Behavioral Neuroscience
2002, Vol. 116, No. 5, 912–917

0735-7044/02/$5.00 DOI: 10.1037//0735-7044.116.5.912

The Union of the State: Myoclonic Twitching Is Coupled With Nuchal Muscle Atonia in Infant Rats

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Active sleep (AS), as measured by the occurrence of myoclonic twitching (MT), is the most prevalent behavioral state in newborn rats. Historically, AS has been considered a developmental precursor of REM sleep, but recently this idea has been questioned. In the present study, the authors assess, in 2-, 5-, and 8-day-old rats, the relationship between MT and nuchal muscle atonia, a widely recognized component of REM sleep. At all ages, muscle atonia preceded MT and persisted until awake behaviors occurred. In addition, muscle tone decreased gradually during transitions from awake behavior to twitching. Thus, MT during infancy occurs against a backdrop of muscle atonia, a result that is consistent with the view that AS is a developmental precursor of REM sleep.

Rapid eye movement (REM), or *paradoxical*, sleep in adult mammals has been classically defined as the appearance of a low-voltage, high-frequency (i.e., desynchronized) electroencephalograph (EEG); low muscle tone; and, of course, REMs (Carskadon & Dement, 2000; Rechtschaffen & Kales, 1968). Other components of REM sleep include respiratory and cardiac irregularity, a hippocampal theta rhythm, pontine–geniculo–occipital (PGO) waves, and myoclonic twitching (MT) of the distal limbs. Although the neurological circuits that modulate REM sleep are still poorly understood, it is now clear that a variety of nuclei within the mesopontine region contribute importantly to the phenomenology of REM sleep (McCarley, Greene, Rainnie, & Portas, 1995; Vertes, 1984). Nonetheless, as with any complex state comprising multiple components, it remains unclear which components of REM sleep, if any, should be viewed as essential for defining this behavioral state (Blumberg & Lucas, 1996).

Strict adherence to the defining features of REM sleep in adults poses a problem when one examines sleep patterns across species, and even at different ages within a species. For example, in newborn rats, most of the components that are used to define REM sleep in adults appear to be absent, including the desynchronized EEG, muscle atonia, REMs, and hippocampal theta (Blumberg & Lucas, 1996; Guilleminault & Baker, 1984; Siegel, 2000). In contrast, MT is perhaps the most prominent behavioral indicator of sleep in the infants of many mammalian species (Corner, 1977; Gramsbergen, Schwartze, & Prechtl, 1970). To distinguish this form of infant sleep from the componentially complex REM sleep of adults, investigators refer to it as *active sleep* (AS). Thus, researchers are left with the question of how to conceptualize the relationship between AS and REM sleep.

On the one hand, many investigators have come to view AS and REM sleep as homologous states (Corner, 1977; Siegel, 2000). For example, Corner (1985) metaphorically likens the development of REM sleep to a rope accumulating strands through time, with each strand representing a different component of REM. Similarly, Siegel (1999), noting the apparently continuous developmental relationships among the components of AS and REM sleep, states that this "continuity makes it easy to accept that the neonatal 'active sleep' state is closely related to the state of REM sleep . . . seen in the adult" (p. 88). On the other hand, although not claiming that the developmental relationship between AS and REM is discontinuous, Frank and Heller (1997) have argued that "AS is best considered as an undifferentiated behavioral state from which both SWS [slow wave sleep] and REM sleep develop" (p. R1792) and, therefore, that AS and REM sleep are not homologous states.

Applying the concepts of developmental continuity and homology to a complex behavioral phenomenon like sleep raises a number of difficult issues that can only be resolved through systematic investigations of a variety of behavioral, anatomical, and physiological variables. As one step toward addressing these larger issues, the present experiment examined the relationship, in 2-, 5-, and 8-day-old rats, between MT and a second, widely accepted component of REM sleep: nuchal muscle atonia. Although there is little empirical evidence that bears directly on this relationship, a number of investigators, including the second author (M. S. B.), have assumed that muscle atonia is not a feature of AS in young infants (Blumberg & Lucas, 1996; Horne, 2000; Siegel, 2000). For example, Siegel (2000) states that in "human infants and in many young animals, active sleep—the ontogenetic precursor of REM sleep—is not accompanied by a low-voltage cortical EEG or by muscle atonia" (p. 112). Similarly, Horne (2000) declares that "[i]mportant neurophysiological characteristics of REM, such as hippocampal theta activity, PGO waves . . . and atonia . . . are not apparent in early AS" (p. 780).

