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Sleep-Waking Shifts and Cerebral Blood Flow in Stable Preterm Infants

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ABSTRACT. Cerebral blood flow was estimated on 60 occasions in 15 well infants, 29–34 wk of gestational age, 5–17 days after birth, using 133-Xenon clearance after intravenous injection. The sleep state of the infants was determined by biparietal electroencephalography, clinical observation, and tracings of heart rate and respiration. Blood flow was 22% higher in the 11 estimations made during wakefulness, when compared to the 17 estimations made during quiet sleep. There was no difference between blood flow in active and quiet sleep. Also there was no difference between blood flow during periods of trace alternant and blood flow during periods of continuous electroencephalographic activity. It is suggested that flow-metabolism coupling is present in stable, preterm infants. The absence of an increase in cerebral blood flow during active sleep as compared with quiet sleep suggests that the neurophysiologic and neurometabolic mechanisms of rapid eye movement sleep are not yet fully developed in preterm infants. (*Pediatr Res* 19: 1156–1159, 1985)

Abbreviations

CBF, cerebral blood flow
EEG, electroencephalography
REM, rapid eye movements

CBF is coupled to cerebral metabolic rate in normal brain. This coupling allows inferences regarding metabolic rate, which may be the more important physiologic variable, from blood flow data. The present study was undertaken to examine the coupling between cerebral activity as judged by clinical observation and EEG, and flow in the preterm infant. Blood flow has previously been found to vary considerably among stable, preterm infants (1). Cerebral blood flow can be estimated in newborn infants by the intravenous 133-Xenon technique (1, 2) which more commonly is used in adults.

MATERIALS AND METHODS

Fifteen infants, 29–34 wk gestation, admitted to the department of Neonatology, Rigshospitalet, were studied at the age of 5–17 days, after parental consent. The infants had suffered no major respiratory distress, perinatal asphyxia, intracranial hemorrhage, or other illness. On the day of study, all the infants were spontaneously breathing in room air, had no clinical evidence of persistent arterial ductus, needed no intravenous fluids, and were considered well.

Study procedure. After placing of a peripheral intravenous line for the administration of 133-Xenon, three disc electrodes for biparietal EEG, and three chest leads for ECG and respiratory wave, the infants were left untouched in a lateral or prone position. The total study procedure lasted 1½–3 h. One exception, infant 519 was initially studied on his mother's lap because he was difficult to calm. Infants who became restless or cried were comforted by holding their hands and/or feet. In a few instances wakefulness was provoked or stimulated by quiet talking or petting. Parents often were present during the procedure.

Measurements. In each infant, three to five estimations of CBF by 133-Xenon clearance were performed. The 133-Xenon was given as intravenous bolus injections (0.2–0.6 mCi/kg), increasing the amount from one estimation to the next. The total whole-body radiation was 30 mRad. The activity over one frontoparietal region was detected by a NaI crystal detector with a 20-mm long cylindrical collimation, 17 mm in diameter. Care was taken to avoid the detector pointing caudally and thus to include the airways in the counting geometry. The activity over the right side of the chest was detected by a NaI detector with a 40 mm long cylindrical collimation, 12 mm in diameter. The windows were set to 55–105 KeV. All detectors were shielded by 2 mm lead in their full length. Expired 133-Xenon was carefully removed from the field by suctioning. The activity was registered for 8 min after the injection. Remaining activity from the previous estimations was accounted for. The 133-Xenon clearance curves from the head and chest (Fig. 1) were analyzed by a computerised procedure as previously described (3). In brief, the chest clearance curve was corrected for contribution from the chest wall and then used as an estimate of the arterial input of 133-Xenon as a function of time. Due to the small amount of 133-Xenon used, the count rate of the cranial clearance curve was insufficient for two-compartment analysis (4). Therefore the least squares fit of a single exponential convoluted with the estimate of the arterial input function was obtained. The slope, the rate constant of the exponential, was calculated from a 3-min segment of the cranial clearance curve starting 30 s after the peak of the arterial input (3). Furthermore, the slope in an earlier 1-minute period was estimated. The period over which this slope was fitted was adjusted to compensate for small differences in arrival of 133-Xenon to the brain, following the intravenous injection and the clearance from the lungs. The starting point was selected, when the preceding accumulated arterial input equaled the accumulated arterial input in the next minute. The two slopes were multiplied by the neonatal brain-blood partition coefficient of 0.8 ml/g, adjusted for blood Hb concentration (3), to obtain a late slope index and an early index expressed in ml/100 g/min. Monoexponential slope indices represent a mix of the highly and the lowly perfused areas of the brain within the counting geometry of the cranial detector (virtually the entire

