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CHAPTER

20 The Form and Function of Infant Sleep: From Muscle to Neocortex

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Abstract

Despite the predominance of sleep during the perinatal period in mammals, most investigations of the mechanisms and functions of sleep continue to focus on adults. This chapter reviews recent progress in our understanding of infant sleep and its development, including developmental transitions in the temporal expression of ultradian and circadian rhythms, the developmental emergence of sleep components (e.g., cortical delta activity), the neural mechanisms of infant sleep, and the contributions of sleep processes to neural development. In addition, it is argued that a thorough understanding of the development of sleep can help us to understand the relations between normal and pathological states as well as the evolutionary modification of developmental mechanisms to shape species-specific features of sleep and wakefulness.

Keywords: sleep, infancy, REM, ultradian rhythms, circadian rhythms, neural development, wakefulness, evolution

Introduction

Everyday experience and decades of research inform us that the infants of many mammalian species, including humans, spend most of their days and nights asleep (Jouvet-Mounier, Astic, & Lacote, 1970; Kleitman & Engelmann, 1953; Roffwarg, Muzio, & Dement, 1966). Nonetheless, our understanding and appreciation of infant sleep have been impeded by a variety of obstacles. These obstacles reflect in part the technical and methodological challenges that small, fragile infants pose to the experimental scientist, as well as the challenges that arise when we attempt to interpret infant behavior using concepts that have emerged from research using adult subjects. Although these issues are very familiar to developmental psychobiologists (Alberts & Cramer, 1988; Hall & Oppenheim, 1987), they are—in our experience—less familiar to the majority of sleep researchers.

In the 1990s, doubts were raised as to whether the state that we identify as sleep in infants is qualitatively similar to the sleep that we recognize in adults (Frank & Heller, 1997b, 2003). Specifically, it was suggested that infant sleep is initially best described as an amalgam of sleep states (called "presleep"). The fine details of the presleep hypothesis and the ensuing debate have been reviewed elsewhere (Blumberg, Karlsson, Seelke, & Mohns, 2005a; Frank & Heller, 2005). Instead, this chapter is devoted primarily to recent studies that have resolved this debate by advancing our understanding of the phenomenology of sleep during the early postnatal period. By way of introduction here, we focus first on several broad issues that are foundational to any attempt to understand infant sleep and its development.

First, it behooves us to appreciate the distinction between descriptions and explanations of sleep. To borrow the famous line from Supreme Court Justice Potter Stewart as he struggled to define pornography, we know sleep when we see it. But good science, like the good law, demands more precise definitions. Accordingly, sleep researchers in the 1960s produced *A Manual of Standardized Terminology, Techniques and Scoring System* to aid communication among laboratories and guide the work of future investigators (Rechtschaffen & Kales, 1968).

Although this manual provided the necessary criteria for describing (or "diagnosing") sleep in adult humans, it did little to explain the causal mechanisms controlling it. Nor did the manual provide a theory of sleep or even hypotheses concerning sleep's functions. Indeed, to their credit, the authors of the manual explicitly advised readers regarding its aims and limitations:

> Although there is considerable comparability of sleep stage manifestations among various species, the differences are sufficiently great to require a separate scoring system for most species. This proposal is designed for adult humans.... [I]t is well known that human infants show combinations of polygraphic features which defy classification by the criteria proposed here. A strict adherence to the proposed system *would not yield* an adequate description of infant sleep. (italics added)

Despite these caveats, the manual would have a pervasive influence on how we interpret sleep in other species and in infants. Perhaps most influential was the adoption of three electrographic measures—the electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG)—for categorizing the major states of sleep and wakefulness. Once these criteria were established, it became difficult to avoid analyzing sleep–wake states in nonhumans and nonadults without reference to them.

An alternative approach—one that grew out of the European ethological tradition—has relied predominantly on behavioral measures alone to categorize behavioral states (Gramsbergen, Schwartze, & Prechtl, 1970; Nijhuis, Martin, & Prechtl, 1984). This approach is particularly useful for investigations of subjects that are not amenable to more invasive approaches. For example, the body size of infant rats and the fragility of preterm human infants have impeded the use of the kinds of instrumentation procedures that are easily accomplished in larger and more robust human and nonhuman animals. In infant rats, investigators were able to distinguish high-amplitude movements of limbs (e.g., stretching, kicking) as indicators of wakefulness and myoclonic twitches of the limbs and tail as indicators of active sleep (Gramsbergen et al., 1970). As we will see, however, such motor activity alone does not fully capture all aspects of behavioral state expression in perinates.

Regardless of whether one relies on behavioral or electrographic criteria (or both) for classifying behavioral states, there is little doubt that state assignments become more reliable (in the sense that interrater reliability increases) the more measures one has at one's disposal. But it is also true that the selection and interpretation of particular criteria reflect underlying assumptions that can critically influence our assessment of sleep.

For example, the traditional reliance on epochs to categorize behavioral states, a reliance codified in Rechtschaffen and Kale's manual, has the practical effect of filtering out events that occur at a temporal scale that is smaller than the epoch (in other words, the epoch technique functions as a low-pass filter). Similarly, although the neocortical EEG can provide valuable information when assessing sleep and wake states under many circumstances, we must be careful not to elevate this single measure to a status that it does not deserve. As we will see, overestimation of the value of the neocortical EEG, particularly delta activity, can be particularly confusing when we are examining animals that do not exhibit easily identifiable state-dependent neocortical activity.

The broader message is simple: Electrographic and behavioral measures are tools for categorizing states to animals, but we must exercise caution so as not to confuse these measures with the inferred state themselves. In effect, these measures are useful for *describing* a state, but for *explanation* we need formal, testable hypotheses that address the mechanistic links among the various measures and their functional significance.

One guiding theme of developmental psychobiology is that infants are adapted to the developmental niche in which they live as well as prepare for the likely niches that are to come. This notion of the "dual infant," connected as it is to the related concept of ontogenetic adaptation, helps us to appreciate the significance of the developmental period as more than a period of stasis and dependency (Alberts & Cramer, 1988). For example, if we wish to understand thermal homeostatic

capabilities in infant rats, then we should not test them under ambient conditions appropriate to adults (Blumberg, 2001). Indeed, when thermal challenges are scaled to the size of the animal under study, infants reveal a regulatory capacity that is otherwise masked.

These lessons are equally important in the field of sleep research as we strive to develop criteria for measuring sleep and wakefulness that are not constrained by those criteria that apply most readily only later in life. Again, the fact that the EEG is useful for assigning behavioral states in adults does not imply that its absence in infants precludes effective descriptions of sleep and wakefulness. Rather than attempt to fit infants into slots custom-built for adults, we should strive to develop descriptive and explanatory tools and that are relevant for and appropriate to our infant subjects.

We note that the field of animal learning made its greatest strides as investigators turned to "simple" animal models of learning in invertebrates (e.g., *Aplysia*) (Kandel & Schwartz, 1982) and well-defined model systems in adult mammals (e.g., eyeblink conditioning) (Gormezano, Kehoe, & Marshall, 1983; Thompson, 1986). Accordingly, sleep researchers are considering the potential benefits of using "simple" animal models, including invertebrates (Hendricks, Sehgal, & Pack, 2000). We believe that the infants of altricial species (e.g., rats, mice), with their strong drive for sleep and their rapidly changing sleep patterns, also offer valuable opportunities for making progress in our understanding of the mechanisms and functions of sleep.

These and other related themes are explored in this chapter as we review recent research relating to the form and function of infant sleep. We first describe the various approaches that have been used to provide a modern description of behavioral states in infants, particularly infant rats. Then, we aim to show how, through developmental analysis, we can move beyond description to explanations of the mechanisms and functions of sleep across the lifespan.

The Phenomenology of Infant Sleep

A behavioral state is an outward manifestation of a stable but reversible and recurring pattern of internal conditions of an animal that exhibits two general features: first, a state must exhibit concordance or coherence among the components comprising it; second, it must exhibit persistence, that is, temporal stability (Nijhuis, 1995). As characterized in adults—and even in older infants—behavioral states such as quiet sleep (QS, or nonrapid eye movement [NREM] or slow-wave sleep), active sleep (AS, or rapid eye movement [REM] or desynchronized sleep), and waking seem to be global phenomena comprising persistent and concordant components regulated by specific regions of the brain (Pace-Schott & Hobson, 2002; Siegel, 2005b). As for younger infants, however, it has been suggested that sleep states comprise relatively few components that are poorly integrated (Adrien & Lanfumey, 1984; Jouvet-Mounier et al., 1970; McGinty et al., 1977) and, further, that the brain does not modulate infant behavioral states (Frank & Heller, 1997b, 2003). Such claims present a challenge to the development of a comprehensive description of sleep across the lifespan.

This challenge becomes particularly acute when sleep is defined using an arbitrary number of privileged components or when it is asserted that a single, "essential" component must be present in order for sleep to be expressed. One response to the latter claim is to suggest alternative names to behavioral states that lack the essential component (Frank & Heller, 2003). Although such a classification strategy is appropriate and even useful in a clinical setting, no classification scheme alone is sufficient for revealing the mechanisms that produce and regulate sleep–wake states.

We suggest that any theory of sleep development must account for the addition and integration of individual sleep components, as well as changes in the persistence of sleep and wakefulness across ontogeny (Blumberg & Lucas, 1996; Corner, 1985; Dreyfus-Brisac, 1970). To that end, it is important that we recognize the role that behavioral assessment can play in providing a firm foundation for further explorations of the mechanisms underlying behavioral states. Thus, we begin with behavior.

The Foundation: Behavior

The earliest behavior of invertebrate and vertebrate animals is characterized by spontaneous movements of the head, limbs, and tail (Corner, 1977). In mammalian and avian embryos, this spontaneous motor activity (SMA) is a ubiquitous feature of behavioral expression and has been a major focus of investigation for behavioral embryologists (Hamburger, 1973; Narayanan, Fox, & Hamburger, 1971; Provine, 1973). In considering these various embryonic and infant movements, Corner (1977) proposed that they exhibit continuity across the life span. Indeed, he maintained

that "sleep motility in its entirety… is nothing less than the continued postnatal expression of primordial nervous functional processes" (p. 292).

The SMA of fetal and infant rats exhibits organization in both spatial and temporal dimensions. One form of spatiotemporal organization that has received relatively little attention is movement synchrony, in which one limb moves in temporal proximity to another (Robinson & Smotherman, 1987). Although these synchronous movements occur predominantly at intermovement intervals of 0.5 s or less (Lane & Robinson, 1998), they are not simultaneous and do not resemble the whole-body startles[1] that have long been recognized (Gramsbergen et al., 1970; Hamburger & Oppenheim, 1967). Furthermore, movement synchrony reflects more than simply a temporal dependence among pairs of limbs; rather, patterns of movements among two or more limbs are organized into discrete bouts[2] (Fagen & Young, 1978). Using this bout-analytic approach (Robinson, Blumberg, Lane, & Kreber, 2000), similarities in bout structure between fetuses (embryonic day [E]17–E21) and infants (P1–P9) become readily apparent, thus providing additional empirical support for Corner's continuity hypothesis for SMA.

To further understand this organization, a computational model of SMA was developed that incorporated spontaneous activity of spinal motor neurons, intrasegmental and intersegmental interactions within the spinal cord, recurrent inhibition within the spinal cord, and descending influences from the brain; this model produced bouts with the same structure that we observed in perinatal rats (Robinson et al., 2000). Moreover, consistent with the model, bouts were not eliminated on embryonic day (E)20 after cervical spinal transection, suggesting that the brain is not necessary to produce bout organization in fetuses. Thus, the organization of limb movements into bouts appears to be a highly robust phenomenon that is consistently expressed by fetal and infant rats, and exhibits systematic changes during prenatal and postnatal development.

When an infant rat is placed in a humidified and thermoneutral environment—that is, an environment that allows for the minimal expenditure of energy[3]—it cycles rapidly between sleep and wakefulness. When awake, the pup often exhibits high-amplitude movements including locomotion, head-lifting, kicking, stretching, and yawning. When this activity ceases, there ensues a period of behavioral quiescence as muscles in the body relax. After this period of QS, AS commences with the onset of myoclonic twitching of the fore and hind limbs, tail, and head. These periods of twitching wax and wane until the pup suddenly reawakens and resumes high-amplitude movements. A typical cycle of waking, QS, and AS exhibit this basic order of expression, with the duration of each bout of sleep and wakefulness varying significantly within and between individuals, as well as across age.

