationship between fluctuations in CBF and those in CPP provides insight into the impact of the regulation of vascular resistance on CBF. The aim of this work was to study sleep-related changes in CBF regulation in newborn lambs, by quantifying the extent to which variability in CBF is related to that of CPP in the different wake-sleep states.

Design: Repeated-measurement within-subject.

Participants: 8 newborn lambs.

Interventions: Chronic instrumentation with electrodes (electrocorticogram, electrooculogram, nuchal electromyogram), an arterial catheter (arterial pressure), a subdural catheter (intracranial pressure), and an ultrasonic flow probe around the superior sagittal sinus (CBF).

Measurements and Results: The CPP (difference between arterial and intracranial pressure) and CBF data sequences during quiet wakefulness, rapid-eye-movement (REM) sleep and non-REM sleep were subject to spectral analysis. The fraction of CBF variability explained by CPP variability (CPP vs CBF squared coherence in the range 0.05-0.3 Hz) was highest in REM sleep (0.653) and lowest in non-REM sleep (0.413). The CBF variability (coefficient of variation due to fluctuations in the range 0.05-0.3 Hz) was higher than CPP variability in all states, albeit not significantly in REM sleep.

Conclusions: Results suggest that synchronized vasomotor fluctuations accounting for a quota of CBF variability not explained by CPP variability occur in all states in newborn lambs. Their relative contribution to CBF variability differs among wake-sleep states, being highest during non-REM sleep and lowest during REM sleep.

Key Words: Sleep; cerebral blood flow; cerebral perfusion pressure; spectral analysis; newborn; sheep

Abbreviations: AP, arterial pressure; CBF, cerebral blood flow; CI, 95% confidence interval for the mean; CPP, cerebral perfusion pressure; ECoG, electrocorticogram; EMGn, nuchal electromyogram; EOG, electrooculogram; ICP, intracranial pressure; LF, low-frequency; NREM, non-rapid-eye-movement; QW, quiet wakefulness; REM, rapid-eye-movement

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INTRODUCTION

THE CEREBRAL CIRCULATION IS REGULATED TO MATCH CEREBRAL BLOOD FLOW (CBF) TO THE VARYING METABOLIC NEEDS OF THE TISSUE PERFUSED, DESPITE POSSIBLE CHANGES IN SYSTEMIC ARTERIAL PRESSURE (AP). The CBF is determined by the ratio between cerebral perfusion pressure (CPP) and vascular resistance. The CPP may be computed as the difference between AP and intracranial pressure (ICP) because blood pressure in the collapsible venous vessels upstream of the dural sinuses is assumed to be equal to ICP (ie, the venous cerebrovascular bed is modeled as a Starling resistor).[1]

The interaction between AP, ICP, and cerebrovascular resistance leads to complex fluctuations in CBF at frequencies lower than the heart rate, as reported in adults[2-8] and in newborn babies.[9-11] Fluctuations in CBF might be essential to physiologic circulatory function and be generated by regulatory mechanisms. Alternatively, homeostasis might be maintained despite such fluctuations, dampened by regulatory mechanisms. Accordingly, the relationship between spontaneous fluctuations in CBF and those in CPP provides insight into the dynamic properties of cerebral autoregulation in health[4,5,7,8,12] and disease.[3,6] Slow fluctuations in CPP are followed by autoregulatory changes in cerebrovascular resistance, which cause a phase shift between oscillations in CPP and those in CBF and result in CBF fluctuations apparently preceding CPP fluctuations.[7] No such phase advance of CBF before CPP is evident at higher frequencies, which supports the view that cerebral autoregulation behaves as a high-pass filter.[3,5,6,12]

To our knowledge, there has been no quantitative assessment during sleep of the physiologic relationship between spontaneous fluctuations in CBF and those in CPP, possibly because of the lack of ICP measurements in physiologic conditions. The aim of this study was to provide insight into the impact that the regulation of vascular resistance has on CBF during sleep, by quantifying the extent to which variability in CBF is related to that of CPP in the different states of the wake-sleep cycle. The CBF was measured by an ultrasonic flow probe around the superior sagittal sinus, which yields continuous and quantitative measurements of blood flow.[13] The work was performed on newborn lambs, a model widely used to investigate sleep physiology during early postnatal development.

The extent to which CPP variability determines variability in CBF during sleep is of substantial importance in newborn life, when both total sleep duration and rapid-eye-movement (REM) sleep duration are at a lifetime maximum.[14] Prominent cardiovascular and ventilatory variability is commonly observed in REM sleep[15] and reflects the impairment of homeostatic regulation, which has been demonstrated in this state.[15,16] In REM sleep, cardiorespiratory disturbances may not be effectively buffered by the developing cardiovascular control system of the newborn,[14,17] particularly in pathophysiologic conditions in which protective arousal, circulatory, and respiratory responses may become inef-

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fective.[18] Moreover, CBF is at a lifetime minimum immediately after birth,[19] a period when AP is also low compared with adult levels (cf[20]). Therefore, local CBF regulation in the newborn represents a critical defense against the risks of brain hypoperfusion and brain capillary hypertension due to pathologic blood-pressure disturbances that may occur during REM sleep.

METHODS

Eight newborn lambs (Merino/Border-Leicester cross) were separated from their ewes within 24 hours of birth and housed within a Plexiglas cage. Once feeding independently (lamb milk replacer: Veanavite, Shepparton, Australia) and gaining weight normally, each lamb was surgically prepared for chronic study. All surgical and experimental procedures were performed in accordance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes established by the National Health and Medical Research Council of Australia and were approved by the Monash Medical Centre Committee on Ethics in Animal Experimentation.