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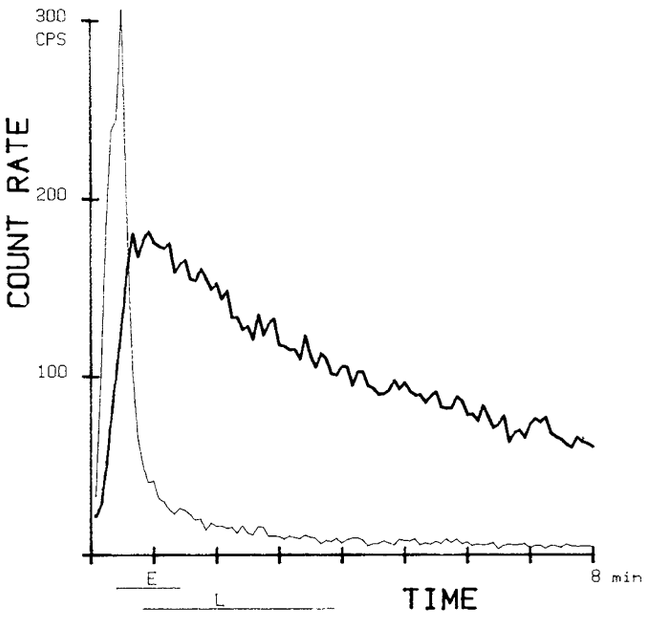

Fig. 1. Cranial (*bold line*) and chest (*thin line*) clearance curves after intravenous 133-Xenon bolus injection in infant 546. The vertical bars indicate the time periods of fitting the early slope index (*E*) and the late slope index (*L*).

brain of small preterm infants). The early slope index is greater than the late slope index since the highly perfused areas, such as cortex, are heavily represented. Also the early slope index is more sensitive to changes in the perfusion of these areas compared to the late slope index (5). The late slope index, on the other hand, represents all areas approximately according to their relative weight and is computationally more stable (3). The scintillation equipment and software were delivered by Novo Diagnostic Systems; the software was modified and extended by ourselves.

EEG input was filtered and the amplitudes integrated and recorded (Cerebral Function Monitor, Devices Ldt or Cerebral Function Monitor, Criticon). Trace alternant was characterized by quiet periods with very low integrated amplitude alternating with bursts of normal or high amplitude (Fig. 2). In all infants studied cyclic changes between this pattern and periods of more continuous EEG activity were apparent.

Instantaneous heart rate and respiratory waves were recorded on paper (Fig. 2). Clinical observations of gross motor activity and face and eye movements were recorded, as well as all interferences with the infant and the timing of CBF estimations.

The sleep state during CBF estimations were classified at a later time, without knowledge of the results. Four sleep states were defined.

1) *Quiet sleep.* The infant was without gross motor activity, eyes were closed, no eye movements were noted, heart rate variability was low, respiratory wave regular or regular periodic and EEG showed predominantly trace alternant.

2) *Active sleep.* The infant was without major gross motor activity, eyes were closed, eye movements were sometimes noted, face movements were often noted, 15 to 45 s periods of heart rate acceleration synchronized to periods of irregular breathing were usually seen; otherwise moderate heart rate variability was noted with regular or periodic breathing. The EEG showed predominantly continuous activity.

3) *Wakefulness.* The infant showed sequences of gross motor activity or face movements often clearly responsive to interferences, the eyes were open or closed, eye movements absent or present, and heart rate and respiration periodically grossly irregular. The EEG showed predominantly continuous activity.

4) *Unclassifiable.* This state was characterized by incompatibility of clinical observations, heart rate, and respiratory wave patterns and EEG, or change of state during the estimation of CBF. Two instances of crying were included in this group.

