

Sleep State Organization in Premature Infants of Less Than 35 Weeks' Gestational Age

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ABSTRACT. To assess sleep organization in premature infants of <35 wk gestational age (w GA), we performed polygraphic recordings in 24 neurologically normal neonates (eight per group): artificially ventilated 27–30 and 31–34 w GA infants and nonventilated 31–34 w GA infants. Sleep states were defined by concordance of EEG and rapid eye movement criteria. Uninterrupted active sleep periods of >13 min and quiet sleep periods of >5 min were observed in all babies, except in one 33 w GA ventilated infant. Intervals from the beginning of recording to the 1st quiet sleep period varied from 0 to 63 min and intervals to the beginning of the longest sleep cycle varied from 5 to 84 min. Nonventilated 31–34 w GA infants had longer sleep cycles ($p < 0.02$), principally because of longer active sleep periods. However, percentages of different states in the cycles were similar in all groups. When body movements were required for state definition, amounts of active and quiet sleep diminished and the percentage of indeterminate sleep was augmented significantly. In conclusion, our study demonstrated that 1) sleep state differentiation is present as soon as 27 w GA; and 2) artificial ventilation, performed in a highly specialized neonatal intensive care unit, does not modify sleep organization of neurologically normal premature infants. We hypothesize that this "earlier" sleep state differentiation, compared with previous data, may be related to improvements in neonatal intensive care over recent years. (*Pediatr Res* 34: 624–628, 1993)

Abbreviations

AS, active sleep
QS, quiet sleep
IS, indeterminate sleep
GA, gestational age
w GA, weeks' gestational age
REM, rapid eye movement

guishable from QS on the basis of different EEG patterns; presence of REM; respiratory, heart rate, and spontaneous skin potential control (2); oxygen consumption, and carbon dioxide production (3).

Little is known about differences between AS and QS in infants under 31 w GA. Dreyfus-Brisac (4) found no cyclic organization of EEG, REM, and body movement or heart and respiratory rate between 24 and 27 w GA. Monod and Garma (5) found only undifferentiated sleep in three infants of 30–31 w GA. On the contrary, in a study based on EEG recordings and visual monitoring of eye and body movements, Karch *et al.* (6) found well-defined AS and QS states in ventilated and spontaneously breathing premature infants from 28–33 w GA, although the study reported no other information on sleep structure at this age. Dittrichova and Paul (7) noted that both states could be observed at 32 w GA.

Because evidence of state-dependent differences in respiratory regulation has been found in artificially ventilated premature infants >28 w GA (8, 9), we considered that sleep organization in very young premature infants needed further study. Consequently, we designed the present study in neurologically normal premature infants. Our aim was principally to answer two questions: 1) Does sleep state differentiation exist in 27–34 w GA premature infants and, in the event of an affirmative answer, what are the appropriate criteria for coding states at this age? 2) Is sleep organization modified by mechanical ventilation used to treat respiratory distress syndrome, which is one of the most common conditions in very premature babies?

MATERIALS AND METHODS

Subjects. This study was carried out on 24 neurologically normal premature infants, separately investigated in three groups of eight subjects each according to GA on the day of recording: mechanically ventilated, 27–30 w GA infants; mechanically ventilated 31–34 w GA infants; and spontaneously breathing 31–34 w GA infants (Table 1). Recordings were performed when infants were clinically stabilized, in many cases on the day before wean-

Sleep states are distinct situations, each having its specific characteristics and reflecting a particular mode of CNS function (1). The time of first appearance of AS and QS is controversial. In most cases, however, comparisons between studies are difficult because of variations in the parameters chosen for state definition. Recent data on differences in physiologic variables between epochs of AS and QS parameters are consistent with the view that state differentiation occurs earlier than suggested by some studies published before 1980. From 31–34 w GA, AS is distin-