Surgery and Experimental Procedures

Each lamb was anesthetized (halothane 1%-2%, nitrous oxide 60%, balance oxygen) and instrumented for study using sterile surgical techniques under artificial ventilation. A transit-time ultrasonic flow probe (2-mm diameter; Transonic Systems, Ithaca, NY) was positioned around the superior sagittal sinus to record CBF as previously described.[13] In brief, a 2-cm × 2-cm section of the skull overlying the intersection of the lambdoid and sagittal sutures was removed. The flow probe was carefully positioned around the superior sagittal sinus, taking care not to damage neural or vascular tissue. A rigid cap of dental acrylic was formed over the probe to stabilize it and to replace the section of skull that had been removed. The technique has been validated for use on the sagittal sinus of the lamb: the technique yields a beat-by-beat measurement of blood flow from 35% of total brain mass, principally from the frontal and anterior parietal lobes.[13] Nonocclusive saline-filled catheters (0.86 mm id, 1.52 mm od) were inserted into the femoral artery for blood-pressure monitoring and blood sampling and into the jugular vein for drug infusion. A catheter (1.57 mm id, 2.41 mm od) was positioned under the dura to record ICP. Pairs of Teflon-coated stainless-steel wires were implanted on the parietal cortex (electrocorticogram, ECoG), at the inner and outer canthus of the left eye (electrooculogram, EOG), and in the dorsal musculature of the neck (nuchal electromyogram, EMGn).

Quiet wakefulness (QW) was identified when the lambs were lying down, when the ECoG displayed a pattern of low-voltage high-frequency activity, and when eye movements and EMGn tone were present. In non-REM (NREM) sleep, the ECoG displayed a pattern of high-voltage low-frequency activity, eye movements were absent, and EMGn tone was reduced compared with that in QW. During REM sleep, the ECoG displayed a pattern of low-voltage high-frequency activity, REMs were present, and EMGn tone was absent.

Conditions of Study

After a minimum of 72 hours postoperative recovery, the lambs were studied for 1 to 3 days, at the age of 18 ± 5 days (mean ± SD). During the study periods, the lambs' cages were partitioned to prevent the lambs from turning around, while still allowing freedom to move forward and backward and to stand up and lie down. Food was available ad libitum throughout the study, and room temperature was maintained between 22°C and 25°C. The flow probe was connected to the flowmeter (model T101 Ultrasonic Blood Flowmeter, Transonic Systems, Ithaca, NY), which, along with the electrodes, was connected to an amplifier and signal conditioner (Cyberamp 380, Axon Instruments; Foster City, Calif). Electrophysiologic signals were filtered with the signal conditioner (0.3-80 Hz, 0.3-80 Hz, and 30-80 Hz, for ECoG, EOG, and EMGn, respectively). Vascular and intracranial catheters were connected to calibrated strain-gauge manometers (Cobe CDX III, Cobe Laboratories; Lakewood, Colo), which in turn were connected to the signal conditioner. All pressures were referenced to the midthoracic level when the lambs were lying down. Pressure and flow signals were low-pass filtered at 100 Hz and 30 Hz, respectively, and along with the electrophysiologic signals, were stored on a computer (486 DX/50) at a sampling rate of 200 Hz, using an analog-digital converting board (model 4801A, ADAC; Woburn, Mass) and acquisition software (CVSOFT Data Acquisition and Analysis Software, Odessa Computer Systems; Calgary, Canada).

Data Analysis and Statistics

After the study was completed, the stored physiologic signals were reviewed to reject artifacts, including movement artifacts, spontaneous awakenings, or sleep-state transitions. Data sequences, 120 seconds long, sampled at a rate of 200 Hz, were selected during stable states of QW, REM sleep, and NREM sleep. The 120-second duration was chosen as a compromise between the length of the sequence and the absence in the same sequence of artifacts. A total of 86 epochs were collected during QW, 218 during NREM sleep, and 95 during REM sleep. In 1 lamb, we were unable to collect artifact-free epochs in QW, and in 2 other lambs, we could not obtain artifact-free epochs in REM sleep. Signal analysis was performed with the software package MATLAB (The MathWorks, Inc, Natick, Mass).

The CPP was computed as AP-ICP. Averages for CPP and CBF were calculated over each sequence. Data were then subject to spectral analysis by the construction of average periodogram.[21] Time series were divided into 5 segments of 60 seconds each, with a 75% overlap.[3] Data in each segment were regressed versus time, and the expected value was subtracted to avoid contribution of the linear trend to low-frequency power. Detrended data were then multiplied by a full cosine-tapered (Hanning) window to minimize spectral leakage and transformed with the discrete Fourier transform algorithm. Spectra obtained for the 5 data segments were averaged to minimize contributions from variable noise and to sharpen reproducible spectral components. An additional scaling factor was applied equal to the mean-squared value of the time-domain window, to maintain windowing operation at unity gain.[21] The lowest frequency bin considered for analysis was 0.05 Hz, to minimize bias in power estimates due to detrending and windowing procedures on short data segments.