Contrary to these expectations, using the combined information provided by behavioral and electromyographic (EMG) data, we found that MT occurs only against a background of muscle atonia

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This research was supported by National Institute of Mental Health Grant MH50701 and National Institute of Child Health and Human Development Grant HD38708. We thank Joy Kreider for her helpful comments on an earlier version of this article.

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and that AS and waking are clearly defined states. By demonstrating in infant rats a strong relationship between two known components of REM sleep that are not associated with SWS, these results support the view that AS is a developmental precursor of REM sleep. These results also emphasize once again the importance of appropriately modifying methods garnered from work with adults when addressing developmental problems (Alberts & Cramer, 1988; Blumberg, 2001; Hall & Oppenheim, 1987).

Method

Subjects

Eighteen 2-, 5-, and 8-day-old male Sprague-Dawley rats from 16 litters were used (hereafter referred to as Postnatal Day [PD]2, PD5, and PD8, respectively). Both sexes were equally represented at each age. Body weights were 6.79–9.69 g at PD2, 8.16–15.73 g at PD5, and 16.77–20.55 g at PD8. Litters were culled to 8 pups within 3 days after birth (day of birth = Day 0). Mothers and their litters were housed in standard laboratory cages (48 cm long × 20 cm high × 26 cm wide) in the animal colony at the University of Iowa, with food and water available ad libitum. All rats were maintained on a 12-hr light–dark schedule with lights on at 0600, and all tests were conducted between 0830 and 1730 (with the exception of one 2-day-old that was tested between 2000 and 2115). The experimental procedures conformed to all local, state, and federal guidelines concerning the care and use of animals.

Surgery

On the day of testing, a pup with a visible milk band was removed from the litter, weighed, and placed on a heating pad. The nose and mouth were covered with a small rubber mask, and isoflurane was administered. Two bipolar stainless steel hook electrodes (50 μm diameter; California Fine Wire, Grover Beach, CA) were inserted with a 27-g needle, through the skin bilaterally into the nuchal muscle. In addition, a ground wire was looped through the back skin.

Procedure and Data Acquisition

While the pup was recovering from anesthesia, it was placed on a soft felt pad and lightly secured in a supine position with two pipe cleaners placed over the thorax and abdomen. The electrode wires ran through a hole in the felt pad. By testing a pup on its back, the experimenter can easily observe MT of the individual limbs (Blumberg & Lucas, 1994; Robinson, Blumberg, Lane, & Kreber, 2000); this procedure also helps to minimize movement artifact in the EMG. Pups appear to be unaffected by this light restraint, as they begin to twitch within seconds of being secured to the pad and they exhibit low rates of awake behavior. Moreover, rates of twitching and awake behaviors have been comparable between experiments in which we have observed pups restrained in a supine position (Kreider & Blumberg, 2000) or unrestrained (Sokoloff & Blumberg, 1998).

After the electrodes were implanted and the pup was secured to the harness, it was placed inside a double-walled glass chamber (height = 17.0 cm, i.d. = 12.5 cm), through which temperature-controlled water was circulated. Air temperature inside the chamber was maintained at 35.25 °C, which is within the thermoneutral range for pups at the ages used here (Spiers & Adair, 1986). Air flow through the chamber was maintained at 300 ml/min. After the electrodes were connected to a differential amplifier (A-M Systems, Model 1700, Carlsborg, WA), a camera was situated above the chamber lid for recording of sleep–wake behaviors. Finally, a Faraday cage (28 cm long × 28 cm high × 40 cm wide) was placed around the chamber and the camera. The pup was allowed 1 hr of acclimation to the chamber, after which EMG and behavioral data were recorded for 1 hr.

The EMG signal was amplified (×10,000), with filter settings at 5 kHz high-frequency cutoff, 300 Hz low-frequency cutoff, and a 60-Hz notch filter. The amplified signals were recorded with a digital recorder system (Model DV8; Vetron, Rebersburg, PA). This system allows raw EMG signals and video signals to be recorded simultaneously to the same tape for later playback and analysis. During data acquisition, the EMG signals were monitored with a data acquisition system (Model MP150; BioPac Systems, Santa Barbara, CA) while the behavior was simultaneously viewed on a monitor.