Table 1. Age and sleep duration in different groups studied (mean \pm SD, range)*

| Group | Gestational age at birth (wk) | Postnatal age (d) | Gestational age when recorded (wk) | | Sleep duration (min) |
|---------|-------------------------------|---------------------------|------------------------------------|--------------------------------|----------------------|
| | | | | | |
| V 27–30 | 28.1 \pm 0.9 (26.3–29.1) | 9.8 \pm 4.3 (3–16) | 29.5 \pm 1.2 (27–30.7) | 110 \pm 25.1 (74.7–152.7) | |
| V 31–34 | 29.1 \pm 1.3 (27–30.7) | 21.4 \pm 15.8 (5–43) | 32.2 \pm 1.2 (31–34.4) | 112 \pm 28.9 (85–165) | |
| N 31–34 | 31.8 \pm 1.7 (30–34.1) | 6.4 \pm 1.9 (4–9) | 33.0 \pm 1.5 (31–34.8) | 122 \pm 26.1 (63.3–145) | |

* V, mechanically ventilated; N, nonventilated infants. Note that days are transformed to weeks using a decimal system.

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ing from mechanical ventilation. Each mother gave her consent after receiving a detailed explanation of the method of recording and aim of the study. Data obtained by polygraphy were part of a routine neurologic status and GA evaluation.

GA was determined on the basis of concordance of the following criteria: 1) first day of the last menstrual period (only babies born to women with regular menstrual periods were included); 2) clinical and neurologic data; 3) weight, body length, and head circumference (10); and 4) EEG pattern (11). Mechanical ventilation was performed because of acute neonatal respiratory distress syndrome related to hyaline membrane disease. Neurologic normality was established on the basis of findings upon neurologic evaluation, EEG, and ultrasound transfontanelle echoencephalography. Recordings were performed in the neonatal department in the morning (from 0900 to 1300 h). All infants were lying in incubators with temperature (30–36°C) adapted to maintain normal body temperature. The study did not influence routine clinical care. All infants were in the supine position.

Polysomnography. Polygraphic recordings (Figure 1) included monitoring of centro-occipital, left and right bipolar EEG channels, REM and limb movements (recorded with piezo-electric crystal transducers), thoracic and abdominal respiratory movements, pulse oxymetry (transcutaneous arterial O₂ saturation), and ECG and cardiographic tracings. Respiratory air flow was recorded by nasal thermistors in normal infants. In mechanically ventilated infants, air flow given by the ventilator was recorded by a Fleisch 00 pneumotachograph connected to the inspiratory circuit. Polygraphic data were simultaneously recorded on paper and magnetic tape. Infant behavior was continuously monitored by two independent observers.

Sleep state analysis. Sleep was defined by eyes being closed (however, brief eye opening and closing related to REM were accepted in AS) and no continuous general body movements lasting more than 1 min. Tracings were interrupted when spontaneous waking or crying lasted >10 min.

Characteristics of three polygraphic parameters were independently coded by 20-s epochs by two independent experienced observers and later related to different sleep states as follows: 1) EEG patterns, characteristic for the given GA (11). AS was characterized by continuous theta plus delta or delta activity. Delta activity present in more than 70% of a given 20-s period was coded as a continuous tracing. QS was characterized by a discontinuous tracing. 2) REM: present in AS, absent in QS. 3) Body movements: present in AS, absent in QS.

We performed two independent codings of sleep states according to the simultaneous presence of two or three criteria, as