From a mathematical viewpoint, the power spectrum of a signal can be regarded as the distribution in the frequency domain of the time-domain signal variance. Therefore, for a given frequency range, we estimated variance, SD, and coefficient of variation of the signals due to fluctuations in that frequency range. Cross-spectral analysis was generated from the same 5 data sets of signals used in average periodogram analysis to estimate a magnitude-squared coherence function and the system gain and phase factors.[21]

The value of the squared coherence function ranges from 0 to 1 and reflects the fraction of power of the output signal (ie, CBF) that can be linearly related to power of the input signal (ie, CPP) at each frequency: a squared coherence of 0.5 implies that 50% of CBF variability is linearly related to CPP variability at a given frequency. Low values of squared coherence may indicate extraneous noise in the measurements, nonlinearity of the system relating CPP to CBF, or dependence of CBF upon other inputs besides CPP.[4,21] The significance of the observed values of coherence was tested by comparison with the random level of coherence computed after shifting the input and output data segments by 60 seconds relative to each other. Before computing averages, Fisher's z-transformation was applied to coherence values, which yielded identical variance of the coherence estimate for all frequency bins.[22] Statistical tests were performed on Fisher's z-transformed coherence values.

The gain factor indicates the magnitude of the change in the output signal (ie, CBF) determined by a unity change in the input signal (ie, CPP) at a given frequency. The phase factor indicates the shift in radians needed to align CPP and CBF changes at a given frequency[3]; we report

a positive phase shift when CBF changes "precede" CPP changes.[7] If CBF contains oscillatory components independent of CPP, random error of both gain and phase factors will arise, its magnitude increasing as squared coherence between CPP and CBF decreases.[21] In the presence of low squared-coherence values, random components would highly affect gain and phase estimates; therefore, average gain and phase relationships between CPP and CBF were computed only on frequency bins in which CPP versus CBF squared coherence was at least 0.5.[3] Moreover, for each wake-sleep state, gain and phase factors were obtained only from lambs in which the mean value of CPP versus CBF squared coherence in the frequency band of interest was at least 0.5.[7]

Mean values for each wake-sleep state were calculated for each animal. Mean values of Fisher's z-transformed coherence and the upper and lower bounds of the 95% confidence interval for the mean (CI) were retransformed, squared, and reported in the text and in the figure. Differences of squared coherence from 0.5 and differences of phase shift from 0 were assessed by 1-sample *t* test. Differences among wake-sleep states were detected by nonparametric repeated-measures analysis of variance on ranks (Friedman test) and isolated by Wilcoxon test. Differences between actual and random coherence level and between coefficients of variation were assessed by Wilcoxon test. The effect of CPP variance on CPP versus CBF coherence was tested by analysis of covariance applied to all data sequences, with wake-sleep state as a factor (3 levels) and with the coefficient of variation of CPP as a covariate. Statistical testing was performed using standard procedures (SPSS, http://www.spss.com) with a *P* value of less than .05 considered to be statistically significant. Data are means ± SD in the text and tables, with n indicating the number of animals.

RESULTS

Arterial blood gases and pH in QW were similar to those previously recorded in healthy lambs[13,23] (pH = 7.43 ± 0.01, arterial PO_2 = 102 ± 12 mmHg, arterial O_2 saturation = 95 ± 4%, arterial PCO_2 = 42 ± 5 mmHg, hemoglobin = 7.8 ± 1.7 g/dL, and base excess = 3 ± 3 mmol). As previously found,[23] there were no differences in arterial blood gas or pH among wake-sleep states in the lamb.

The mean value of CPP did not differ among wake-sleep states, but behavioral state differences did exist in the mean value of CBF (Table 1), which was significantly lower in NREM sleep than in REM sleep and QW. Variances of CPP and CBF showed a similar frequency distribution in all behavioral states, with a substantial contribution at frequencies at the lower end of the spectrum and a minimum around 0.3 Hz. A high-frequency peak was observed at frequencies between 0.3 and 1.2 Hz, comparable with values of breathing rate reported in the lamb.[24] A peak at heart rate was observed in both signals (Figure 1).

The following analysis focused on fluctuations in the low-frequency band (0.05-0.3 Hz) to study the relationship between CPP and CBF assuming effective cerebral autoregulation: autoregulation behaves as a high-pass filter, its effectiveness subsiding as the frequency of pressure oscillations increases.[3,5,6,12]

Table 1 shows that CPP variance was higher in REM sleep than in either QW or NREM sleep ($P < .05$). The CBF variance was lower in NREM sleep than in either QW or REM sleep ($P < .05$). The CPP and

Table 1—Cerebral perfusion pressure and cerebral blood flow across wake-sleep states

	QW (n = 7)	NREM sleep (n = 8)	REM sleep (n = 6)
Mean values			
CPP	57.5 ± 3.5	58.9 ± 4.4	58.7 ± 7.7
CBF	19.4 ± 5.5*	16.0 ± 5.6	20.5 ± 5.4*
LF variances			
CPP	1.53 ± 0.52	1.13 ± 0.50	4.58 ± 2.36*†
CBF	0.90 ± 0.67*	0.38 ± 0.26	0.91 ± 0.57*

Values represent mean ± SD.
QW refers to quiet wakefulness; NREM, non-REM sleep; REM, rapid-eye-movement sleep; LF, low frequency (0.05-0.3 Hz); CPP, cerebral perfusion pressure (mean values, mmHg; variance, $mmHg^2$); CBF, cerebral blood flow (mean values, $mL \cdot min^{-1}$; variance, $mL^2 \cdot min^{-2}$).
*$P < .05$ vs NREM sleep
†$P < .05$ vs QW