Data Analysis

Recordings were played back from the videotape in 15-min sections and the EMG signals were stored digitally to computer by means of the BioPac system. As the EMG signals were digitized, a trained observer viewed the videotape of the pup's behavior on a monitor and, using an event recorder, pressed one of two keys when MT or coordinated movements (CM) were detected. In this way, a synchronized digital record of the pup's behavior and EMG activity was produced for later analysis. MT was defined as phasic, rapid, and independent movements of the limbs and tail (Blumberg & Lucas, 1996; Blumberg & Stolba, 1996; Gramsbergen, Schwartze, & Prechtl, 1970). CM was defined as coordinated motor activities such as stretching, kicking, and yawning, as well as postural elevation of the head and/or torso. During sustained periods of these behaviors, the observer repeatedly hit the key at a rate of at least once per second until the behavior ceased.

Three types of analyses were performed. First, the time spent in a state of high versus low muscle tone and the duration of MT and CM states were determined for each pup. Using the digitally stored record, we determined the time spent in each behavioral state by adding the duration of all bouts of CM and all bouts of MT during a 15-min period. For this analysis, a bout of CM was defined as an uninterrupted series of coordinated movements, and its duration was defined as the time elapsing from the first movement to the last. Similarly a bout of MT was defined as an uninterrupted series of twitches, and its duration was defined as the time elapsing from the first twitch to the last. The total durations of high and low muscle tone were determined for the same 15-min period.

For the second analysis, muscle tone was assessed and compared during clearly defined bouts of MT and CM. The scored record was used to define two clear behavioral states that could be compared for EMG activity. The following criteria were used: (a) A given bout of CM was defined as beginning with the first coordinated movement and ending with the last, with no more than 2 s intervening between successive CM events. (b) A given bout of MT was defined as beginning with the first twitch and ending with the last, with no more than 2 s intervening between successive twitches. (It follows from these criteria that, to be considered a bout of CM or MT, a minimum of two events must have been observed.) After scoring the behavioral states, the two nuchal EMG signals were summed and full-wave rectified. Muscle tone was measured as the average EMG value of the rectified signal over a specified time period. Care was taken to measure tone only during periods that were absent of noise (i.e., spikes, movement artifact). The minimum period of measurement of tone was at least 1.5 s. Ten independent measurements were made for each subject from 10 distinct episodes of each behavioral state. The means of these 10 samples were calculated for each individual pup. (If a 15-min scored session for a pup did not provide sufficient data for this analysis, a second 15-min period was scored.) These means were then imported into StatView 5.0 for analysis (SAS, Cary, NC). A two-way analysis of variance (ANOVA), with age and behavioral state as variables, was used.

The third and final analysis aimed to determine changes in muscle tone during transitions from bouts of CM to bouts of MT. A transition from CM to MT was defined as the time from the last movement of a bout of CM to the first twitch of a bout of MT. The transition period was divided into quartiles, and muscle tone was measured during each quartile. The minimal length for a transition period was 4 s, and thus the minimum size of each quartile was 1 s. Muscle tone was also measured during the last 1.5 s of the CM period and the first 1.5 s of the MT period. For each pup, 10 transitions

were identified, muscle tone was measured for each of the six periods (CM + four quartiles + MT) during each transition, and means for each period were calculated. (Again, if a 15-min scored session for a pup did not provide sufficient data for this analysis, a second 15-min period was scored.) These means were then imported into StatView. A one-way repeated measures ANOVA was performed with age as the variable and time (period) as the repeated measure. Paired *t* tests were used to test for differences in muscle tone between successive periods.

Results

Durations of MT, CM, and high and low muscle tone during a 15-min test period are shown in Table 1. Approximately one third to one half of the period was spent in states of low and high muscle tone. As expected, the total duration of bouts of MT at each age was similar to the total duration of low muscle tone. In contrast, the total duration of bouts of CM underestimated the duration of high muscle tone periods. In other words, there were many instances when the pup was behaviorally quiet but was exhibiting high muscle tone.