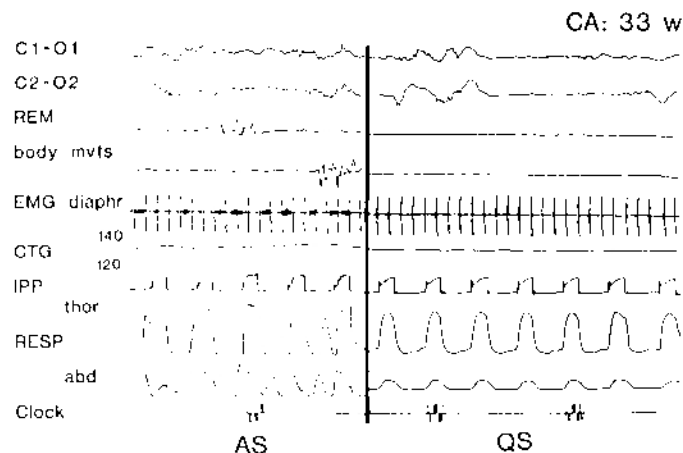


Fig. 1. Example of polygraphic recording from a 33 wk conceptual age (C1) mechanically ventilated infant. C1-O1, C2-O2, bipolar EEG; mvts, movements; EMG, surface diaphragmatic electromyogram; CTG, cardiographic; RESP, respiratory movements recorded at the thoracic (thor) and upper abdominal (abd) level; IPP, mechanical intermittent positive pressure ventilation.

follows: 1 = EEG patterns + REM, and, 2 = EEG patterns + REM + body movements.

The beginning of an AS or QS state was defined by the simultaneous presence of the relevant parameters for at least 1 min (12). Interruption of concordance between the selected parameters for 1 min or more was considered as denoting interruption of the state. Periods that did not fulfill criteria for either AS or QS were coded as IS. Periods for which interobserver agreement concerned less than 90% of the duration of a given parameter were also classified as IS.

We studied the following sleep parameters: 1) duration, percentage, and number of epochs of AS, QS, IS, and waking in the total tracing; 2) duration of the longest uninterrupted AS, QS, and IS period observed in the total tracing; 3) duration of the longest complete sleep cycle (including one AS and one QS period) in a given tracing; 4) duration, percentage, and number of epochs of AS, QS, and IS in the longest sleep cycle; and 5) latencies from the beginning of recording to the beginning of the first QS and the longest sleep cycle.

Data were statistically tested by Mann-Whitney nonparametric analysis of variance and Wilcoxon's matched-pairs signed-ranks test.

RESULTS

Total tracing duration and total sleep duration in a given tracing were not significantly different in the three groups studied. All infants fell asleep during electrode fixing. Thus, we have no polygraphic information on mode of sleep onset. All polygraphic tracings began by AS or IS.

A. Data on Total Tracing Duration. Mean percentage of recording time spent awake was 2.1 to 4.2% with no statistically significant differences between groups.

a. Scoring by concordance of EEG + REM criteria. The mean number of state epochs recorded was similar in all groups, variable across tracings, and dependent on sleep state. We found one to three AS and QS epochs and one to five IS epochs per tracing. Table 2 shows the longest uninterrupted AS, IS, and QS periods observed. In all infants, we found at least one continuous AS period of more than 13 min duration. In all but one subject, a 33 w GA mechanically ventilated infant, QS periods of more than 5 min duration were observed. In all groups, longest AS was significantly longer than longest QS ($p < 0.02$) and IS ($p < 0.05$).

Latency of the first period of QS, which is the state in which EEG abnormalities are most striking, ranged from 1 to 84 min (Table 3).

Sleep duration and percentage of different sleep states in total tracings are given in Table 4. There were large between-subject differences ($p < 0.001$ for all comparisons). AS percentage in total tracing was significantly higher than QS percentage in mechanically ventilated 31–34 w GA ($p < 0.01$) and nonventilated 31–34 w GA infants ($p < 0.05$). No significant between-group differences were found, except for a higher percentage of AS in mechanically ventilated 31–34 w GA infants as compared with nonventilated 31–34 w GA infants ($p < 0.03$).

b. State scoring by concordance of EEG, REM, and body movement criteria. Body movements were recorded in all non-

Table 2. Longest uninterrupted periods of AS, IS, and QS in total tracing in min (coding by EEG + REM criteria)*

| Group | AS | IS | QS |
|---------|----------------------------|--------------------------|--------------------------|
| V 27–30 | 23.6 ± 7.7 (13.6–38.3) | 8.9 ± 2.8 (5.3–14.0) | 11.0 ± 4.1 (6.3–16.6) |
| V 31–34 | 30.9 ± 16.6 (15.3–67.3) | 9.5 ± 6.5 (1.3–23.0) | 8.3 ± 4.2 (1.6–15.0) |
| N 31–34 | 30.0 ± 10.9 (13.0–44.3) | 15.3 ± 7.5 (4.3–25.3) | 17.6 ± 8.2 (7.6–33.6) |

* V, mechanically ventilated; N, nonventilated.