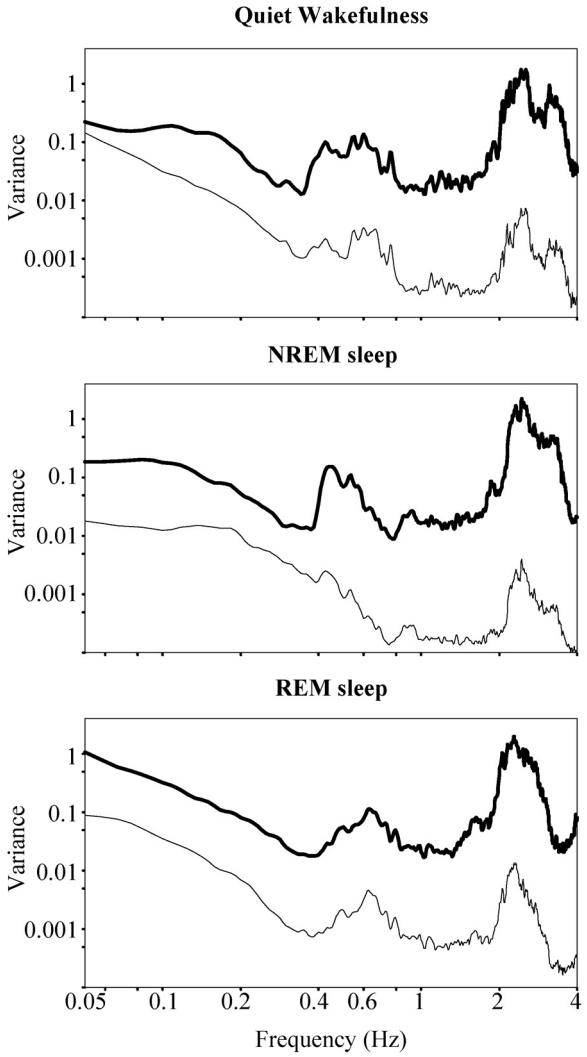

Figure 1—Frequency distribution of the variance in cerebral perfusion pressure and in cerebral blood flow. Data are averages in 1 lamb. Upper lines, variance of cerebral perfusion pressure ($mmHg^2$); lower lines, variance of cerebral blood flow ($mL^2 \cdot min^{-2}$). Frequency distribution of variance shows a minimum around 0.3 Hz, a high-frequency peak at frequencies between 0.3 and 1.2 Hz, and a peak at heart rate between 2.5 and 3.5 Hz. REM refers to rapid eye movement sleep; NREM, non-rapid eye movement sleep.

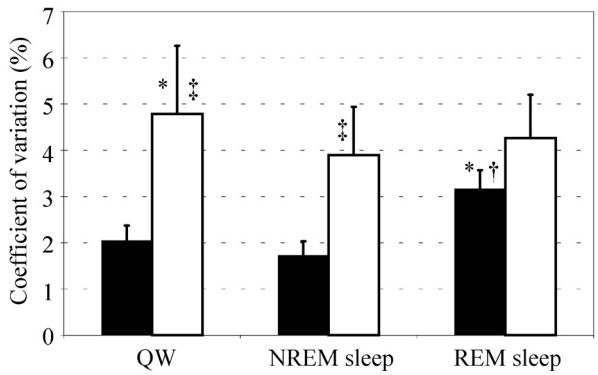

Figure 2—Coefficients of variation of cerebral blood flow (white bars) and cerebral perfusion pressure (black bars) due to fluctuations in the range 0.05-0.3 Hz. Mean values are shown. Error bars represent 1 SD. QW (n = 7) refers to quiet wakefulness; NREM (n = 8), non-REM sleep; REM (n = 6), rapid-eye-movement sleep. *$P < .05$ vs NREM sleep; †$P < .05$ vs QW; ‡ $P < .05$ vs coefficient of variation of cerebral perfusion pressure.

CBF coefficients of variation are presented in Figure 2. The coefficient of variation of CPP was higher in REM sleep than in either QW or NREM sleep ($P < .05$); the coefficient of variation of CBF was lower in NREM sleep than in QW ($P < .05$). The coefficient of variation of CBF was higher than that of CPP in all states, the difference being significant in QW and in NREM sleep ($P < .05$) but not reaching statistical significance in REM sleep ($P = .075$). Accordingly, a greater variability in CBF with respect to that in CPP was readily evident from recordings of physiologic data, particularly in QW and NREM sleep (Figure 3).

Average squared coherence between CPP and CBF in the low-frequency band was 0.489 (CI, 0.427-0.547) in QW, 0.413 (CI, 0.371-0.454) in NREM sleep, and 0.653 (CI, 0.501-0.768) in REM sleep (Figure 4). Random level of CPP versus CBF squared coherence was 0.295 (CI, 0.271-0.319), 0.328 (CI, 0.303-0.353), and 0.257 (CI, 0.218-0.298) in QW, NREM sleep, and REM sleep, respectively. Mean values of coherence were higher than the corresponding random level in all states ($P < .05$). Coherence between CPP and CBF was highest in REM sleep and lowest in NREM sleep ($P < .05$). Squared coherence between CPP and CBF was significantly higher than 0.5 in REM sleep and lower than 0.5 in NREM sleep, whereas the difference from 0.5 was not significant in QW. A value of squared coherence higher than 0.5 indicates that more than 50% of the variability in CBF may be explained by the variability in CPP (see Methods). To investigate the role of changes in CPP variability on the differences we found in CPP versus CBF coherence among states, the relationship between CPP versus CBF coherence and CPP variability was also assessed on all data sequences. An analysis of covariance was performed, assuming CPP versus CBF coherence as a dependent variable, wake-sleep state as a factor, and CPP coefficient of variation as a covariate. After accounting for changes in CPP coefficient of variation, CPP versus CBF coherence still depended on the wake-sleep state ($F = 45.38$, $P < .001$).