Careful analysis of the behavior of infant rats indicates that they conform to many of the standard criteria used by other researchers to assess the existence of sleep in a variety of vertebrate and invertebrate species (Campbell & Tobler, 1984; Hendricks et al., 2000). These criteria include (a) an absence of high-amplitude movements (often designated in the literature as voluntary, coordinated, or purposeful), (b) spontaneity, as indicated by transitions between behavioral states that occur in a protected environment and are therefore not triggered by exogenous stimuli, and (c) reversibility, a criterion that helps to distinguish sleep from irreversible pathological states (e.g., coma). Other criteria for defining sleep, including circadian rhythmicity, increased sensory and/or arousal thresholds, homeostatic regulation, and neural control are addressed later in this chapter.

We have used behavior alone to examine the effects of air temperature and endogenous heat production on the expression of sleep and wakefulness (Blumberg & Stolba, 1996; Sokoloff & Blumberg, 1998). In addition, as discussed above, behavioral analysis was used to assess the temporal structure of twitching in fetal and neonatal rats (Robinson et al., 2000), as well as the contributions of the spinal cord to twitching (Blumberg & Lucas, 1994) and the reliance of twitching upon mesopontine neural circuitry (Kreider, 2003). Nonetheless, there are limitations to complete reliance upon behavioral measures. For example, using this method, it is not possible to discern the transition between quiet wakefulness and QS, both of which are marked by behavioral quiescence (Gramsbergen et al., 1970). Thus, demonstrating a stable relationship between the expression of sleep–wake behaviors and a second component would provide an important step forward in our understanding of state organization in infants.

Beyond Behavior: Nuchal Muscle Activity

As we began our search for a second measure of sleep and wakefulness to complement behavior in our infant subjects, we turned to the "trio"

of electrographic measures—EMG, EOG, and EEG—that had been codified in the manual of Rechtschaffen and Kales. Of these three, state-dependent EEG is not expressed in rats younger than P11 (Corner & Mirmiran, 1990; Frank & Heller, 1997b; Gramsbergen, 1976; Jouvet-Mounier et al., 1970; Seelke & Blumberg, 2008; Seelke, Karlsson, Gall, & Blumberg, 2005). Of the other two, we initially doubted our ability to measure the EOG, in part because we doubted that rapid eye movements occur early in infancy. So, we chose to measure EMG activity in the nuchal muscle, the primary elevator muscle of the head. To do this, we implanted fine-wire bipolar hook electrodes into the nuchal muscles of infant rats at P2, P5, and P8 (Karlsson & Blumberg, 2002) and recorded nuchal EMG activity and behavior, including twitches of the limbs and tail.

Figure 20.1A depicts a sleep–wake cycle in a 1-week-old rat, illustrating the progression from wakefulness to QS as indicated by the transition from high muscle tone to atonia. AS commences with the onset of twitching, as determined by behavioral analysis as well as the presence of twitch-related spikes in the nuchal EMG. Finally, with the onset of wakefulness, twitching is replaced by high-amplitude movements and nuchal atonia is replaced by high muscle tone, thus completing the cycle.

We noted that isolated spikes in the nuchal EMG occur only against a background of atonia and often result in noticeable twitch-like movements of the head. The relationship between these spikes and behaviorally scored twitches of the limbs and tail was examined, and we found a strong temporal relationship between them (Seelke & Blumberg, 2005). Specifically, during atonia periods, the onset of spikes in the nuchal EMG coincides with the onset of twitching in the limbs and tail (Figure 20.1B) and the two categories of twitching are highly correlated with each other (Figure 20.1C). Finally, bouts of twitching in the nuchal muscle and limbs are temporally linked across atonia periods, producing bouts of synchronized phasic activity interspersed with bouts of quiescence (Figure 20.1D) (Seelke & Blumberg, 2005; Seelke et al., 2005). Based on these and other observations, we can see that sleep and wakefulness in infant rats can be defined accurately using two measures—nuchal EMG and behavior—and, surprisingly, these measures are highly concordant at a very early age in this altricial species.

Using EMG and behavior, we were able to explore further whether the infant sleep state meets other standard criteria in the field (Hendricks et al., 2000). For example, to assess changes in sensory threshold during sleep, P8 rats were instrumented with nuchal EMG electrodes as well as electrodes for measuring respiration. Then, dimethyl disulfide, an olfactory stimulus, of various concentrations was presented to these subjects during periods of AS or wakefulness (Seelke & Blumberg, 2004). When awake, the threshold to exhibit polypnea (i.e., bursts of increased respiratory rate indicative of sniffing) was lower than when pups were in AS, suggesting a heightened sensory threshold during this sleep state.

Still another traditional criterion of sleep concerns the regulatory response to deprivation—commonly referred to as sleep homeostasis. Sleep homeostasis is typically assessed by depriving a subject of sleep and monitoring corrective responses (Bonnet, 2000; Rechtschaffen, Bergmann, Gilliland, & Bauer, 1999). Specifically, sleep deprivation is thought to evoke two compensatory responses: sleep pressure, which occurs during the period of deprivation and is indicated by an increase in the number of attempts to enter sleep (and a corresponding increase in the difficulty of producing and maintaining arousal), and sleep rebound, which occurs when sleep is permitted after a period of deprivation and is indicated by a compensatory increase in sleep.

To explore sleep regulation in early infancy, P5 rats were deprived of sleep for 30 min by delivering brief flank shocks whenever the nuchal muscle became atonic (Blumberg, Middlemis-Brown, & Johnson, 2004). Because it was increasingly difficult to maintain arousal over the period of sleep deprivation—as indicated by the need to increase the number of shocks and their intensity—we concluded that the procedure was inducing increased sleep pressure. In contrast to sleep pressure, sleep rebound was not detected at P5 in this study, consistent with an earlier study that suggested that rebound does not occur until after P14 (Frank, Morrissette, & Heller, 1998).

However, more recently (Todd & Blumberg, 2007), we developed an alternative method for depriving pups of sleep—one that allowed us to assess with greater confidence the effectiveness of the sleep deprivation procedure.[4] This method entailed the application of a cold stimulus to the snout, which possesses a high concentration of cold receptors (Dickenson, Hellon, & Taylor, 1979). Using this method in P2 and P8 rats, we again saw pronounced increases in sleep pressure. Moreover,

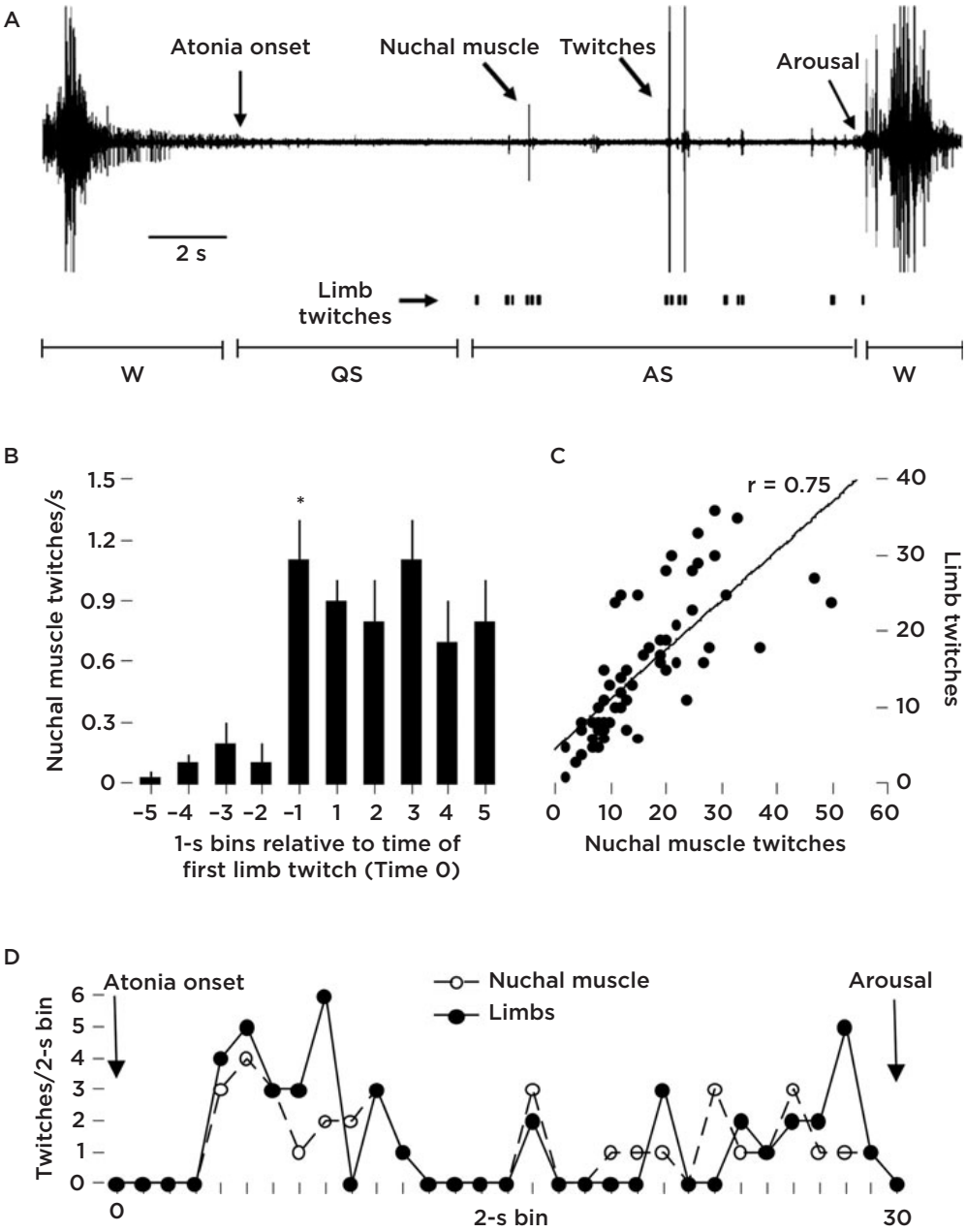

Figure 20.1 Relationship between myoclonic twitching as measured by visual observation of the limbs and activity in nuchal EMG. (A) Representative cycle of high nuchal muscle tone and atonia in a P8 rat tested at thermoneutrality (i.e., 35°C). Nuchal muscle twitches against a background of atonia are indicated, as are instances of visually scored limb twitches. This cycle has been divided into periods of wakefulness (W), quiet sleep (QS), and active sleep (AS). (B) Perievent histogram indicating increase in nuchal muscle twitching within 1 s of the first visually scored limb twitch of an atonia period. Data are from 10 atonia periods across 6 P8 rats . * Significant difference from previous time bin. (C) Regression relating the number of behaviorally scored limb twitches and the number of nuchal muscle twitches during periods of atonia. Data are from the same atonia periods as in (B). $N = 60$ data points. The best-fit line is shown. (D) Representative data from a single period of atonia in a P8 rat showing the number of twitches measured during successive 2-s bins. Behaviorally scored limb twitches (filled circles) and nuchal muscle twitches (open circles) are shown separately and indicate synchronized bursts of phasic activity during the atonia period. (From Seelke & Blumberg, 2005.)

when the deprivation period was over and the pups were allowed to sleep without disturbance, they exhibited pronounced increases in sleep duration, consistent with sleep rebound. Finally, when precollicular transections were performed at P2, sleep pressure increased significantly during the deprivation period, but sleep rebound was now prevented. This dissociation between pressure and

rebound with precollicular transection has also been reported in adult cats (de Andres, Garzon, & Villablanca, 2003).

The studies just described are the first to address the effects of sleep deprivation before P12 in rats (Feng, Ma, & Vogel, 2001; Frank et al., 1998; Mirmiran et al., 1983; Mirmiran, Van De Poll, Corner, Van Oyen, & Bour, 1981). Clearly, important questions remain and more work in this area is needed.

Rapid Eye Movements and Extraocular Muscle Activity

During AS in adults, REMs occur along with other forms of phasic activity, such as myoclonic twitching. Jouvet-Mounier et al. (1970) reported that REMs occur as early as P6, but they did not mention whether REMs are present at earlier ages. Other researchers, including van Someren et al. (1990), reported the presence of REMs at P8 that exhibited an "adult-like appearance" by P15, that is, around the time of eye opening. These reports left several unanswered questions. First, do the eyes exhibit state-dependent movements, or indeed any activity, before P6? Second, what is the mechanism that generates the sleep-related rapid eye movements?