Table 3. Interval in min between beginning of tracing and beginning of 1st QS state and longest sleep cycle in given tracing*

| Group | 1st QS | Longest sleep cycle |
|---------|---------------------------|---------------------------|
| V 27-30 | 18.6 ± 20.6 (1.0-63.6) | 39.7 ± 18.6 (10-59.6) |
| V 31-34 | 26.1 ± 11.2 (1.6-39.0) | 35.0 ± 17.2 (9.3-71.3) |
| N 31-34 | 22.7 ± 16.8 (0-43.6) | 30.0 ± 27.6 (5.0-84.6) |

* V, mechanically ventilated; n, nonventilated.

Table 4. Sleep duration and percentage of sleep states (coded by EEG + REM criteria) in total tracing*

| Group | AS (%) | IS (%) | QS (%) |
|---------|----------------------------|---------------------------|---------------------------|
| V 27-30 | 42 ± 11.2 (27.3-58.8) | 31.8 ± 4.5 (23.4-38.8) | 27.3 ± 11 (14.8-47.5) |
| V 31-34 | 57.1 ± 27.7 (22.6-90.3) | 27.1 ± 19.0 (6.4-65.2) | 15.7 ± 8.3 (3.3-25.1) |
| N 31-34 | 45.1 ± 14.2 (27.1-73.3) | 29.7 ± 10.8 (7.4-39.5) | 25.1 ± 7.5 (11.7-33.4) |

* V, mechanically ventilated; N, nonventilated.

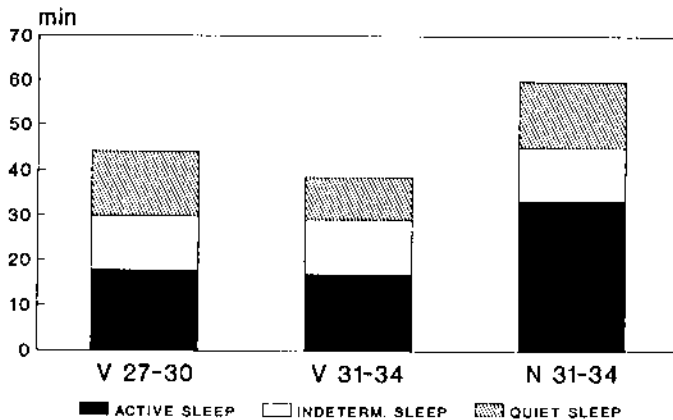


Fig. 2. State duration in min in the longest sleep cycle observed in individual babies. V, mechanically ventilated; N, nonventilated infants. The numbers under the bars indicate gestational age.

ventilated infants and in six of eight infants in each of the mechanically ventilated groups. In every case, we found movement-free AS periods of variable duration, whereas movements occurred during many QS periods. Consequently, AS and QS were significantly shortened ($p < 0.02$). IS was augmented by 45.6% in 27-30 w GA, 84.5% in mechanically ventilated 31-34 w GA infants, and 40% in nonventilated 31-34 w GA infants. In spite of total AS and QS reduction, in all groups percentage of AS remained significantly higher than percentage of QS ($p < 0.05$). No significant between-group differences were found.