In REM sleep, mean value of squared coherence in the frequency range 0.05 to 0.3 Hz was ≥ 0.5 in 5 out of 6 lambs, allowing computation of gain and phase factors (Figure 5). In the frequency range 0.05 to 0.1 Hz, phase shift was positive (0.40 ± 0.30 radians, $P < .05$), and gain tended to increase with frequency. Mean value of CPP versus CBF squared coherence in the frequency range 0.05 to 0.3 Hz was less than 0.5 in all lambs in NREM sleep and in 5 out of 7 lambs in QW, thus not allowing meaningful analysis of gain and phase data in these states.

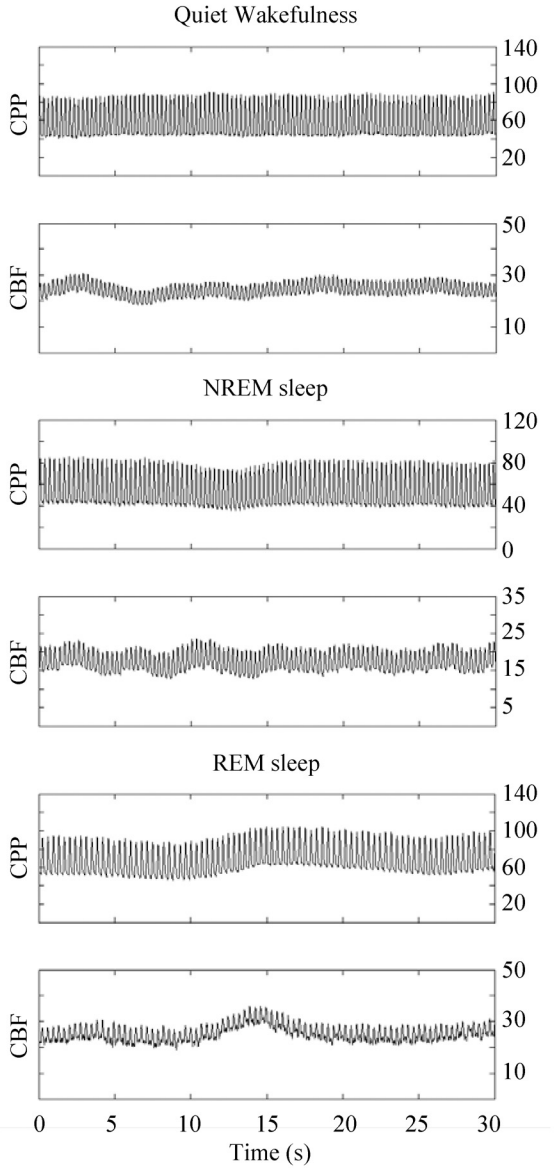

Figure 3—Representative tracings of cerebral perfusion pressure (CPP, mmHg) and of cerebral blood flow (CBF, mL·min^{-1}). NREM refers to non-REM sleep; REM, rapid-eye-movement sleep. In quiet wakefulness and NREM sleep, changes in CBF are evident in the absence of corresponding changes in CPP. In REM sleep, oscillations in CBF are more tightly bound to those in CPP.

DISCUSSION

The relationship between slow fluctuations in CBF and those in CPP was quantitatively assessed by spectral analysis in newborn lambs. Our study yielded 2 main findings. First, a significant fraction of CBF variability due to slow fluctuations is not explained by CPP variability but, rather, by cerebral vasomotion. Second, the relationship between slow fluctuations in CBF and those in CPP varies across the wake-sleep cycle, with CBF variability having a greater dependence on CPP variability in

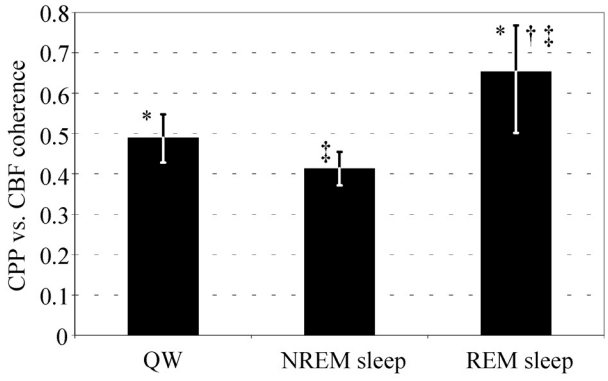

Figure 4—Squared coherence between cerebral perfusion pressure (CPP) and cerebral blood flow (CBF) in the frequency range 0.05 to 0.3 Hz. Retransformed and squared mean values and 95% confidence intervals for the means (upper and lower bounds) of Fisher's z-transformed coherence are shown. QW (n = 7) refers to quiet wakefulness; NREM (n = 8), non-REM sleep; REM (n = 6), rapid-eye-movement sleep. *$P < .05$ vs NREM sleep; †$P < .05$ vs QW; ‡$P < .05$ vs squared coherence value of 0.5.