A novel perspective on the mechanisms of REM generation was proposed by Chase and Morales (1983, 1990). Specifically, in their 1983 paper examining the origin of myoclonic twitches, they asked whether the

> mechanisms responsible for the phasic contraction of the peripheral musculature during REM periods may reflect a general pattern that affects other somatomotor functions as well. For example, the striated muscles that move the orbits are active during REM periods. . . . It is possible that the central neural areas that give rise to myoclonic activation of the limb muscles during REM periods also initiate a pattern of twitches and jerks that affect all striated muscles. REMs are an example. . . . (p. 1198)

This hypothesis—that the same mechanisms responsible for generating myoclonic twitches of the limbs could also be responsible for generating twitches of the eyes—provided us with a framework for examining the development of REMs in infant rats. We wondered: If REMs are indeed produced by twitches of the extraocular muscles, and if these extraocular muscle twitches are phenomenologically similar to the twitches produced by other striated muscles, then perhaps direct measurement of extraocular muscle activity would reveal ontogenetic precursors of REMs that had heretofore gone unnoticed.

Thus, we examined extraocular EMG activity in rats at P3, P8, and P14 (Seelke et al., 2005). EMG electrodes were implanted in the medial and lateral rectus muscle of each eye and the signals from the electrodes were filtered to allow for the examination of both gross eye movements and extraocular muscle activity. EMG electrodes were implanted in the nuchal muscle of each subject and, in P14 subjects, EEG activity was also measured.

As shown in Figure 20.2, each extraocular EMG record could be filtered in two ways to reveal two kinds of activity. First, filtering for high-frequency activity (i.e., 300–5000 Hz, top row of Figure 20.2) revealed spiking in the EMG record indicative of muscle twitching. At all three ages—P3, P8, and P14—distinct twitching was observed in the extraocular EMG. Second, filtering for low-frequency activity (i.e., 1–35 Hz, bottom row of Figure 20.2) revealed movements of the eyeball, similar to the information provided by the EOG. Sleep-related rapid eye movements were most easily identified

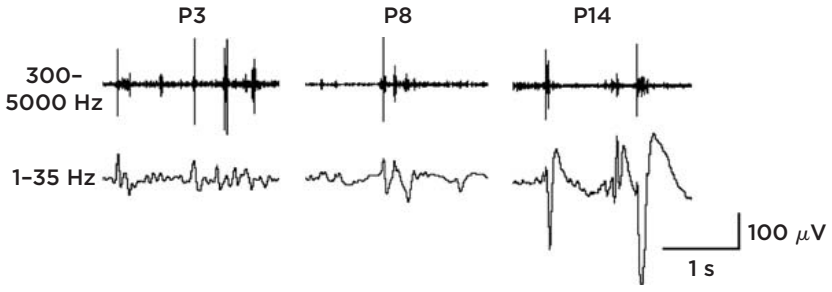

Figure 20.2 Representative extraocular EMG activity and eye movements at P3, P8, and P14. Top row: High-pass (300–5000 Hz) filtering to reveal myoclonic twitching. Bottom row: Low-pass (1–35 Hz) filtering to reveal eye movements. (From Seelke et al., 2005.)

at P14, but some evidence of such movements was found at P8 and even P3. Significantly, these REMs were typically accompanied by twitches of the extraocular muscles, thus providing direct support for the Chase and Morales hypothesis. Moreover, because twitching was easily identified as early as P3, an age when eye movements were not robust, it was apparent that twitching in the EMG record reveals features of early oculomotor activity that are not easily detectible using conventional EOG techniques.

Although REMs are often afforded privileged status by sleep researchers, our findings indicated that the eye muscles are, like those controlling any limb, prone to twitching during AS. One implication of this interpretation is that conventional assessments of the EMG and EOG may reveal tonic and phasic aspects of muscle activity, respectively, but a single record of skeletal muscle activity—when less restrictive filtering and sampling methods are used—can capture both tonic fluctuations in muscle tone as well as occurrences of phasic twitching (Seelke et al., 2005).

Having established that REMs are produced by twitches of the extraocular muscles, we next turned our attention to the relationship between these twitches and other forms of sleep-related phasic activity. As described above (see also Figure 20.1D), atonia periods begin with a bout of behavioral quiescence, soon followed by the onset of twitches as detected from the extraocular and nuchal EMGs as well as behavioral observations of the limbs and tail. Perhaps most striking, we found that as early as P3 (see Figure 20.3A), all of the phasic movements occur together to define a coherent period of AS. Moreover, when these movements are analyzed in greater detail, we see again that they are expressed as synchronized "waves" of phasic activity occurring in the extraocular muscles, nuchal muscle, and limbs. This highly organized structure of AS contrasts sharply with the perspective of infant sleep as diffuse, primitive, and dissociated (Adrien & Lanfumey, 1984; Frank & Heller, 1997b, 2003).

Completing the Trio: Neocortical EEG

As already mentioned, it is widely accepted that the neocortical EEG in rats does not exhibit state-dependent differentiation, especially delta (or slow-wave) activity, until approximately P11 (Frank & Heller, 1997b; Gramsbergen, 1976; Mirmiran & Corner, 1982). In other species, this milestone is reached at 115–120 days postconception in sheep (Clewlow, Dawes, Johnston, & Walker, 1983; Szeto & Hinman, 1985), 50 days postconception in guinea pigs (Umans et al., 1985), and approximately 32 weeks postconception in preterm human infants (Dreyfus-Brisac, 1975). Despite the fact that neocortical activity is a noncausal correlate of sleep and wakefulness and not an integral component of the neural circuitry that modulates state, its inclusion as one of the critical criteria in the Rechtschaffen and Kales sleep manual elevated its status among sleep researchers (e.g., see Frank & Heller, 2003).

The absence of the EEG as a reliable measure of behavioral state in infants had forced earlier investigators to rely on other measures, including body movements, respiration, heart rate, and muscle tone (Gramsbergen et al., 1970; Nijhuis et al., 1984; Parmelee, Wenner, Akiyama, Schultz, & Stern, 1967). Perhaps inevitably, disagreement and confusion emerged as different investigators relied on different measures and adopted different criteria for categorizing sleep at these early ages (Dreyfus-Brisac, 1970; Prechtl, 1974). One of the aims of our research program was to avoid conceptual and semantic confusion through systematic and unbiased analysis of the components of sleep as they are elaborated through developmental time (Blumberg & Lucas, 1996). As detailed until this point, our research had indicated the presence of an ultradian rhythm comprising bouts of high and low muscle tone linked with other state-related phenomena (e.g., myoclonic twitches).

The relatively late postnatal emergence of delta activity in rats provides an opportunity to observe the real-time linking of a new sleep component with those that have already developed. We did this by examining the relationship between the neocortical EEG, nuchal and extraocular EMGs, and limb movements (Seelke et al., 2005). First, as shown for the P3 subject in Figure 20.3A, note once again how wakefulness is followed by a period of QS which, in turn, is followed by an AS period defined by the presence of phasic muscle activity. The question we addressed was whether delta activity, once it emerged, would occupy the "location" designated as QS in younger subjects. As shown for the P14 subject in Figure 20.3B, the period of nuchal atonia before the onset of twitching was accompanied by high-amplitude EEG activity; this EEG activity exhibited a dominant frequency of 2.5–4 Hz, characteristic of delta activity. Because the records at the two ages are, with the exception of EEG activity and timescale, nearly identical, we concluded that the states designated as QS and AS

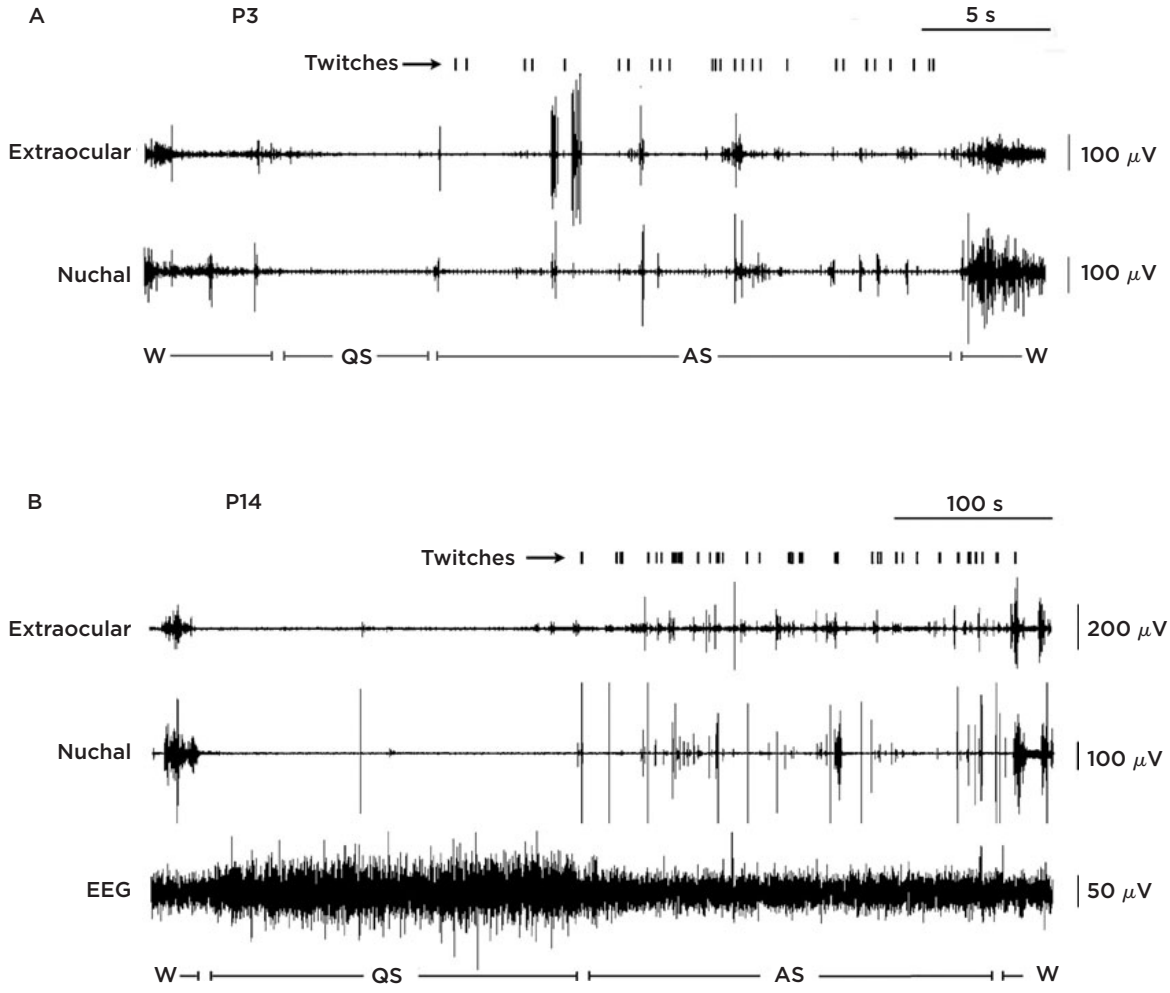

Figure 20.3 Phasic and tonic behavioral and electrographic events across a complete sleep–wake cycle in a P3 and P14 rat. (A) Behaviorally scored limb twitches and extraocular and nuchal EMGs for a P3 rat. Periods of wakefulness (W), quiet sleep (QS), and active sleep (AS) are indicated. (B) Same as (A) except the subject is a P14 rat and neocortical EEG is also recorded. The twitch occurring in the middle of the QS period is actualy due to a startle. Note the different timescales for (A) and (B). (From Seelke et al., 2005.)

before the emergence of delta activity are homologous with those that come later.

In subsequent work, we assessed the structure of sleep bouts in P9, P11, and P13 rats—that is, before, during, and after the emergence of delta activity (Seelke & Blumberg, 2008). At all three ages, using EMG and behavioral measures alone, we found that QS predominates during the first third of each sleep period. At P11 and P13, delta activity similarly predominates during the first third of each atonia period, declines during the second third, and is rare during the final third. When delta occurs during the final third of a sleep period, it occurs during those brief interludes between bursts of myoclonic twitching. Thus, with the developmental emergence of delta activity, we can positively identify multiple cycles of QS and AS, as in adults (Zepelin, Siegel, & Tobler, 2005).