In Figure 1, representative 100-s records of nuchal muscle activity and behavior are shown in separate plots for PD2, PD5, and PD8 rats. For each plot, the raw EMG trace is situated above the behaviorally scored record, on which spikes above the midline represent individual twitches and spikes below the midline represent instances of CM. The behavioral records are slightly delayed relative to the EMG records because of the reaction time of the scorer.

From the plots in Figure 1, it is clear that MT occurred only when the pup was in a state of low muscle tone and that CM occurred when the pup was in a state of high muscle tone. During a state of low muscle tone, twitches were typically observed throughout the low-tone period. In contrast, during a state of high muscle tone, CMs typically occurred at the beginning of the high-tone period and then ceased. These observations are reflected in the data in Table 1. It is also clear from these data that pups cycled rapidly between periods of high muscle tone and low muscle tone.

Figure 2 presents nuchal muscle tone during bouts of MT and CM for the PD2, PD5, and PD8 rats. As noted earlier, for measurements of muscle tone, care was taken to include only noise-free segments of EMG data. There was a significant effect of behavioral state on muscle tone, $F(1, 30) = 53.5$, $p < .0001$, but

Table 1
Mean (± SEM) Durations of Sleep–Awake Activity as Determined With Behavioral and EMG Measures

	Active sleep		Awake	
Age	MT	Low muscle tone	CM	High muscle tone
PD2	**5.4** ± 1.1	**5.1** ± 1.0	**3.0** ± 1.1	**6.4** ± 1.1
PD5	**8.2** ± 0.5	**7.9** ± 1.1	**2.7** ± 0.5	**4.1** ± 0.7
PD8	**6.3** ± 0.5	**6.1** ± 0.7	**3.5** ± 0.7	**5.7** ± 1.3
Total	**6.7** ± 0.5	**6.4** ± 0.6	**3.1** ± 1.9	**5.4** ± 2.7

Note. Durations (in minutes) of myoclonic twitching (MT), coordinated movements (CM), and high and low nuchal muscle tone during 15-min test periods in Postnatal Day (PD)2, PD5, and PD8 rats. $n = 6$ at each age. Boldface indicates means. EMG = electromyographic.