Transcutaneous pCO_2 was continuously monitored in five infants (Radiometer); in all infants $PaCO_2$ and Hb were determined on arterialised capillary blood at the end of the procedure. In seven infants arterial blood pressure was determined by occillometry (Dynamap) during all the CBF estimations; in the remain-

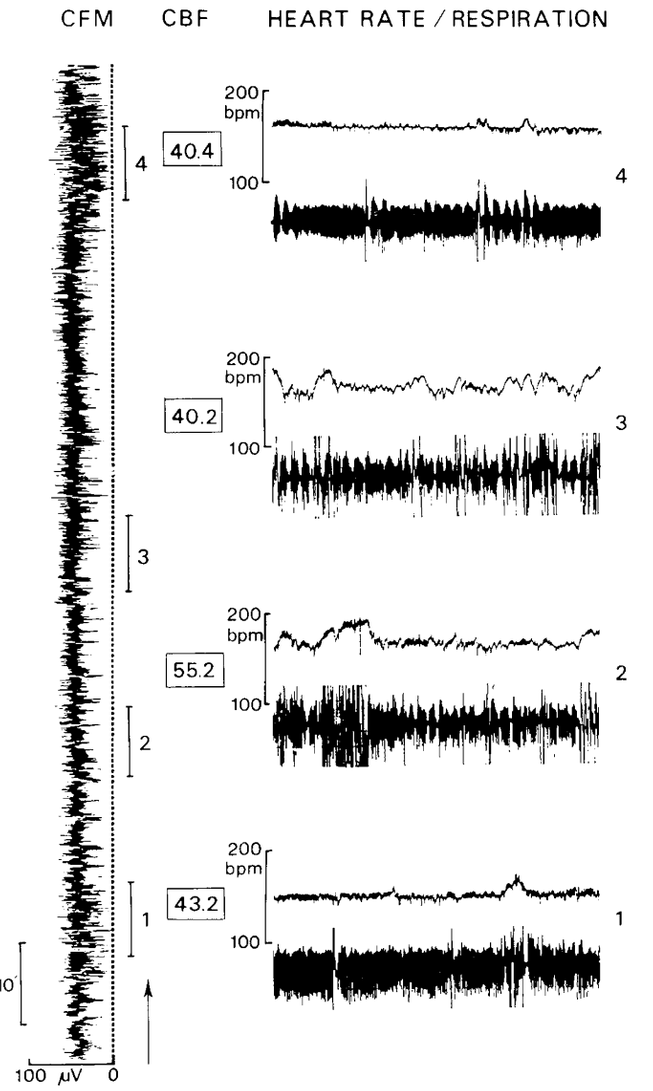

Fig. 2. Tracings of EEG, heart rate, and respiration in infant 546 at four CBF estimations. EEG was continuously monitored by a cerebral function monitor (*CFM*). The tracing is read from bottom up, note the slow time scale. The periods of the four CBF estimations are indicated by numbers. The heart rate and respiration during the entire length of each CBF estimation are shown to the right together with the CBF value (early slope index). The activity of the infant was described as follows: *1.* (Quiet sleep): closed eyes, no movements except for one startle, regular breathing except for one period of transient increase. EEG discontinuous. *2.* (Awake): eyes open and shut, gross movements present, respiration irregular. Heart rate irregular. EEG continuous. *3.* (Awake): appears somewhat drowsy, a burst of suckling movements at the beginning, gross movements present. Eyes open and shut. Gently stimulated during the estimation. Periodic breathing at the beginning of CBF estimation, irregular at the end. EEG continuous. *4.* (Quiet sleep): eyes closed, no movements, regular or periodic respiration. Regular heart rate. EEG discontinuous.

ing infants blood pressure was determined at the end of the study procedure. In all infants $Paco_2$, blood Hb, and arterial blood pressure were within normal ranges. In the infants monitored with transcutaneous pco_2 or multiple blood pressure determinations, no significant differences were seen during the study procedure.

Six infants received theophyllamine for apnea, no other drugs thought to influence CBF were used. Three infants received phototherapy for mild, uncomplicated jaundice, and three infants had subependymal hemorrhage. None of these developed subsequent ventricular dilatation (Table 1).

Statistics. Means and SDs of the slope indices for the states 1 to 4 were calculated. The distribution of the slope indices were skewed upward. These variables were therefore transformed logarithmically before their use as dependent variables in linear regression. To take account of the interindividual variation, a factor with 15 levels (one for each infant) was entered into the regression (GENSTAT). Then, the effect of sleep state (factor of 4 levels) and the effect of EEG (factor of 3 levels) were analyzed in the presence or absence of a factor representing stimulation to provoke wakefulness. To test differences between regression coefficients, Student's t function was calculated; probabilities less than 0.05 were considered significant. The regression coefficients and their 95% confidence limits were retransformed to the normal scale to obtain the effect of sleep states relative to quiet sleep, and of EEG relative to trace alternant. The variance associated with the sleep states was compared to the residual variance of the regression when the infant factor was considered alone (the intraindividual variance).

