Yawning and Behavioral States in Premature Infants

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Fiorenza Giganti Psychology Department University of Florence Florence, ltaly
Marie J. Hayes Psychology Department University of Maine Orono, ME 04469-5742
Manjapra R. Akilesh Neonatology Department Eastern Maine Medical Center Sangor, ME 04402
Piero Salzarulo Psychology Department University of Florence Florence, ltaly

Human, adult observational studies have established that yawning is a stereotyped action pattern that occurs at low frequency throughout the waking period (Provine, 1986a, 1986b). It is a complex behavior that is characterized by gaping of the mouth, a long inspiration followed by a shorter expiration. Like other stereotyped patterns, when initiated, it proceeds to completion with minimal influence from sensory feedback. Yawning often is accompanied by stretching, chewing, and suppression of locomotor activity. The frequent inclusion of whole body stretching and pende erection in animal studies has led te, the characterization of the behavior pattern as the "stretching-yawning syndrome" (Argiolis & Melis, 1998).

Yawning is facilitated by DA, acetylcholine, serotonin, nitric oxide, and adrenocorticotropic peptides which have been shown to stimulate oxytocinergic, paraventricular nucleus neurons and projections to hippocampus, pons, and medulla. Central nervous system (CNS) injection of adrenocorticotropic hormone (ACTH) or alpha- mel anocyte-stimul ating hormone and related peptides increases yawning which continues for several hours; induced yawning is suppressed by opioid peptides and gamma aminobutyric acid (Argiolas & Melis, 1998; Ferrari, Gessa, & Vargiu, 1963; Gessa, Pisano, Vargiu, Crabai, & Ferrari, 1967).

In human adults, yawning bas been related to a change in activity or arousal state and is, therefore, tied to CNS arousal modulation. Yawning increases in the hour before bedtime and after waking (Provine, Hamernik, & Curchack, 1987c) and when subjects are watching or participating in uneventful, repetitious activities (Provine & Hamernik, 1986a). In a study of cab drivers working long shifts, yawning increased along with head, neck, and leg movements toward the end of the shift in the early morning hours (Sakai & Takahashi, 1975). Cortical electroencephalographic activity (a marker of increased arousal) increases during yawning (Bertolini & Gessa, 1981). These findings have been interpreted as evidence that yawning may be potentiated when an increase in arousal is needed to maintain alertness and vigilance in a waking state.

Yawning is a precocious behavior that is observed sporadically in fetuses as early as 12 to 14 weeks of gestational age (De Vries, Visser, & Prechtl, 1985). No changes in the incidence of yawns between 20 and 36 weeks of gestational age have been observed in the fetus by Roodenburg, Wladimiroff, van Es, and Precht (1991). In full-term infants, yawns were reported on the first day of life (Gesell, 1928). However, yawning in neonates is not reported in studies of spontaneous behavior in neonates, perhaps, in part, due to its exceptionally low incidence (e.g., Korner, 1969, 1973; Korner & Beason, 1972). In the only study to describe neonatal yawning in some detail, Wolff (1987) observed that infant yawns occur mainly rieur, or possibly anticipating, the onset of sleep, together with reddening of the eye sclera and drooping of the eyelids. State Il sleep follows yawning with a mean latency of 7 min.

The present study examined yawning during very early postnatal development in relation to contextual behaviors and spontaneous alterations in behavioral state. Since yawning is a precocious behavior during early fetal life, appearing many weeks before discemable sleep-wake states, its expression gradually may become linked to state. Further, since adult yawning is increased during sleep-wake transitions, we aimed to

investigate if yawning may be a corollary of state transition and/or temporally close to sleep state during early development. We also examined the temporal association of yawning and the frequent bursts of generalized activity expressed by the neonate particularly during active sleep (AS) (Hayes, Plante, Fielding, Kumar, & Delivoria-Papadopoulos, 1994; Robertson, 1987).

METHOD

Subjects

This study was approved by Eastern Maine Medical Center Institutional Review Board, and consent was obtained from parents for participation. Infants (N= 8) between 30 to 35 weeks post-conceptual age (PCA) were evaluated for behavioral state, motility, and qualitative movements in the Neonatal Intensive Care Unit (NICU). Recruited infants were low in neurological risk (IVH < grade II), had 5-min Apgar score > 6, absence of congenital anomalies, and had an unremarkable postnatal medical course (e.g., no recent infection episodes, not currently on antibiotics or methylxanthine medication for apnea, etc.). The demographic characteristics of the subjects are summarized in Table 1.