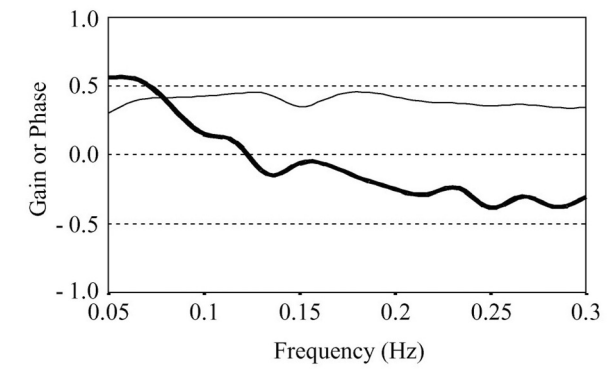

Figure 5—Group-averaged transfer function gain and phase in rapid-eye-movement sleep. Thin line, gain (mL·min^{-1}·mmHg^{-1}); bold line, phase shift (radians). n = 5. At frequencies lower than 0.1 Hz, an apparent phase lead of cerebral blood flow before cerebral perfusion pressure (ie, a positive phase shift) is evident.

REM sleep (0.653) than in the other behavioral states (QW, 0.489, and NREM sleep, 0.413).

These data show that CBF variability in REM sleep is strongly dependent on CPP variability in physiologic conditions. The highest values of CPP variability in REM sleep (Table 1) support the hypothesis that systemic cardiovascular regulation is altered in this state.[15,16] In pathophysiologic conditions, further increases in CPP variability in REM sleep would place the brain at risk of hypoperfusion or of excessive capillary pressure because of the tight link between CPP variability and CBF variability in this state. Arousal from sleep may represent a protective response.[24] However, specific pathophysiologic conditions may determine both cardiovascular disturbances and arousal depression in the newborn. Accordingly, repeated hypoxia in newborn lambs becomes ineffective in evoking protective arousal, circulatory, and respiratory responses during REM sleep.[18]

Both mean value and low-frequency variance of CBF were the lowest in NREM sleep, whereas values in REM sleep were similar to those in QW. These data agree with the hypothesis that, during NREM sleep, tight sleep homeostasis[15] is maintained, with the least need for the operation of local regulatory mechanisms. Few data are available in humans concerning variability in flow velocity in the middle cerebral artery during sleep. In adults, a periodicity of 20 to 75 seconds has been shown to be most prominent during REM sleep.[2] In healthy preterm infants, the coefficient of variation of fluctuations at a frequency from 0.5 to 6 cycles per minute was higher in REM sleep than in NREM sleep in a study by Rehan et al.[10] Conversely, in full-term newborn babies, amplitude of fluctuations at frequencies between 2 and 6 cycles per minutes has been shown to be higher in quiet sleep than in active sleep.[11] It is hard to reconcile such divergent results, notwithstanding differences in indexes analyzed; developmental differences and heterogeneity in the vegetative phenomenology of REM sleep[15] may partly account for the discrepancy.

By spectral analysis, we computed a coherence function between CBF and CPP as a quantitative index of the extent to which variability in CBF is related to that of CPP. Spectral analysis yields a linear approximation of the system studied, which is an accepted first step to its description: the technique has been widely used in the study of slow spontaneous fluctuations in cerebral hemodynamics.[3,4,6-9,12] In QW, NREM sleep, and REM sleep, we found that coherence in the low-frequency range between CBF and CPP was higher than its random value, indicating that a significant fraction of CBF variability at low frequencies is determined by CPP. In NREM sleep and in QW, however, squared coherence in the low-frequency range was lower than or not significantly different from 0.5, indicating that less than or almost 50% of CBF variability due to slow fluctuations was linearly related to that of CPP. In REM sleep, on the other hand, coherence analysis showed that, on average, 0.653 of the variability in CBF was linearly related to that of CPP.

In adult men during wakefulness, squared coherence between blood-flow velocity in the middle cerebral artery and AP was high (> 0.6) in the low-frequency and high-frequency range but not at very-low frequencies (lower than 0.04 Hz[4] or 0.07 Hz[5]). In newborn babies, slow oscillations (0.025-0.083 Hz) in flow velocity in the middle cerebral artery have been reported without similar variability in AP.[25] Species and technical differences (ie, analysis of venous volume flow vs analysis of arterial flow velocity) may account for the wider frequency range in which coherence was low in our study. Further work on coherence between CPP and CBF during early postnatal development is needed to understand whether developmental differences also play a role. We computed CPP as the difference between AP and ICP and assumed that low-frequency fluctuations in CBF are driven by fluctuations in CPP rather than by those in AP.[1,3,5] The CBF would not be independent of downstream venous pressure in case of primary blood-pressure increases at the dural sinuses to values higher than those of ICP, which would occur following jugular vein compression or Valsalva maneuver.[1] Because we studied low-frequency fluctuations in CBF during undisturbed wake-sleep states, when major changes in venous pressure are not expected, we assumed CBF was independent of changes in venous pressure.

At variance with transcranial Doppler sonography, the transit time flowmetry technique gives a quantitative measurement of mean volume flow.[13] We computed coefficients of variation of CBF and CPP to allow a direct comparison between amplitudes of fluctuations in the 2 variables. In all states, the coefficient of variation of CBF was higher than that of CPP; the difference was significant in QW and in NREM sleep but not in REM sleep ($P = .075$).