RESULTS

Sixty determinations of CBF were obtained in the 15 infants. In the 17 determinations obtained during quiet sleep, the mean early slope index was 49.7 ± 9.7 SD ml/100 g/min, and the mean late slope index was 17.4 ± 4.0 SD ml/100 g/min (Table 2). The SD of both slope indices during quiet sleep was less than among determinations obtained during other states, but the differences were not significant. Twelve and 11 CBF estimations were done in active sleep and wakefulness, respectively, while the state was unclassifiable in the remaining 20 estimations. In 13 infants estimations of CBF in more than one defined state were obtained (Fig. 3).

The early slope index was significantly higher during wakefulness than during quiet and active sleep, while there was no difference between quiet and active sleep (Table 3). These findings were supported by the use of the late slope index, although the difference between wakefulness and quiet sleep did not achieve statistical significance. The intraindividual variance of the early slope index was 83% of the total variance; whereas the variance associated with sleep state was 13% of the intraindividual variance ($p < 0.025$). For the late slope index the intrain-

Table 1. *Summarized clinical data*

Infant	Gestational age (weeks)	Postnatal age (days)	Clinical data
327	32	14	
368	32	17	Theophyllamine
405	32	8	Phototherapy
415	31	5	Theophyllamine, phototherapy
441	34	8	Phototherapy
498	33	7	Subependymal hemorrhage
519	33	12	
535	30	8	Subependymal hemorrhage
546	32	8	
549	32	10	Theophyllamine
685	29	6	Theophyllamine
861	33	8	
870	33	12	Theophyllamine, subependymal hemorrhage
921	31	7	
922	31	7	Theophyllamine

Table 2. *CBF, as estimated by 2 slope indices of 133-Xenon clearance in 15 stable, preterm infants according to sleep state (means ± SD)*

State	Early slope index (ml/100 g/min)	Late slope index (ml/10 g/min)
Quiet sleep ($n = 17$)	49.7 ± 9.7	17.4 ± 4.0
Active sleep ($n = 12$)	45.8 ± 13.5	17.0 ± 4.2
Wakefulness ($n = 11$)	57.7 ± 12.5	21.8 ± 5.6
Unclassifiable ($n = 20$)	46.5 ± 13.2	16.8 ± 4.3

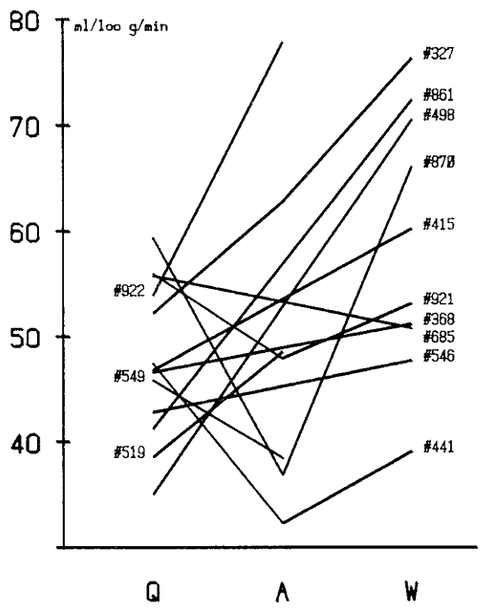

Fig. 3. Cerebral blood flow as estimated by the early slope index of 133-Xenon clearance in 13 preterm infants in whom more than one defined state was studied (Q, quiet sleep; A, active sleep; W, wakefulness). When more than one CBF estimation was done in the same state in an infant, the mean value was plotted.

Table 3. *Relationship between sleep state and CBF, as estimated by 2 slope indices of 133-Xenon clearance in 15 stable, preterm infants; the CBF in active sleep, wakefulness, and unclassifiable state was compared to the CBF in quiet sleep*

State	Early slope index	Late slope index
Quiet sleep ($n = 17$)	Baseline	Baseline
Active sleep ($n = 12$)	−6% (−24 to +14)*	−4% (−20 to +16)
Wakefulness ($n = 11$)	+22% (+1 to +48)†	+18% (−1 to +41)
Unclassifiable ($n = 20$)	−5% (−18 to +10)	0% (−13 to +15)

Note that the confidence intervals are asymmetric as the regression analysis was performed on logarithmically transformed slope index values.

* Confidence limits.

† $p < 0.05$.