Based on previous studies, it is clear that QS dominates the first third of sleep periods in rats as early as P3 (Seelke et al., 2005) and that this pattern continues up until the emergence of delta activity at P11 (Seelke & Blumberg, 2008). These results seem inconsistent with the findings of Jouvet-Mounier et al., who reported that QS in infant rats is virtually nonexistent before P10 and increases explosively thereafter (Jouvet-Mounier et al., 1970). The discrepancy between these findings is likely due to the restrictive criteria used by those earlier investigators for defining QS. Specifically, in the Jouvet-Mounier et al. study, 30 s of behavioral quiescence were required before the designation of QS was applied. When we consider that the mean

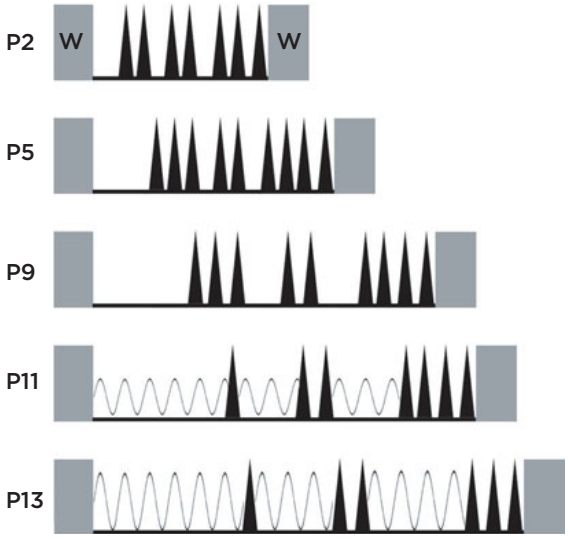

Figure 20.4 Schematic depiction of developmental changes in the temporal organization of sleep and wakefulness and the emergence of delta activity in infant rats. Gray rectangles represent periods of high muscle tone, indicative of wakefulness (W). Interposed periods of sleep are defined by nuchal atonia, depicted as black lines. Phasic bursts of myoclonic twitching, indicative of active sleep, are depicted as black triangles. At P9 and earlier, each sleep period comprises an initial bout of quiet sleep followed by bursts of phasic activity interrupted by brief bouts of behavioral quiescence. By P11, delta activity (depicted as sinusoidal waves) is detected during the first quiet sleep episode as well as during some of the subsequent periods of quiescence between bouts of twitching. By P13, delta power has increased and is more reliably expressed during periods of quiescence. Overall, with age, sleep durations increase and the intervals between bouts of twitching also increase, thus providing greater opportunity for the expression of delta activity during the final third of a sleep bout. (Adapted from Seelke & Blumberg, 2008.)

sleep bout duration during the first postnatal week ranges from 20 s at P3 to 45 s at P8 (Blumberg, Seelke, Lowen, & Karlsson, 2005b), and that mean QS duration only exceeds 30 s by P13 (Seelke & Blumberg, 2008), a 30-s criterion for the identification of QS effectively precluded its detection in that earlier study.

Thus, using the methods and criteria outlined above, within days after birth we can identify sleep periods comprising periods of quiescence interspersed with bursts of myoclonic twitching (see Figure 20.4). These bursts, comprising synchronized activity in multiple muscle groups throughout the body, begin shortly after the onset of atonia and continue throughout the duration of the sleep period. The periods of quiescence are initially very brief—during the first postnatal week they often last less than 2 s. The duration of these periods of quiescence increases with age and, by P11, are often accompanied by delta activity.

We should stress that no classification scheme, including ours, provides perfect assessments across all spatial and temporal scales. Accordingly, it has long been appreciated that the inclusion of as many components as possible into a scheme helps to resolve uncertainties. However, given the fluidity of state development and the unavailability of certain state components at earlier ages, our goal should be to devise classification schemes that capture the full range of developmental phenomena in an unbiased fashion. Then, to move beyond classification toward explanation, we should seek to understand the neural mechanisms that produce the components as well as their functional relations.

State-dependent Neocortical Activity Revisited

The traditional reliance on surface EEG recordings and the focus on delta activity gave rise to the notion that the EEG is undifferentiated prior to the emergence of delta activity around P11 in rats. For a variety of reasons, however, this perspective is no longer accurate. First, the use of electrodes that can detect infraslow activity has revealed bursts of sleep-related "slow activity transients" in premature human infants during sleep that disappear around 40 postconceptional weeks (Vanhatalo et al., 2005; see Chapter 6).

Second, while recording from the primary sensory areas of newborn rats, Khazipov and colleagues detected brief oscillations that they call "spindle-bursts" (Khazipov et al., 2004; see Chapter 8). In somatosensory cortex, these bursts occur in response to spontaneous or evoked peripheral stimulation in a somatotopic manner. Subsequent work indicates that spindle-bursts within the forelimb region of primary somatosensory cortex are closely associated with the proprioceptive stimulation that accompanies twitching of the forelimb during AS (Marcano-Reik & Blumberg, 2008). Thus, already at P2–6, the infant neocortex exhibits somatotopic organization and a distinct neurophysiological response to discrete sensory stimulation. Because spindle-bursts occur in response to self-generated movements, they will occur predominantly during wakefulness or AS; in the nest, sensory stimulation from the dam and from other pups will also evoke them.

Spindle-bursts have also been detected in barrel and visual cortex after whisker stimulation and retinal waves, respectively (Hanganu, Ben-Ari, &

Khazipov, 2006; Hanganu, Staiger, Ben-Ari, & Khazipov, 2007; Minlebaev, Ben-Ari, & Khazipov, 2007). Thus, in conjunction with the previous findings in somatosensory cortex, it is clear that the neocortex of the altricial infant rat exhibits complex activity soon after birth. Some forms of this activity—as with spindle-bursts—are detected exclusively within primary sensory areas, whereas others may be found predominantly outside these areas. Regardless, it now appears that the infant neocortex exhibits state-dependent activity long before delta activity emerges. This early activity may play a critical role in the establishment and refinement of cortical connections, including those necessary for somatotopy (Khazipov et al., 2004; Seelke et al., 2005).

In P9, P11, and P13 rats (Seelke & Blumberg, 2008), we found evidence of very low-frequency cortical events that appear similar to the slow activity transients (SATs) described in premature human infants (Vanhatalo & Kaila, 2006; Vanhatalo et al., 2005). Also, as with SATs in humans, we found that SATs in infant rats are sleep-related. Specifically, we found that SATs predominate during the first third of the sleep period (Seelke & Blumberg, 2008), thus suggesting a close association with QS (although SATs also occur during wakefulness and in temporal proximity to AS-related twitching).

The developmental disappearance of SATs has been associated with the upregulation of the neuronal chloride extruder K^+–Cl^- cotransporter 2 (KCC2) and the associated emergence of the hyperpolarizing effects of GABA (Vanhatalo et al., 2005; see Chapter 6), which occurs during the second postnatal week in rats (Payne, Rivera, Voipio, & Kaila, 2003). Using calcium imaging, similar high-amplitude, low-frequency events have been observed in infant rat cortical tissue in vitro (Garaschuk, Linn, Eilers, & Konnerth, 2000) and neonatal mice in vivo (Adelsberger, Garaschuk, & Konnerth, 2005) and are referred to as "early network oscillations" (ENOs). As with SATs, the developmental disappearance of ENOs also appears to depend upon emerging GABAergic inhibition (Garaschuk et al., 2000). Thus, the disappearance of SATs/ENOs is mirrored by the appearance of delta activity, which may also depend upon GABAergic inhibition (Steriade, Curro Dossi, & Nunez, 1991; Terman, Bose, & Kopell, 1996).

Neural Substrates of Infant Sleep and Wakefulness

The successful integration of sleep research with neuroscience has contributed to the belief that sleep is "of the brain, by the brain, and for the brain" (Hobson, 2005). As a consequence, it is now expected that true sleep processes will be reflected in discernible neural activity, so much so that the identification of state-dependent neural activity has been mentioned as one criterion of sleep (Hendricks et al., 2000).

With regard to sleep in infant rats, technical obstacles have hampered the search for state-dependent neural activity. In only two early studies was it reported that the infant brain—specifically, the pontine and mesencephalic reticular formation—exhibits state-dependent activity (Corner & Bour, 1984; Tamásy, Korányi, & Lissák, 1980). Nonetheless, some had come to believe that the sleep–wake cycles of infant rats are not regulated by the brain, or at least are not controlled by the kinds of specific brain nuclei that have been identified in adults. Thus, in 1984, it was written that infant sleep comprises "a very primitive system of diffuse activation within the whole central nervous system" (Adrien & Lanfumey, 1984). More recently, Frank and Heller (2003) declared that infant sleep "is not controlled by executive sleep centers." In contrast with these views, we now review recent work documenting the contributions of central neural mechanisms to the modulation of infant sleep–wake states and the components that comprise them.

Spinal Cord

Myoclonic twitching has often been compared with other forms of SMA that are prevalent in vertebrate embryos, including birds and mammals (Corner, 1977; Narayanan et al., 1971; Robinson et al., 2000). Indeed, as already discussed, spontaneous activity in rats exhibits temporal structure that varies continuously across the prenatal-to-postnatal period (Robinson et al., 2000). Because this spontaneous activity provides useful information concerning the organization of behavioral states in early infancy, it is important to understand the neural mechanisms that produce it.

In order to investigate the mechanisms underlying the generation of myoclonic twitches, newborn rats received midthoracic spinal transections within several days after birth. When they were examined 1 week later, it was found that the hindlimbs (i.e., the limbs caudal to the transection) exhibited 50% fewer twitches than control pups (Blumberg & Lucas, 1994). Moreover, activity of the forelimbs (i.e., the limbs rostral to the transection) were unaffected by the spinal transection. It was

concluded that spinal mechanisms alone can produce twitching, especially in fetuses and soon after birth in rats, but that there are increasing contributions with age from more rostral structures, including cervical spinal cord and brain.

Subsequent work demonstrated that the mesopontine region is a likely area of importance for the expression of twitching in rats within 1 week of birth. This was demonstrated by transecting the brain at various levels just caudal and rostral to the mesopontine region (Kreider & Blumberg, 2000). When the transections were placed anterior to the mesopontine region, twitching was no longer affected.

Therefore, both the brain and spinal cord contribute to the production of spontaneous activity, and specifically myoclonic twitching, soon after birth in rats. Furthermore, it appears that myoclonic twitching is initially produced independently by neural circuits within the spinal cord and that those circuits gradually come under the control of rostral brain structures (Stelzner, 1982).

Medulla

The demonstrated linkage between motor behavior (including myoclonic twitching) and nuchal EMG provided additional opportunities for exploring the neural bases of behavioral state organization in early infancy, provided that certain technical difficulties could be overcome. In particular, the soft, uncalcified skull of infant rats precludes many of the techniques that are readily used in larger and less fragile subjects. Over time, we developed a method for stimulating and recording from the brain of unanesthetized, head-fixed infants as nuchal EMG activity and behavior are also monitored.

The sleep-related nuchal atonia observed in infant rats could result from two distinct and mutually exclusive processes: the active inhibition of spinal motoneurons, as in adults (Chase & Morales, 1990), or from mere passive withdrawal of excitation to spinal motoneurons. If infant and adult atonia are produced by similar mechanisms, then the infant brain stem should contain neurons within the ventromedial medulla that produce atonia when stimulated (Hajnik, Lai, & Siegel, 2000) and exhibit atonia-related discharge properties (Sakai, 1988; Siegel et al., 1991); moreover, lesions of this area should produce a state reminiscent of "REM without atonia" (Schenkel & Siegel, 1989).

Using P7–10 rats, we found support for each of these predictions (Karlsson & Blumberg, 2005). First, we used electrical stimulation to identify an inhibitory area within the medial medulla. As shown in Figure 20.5A, muscle tone inhibition was consistently found on or near the midline within the ventromedial medulla in an area that includes nucleus gigantocellularis, nucleus paramedianis, and raphe obscurus. Figures 20.5B and C depict the effect of electrical stimulation within this region on nuchal muscle tone in a representative subject. It is clear that each pulse of electrical stimulation produces a discrete and reversible inhibition of nuchal tone. To ensure that these observed effects were not due to stimulation of fibers of passage, we infused the glutamate agonist quisqualic acid into this same region and produced rapid inhibition of muscle tone (similar infusions of the cholinergic agonist carbachol or corticotropin-releasing factor had no effect).