Procedure

All infants resided in the intermediate care division of the NICU. Formal recording of video and actigraphic data was standardized to occur from 2400 to 0500 hr and occurred in the infant's home incubator. The spatial position of the incubator was not moved to preserve undisturbed conditions. The equipment was set up in the early evening (ca. 2000 hr), and formal recordings were begun at 2400 hr following the 2300 hr feeding and vital signs nursing intervention. Nurses were requested to perform any significant care procedures earlier in the shift before testing. A second feeding during the recording period occurred between 0300 to 0400 hr. Nursing interventions besides this episode were very brief to allow for spontaneous sleep-wake alterations.

Instrumentation

Subjects were filmed during the night in a semidarkened hospital room with lighting at each incubator emanating from the lower third of the wall regulated by the staff, Behavior was recorded using a time-lapse video recorder (four frames/s) and a highresolution, low-light camera with an infrared light source.

Movements were recorded electronically using a piezo-electric recording pad (10 x 10 x 1 cm) positioned under the bottom bed sheet with tape. One sensor was placed beneath the head and shoulder region and extended to the buttocks. The equipment was set up by the experimenters several hours before sleep to insure reliability of the actigraphy signal. The subject lay on the sensor for several minutes to set the gain of the sensor amplifier. The lowest gain setting capable of reflecting the complete range of movement intensity for each subject was used. Sensor output was sampled nine times per second, and digitized. The data were stored on a portable personal computer and analyzed at a later time.

Behavior Measures

Yawn Coding. Yawning is a low-incidence, highly stereotyped, discrete, and discriminable behavior. Yawns was defined as opening of the mouth to its full extension in a dramatic stretch movement that included all facial muscles below the eyes, frequent closing of the eyes, and brow and forehead. contraction. In some cases, yawning was accompanied by a general body stretch involving synchronized, bilateral arrn and trunk extension movements occuring over several seconds. However, this "larger" set of movements was not usual and not a required component in our yawn coding criteria.

Yawning bout structure was confined to a single, isolated event in 90% of pilot and study observations. Bouts of >1 yawn never exceeded two yawns per 3-min period. When more than one yawn occurred, multiple yawns were temporally contiguous and occurred as a burst. These multiple events were coded

separately. The entire 5-hr recording was divided into 100 consecutive 3-min epochs. Each yawn was coded temporally in the 3-min bin in which it occurred. Yawn and behavioral states were coded separately by different observers unaware of the hypotheses of the study.

State Coding. For state coding, four behavioral states were determined according to the criteria in Table 2. Behavioral categories for coding sleep states are based on the criteria from Anders, Emde, and Parmalee (1971), Prechtl (1965), and Thoman and Holditch-Davis (1985). These criteria are summarized in Table 2.

A 3-min epoch size was adopted for state determination. Criteria for each state must be met consecutively for a minimum of 1.5 min of each 3-min bin. This time window for neonates is informed by Anders et al. and Prechtl. Our method uses longer epochs than the 1 -min criterion used by Stefanski et al. (1984), but similar to the 3-min smoothing used by Myers et al. (1998) for premature neonates.

The Anders et al. (1971) sleep state coding manual addresses sleep state coding intervals in neonates in the following manner: "Since ongoing states are frequently interrupted by brief periods of another state ... many find it useful to smooth the record by using a criterion of one minute ... that is, if the interruption is less than one minute in duration, the epochs are scored as part of the ongoing state and no state change is considered to have occurred ... If the interruption represents one minute or longer of a single, new state, then the epochs are scored as belonging to that state. (p. 6-7)"

The 3-min window with the criterion of 1.5 min of a single state for coding allows for transient states to be smoothed while retaining ongoing stable states, which is a particular problem in state coding of premature infants (Curzi-Dascalova et al., 1985).