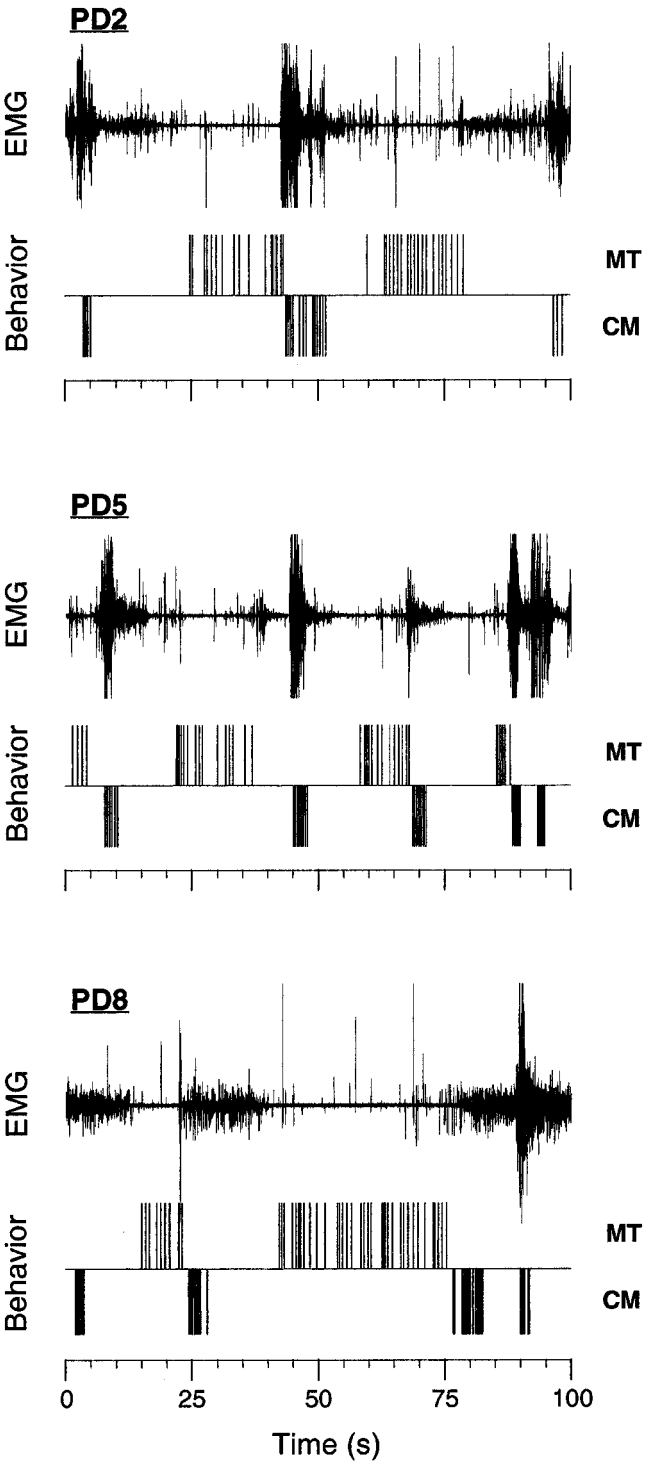

Figure 1. Representative 100-s records of electromyographic (EMG; arbitrary units) activity and behavior for Postnatal Day (PD)2, PD5, and PD8 rats. Records were derived by summing raw EMG signals from two bipolar electrodes implanted bilaterally in the nuchal muscle. Behavioral data were scored from video records that were synchronized with the EMG activity. The observer scored the presence of myoclonic twitching (MT) in individual limbs and coordinated movement (CM) among limbs. At all three ages, it is evident that MT occurs against a background of low muscle tone and CM occurs against a background of high muscle tone. Data were downsampled from 5000 Hz to 250 Hz for these figures. $n = 6$ at each age.

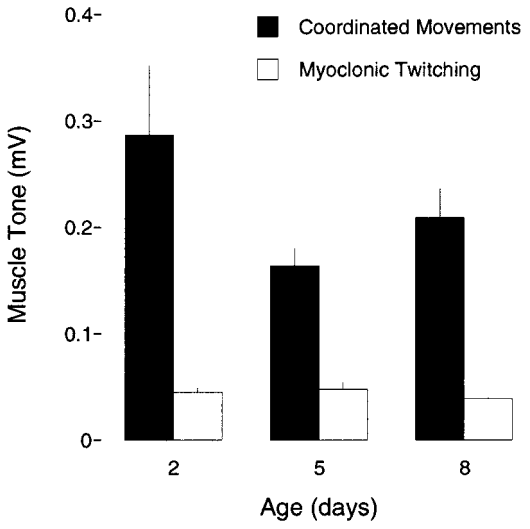

Figure 2. Mean (± *SEM*) nuchal muscle tone during bouts of coordinated movement (CM) and myoclonic twitching (MT) for Postnatal Day (PD)2, PD5, and PD8 rats. Measures of tone were restricted to periods of at least 1.5 s during bouts of CM and MT. For each subject, muscle tone was averaged from 10 observations for each behavioral state. $n = 6$ at each age. Muscle tone was significantly greater during CM than during MT; there were no significant differences between ages.

there was neither a significant effect of age, $F(2, 30) = 2.2$, nor a Behavioral State × Age interaction, $F(2, 30) = 2.3$.

Changes in nuchal muscle tone during the transition from a bout of CM to a bout of MT are shown in Figure 3. Again, care was taken to include only noise-free segments of EMG data. As shown in the insert to Figure 3, muscle tone decreased similarly across the transition from CM to MT at each age. A one-way repeated measures ANOVA indicated a significant effect of time, $F(5, 75) = 75.6$, $p < .0001$, but neither a significant effect of age, $F(2, 15) = 1.9$, nor a significant Time × Age interaction, $F(10, 75) = 1.1$. Finally, post hoc tests revealed that, collapsed across age, muscle tone decreased significantly during each transition period except the last.

Discussion

In this experiment, we found that MT coupled with muscle atonia represents a unified behavioral state, AS. The bouts of MT took place only against a background of atonia and ceased immediately when muscle tone increased. The decrease in tone during the transition period from CM to MT highlights the close relationship between muscle tone and behavioral state. Specifically, we found that muscle tone decreased significantly during each period of the transition from a bout of CM to a bout of MT except the last. The way in which these two known components of REM sleep—MT and muscle atonia—coincide in the infant rat is consistent with the view that AS is homologous to REM sleep. We found no evidence for a developmental trend in the temporal relationship between periods of muscle atonia and MT. Thus, it would be interesting to examine the relationship between these two phenomena in fetal rats.