Second, as shown in Figure 20.5D**,** extracellular recordings from this same area in the medulla revealed the presence of neurons exhibiting "atonia-on" profiles, that is, they became active during periods of nuchal atonia and went silent during period of high nuchal muscle tone (neurons with "EMG-on" profiles were also found during periods of high muscle tone, but at more lateral sites). Finally, chemical lesions within the inhibitory area resulted in significant reductions in atonia durations, as well as decoupling of atonia from myoclonic twitching; specifically, twitches occasionally occurred during periods of high muscle tone, a condition reminiscent of "REM without atonia" as described in adults (Morrison, 1988; Schenkel & Siegel, 1989).

Based on the results of this study employing three experimental approaches—stimulation, recording, and lesioning—we concluded that the brain of infant rats, like that of adults, contains a medullary inhibitory area (MIA) that must be activated in order to produce the atonia of sleep. These results were then used as a foundation for further investigations of the neural contributions to infant sleep and wakefulness at other levels of the neuraxis.

Mesopontine Region

Having established a foothold in the medulla with the identification of the MIA, we used a variety of experimental approaches to delineate the components of the neural circuit mediating behavioral state in 1-week-old rats (Karlsson, Gall, Mohns, Seelke, & Blumberg, 2005). First, to establish the presence of efferents to the MIA, we

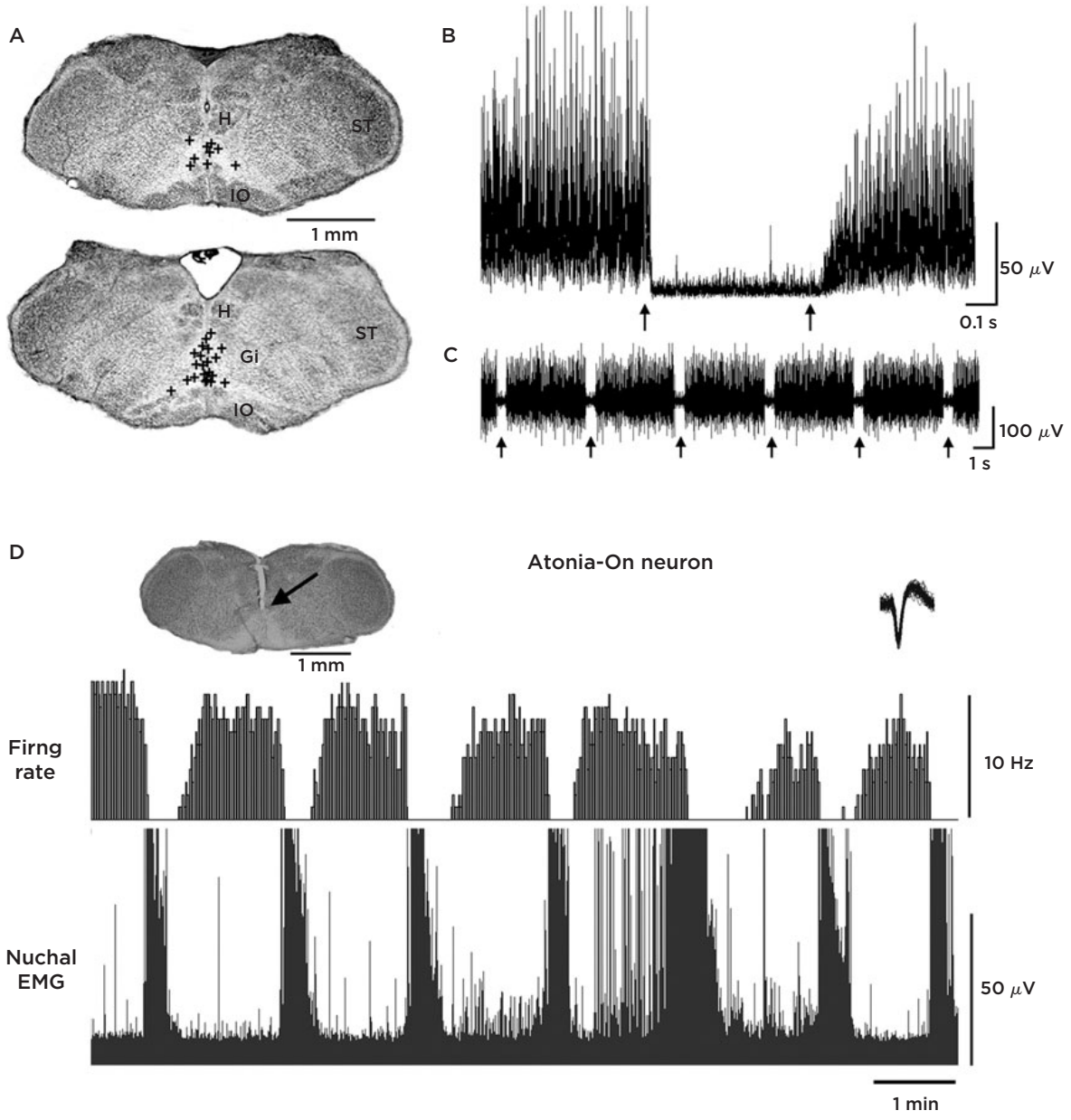

Figure 20.5 Inhibitory sites in the ventromedial medulla identified using electrical stimulation in P8–10 rats. (A) Coronal sections of the medullary inhibitory area. Each symbol (+) represents one inhibitory site from one pup (H, hypoglossal nucleus; ST, spinal trigeminal nucleus; Gi, nucleus gigantocellularis; IO, inferior olive). (B) Averaged nuchal EMG trace from 10 stimulus pulses applied to the inhibitory area of one pup; the arrows depict pulse onset and offset. (C) Representative sample of raw nuchal EMG responses to stimulation pulses; the arrows indicate periods of stimulation-induced atonia. (D) The discharge profile of a representative atonia-on unit in the ventromedial medulla (top) and its associated nuchal EMG trace (bottom). In the photomicrograph, the arrow indicates the tip of the electrode. The inset in the upper-right corner depicts 50 superimposed action potentials of the sorted unit. For purposes of illustration, the EMG trace is full-wave rectified. (From Karlsson & Blumberg, 2005.)

infused the fluorescent tracer DiI into that region and searched for the presence of cell bodies projecting to it. As expected, we found inputs to the MIA from medullary and mesopontine structures—including the nucleus subcoeruleus (SubLC), nucleus pontis caudalis (PC), and laterodorsal tegmental nucleus (LDT)—that are similar to those reported in adults (Cobos, Lima, Almeida, & Tavares, 2003; Malinowska & Kubin, 2004; Vertes, Martin, & Waltzer, 1986).

Using the tracing study as a guide, we next searched within the medulla and mesopontine region

for sites that exhibited state-dependent activity. In particular, we were searching for neurons exhibiting firing patterns that mirrored sleep–wake states identified using the nuchal EMG, including atonia-on neurons (indicative of sleep, such as those identified in the MIA), EMG-on neurons (indicative of wakefulness), as well as other patterns that have been identified in adults. For example, as shown in Figure 20.6, atonia-on and EMG-on neurons, and even neurons associated particularly with AS ("AS-on"), were found within the SubLC and PC.

In contrast, the LDT contained many EMG-on neurons. Also, and perhaps most surprising was the identification of neurons within the LDT that exhibited a burst of activity in anticipation of myoclonic twitches; this finding is consistent with our earlier claim (Kreider & Blumberg, 2000; see discussion above) that neurons within the mesopontine region of 1-week-old rats contribute to twitching. All together, these findings demonstrate a remarkable diversity of state-dependent neural activity in neonatal rats.

Next, building on this neurophysiological evidence, we performed electrical and chemical lesions within the mesopontine region. Consistent with the recording data, we demonstrated that atonia durations are decreased after lesions of SubLC or nucleus pontis oralis (PO) and myoclonic twitching is reduced after lesions within the dorsolateral pontine tegmentum (DLPT), despite a dramatic increase in atonia duration. Moreover, lesions of SubLC and PO decoupled myoclonic twitching from nuchal atonia, producing a condition resembling "REM sleep without atonia" similar to that found after lesions within the MIA (Karlsson & Blumberg, 2005).

More recently, we tested the hypothesis that nuchal muscle tone is modulated, at least in part, by cholinergically mediated interactions between the DLPT and PO (Gall, Poremba, & Blumberg, 2007). Again using unanesthetized P8–10 rats, we found that chemical infusion of the cholinergic agonist carbachol within the DLPT activated high muscle tone. Next, chemical lesions of the PO were used to produce a chronic state of high nuchal muscle tone, at which time the cholinergic antagonist scopolamine was infused into the DLPT. Scopolamine effectively decreased nuchal muscle tone, suggesting that lesions of the PO increase muscle tone via cholinergic activation of the DLPT. Indeed, activation of the DLPT after PO lesions was effectively visualized using 2-deoxyglucose (2-DG) autoradiography. Finally,

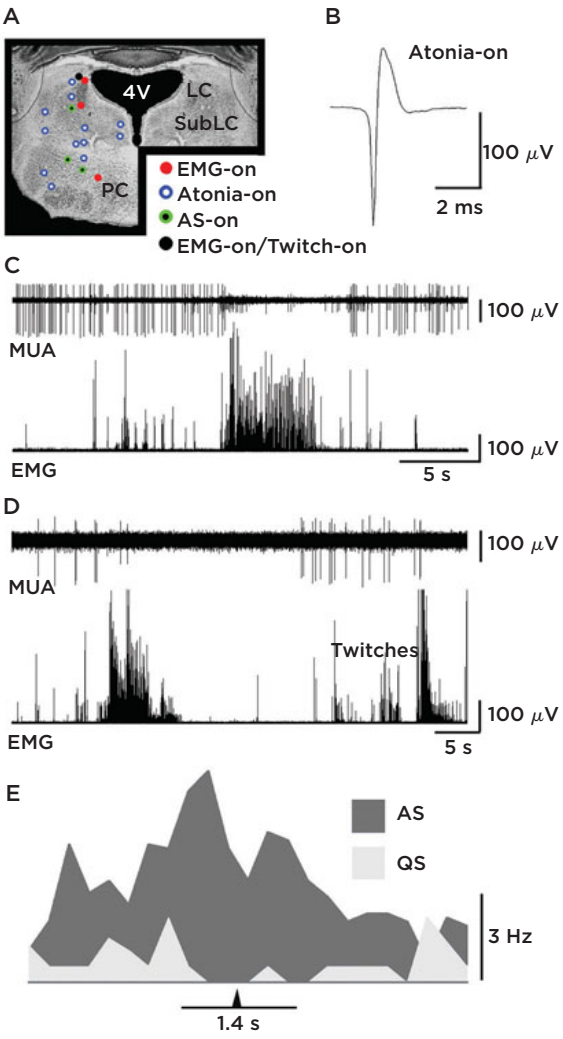

Figure 20.6 State-dependent neural activity within the mesopontine region. (A) Recording sites of state-dependent neurons reconstructed on a coronal section at the mesopontine level of a P8 rat. Note the predominance of atonia-on neurons. (B) Averaged waveform of a representative atonia-on neuron. (C) Upper trace: multiunit activity. Lower trace: concurrently recorded nuchal EMG. Spike sorting revealed 2 units that are easily distinguished by their amplitudes. The higher-amplitude unit is atonia-on; note its tonic discharge throughout the atonia period. (D) Upper trace: multiunit activity. Lower trace: concurrently recorded nuchal EMG. Spike sorting revealed 2 units that are easily distinguished by their amplitudes. The higher-amplitude unit is AS-on; note the absence of multiunit activity at the onset of the atonia period and then the increase in activity coinciding with the appearance of nuchal twitches. (E) Mean discharge rates of a representative AS-on neuron during bouts of AS and QS as defined, respectively, by the presence or absence of phasic nuchal twitches during periods of atonia. The arrowhead indicates the midpoint of the AS and QS bouts. 4V: fourth ventricle; LC: locus coeruleus; SubLC: subcoeruleus; PC: nucleus pontis caudalis. (From Karlsson et al., 2005.)

consistent with the hypothesis that PO inactivation produces high muscle tone, infusion of the sodium channel blocker lidocaine into the PO of unanesthetized pups produced a rapid increase in muscle tone. Thus, it appears that, even early in infancy, the DLPT is critically involved in the regulation of muscle tone and behavioral state and that its activity is modulated by a cholinergic mechanism that is directly or indirectly controlled by the PnO.