B. Organization of Longest Sleep Cycle. Latency from the beginning of recording to the beginning of the longest sleep cycle was variable, from 5 to 84 min (Table 3).

a. Data obtained by scoring according to EEG and REM criteria. One to four sleep cycles per tracing were recorded. Cycle duration, including AS, QS, and in most cases IS, ranged from 18.7 to 63 min in mechanically ventilated infants and from 40 to 85 min in nonventilated infants. Mean cycle and sleep state durations are given in Figure 2. Mean duration of the longest sleep cycle was 44 min in mechanically ventilated 27-30 w GA infants, 39 min in mechanically ventilated 31-34 w GA infants, and 60 min in nonventilated infants ($p < 0.03$ between mechanically ventilated and nonventilated 31-34 w GA infants). The longer sleep cycle duration in nonventilated 31-34 w GA infants was principally due to longer AS periods in this group compared

with the two groups of mechanically ventilated infants ($p < 0.01$). However, in spite of cycle duration differences, structure of sleep cycles was similar, with closely similar percentages (no significant differences) in all three groups.

b. Data obtained by scoring according to EEG, REM, and body movement criteria. The significant decreases in AS and QS and the increase in IS were of similar magnitude as in the total tracing analysis. Cycle duration was slightly modified because of IS augmentation. However, in all but one 28 w GA infant (in whom AS nearly disappeared), cyclic organization of sleep persisted.

DISCUSSION

The present study demonstrates that, as early as 27 w GA, stable concordance between EEG patterns and presence or absence of REM allows differentiation of AS and QS states.

To the best of our knowledge, similar quantitative polygraphic investigations of sleep organization before 31 w GA have never before been performed. The study by Monod and Garma (5) focused on auditory responsiveness in premature infants. These authors found IS only before 31 w GA, but this finding was possibly due to sleep disturbance by repetitive auditory stimulation. In another study, Karch *et al.* (6) mentioned that they detected AS and QS in infants of 28 to 33 w GA but gave no further information on this topic. In these two studies, as well as in the work by Dreyfus-Brisac (4), EEG was the only parameter recorded, whereas REM and body movement parameters were visually monitored. It is known that use of piezo-electrical quartz transducers provides more accurate and quantitatively different detection of REM, as compared with visual or oculographic monitoring (13).

Sleep organization, evaluated by the concordance of recorded EEG and REM parameters, showed virtually no changes from 27 to 34 w GA. At 27-30 w GA as well as at 31-34 w GA, the amount of AS was significantly higher than the amount of QS, and IS accounted for a substantial proportion of sleep. In this work, we studied state distribution in the best organized and longest sleep cycle observed in a given recording. Some of the data thus obtained in normal 31-34 w GA infants were slightly different from our data obtained in a previous study involving analysis of the first sleep cycle (12): the sleep cycle analyzed was longer, with a mean duration of 60 min *versus* 46 min previously, and the percentage of IS was lower, *i.e.* 18.5% *versus* 31.4% previously. These differences illustrate the difficulty of comparing data from different studies that are not strictly identical with respect to recording method, state coding or, as in the present case, choice of the periods studied. The aim of our present study was to analyze the best state differentiation that can be achieved by premature infants: this goal explains why we modified our methods of cycle analysis. Our data differ in many aspects from findings reported in 1960s and 1970s. In a review of the literature (mainly of reports published before 1980), Lombroso (14) reported that before 29 w GA "the EEG patterns are invariably discontinuous" and "organization of states cannot be recognized." In all 27-30 w GA infants, we observed periods of uninterrupted AS of more than 13 min duration, *i.e.* periods accompanied by continuous or near-continuous EEG tracing. In all these infants, we detected stable QS periods of more than 6 min duration. Because the authors cited by Lombroso are well-known authorities on sleep in infants, and because the occurrence of changes in CNS structure over the last 20 y is improbable, we conclude that very young premature infants sleep better now than before and hypothesize that this improvement is due to improved care and control of homeostasis in intensive care units. This hypothesis is supported by our personal experience: premature infant recordings used in the present study, which were obtained between 1986 and 1991, demonstrated better sleep organization than premature infant recordings obtained by some of us between 1968 and 1971 (unpublished data).

Since the description of the rest-activity cycle and its age-related evolution in animals (15, 16), rest-activity cycle and sleep state organization have sometimes been confused. Rest-activity cycle organization studies are based only on body movement evaluation. The present study shows that body movements are not perfectly correlated with EEG and REM parameters. Although percentage of time spent with movements is higher in AS than in QS (17), presence or absence of body movements is unreliable for defining sleep states in very premature infants (the present study), as well as in newborns up to normal term (12); in both premature and full-term infants, body movements are present during more than 50% of QS and continue to be present during many QS periods during the first months of life (18).