The representative data in Figure 1 clearly demonstrate how rapidly infant rats cycle between behavioral states. For example, the PD5 rat in that figure exhibits seven transitions in the 100-s period depicted. Thus, although there were no developmental differences found with regard to the relationship between muscle atonia and twitching, it may be the case that the cycling between behavioral states differs across age. Longer periods of data collection are needed to address this question adequately.

Although MT is not considered an essential component of REM sleep in adults, it is nonetheless a prominent feature that has received some attention from sleep researchers (Chase & Morales, 1990; Gassel, Marchiafava, & Pompeiano, 1964). In infants, especially altricial infants, MT is less easily ignored. Moreover, it has been used quite effectively for assessing the effect of cold exposure on behavioral state in infant rats, exhibiting reliable associations with other behavioral and physiological measures (Blumberg & Sokoloff, 1998; Blumberg & Stolba, 1996; Sokoloff & Blumberg, 1998). Consistent with the argument that AS is a developmental precursor of REM sleep, it appears that circuits

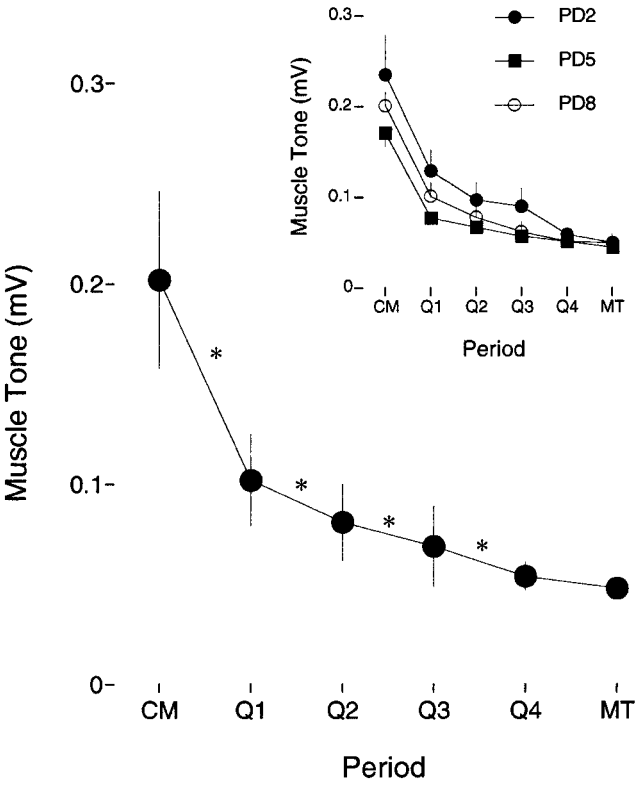

Figure 3. Mean (± *SEM*) changes in nuchal muscle tone, collapsed across age, during the transition from wakefulness to sleep in Postnatal Day (PD)2, PD5, and PD8 rats. Data are also shown for each age separately (insert). Transitions from bouts of coordinated movement (CM) to bouts of myoclonic twitching (MT) were identified, and muscle tone was measured at six time periods: the final 1.5 s of the bout of CM; the first 1.5 s of the bout of MT; and the intervening transition period, divided into quartiles (Q1–Q4). The transition periods used in this analysis were always at least 4 s in duration. $n = 6$ at each age. There were no significant differences between ages. Asterisks indicate significant differences between adjacent points ($p < .01$, paired t test).

within the mesopontine region are necessary for the normal expression of MT (Kreider & Blumberg, 2000).

As mentioned earlier, Frank and Heller (1997) have argued that AS and REM sleep are not homologous behavioral states. Using 9.5- to 20.0-day-old rats, they recorded EEG and EMG during sleep and related changes in these measures to the occurrence of MT. First, consistent with previous reports (Jouvet-Mounier, Astic, & Lacote, 1970), they did not detect desynchronization of the EEG until pups were 12–14 days of age. Second, they did find evidence of synchronized EEG activity, indicative of SWS, during periods of MT by 11.5 days of age. Because the degree of overlap between these behaviorally and electrophysiologically determined states decreased with age, they concluded that AS is not a precursor to REM sleep but is an independent "protostate" from which both SWS and REM sleep emerge.