Forebrain

In light of recent doubts concerning medullary and mesopontine contributions to behavioral state organization in infant rats, the notion that the forebrain would make its own contributions would appear even more unlikely. In addressing this issue, an examination of the representative data presented in Figure 20.7A is particularly instructive. In noting the durations of sleep and wake bouts in that figure, it is apparent that the atonia periods depicted for the P2 subjects are substantially shorter in duration than that for the P8 subject; in contrast, the high-tone durations are similar at these two ages. When we quantified these durations across P2 and P8 subjects, as shown in Figure 20.7B, we found that mean atonia durations (i.e., sleep durations) increase from approximately 15 to 40 s between P2 and P8, whereas mean tone durations (i.e., wake durations) increase from approximately 5 to 15 s (Karlsson, Kreider, & Blumberg, 2004).

We hypothesized that the increasing durations of sleep and wake bouts across this early developmental period result, at least in part, from increasing modulatory effects of the forebrain on brainstem

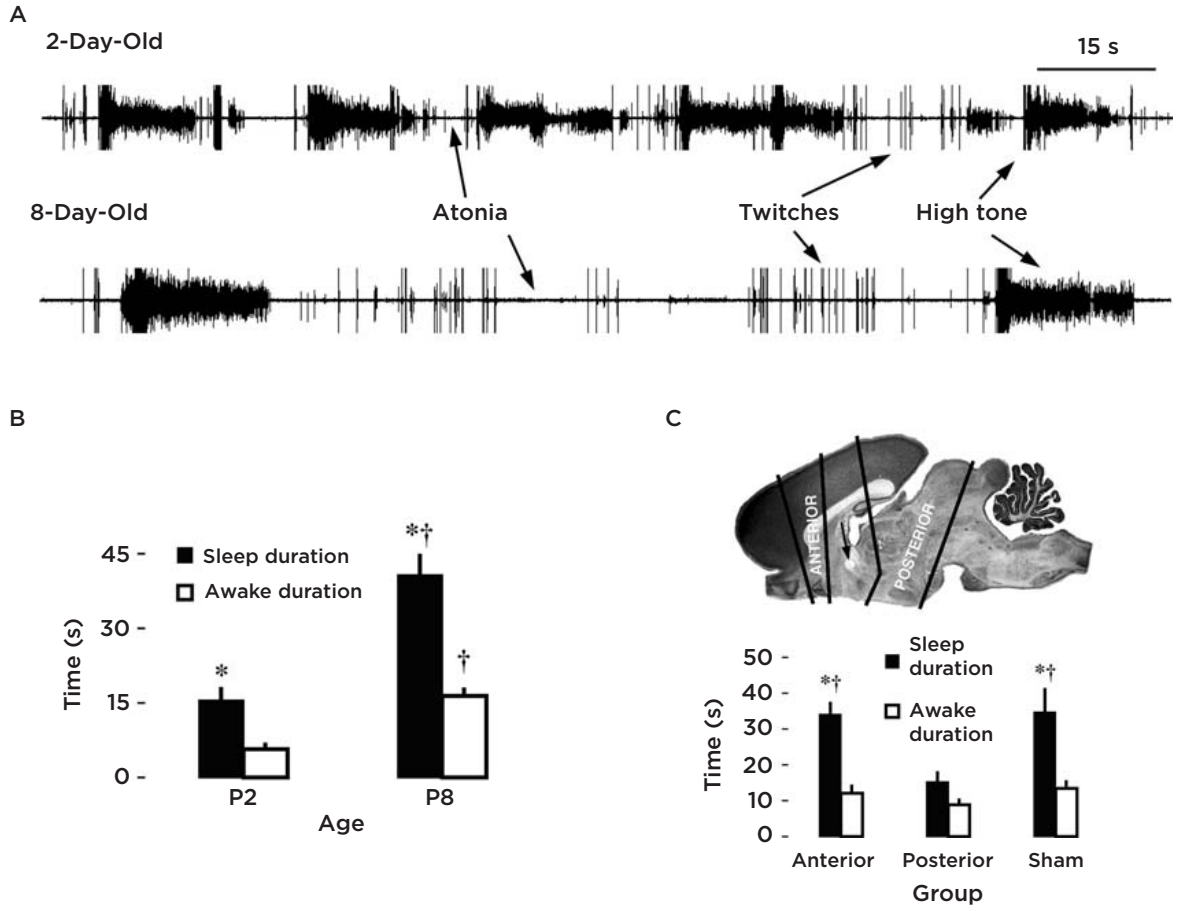

Figure 20.7 (A) Nuchal EMG data from representative P2 (top) and P8 (bottom) rats. Each segment is 2.4 min long. Atonia (i.e., sleep) and high-tone (i.e., awake) periods are indicated by arrows. The P2 rat cycles between the two EMG states more rapidly than the P8 rat. Instances of myoclonic twitching against a background of atonia are also indicated. (B) Mean sleep and wake bout durations for unrestrained P2 and P8 rats. * Significant difference from Awake Duration. † Significant difference from Sleep Duration. (C) Top: Mid-sagittal section in a P8 rat, with the rostral-caudal range of anterior and posterior transections depicted. Arrow indicates anterior commissure. Bottom: Mean sleep and wake bout durations for pups with anterior and posterior transections and for sham controls. * Significant difference from Awake Duration. † Significant difference from posterior transection group. (From Karlsson et al., 2004.)

structures. We initially tested this hypothesis by performing transections at two different levels of the neuraxis in P8 rats. As illustrated in Figure 20.7C, one set of "posterior" transections was placed ventrally within the caudal hypothalamus, and another set of "anterior" transection was placed rostral to the preoptic hypothalamus (POA). Figure 20.7C also presents the effects of these transections on mean sleep and wake bout durations. Whereas the anterior and sham transections exhibited similar bout durations, the posterior transections significantly decreased the sleep durations, resulting in pups that cycled very rapidly between sleep and wakefulness.

In a subsequent study (Mohns, Karlsson, & Blumberg, 2006), we identified the regions between the posterior and anterior transections that were responsible for the effects of transection on sleep and wake bouts. We focused on the ventrolateral preoptic area (VLPO) and basal forebrain because of their roles in adult sleep–wake regulation (Saper, Chou, & Scammell, 2001; Szymusiak, Steininger, Alam, & McGinty, 2001) and because these structures were spared by the most rostral posterior transections and the most caudal anterior transactions in the earlier transection study. Electrolytic lesions placed selectively in the VLPO or basal forebrain produced discrete changes in sleep and wakefulness. Critically, however, only combined lesions of the two regions reproduced the reduced sleep and wake bouts produced by the posterior decerebrations in the earlier study.

Interestingly, the P8 subjects with posterior transections, described above, exhibited bout durations that resemble those of intact P2 subjects. Such a finding could suggest that sleep–wake regulation at P2 occurs without any contribution from forebrain structures. However, when similar transections were performed in P2 subjects, mean sleep durations were also significantly decreased (Mohns et al., 2006). Thus, it is not the case that the consolidation of sleep bouts across the first postnatal week arises entirely from the onset of a functional POA. Rather, it appears that both brainstem and forebrain mechanisms contribute.

In this same study, we also recorded extracellular neural activity within the preoptic area and basal forebrain of unanesthetized P9 subjects, the first such recordings in infant rats. Both sleep- and wake-on neural activity was found, consistent with a role for these forebrain areas in the modulation of sleep and wakefulness.

To investigate the possibility that the POA's modulatory effects on sleep and wakefulness change across early development, we administered the wake-promoting drug modafinil at P2 and P9. Although modafinil's wake-promoting (and sleep-inhibiting) mechanisms are not completely understood, this drug has been shown to potentiate the inhibition of GABAergic POA neurons in vitro (Gallopin, Luppi, Rambert, Frydman, & Fort, 2004). In our infant subjects, we found that modafinil had a strong wake-promoting effect at both P2 and P9, whereas the drug's ability to suppress sleep was significantly greater at P9 (perhaps resulting in part from the existence of longer sleep bout durations at P9). These results further support the hypothesis that the POA, through its modulation of more caudal regions, contributes to increasing sleep bout durations across the first postnatal week.

Summary

As reviewed here, and contrary to the predictions of the presleep hypothesis, the infant brainstem and forebrain are intimately involved in the generation of cyclic changes in muscle tone and the production of the phasic activity that defines AS. We still have much to learn about the neural circuit involved in infant sleep and how it changes across development. Nonetheless, it is notable that rapid progress in this area of research followed upon the development of the nuchal EMG as a measure of tonic and phasic changes in behavioral state. Used in its proper context, and divorced from expectations imported from work with adults, the nuchal EMG has proven a reliable indicator of sleep and wakefulness. Its usefulness has proven itself further as we developed new methods for acquiring neurophysiological data from unanesthetized infant subjects. Such methods have been, and are being developed in several laboratories (Karlsson et al., 2005; Khazipov et al., 2004; Lahtinen et al., 2001; Leinekugel et al., 2002; see Chapters 6 and 8). As these methods improve, they should provide an impetus to further investigations of the neural bases of infant behavior in general, and infant sleep in particular.

The Developing Temporal Structure of Sleep and Wakefulness

As discussed above, differing perspectives of the organization of infant behavioral states have largely concerned the brain and its role in that organization. The research reviewed above clarified this issue by showing that the brain plays a significant role in the organization of sleep and wakefulness

in infants. But over the last several years, what has been most striking to us is not the presence or identity of the neural mechanisms involved in infant sleep, but the temporal structure of infant sleep and how it changes with age. For example, perhaps the most striking difference between the P3 and P14 records in Figure 20.3 concerns the timing of the sleep–wake cycles. Specifically, note how the timescale bar for the P3 subject indicates 5 s, whereas the time scale bar for the P14 subject indicates 100 s. Similar differences in timing can be seen for the P2 and P8 subjects in Figure 20.7.

Such dramatic developmental shifts in the temporal structure of sleep and wakefulness are well known (Gramsbergen et al., 1970; Kleitman & Engelmann, 1953; Roffwarg et al., 1966). Recently, using analytical procedures that are relatively new to the field, we have sought to fully describe the statistical structure of sleep and wake bout durations across development. Such analyses have revealed previously unsuspected statistical structure in sleep and wake bouts and provide a foundation for future developmental, comparative, and neurophysiological investigations of sleep–wake organization.

Statistical Distributions of Sleep and Wake Bouts across Normal Development

Total amounts of sleep and wakefulness are accumulated in short bouts—produced by transitions between states—such as those depicted in Figure 20.8A for an infant rat. When such bouts are plotted to depict their distribution in real-time, as in Figure 20.8B for a P2 and P21 rat, we see highly variable distributions of sleep and wake bouts. Note also how this variability seems to change with age (and note the different timescales in the two plots). The issue to which we now turn concerns the statistical distributions that best describe this variability. A guiding assumption underlying this discussion is that a better understanding of the rules guiding transitions between behavioral states will provide valuable insight into the mechanisms that modulate sleep and wakefulness, including their development.

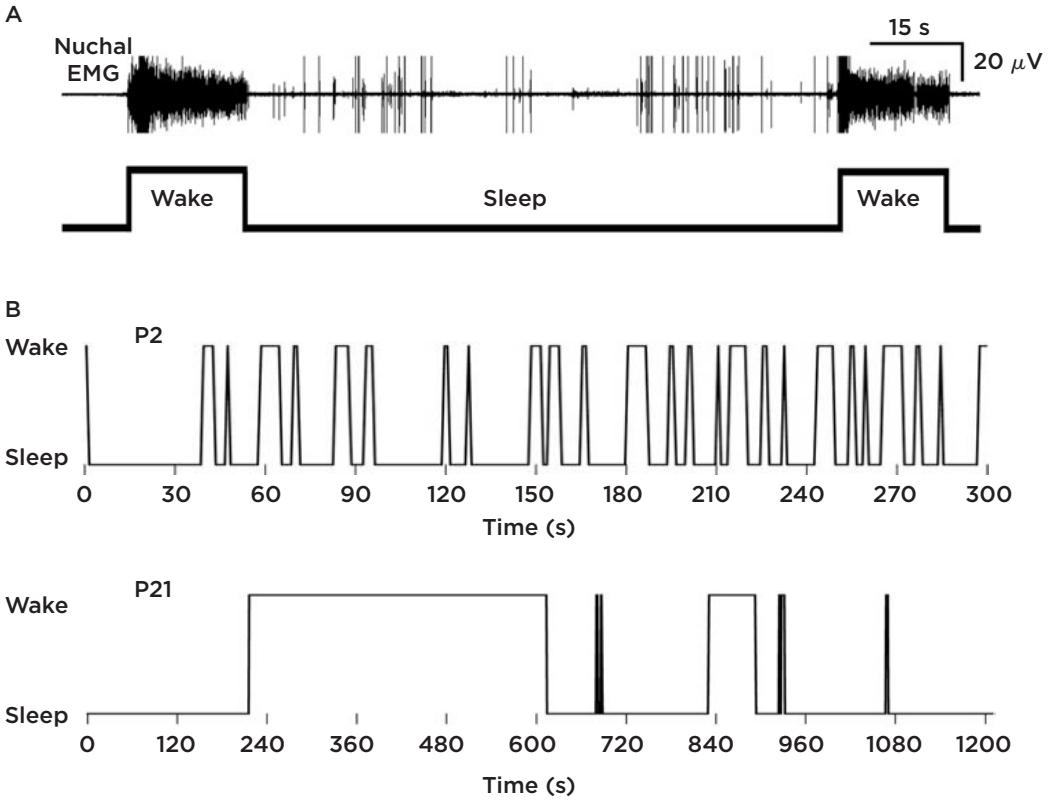

Figure 20.8 (A) A 2.4-min record in a P8 rat of nuchal EMG (upper trace) showing two brief periods of high muscle tone, indicative of wakefulness, separated by a longer period of muscle atonia, indicative of sleep. These dichotomous states are also depicted (lower trace). (B) Cycling between sleep and wakefulness in a P2 (upper trace) and P21 (lower trace) rat. Note the different time scales in the two traces. (From Blumberg et al., 2005.)