Table 1. Characteristics of the Sample

Subject	Gender	PCA (weeks)	Weight (g)	Length (cm)	Gestational Age (weeks)	Apgar (5mn)
2	F	32.0	1,710.0	43.0	31.5	6.0
10	F	32.6	920.0	35.5	29.7	8.0
11	M	33.4	2,070.0	46.0	32.0	6.0
13	M	33.6	1,770.0	43.3	33.0	7.0
15	F	34.4	1,850.0	46.0	33.0	10.0
22	M	31.1	1,280.0	37.5	28.0	9.0
24	F	34.5	1,180.0	38.0	32.0	9.0
39	M	34.5	2,670.0	51.0	34.5	7.0
M	%F=50	33.3	1,681.3	42.5	31.7	7.8
SD		1.3	555.2	5.2	2.0	1.5

Nursing interventions were coded in the saine fashion as behavioral states. Behavior during nursing interventions was not clearly observable and was driven by this interaction to a great extent and therefore not included in the analyses. Nursing interventions accounted for no more than 5% of recording time (Symanski, Hayes, & Akilesh, in press).

Data Analyses. Videorecordings were analyzed in Our laboratory by trained undergraduate assistants. State coding and yawn coding were done on separate occasions by different observers, and Cohen's r. interrater reliability measures were calculated on 50% of the data. Results for smoothed behavioral states were AS = 0.87, W = 0.83, D = 0,88, QS = 0.63, and for yawns, K = 0.95.

The number of yawns was recorded for each 3-min block and for each infant. Yawns were separated by the behavioral state in which they occurred ("yawn state"). The total duration (minutes) of each yawn state was compared to the total for that state across the 5-hr recording time. A quotient of these two measures was computed for each state and an average computed across subjects. This measure was used to assess the probability of yawn occurrence in each state category independent of the inequalities in the proportion of each state. The Friedman test was used to show differences among the probabilities of yawning from each of the behavioral states. All possible pairings of the behavioral state quotients were examined with the Wilcoxon test.

To examine the likelihood of state change in relation to yawn events, a cross-tabulation statistic was computed which compared the probabilities of preceding and following states for yawn versus nonyawn 3-min epochs of each state type.

RESULTS

Yawning Incidence

Spontaneous yawning is rare. The rate of yawning across all infants in the 5-hr nighttime recording period averaged approximately one yawn/hr (median = 4.5 yawns/night). Table 3 shows that, using the quotient statistic described earlier, there were significant differences in the probabilities of yawning across the behavioral states, Friedman's X 2 (3) = 8. 0 1, p = .04. The highest prevalence of yawning was in the D state. Using the Wilcoxon test to compare all possible pairings of behavioral states revealed that a significantly lower quotient of yawning is present in QS coinpared to W (p = .04), D (p = .04), and AS (p = .04), No significant differences were found for the other quotient comparisons. Only 1 infant, the youngest (30 weeks of PCA), showed any yawning in QS.

Table 2. Coding Criteria

State	**Description**
Active sleep	The infant's eyes are closed with periodic REMs occurring. Occasionally, eyes will be open during REMs. Motor activity occurs in bursts of 10 to 60 s (generalized whole body movements including facial, mouth, and head movements), with muscle tone remaining low between movement bures. Typically, respiration is irregular and costal, which is evident by observing the thorax and chest.
Quiet sleep	The infant's eyes are shut. Motor activity is low, and muscle tone is moderate. Occasional mouthing or sucking movements may occur. The infant's respiration is even and abdominal in nature.
Wake	The infant's eyes are open, focused (i.e., but not rapid REM movements, often accompanied by head and arm movements; whole sequence resembles an orientation response), and scanning the environment. State may include crying or fussing. Motor activity may be high or low. Respiration is regular and tracks motor activity.
Drowse	The infant's eyes open and close slowly. When open, the eyes are unfocused with a "heavy-lidded" appearance. Characteristics of both sleep and wake states are present, and respiration is even.

Note : A state is coded if it occurs for >1.5 min of the 3-min epoch.

Table 3. Values of the Quotient: Number of Yawns

From a Given Behavioral State/Total Duration of the Same State (min)

Behavioral State		Median	Mean	SD
Wake	0.042	0.088	0.116	
Drowse	0.056	0.127	0.224	
Active sleep	0.006	0.020	0.026	
Quiet sleep	0.000	0.006	0.016	

Yawn State

The distribution of sleep states for the infants in this study was typical for premature infants of this age (Giganti et al., 2001-, Holditch-Davis & Thoman, 1990; Meyers et al., 1998; AS: mean=48.9%, SD = 13.2; QS: mean = 26.4l'/c, SD = 8.9; D: mean = 11. 1 %, SD = 6.4; W: mean = 12%, SD = 6.6). Nursing interventions occurred on average for 4.8% (SD = 5.3) of the session.