Our data can be interpreted in the sense that fluctuations in CBF result not only from fluctuations in CPP, but also from fluctuations in cerebrovascular tone, the relative contribution of the 2 varying among the states of the wake-sleep cycle. In QW and particularly in NREM sleep, low-frequency variability in CBF is mainly driven by variability in cerebrovascular tone, rather than in CPP, as suggested by a weak linear relationship (low squared coherence value) between CPP and CBF and by the finding that, after normalizing the respective amplitudes of fluctuations to CPP and CBF mean values, CBF fluctuated more than CPP. In REM sleep, CPP versus CBF squared coherence was higher than 0.5 and highest among wake-sleep states, and the difference between the coefficient of variation of CBF and that of CPP was positive, albeit not significantly. Thus, in REM sleep, the role of CPP in controlling CBF fluctuations prevails over that of variability in cerebrovascular tone.

The results of the present study suggest that synchronized vasomotor fluctuations occur in the cerebral circulation, independent of CPP variability. The occurrence of CBF fluctuations in the absence of corresponding changes in CPP was clearly visible in recordings in QW and NREM sleep (Figure 3). Arterial[26] and arteriolar[27] vasomotion have been observed in the cerebral circulation in various animal models. A synchronizing mechanism must be hypothesized to explain slow spontaneous oscillations recorded in CBF, as asynchronous oscillations originating from different anatomic locations would prevent the occurrence of oscillations in total CBF. Studies in the rat have led to the conclusion that arteriolar vasomotion in the brainstem is synchronous with that of the basilar artery.[26] It has been hypothesized that rhythmic vasomotion increases the range of oxygen diffusion through the tissue.[28] The regulation of vasomotion may thus finely match perfusion to the spatial and temporal pattern of tissue metabolic needs.

Analysis of covariance on all data sequences with CPP versus CBF coherence as the dependent variable, wake-sleep state as a factor, and CPP coefficient of variation as a covariate showed that, even after compensating for differences in CPP variability, significant differences existed among wake-sleep states ($P < .001$). This suggests that changes in CPP versus CBF coherence among states are driven not only by state-dependent differences in CPP variability, but also by differences in cerebrovascular-tone variability.

By inducing a 50% reduction in CPP, Grant et al[23] demonstrated that cerebral autoregulation is effective during sleep in newborn lambs, the speed and magnitude of vasodilatory reserves available for autoregulation being lower in REM sleep than either in NREM sleep or QW. The dynamic properties of cerebral autoregulation may be studied by analysis of coherence, gain, and phase shift between spontaneous fluctuations in CPP and CBF.[3-8,12] In NREM sleep and in QW, results of the present study suggest a substantial cerebral-vasomotor component independent of CPP; nonetheless, low CPP versus CBF coherence in NREM sleep and in QW is compatible with effective cerebral autoregulation in these states.[5,7] Conversely, high values of CPP versus CBF squared coherence in REM sleep suggest lower effectiveness of cerebral autoregulation in this state, in agreement with previous results.[23] Cerebral autoregulation to spontaneous CPP fluctuations is not completely absent in REM sleep, however, as shown by gain and phase data in this state (Figure 5). We found a positive phase shift at frequencies lower than 0.1 Hz, indicating that CBF fluctuations apparently lead CPP fluctuations in this frequency range. Previously, a similar phase lead of CBF before CPP was associated with autoregulatory adjustments in vascular resistance, which lagged behind fluctuations in CPP[7] in humans. In this respect, just as in the higher mean CBF and lower cerebrovascular resistance of REM sleep compared with NREM sleep,[16] there appear to be substantial similarities

between basic features of cerebral perfusion across species.

Other sleep-state related neural and metabolic factors may alter the effect that fluctuations in cerebrovascular resistance exert on CBF. Cerebral metabolic rates of oxygen and glucose consumption decrease from wakefulness to NREM sleep and return during REM sleep to values similar to or greater than those in wakefulness. These changes are accompanied by changes in the tonic level of vascular resistance and, hence, in CBF.[16] The activity of the locus coeruleus and the dorsal raphe nucleus is implicated in neurogenic vascular control and decreases from wakefulness to NREM sleep to reach the lowest level in REM sleep.[29,30] A significant tonic role of autonomic neural control in the regulation of CBF has elegantly been demonstrated in humans by Zhang et al.[8] Muscle sympathetic nerve activity decreases in NREM sleep and increases in REM sleep with respect to wakefulness,[16,31] although the concept of a uniform sympathetic tone is inadequate to explain the complexity of vegetative regulation during sleep,[16,31] and sleep-related changes in sympathetic outflow to the brain circulation await investigation.

In conclusion, CBF regulation undergoes changes across the wake-sleep cycle, in that CBF variability has a greater dependence on CPP variability in REM sleep than in QW and NREM sleep. By contrast, in NREM sleep, CBF mainly depends on synchronized oscillations in cerebrovascular tone. Studies investigating the functional implications of these differences between REM sleep and NREM sleep may shed light upon the mechanisms that regulate brain homeostasis in these 2 sleep states.