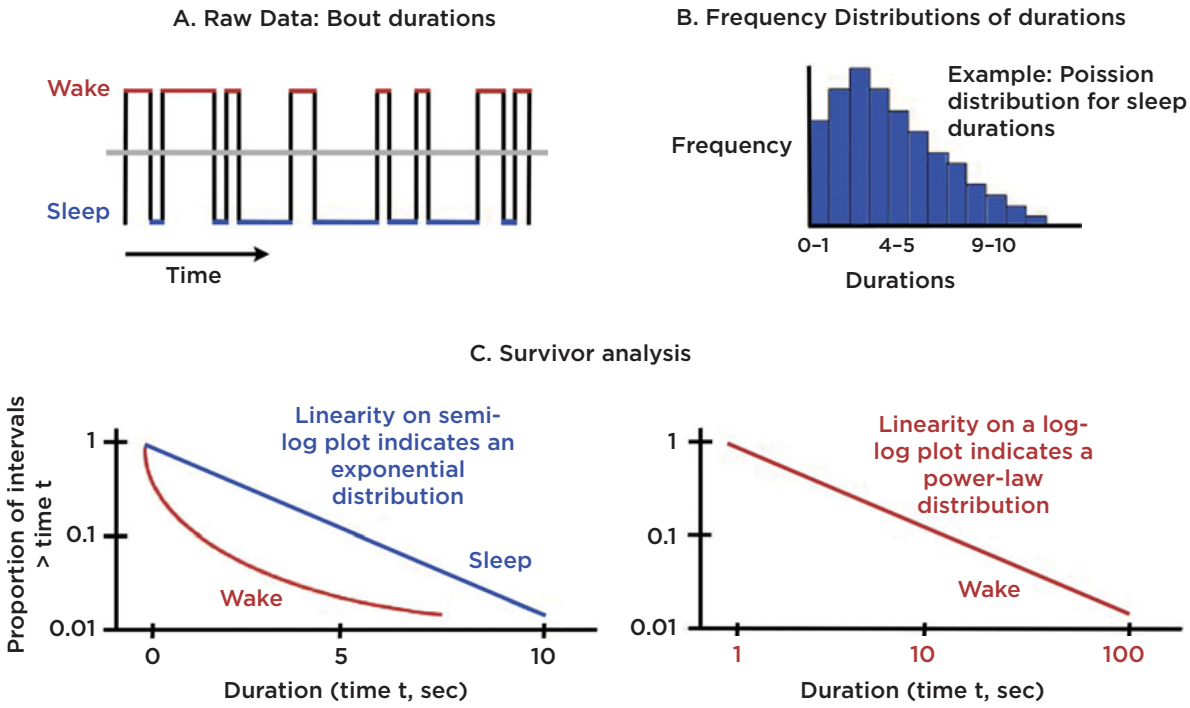

Figure 20.9 Illustration of method for converting raw nuchal EMG data to sleep–wake states in preparation for log-survivor analysis. (1) EMG amplitude is dichotomized into sleep (blue) and wake (red) states. (2) After bout durations are derived, frequency distributions can be produced. Illustrated here is the frequency distribution for sleep bouts for the case in which they follow a Poisson distribution. (3) Left: Plot of sleep and wake bouts on a semi-log plot. When sleep bouts follow a Poisson distribution (blue), they fall on a straight line on a semi-log plot. If wake bouts follow, for example, a power-law distribution, then they do not fall along a straight line on a semilog plot. Right: When power-law wake bouts are replotted on a log-log plot, they now fall along a straight line.

Figure 20.9 illustrates our approach to assessing these distributions. First, as in the previous figure, a continuous record of nuchal EMG is analyzed and dichotomized into sleep and wake bouts (Figure 20.9A). Second, without concern for the ordering of these bouts, frequency distributions are constructed (Figure 20.9B). Finally, the frequency distributions are converted to log-survivor plots. (Log-survivor analysis was originally devised for epidemiological assessment of medical treatments. Rather than assessing the survival of, for example, cancer patients, we are assessing the "survival" of sleep and wake bouts. That is, at each successive bout interval—i.e., 1 s, 2 s, etc.—we ask what percentage of the entire distribution "survived.") As shown in Figure 20.9C, an exponential distribution (such that the frequency distribution $f(t)$ of bout durations of duration t was proportional to $e^{(-t/\tau)}$, where τ is the characteristic timescale) falls along a straight line on a semilog plot. In contrast, a power-law distribution (such that $f(t) \sim t^{-\alpha}$, where α is a characteristic power-law exponent) falls along a straight line on a log–log plot.

Using this basic approach, Lo and colleagues analyzed the distributions of sleep and wake bouts in human adults (Lo et al., 2002). They found that sleep bouts exhibited an exponential distribution, whereas wake bouts exhibited a power-law distribution. In a subsequent report (Lo et al., 2004), similar findings were reported in adult rats, cats, and mice. In addition, these investigators found that the exponential timescale, τ, for sleep bout durations increases with body size, thus possibly implicating a constitutional variable (e.g., metabolic rate) in the regulation of sleep bouts. In contrast, the power-law exponent, α, for wake bout durations did not vary across species.

We suspected that data from developing animals could provide additional critical information for testing the generalizability of Lo et al.'s claims. Indeed, we had found earlier that both sleep and wake bout durations of P2 and P8 rats are better captured by exponential, not power-law, distributions (Karlsson et al., 2004). Because wake bout durations do not exhibit power-law behavior in early infancy, we inferred that this feature develops

after P8. In addition, because the precise nature of these distributions critically shapes the models that we adopt to describe the temporal dynamics of sleep and wakefulness (Lo et al., 2002), we knew that establishing the statistical properties of these bout durations across development was important. Therefore, using archival and specially collected data from rats at P2, P8, P10, P14, and P21, we assessed the statistical behavior of sleep and wake bout durations (Blumberg et al., 2005b).

Survivor distributions for data at P2 and P21 are presented in Figure 20.10 for pooled data and for individual representative subjects. Again, survival data that follow an exponential distribution fall along a straight line on a semilog plot and those that follow a power-law distribution fall along a straight line on a log–log plot. For sleep durations, the data for both the P2 and P21 rats are best described by an exponential function, as they follow a straight line on the semilog plot. This is also true of the data for the wake durations at P2; in contrast, by P21, these data are now linearly distributed on a log–log plot, thus indicating a shift from an exponential distribution at P2 to a power-law distribution at P21.

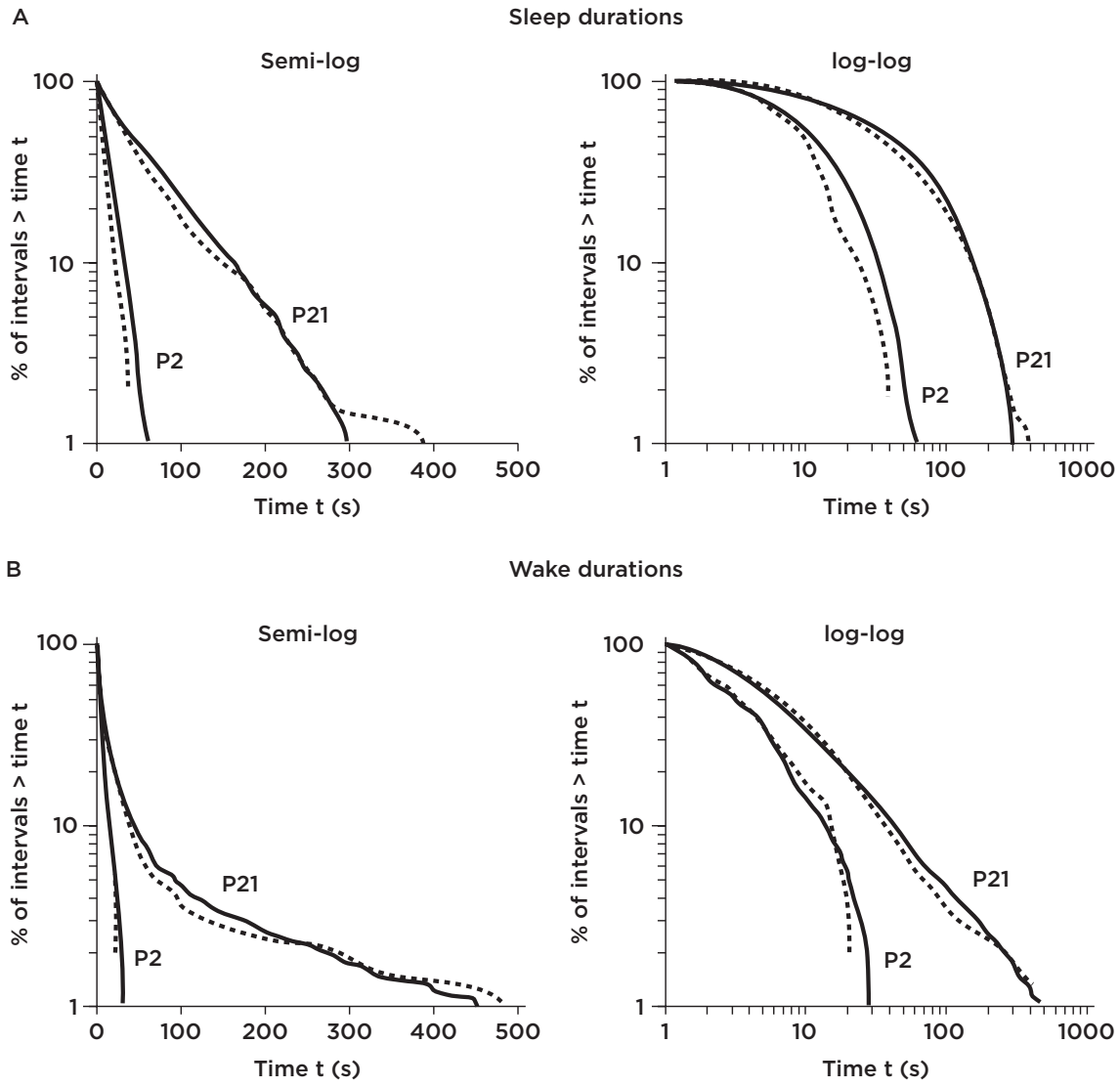

Figure 20.10 Survivor plots of sleep (A) and wake (B) bout durations for rats at P2 and P21. Each plot was constructed using pooled data at each age (solid lines) and data from one representative pup at each age (dotted line). The plots on the left were constructed using semi-log coordinates; straight lines on these plots indicate that the data follow an exponential distribution. The plots on the right were constructed using log-log coordinates; straight lines on these plots indicate that the data follow a power-law distribution. (From Blumberg et al., 2005.)

To determine whether the functions of the sleep and wake bout durations are best described as exponential or power-law distributions at each age, regression analyses were performed for each pup when data were plotted using semilog and log–log coordinates. Then, r^2 values were computed, averaged across subjects, and plotted across age. As shown in Figure 20.11A, sleep bout durations are best fit to an exponential distribution at all ages. In contrast, as shown in Figure 20.11B, wake bout durations are best fit to an exponential distribution through P10 but to a power-law distribution thereafter.