To evaluate the temporal organization of yawning and state, yawns in each state were examined in relation to the preceding or following state (Table 4). QS was not included because there was only one example of a QS yawn in the dataset. Each yawn state (e.g., AS, D, W with at least one yawn) was examined for the state of the preceding or following 3-min epoch. The relative probability of the preceding or following state was examined for its relation to state stability or state change.

Preceding State

The preceding state for each yawn state was compared To nonyawn states from the same subjects. A two-way cross-tabulation statistic was used to examine the expected frequencies of each preceding state given each yawn state. Preceding state probabilities were found to be nonrandom and predicted by the yawn state, X2 = 85.7, df = 12, p <.000 1. Follow-up comparisons found that yawn states AS, X2 = 16.2, df = 2, p<.0001, or W, X = 11.6, df=2, p<.0001, were generally preceded by the same state. That is, preceding state and yawn state were stable. As shown in Figure 1, the preceding state to D yawn state was not stable, X2 = 2.6, df = 2, p <.27. That is, for D yawn state, the preceding state was either D (0.55) or W (0.40).

Next, AS, W, and D nonyawn states were examined similarly. These data were drawn randomly from the same sleep recording for each subject as the yawn-state analysis. As was found for the yawn states, nonyawn states predicted preceding state, X2 = 98.2, df = 12, p <.000 1. AS and W nonyawns states were stable in the preceding 3-min epoch, AS: X2 = 19.6, df=2, p<.0001; W: 100% of all preceding states were W. Unlike yawn D state, nonyawn D state was stable, i.e., usually preceded by D state, X 2 = 6.25, df = 2, p <.04.

Following State

Following states for yawn and nonyawn states were examined in the same manner. Overall, yawn state predicted following state, X2 = 108, df = 12, p < .0001. Follow-up comparisons revealed that yawn states predicted following states for all three states, AS: X 2 = 28.9, df = 2, p <.00 1; W: X2 = 7.36, df = 2, 2 p <.007; D: X = 6.4, df = 2, p <.O 1. These findings indicate that, unlike preceding states, the following states of yawn D state are not characterized by state change.

Nonyawn states also predicted following states, X2 = 103, df= 12, p <.000 1. Follow-up comparisons revealed that nonyawn states predicted following states for AS, X 2 = 9.8, df = 1, p >.001, and W (100% of the cases), but nonyawn D states were not stable, X2 = 3.25, df = 2, p <.20. As shown in Figure 2, the probabilities for following states in nonyawn D state are D=0.70, AS=0.20, and W=0.10.

Table 4. Preceding and Following State Analysis

Yawn State	Preceding State		Following State	
	x 2	p	x 2	p
D	2.6	0.27	6.4	0.01
W	11.6	0.0001	7.36	0.007
AS	16.2	0.0001	28.9	0.001

Nonyawn State	Nonyawn Preceding State		Nonyawn Following State	
	x 2	p	x 2	p
D	6.25	0.04	3.25	.20
W				
AS	19.6	0.0001	9.8	.001

*Wake nonyawn state was preceded in 100% of the cases by wake.

Contextual Behaviors

The type of behaviors that were coded are defined in Table 5. These categories have been used previously to categorize movement type in premature infants under similar conditions (Hayes et al., 1994; Hayes, Smith, Herrick, Roberts, & Swanson, 1997) and are inspired by previous work by Smotherman and Robinson (1988).

In the analysis of contextual behaviors, the 30 s prior to and following each yawn were examined and compared to nonyawn periods. Nonyawn periods were matched to yawn periods for subject and state type. The results show that the 1 -min yawn and nonyawn periods matched for state were different in the total frequency of behaviors, yawn: X2 =17.33, df=4, p <.002; nonyawn: X = 8.00, df = 4, p <.09. Yawn periods were more behavior-rich than nonyawn periods. This difference was in the total frequency of behaviors only. It was not found when examining before and after periods separately or when the data were examined according to state type.