Table 4. *Relationship between EEG and CBF, as estimated by 2 slope indices of 133-Xenon clearance in 15 stable, preterm infants; the CBF during continuous and unclassifiable activity was compared to trace alternant*

EEG	Early slope index	Late slope index
Trace alternant ($n = 17$)	Baseline	Baseline
Continuous ($n = 33$)	+6%	+6%
	(−9 to +22)*	(−7 to +21)
Unclassifiable ($n = 20$)	−8%	−5%
	(−30 to +21)	(−27 to +23)

Note that the confidence limits are asymmetric as the regression analysis was performed on logarithmically transformed slope index values.

* Confidence limits.

dividual variance was 65% of the total; whereas the variance associated with sleep state was only 7% ($p > 0.05$).

Seventeen determinations of CBF were obtained while the EEG showed uninterrupted trace alternant (only one of these were not classified as quiet sleep); 33 determinations were obtained during uninterrupted continuous activity of the EEG. There was no significant difference in the slope indices, although the confidence interval was wide (Table 4).

Stimulation of the infant by talking or petting to provoke wakefulness did not significantly change the relationship of state or EEG to the slope indices.

DISCUSSION

Coupling of blood flow to cerebral activity has been demonstrated in experimental animals and adult man in a range of situations from deep hypothermia to seizures. Such coupling depends on the coupling of blood flow to cerebral metabolic rate. Local glucose utilisation was found decreased in all parts of the brain during non-REM sleep compared to wakefulness in the rhesus monkey (6). The lower blood flow during deep sleep compared to wakefulness found in the present study supports that cerebral metabolic rate is also decreased in deep sleep in stable preterm infants, and also thereby suggests that flow-metabolism coupling is present. An instance of seizure activity occurring in a preterm infant during positron emission tomography has recently been reported, where a localized 2-fold increase corresponded to the focal clinical signs (7).

In adults, CBF in quiet sleep is lower than in resting wakefulness, which again is lower than in REM sleep (8). In term, newborn infants CBF as estimated by venous occlusion plethysmography was higher in REM sleep than in quiet sleep (9, 10). In the present study blood flow during active sleep was significantly lower than during wakefulness and did not differ from blood flow during quiet sleep. Several terms have been coined to identify what in the present study was labeled active sleep, and classifying criteria have differed somewhat (11). However, it is agreed that this kind of sleep is ontogenetically older than quiet sleep. Quiet sleep becomes manifest in the human fetus at about 26 wk of gestation, and active sleep comprises a decreasing part of sleep as the individual matures (12).

The results of the present study suggest that cerebral metabolism is rather low in preterm infants during active sleep, which may thus be different in form or content at this early level of cerebral organization. This hypothesis is supported by the change of EEG in REM sleep observed when preterm infants grow older (13). Until 36 wk postconceptional age, slow wave bursts are dominating in REM sleep as well as in non-REM sleep. Furthermore, until several months postterm, the waking-sleep shift is wakefulness—REM sleep—non-REM sleep, as opposed to the pattern of wakefuness—non-REM sleep—REM sleep found in older infants, children, and adults. On the other hand, the cortex is poorly developed in preterm infants and cortical activity, detached from the activity of the other parts of the brain, would only minimally affect global metabolic rate and thus blood flow. The early slope index is more sensitive to change in cortical blood flow compared to the late slope index; however, there was no indication of the early slope index rising in active sleep. But this finding needs to be confirmed, perhaps in experimental animals, by methods giving spatial resolution, allowing differentiation of cortical and subcortical structures. Furthermore, the 133-Xenon method used in this study suffered from inflexibility in timing of CBF estimation, each one resulting in a mean value over several minutes. The result was that many estimations were done during unclassifiable or changing states.

The EEG reflects cerebral activity, at least in the extremes such as deep hypothermia and seizures. On the cellular level, the level of electrical activity is the major factor determining the metabolic rate. Surface recorded EEG activity, however, reflects the net result of countless opposing electrical fields. CBF was found to correlate with mean EEG frequency in human adults (14) and to mean EEG amplitude during anaesthesia in adult rabbits (15). We could not demonstrate that periods of trace alternant were associated with reduced blood flow. Trace alternant appears in prematurity when the background activity is of very low amplitude. This may represent reorganization of neuronal patterns of activity rather than a net decrease of activity.

In summary, cerebral activity, as reflected in EEG and behavior, seems to account for only a minor, but definite, proportion of the variance of CBF in stable, preterm infants. Sleep state organization is not yet fully developed and is fragile.

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