For a variety of reasons, including convenience, it has become conventional practice among sleep researchers to segment sleep/wake data into 10-s (Frank & Heller, 1997) or 30-s (Vogel & Feng, 2000) epochs and categorically assign a behavioral state to each epoch. The use of epochs to score behavioral states can be useful when the states are well defined and agreed on, as when a clinician is diagnosing a sleep disorder. When a phenomenon is not well understood, however, assigning a single value to each epoch, regardless of the behavioral and electrophysiological complexity residing within it, can obscure the very complexity that we wish to describe and explain. Thus, the use of the epoch technique is comparable to applying a temporal filter. The practical effect of this approach can be seen by applying epochs of 10, 20, or 30 s to the representative data in Figure 1 and noting how the relationship between muscle atonia and MT would be obscured. Similarly, it is possible that Frank and Heller's (1997) interpretation of their data would have been different had they used real-time analysis. Although the epoch technique has dominated both basic science and clinical approaches to sleep, its usefulness for understanding sleep ontogeny (and phylogeny) is clearly limited. Thus, the present results should give us pause when imposing, on the developing infant, sleep definitions derived from work with adults (Blumberg & Lucas, 1996).

For reasons that differ from ours, Vogel and Feng (2000) have criticized the methodological approach of Frank and Heller (1997), most particularly the validity of using MT as a behavioral indicator of REM sleep processes. Although they remain ambivalent as to what AS represents, Vogel and Feng claim that, in the absence of more reliable indicators (i.e., EEG desynchrony), one cannot be certain that behaviorally scored epochs of AS are indeed undifferentiated vigilance states with both REM sleep and SWS properties (Vogel & Feng, 2000). Indeed, they go so far as to state that Frank and Heller's "use of muscle twitch as the defining characteristic of REM sleep constituted an idiosyncratic scoring system which would destroy the empirical relationships that have been established between sleep state and other variables" (p. 1006). The disagreement between Frank and Heller and Vogel and Feng highlights how the interpretation of the significance of myoclonic twitching vitally shapes one's view of sleep development.

In their debate over the significance of myoclonic twitching and the relationship between AS and REM sleep, it is significant that both Vogel and Feng (2000) and Frank and Heller (2000) ultimately rely on the EEG for addressing the nature of infant sleep. As a consequence, both have ignored sleep processes in infant rats younger than 10 days of age. Although the EEG is clearly one of the three pillars of sleep state definition, there is no a priori justification for assigning it special status when assessing sleep states in infants. First, even in adults, EEG activity is a noncausal correlate of other sleep parameters; accordingly, Siegel (1999) emphasizes the fact that "the EEG derives its value because of its correlation with behavioral measures of sleep" (p. 88). Second, the developmental emergence and coalescence of multiple sleep state components, not the presence or absence of any single component, represents the true puzzle of sleep development. Thus, it is striking that, in rats, MT and other forms of spontaneous activity are present before birth (Robinson et al., 2000), that muscle atonia is synchronized with twitching as early as 2 days of age (as shown here), that REMs emerge by 6 days of age (Van Someren et al., 1990), that hippocampal theta rhythms appear associated with AS by 10 days of age (Leblanc & Bland, 1979), and that not until 12 days of age does one see desynchronization of the EEG (Frank & Heller, 1997). Within this broader developmental context, it seems arbitrary to focus on the EEG as the sine qua non of AS or REM sleep.

To summarize, we have shown in infant rats that MT is tightly coupled with muscle atonia in infants that are far younger than would have been suspected, on the basis of recent studies (Frank & Heller, 1997; Vogel, Feng, & Kinney, 2000), to exhibit such coupling. If, as Frank and Heller argue, AS is an undifferentiated protostate from which both REM sleep and SWS evolve, then it is difficult to explain the highly differentiated REM sleep-like states that we observed in our subjects. Furthermore, the present results illustrate how the use of conventional methods for scoring infant sleep can lead to significant errors of interpretation. Thus, these results reinforce the view that any satisfactory description of the development of infant sleep must be derived from high-resolution data and must account for the diversity of tonic and phasic components that compose the sleeping state, including, but not limited to, the EEG (Blumberg & Lucas, 1996).

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Received November 7, 2001
Revision received February 18, 2002
Accepted March 29, 2002 ■