The only finding specific for behavioral typology was that facial movements were proportionally more common in AS yawn versus nonyawn 1-min contextual periods, X 2 = 7, df = 2, p <.03. D and W yawn states, as well as nonyawn AS, W, or D state, showed no différences in the proportion of separate behavior types.

DISCUSSION

In premature infants, the rare behavior of yawning is not randomly distributed with regard to behavioral state across the night. Thirty to 35 PCA infants are Ilemergent" with regard to state, Èe., rapidly developing the capacity to express reliable and stable state organization (Curzi-Dascalova, Peirano, & MorelKahn, 1988; Parmalee, Wenner, Akiyama, Schultz, & Stem, 1967). Nonetheless, yawns were observed in all states except QS. This result suggests that the necessary and sufficient conditions for the elicitation of yawns are not present, or may be actively inhibited, during QS.

As has been observed in adults, yawning prevalence in these infants showed a trend towards highest levels during D state (Provine, Tate, & Geldmacher, 1987b). One possibility suggested by earlier work is that yawning is seen during the D state and is associated with efforts to remain awake. In animal studies, yawning is associated with EEG activation and is triggered by peptides such as ACTH. In this study, our approach to examining the question of the effect of yawning on ongoing states of arousal was to examine yawning placement in the strearn of state organization.

We found that preceding state stability was temporally associated with yawn events in D state, but not in AS or W states. When compared to epochs of nonyawn D state, yawns in D state had a higher incidence of unstable preceding state. In yawn D state, the preceding state was either D or W. This finding suggests that when the preceding state is W state transitions to D may be associated with an increased probability of yawning.

Following states to a yawn state (whether D, W, or AS) were characterized by state stability. However, D state was unstable when yawns were not present. Under these conditions, the following state was either AS or W when state change occurred. It is proposed that the absence of yawning in D state may increase the probability of a state transition in the following 3-min epoch. The sequence analysis suggests that some yawning effects are state-specific. In D state, yawning is associated with recent state change (i.e., "preceding" state instability), but state stability in the following state epoch is sound for all yawn states. Interestingly, al! nonyawn states are stable for preceding state.

D state bas been viewed as a transitional state between wake and sleep (Salzarulo, 1973; Salzarulo, Giganti, Fagioli, & Ficca, in press). Yawning may serve as an arousal modulator in D that is potentiated by an unstable preceding state. Yawning under these conditions may increase arousal sufficiently to stabilize the D state, which is consistent with the position that nonyawn (but not yawn) D epochs were unstable for the sequence of following states. In total, these findings suggest that D state is stabilized by yawn events.

The temporal analysis of yawning revealed that behavior rate was increased in the 1-min window of yawns compared to nonyawn periods. This result is reminiscent of the finding that cab drivers yawned more and were more behaviorally active at the end of their shift. Both findings suggest that yawning is associated with an increase in nonspecific motoric activation.

Premature infants in this study were in AS between 50 and 70% of the time in the 5-hr recording period. W and D contextual behaviors were similar in type to AS periods, although lower in both frequency and variety. AS motor bursts are characterized by a rich variety of behaviors: stretching, facial movements, general movements, and so on. The temporal window of yawning in AS had more facial movements than AS periods without a yawn. This result suggests a spreading activation of facial motor patterning, as has been suggested during movement analyses in our earlier work (Hayes et al., 1994).

Table 5. Behavioral Categories

Movement Pattern	Description
General movement	Whole body stretching and limb movements that continue for > 2 s, coded dichotomously (0 or 1 in each time block) and for duration
Eye movement	Rapid or slow eye rotations, may be observed with open or closed eyelids
Facial movement	Includes all observable muscle movements in the facial area: Subcategories for specific regions
Jaw/tongue	of the face are listed. (Jaw/tongue refers to "mouthing" movements.)
Mouth open	
Eyebrows contract	
Eyebrows extend	
Other	
Hand to mouth	Hand contact to mouth
Twitch	Instantaneous tremors of the hand, foot, or head
Upper/lower body	
Arm movement	Characterized by the predominant result of the muscle activity (extension vs. contraction).
Stretch	Flexion of the back muscles surrounding the thoracic vertebrae with abdominal girdle extension
Startle	An instantaneous contraction of the trunk and limbs
Quiescence	Immobility or > 5 s with the exception of occasional twitches or mouth movements, eyes closed,(Variability in respirations and heart rate is usually low, but not required.)

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