REFERENCES

1. Ursino M, Lodi CA. A simple mathematical model of the interaction between intracranial pressure and cerebral hemodynamics. J Appl Physiol 1997;82:1256-69.
2. Droste DW, Berger W, Schuler E, Krauss JK. Middle cerebral artery blood flow velocity in healthy persons during wakefulness and sleep: a transcranial Doppler study. Sleep 1993;16:603-9.
3. Blaber AP, Bondar RL, Stein F, et al. Transfer function analysis of cerebral autoregulation dynamics in autonomic failure patients. Stroke 1997;28:1686-92.
4. Kuo TB-J, Chern C-M, Sheng W-Y, Wong W-J, Hu H-H. Frequency domain analysis of cerebral blood flow velocity and its correlation with arterial blood pressure. J Cereb Blood Flow Metab 1998;18:311-8.
5. Zhang R, Zuckerman JH, Giller CA, Levine BD. Transfer function analysis of dynamic cerebral autoregulation in humans. Am J Physiol 1998;274:H233-41.
6. Hu H-H, Kuo TB-J, Wong W-J, et al. Transfer function analysis of cerebral hemodynamics in patients with carotid stenosis. J Cereb Blood Flow Metab 1999;19:460-5.
7. Hughson RL, Edwards MR, O'Leary DD, Shoemaker JK. Critical analysis of cerebrovascular autoregulation during repeated head-up tilt. Stroke 2001;32:2403-8.
8. Zhang R, Zuckerman JH, Iwasaki K, Wilson TE, Crandall CG, Levine BD. Autonomic neural control of dynamic cerebral autoregulation in humans. Circulation 2002;106:1814-20.
9. Reynolds KJ, Panerai RB, Kelsall AWR, Rennie JM, Evans DH. Spectral pattern of neonatal cerebral blood flow velocity: comparison with spectra from blood pressure and heart rate. Pediatr Res 1997;41:276-84.
10. Rehan VK, Fajardo CA, Haider AZ, et al. Influence of sleep state and respiratory pattern on cyclical fluctuations of cerebral blood flow velocity in healthy preterm infants. Biol Neonate 1996;69:357-67.
11. Ferrarri F, Kelsall AWR, Rennie JM, Evans DH. The relationship between cerebral blood flow velocity fluctuations and sleep state in normal newborns. Pediatr Res 1994;35:50-4.
12. Panerai RB, Dawson SL, Potter JF. Linear and nonlinear analysis of human dynamic cerebral autoregulation. Am J Physiol 1999;277:H1089-99.
13. Grant DA, Franzini C, Wild J, Walker AM. Continuous measurement of blood flow in the superior sagittal sinus of the lamb. Am J Physiol 1995;269:R274-9.
14. Walker AM, Horne RSC, Bowes G, Berger P. The circulation in sleep in newborn lambs. Aust Pediatr J 1986;22 (suppl. 1):71-4.
15. Parmeggiani PL. Behavioral phenomenology of sleep (somatic and vegetative). Experientia 1980;36:6-11.
16. Franzini C. Cardiovascular physiology: the peripheral circulation. In: Kryger MH, Roth T, Dement WC, eds. Principles and Practice of Sleep Medicine, 3rd ed. Philadelphia: WB Saunders; 2000:193-203.
17. Gaultier C. Cardiorespiratory adaptation during sleep in infants and children. Pediatr Pulmonol 1995;19:105-17.
18. Johnston RV, Grant DA, Wilkinson MH, Walker AM. Repetitive hypoxia rapidly depresses cardio-respiratory responses during active sleep but not quiet sleep in the newborn lamb. J Physiol 1999;519 Pt 2:571-9.
19. Volpe JJ. Neurology of the newborn. 4th ed. Philadelphia: WB Saunders; 2001.
20. de Swiet M, Fayers P, Shinebourne EA. Blood pressure in first 10 years of life: the Brompton study. BMJ 1992;304:23-6.
21. Bendat JS, Piersol AG. Engineering applications of correlation and spectral analysis. 3rd ed. New York: John Wiley & Sons; 1993.
22. Achermann P, Borbely AA. Coherence analysis of the human sleep electroencephalogram. Neuroscience 1998;85:1195-208.
23. Grant D, Franzini C, Wild J, Walker A. Cerebral circulation in sleep: vasodilatory response to cerebral hypotension. J Cereb Blood Flow Metab 1998; 18:639-45.
24. Horne RSC, De Preu ND, Berger PJ, Walker AM. Arousal responses to hypertension in lambs: effect of sinoaortic denervation. Am J Physiol 1991;260:H1283-9.
25. Anthony MY, Evans DH, Levene MI. Cyclical variations in cerebral blood flow velocity. Arch Dis Child 1991;66:12-6.
26. Fujii K, Heistad DD, Faraci FM. Role of the basilar artery in regulation of blood flow to the brain stem in rats. Stroke 1991;22:763-7.
27. Auer LM, Gallhofer B. Rhythmic activity of cat pial vessels in vivo. Eur Neurol 1981;20:448-68.
28. Secomb TW, Intaglietta M, Gross JF. Effects of vasomotion on microcirculatory mass transport. Prog Appl Microcirc 1989;15:49-61.
29. McGinty D, Harper RM. Dorsal raphe neurons: depression of firing during sleep in cats. Brain Res 1976;101:569-75.
30. Rasmussen K, Morilak DA, Jacobs BL. Single unit activity of locus coeruleus neurons in the freely moving cat. I. During naturalistic behaviors and in response to simple and complex stimuli. Brain Res 1986;371:324-34.
31. Somers VK, Dyken ME, Mark AL, Abboud FM. Sympathetic nerve activity during sleep in normal subjects. New Engl J Med 1993;328:303-7.