Advances in real-time ultrasonography since 1980 (19) have allowed the demonstration of the presence of behavioral states in the human fetus resembling those described in neonates. The time of first appearance of behavioral states *in utero* is controversial, as it is for premature infants. Okai *et al.* (20), simultaneously using three ultrasonic real-time scanners, observed stable >3 min duration REM and non-REM periods between 28 and 31 w GA. The correlation between the occurrence of REM and breathing movements was high after 27 w GA. Nijhuis *et al.* (21) reported that behavioral states in human fetuses developed between 36 and 38 w GA. Criteria for state definition were quite different in these two studies. Nijhuis *et al.* emphasized "that the existence of behavioral states can only be accepted if three requirements are satisfied: 1) coincidence of parameters, 2) duration of stable association, and 3) simultaneity of changes at state transition." The first two conditions are without a doubt fundamental for state recognition in both fetuses and newborns, but simultaneity of parameter changes is difficult to satisfy, at least in newborns. As described in full-term newborns (22), transition from one sleep state to another results from progressive, usually nonsimultaneous modifications in parameters characteristic for a given state.

Choice of behavioral and polygraphic criteria for state definition is inevitably arbitrary. Our present study was principally based on evaluation of concordance between age-specific EEG patterns and REM parameters. These two parameters are routinely used for categorical classification of sleep states in adults (23) and older infants (2). We believe that use of a similar methodology facilitates evaluation of maturative, age-related modifications in sleep states. Many data from the literature suggest that sleep state maturation consists of progressive elaboration of concordance between specific parameters characteristic of sleep states in older infants and adults. Concordance between EEG patterns and REM is the earliest means of contrasting AS and QS. Differentiation of sleep states according to these two variables is accompanied by sleep-related modulation of respiratory, motor, and cardiac function control. In mechanically ventilated infants, beyond 28 w GA, ventilator dependency is greater during QS than during AS (8, 9). Spontaneous skin potential responses become more marked in AS compared with QS at 31 w GA (24). At 31–34 w GA (lower age limit of the studies), significant between-state differences have been observed for numerous parameters. Age-related modifications in between-state differentiation have been previously reviewed in one of our papers (2). The recent study by Easton *et al.* (25) confirmed that preterm infants, beyond 35 w GA, show stability in their sleep states, REM, and respiratory characteristics.

The influence of the neonatal intensive care environment on the infant brain and behavior has been addressed by many investigators (26–28). In a recent study, Glotzbach *et al.* (29) showed that biologic rhythmicity in preterm infants before discharge to home was dominated by ultradian components that correlated highly with feeding. Our data demonstrated that, in neurologically normal, clinically stabilized premature infants, mechanical ventilation per se did not prevent sleep state organization. Normal 31–34 w GA premature infants showed the ability to achieve longer sleep cycles, but sleep state organization

in a cycle was observed in both mechanically ventilated and spontaneously breathing infants, who had similar percentages of AS, QS, and IS.

Finally, our study emphasizes that long-duration polygraphic recordings are essential for the evaluation of EEG and sleep organization in premature infants. The presence of two states is considered a good prognosis in newborns when neurologic damage is suspected (30–32). Some EEG abnormalities appear mainly in QS. In some of our subjects, the first QS appeared after 63 min and the longest sleep cycle began after 84 min of recording.

In conclusion, the present study demonstrates that sleep state differentiation appears early in human ontogenesis. On the basis of EEG and REM concordance, well-defined stable periods of AS and QS are identifiable starting at 27 w GA in non-brain-damaged infants. Mechanical ventilation, performed in specialized neonatal intensive care units, does not significantly modify sleep organization. Knowledge of state differentiation in very premature infants is important for the understanding of state modulation of vital functions at this age.

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Announcement

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