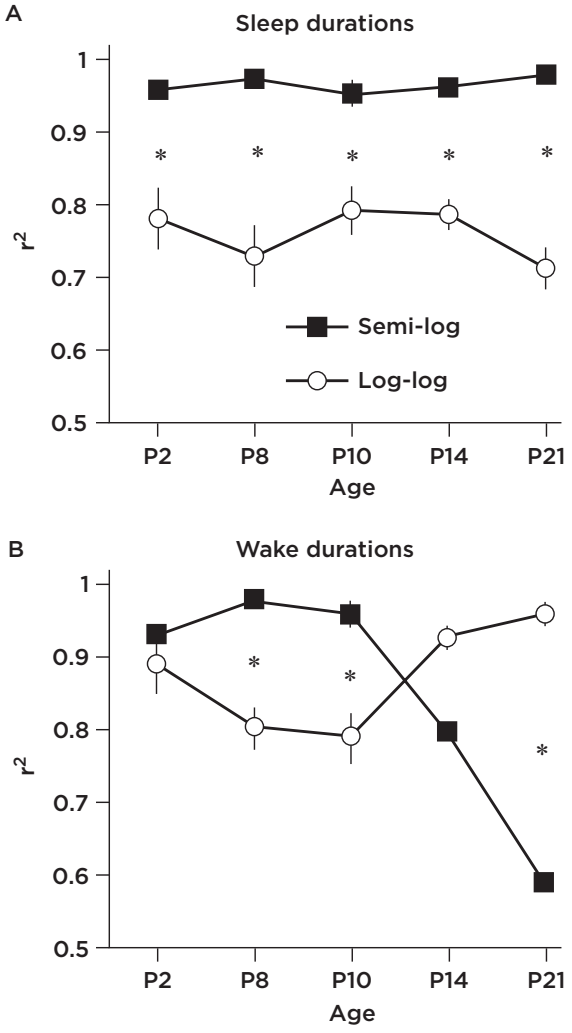

Figure 20.11 Values of r^2 produced using regression analysis of survivor data at five postnatal ages in infant rats. For each individual pup, the degree of fit of the data to power-law and exponential distributions was determined, yielding a value of r^2 that was then averaged across subjects at each age. (A) Values of r^2 for sleep bout durations showing that the data follow an exponential distribution. (B) Values of r^2 for wake bout durations showing that the data follow an exponential distribution at P8 and P10 and a power-law distribution at P21. * Significant within-age difference. Mean + S.E. (From Blumberg et al., 2005.)

Based on these and other analyses, we concluded that as infant rats cycle between sleep and wakefulness, there is no memory for the duration of previous intervals. For example, whether an infant rat exhibits a long or short sleep bout is completely uninfluenced by the length of its previous wake or sleep bouts. Accordingly, we concluded that transitions between sleep and wakefulness most closely resemble an alternating renewal process (Lowen & Teich, 1993a, 1993b); at the youngest ages tested, where both sleep and wake durations distribute exponentially, the sleep–wake state model further specializes to a two-state Markov process (a subset of alternating renewal processes). In other words, the system resets every time the animal wakes up or goes to sleep; no memory of the past persists beyond these events. However, some memory can exist *within* intervals, particularly for those that exhibit a power-law distribution. For example, a wake bout that has already persisted for a long time is likely to persist even longer, producing a relative lack of intermediate-duration wake times. Thus, as these older pups stay awake, they are more likely to stay awake longer. That this phenomenon occurs after P15 suggests a connection between the onset of sustained wakefulness and the initiation of weaning.

Development of Circadian Sleep–Wake Activity

During the early postnatal period in rats, several physiological and behavioral systems are known to exhibit circadian rhythmicity, including body temperature, metabolism, and pineal serotonin *N*-acetyltransferase (Ellison, Weller, & Klein, 1972; Kittrell & Satinoff, 1986; Nuesslein-Hildesheim, Imai-Matsumura, Döring, & Schmidt, 1995; Spiers, 1988). But in comparison to the vast literature detailing circadian rhythms of sleep and wakefulness in adults (Fuller, Gooley, & Saper, 2006), very little is known about these rhythms in infants. In one study, it was reported that nocturnal wakefulness is established in rats during the third postnatal week (Frank & Heller, 1997a).

As described already in this chapter, nuchal EMG has proven a very sensitive measure of sleep–wake cyclicity beginning soon after birth in rats. Thus, we wondered whether we could use this measure to reveal day–night differences in sleep–wake cyclicity and relate these differences to early suprachiasmatic nucleus (SCN) function. To explore

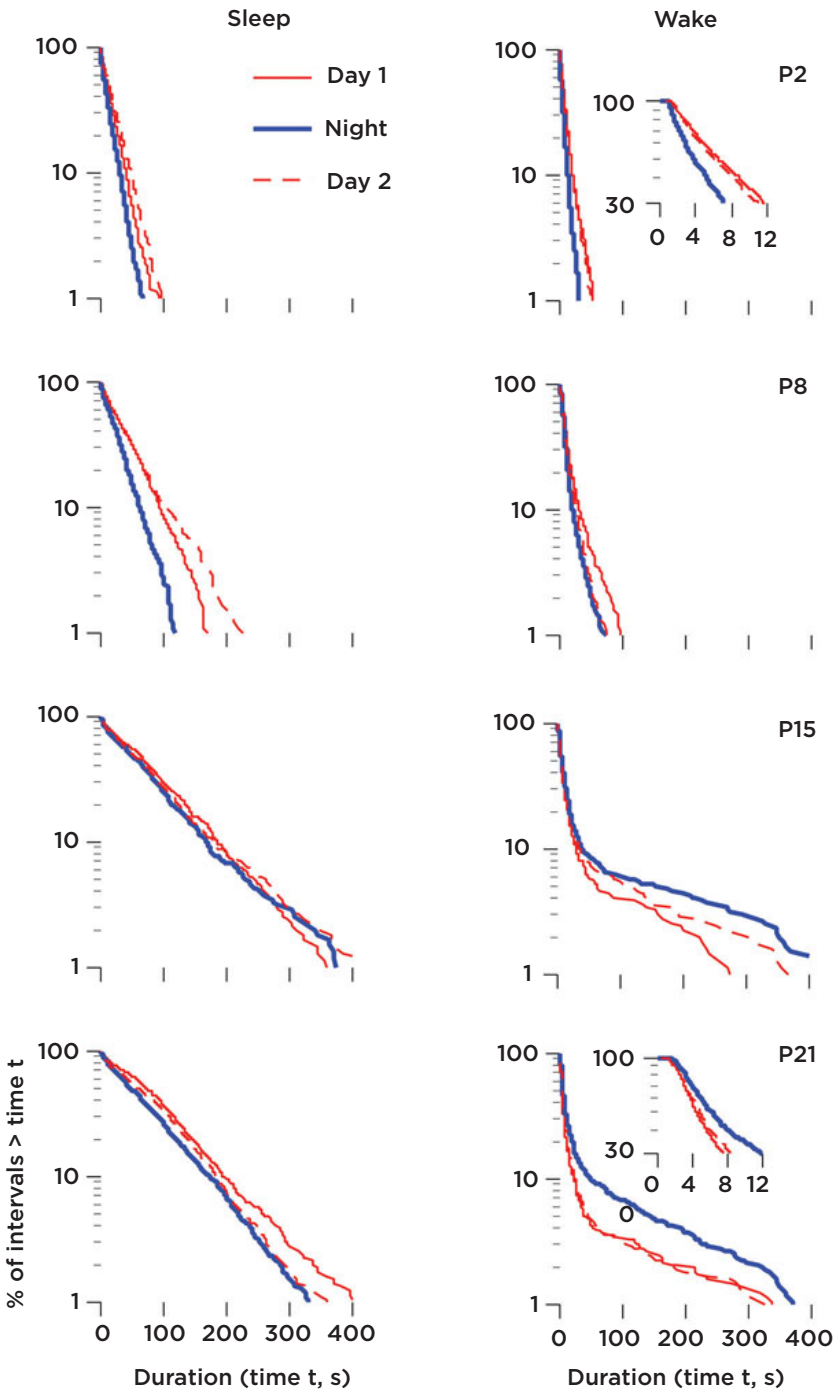

Figure 20.12 Log-survivor plots of (A) sleep and (B) wake bout distributions for rats at P2, P8, P15, and P21 on day 1 (red solid line), at night (blue line), and on day 2 (red dashed line). Each plot is constructed from pooled data. Straight lines on these semi-log plots indicate that the data follow an exponential distribution. Insets provide magnified views at shorter durations to reveal developmental switch in circadian organization of wake bout durations. (From Gall et al., 2008.)

this possibility, we examined day–night differences in sleep–wake cyclicity in rats at P2, P8, P15, and P21 (Gall, Todd, Ray, Coleman, & Blumberg, 2008). At each age, data were collected from three littermates in succession about noon, midnight, and noon on the next day. Pups were always tested at thermoneutrality.

Figure 20.12 presents log-survivor data for the subjects in this study. Surprisingly, day–night differences in sleep–wake activity were detected as early as P2; specifically, pups at this age exhibited significantly more sleep–wake cycles at night than during the day (due to shorter bout durations of both sleep and wakefulness at night). By P15 and especially by P21, the wake bouts were now significantly longer at night, revealing the emergence of the nocturnal pattern of wakefulness that characterizes the adults of this species.

The SCN of the hypothalamus plays a major role in the regulation of circadian rhythms in mammals (Moore, 1983; Rusak & Zucker, 1979). In rats, nearly all neurons in the SCN are formed by embryonic day E18 (Ifft, 1972) and, by E19, the fetal SCN is more metabolically active during the day than during the night and is synchronized to the dam's SCN activity (Reppert & Schwartz, 1984; Reppert, Weaver, & Rivkees, 1988). The entraining effect of the dam on her pups continues through the first postnatal week, at which time light becomes the predominant entraining stimulus (Duncan, Banister, & Reppert, 1986; Ohta, Honma, Abe, & Honma, 2002; Takahashi & Deguchi, 1983). Thus, it is plausible that the pup's SCN circadian activity, entrained to that of the dam's, modulates the sleep–wake rhythmicity detected during the first postnatal week.

The transition from maternal to light entrainment parallels the development of the retinohypothalamic tract's (RHT) connections with the SCN (Felong, 1976; Speh & Moore, 1993). Within the retina, the RHT arises from intrinsically photosensitive ganglion cells that contain the photopigment, melanopsin (Berson, Dunn, & Takao, 2002; Hattar, Liao, Takao, Berson, & Yau, 2002). These melanopsin-containing cells respond to light passing through the eyelids, which in rats do not open until P15. It now appears that this nonimage-forming irradiance detection system is able to modulate SCN activity at birth (Hannibal & Fahrenkrug, 2002; Leard, Macdonald, Heller, & Kilduff, 1994; Sekaran et al., 2005; Sernagor, 2005). Thus, this system is poised to play an important role in the early development of circadian rhythms, including sleep–wake rhythms, and underlies the transition to light entrainment over the first postnatal week.

We hypothesized that eliminating RHT–SCN connectivity during the early postnatal period in rats—before and after SCN entrainment to light—would alter the later emergence of nighttime wakefulness in this nocturnal species. We investigated this possibility by enucleating pups at P3 or P11 and testing their subsequent sleep–wake patterns at P21 (Gall et al., 2008). Pups enucleated at P3 or P11 were similar to the extent that both exhibited power-law wake behavior at P21. In contrast, whereas enucleation at P11 did not prevent P21 rats from exhibiting the normal pattern of longer wake bout durations at night, enucleation at P3 resulted in subjects exhibiting longer wake bout durations during the day. To ensure that pups enucleated at P3 were not free-running and that the observed daytime wakefulness was reliable, we repeated the study but tested weanlings at P28 and P35—with similar results.

This experiment suggests that enucleated infant rats differentially entrain to zeitgebers within the nest environment depending on when—in relation to the development of the RHT—the enucleation takes place. Specifically, it is possible that visual system stimulation—from light and/or spontaneous activity within the retina—transmitted through the RHT to the SCN, induces functional changes in SCN interactions with its downstream neural structures. Interestingly, in adults, the effect of light as a zeitgeber is to stimulate upregulation of growth factors (e.g., NGF1-A, BDNF) in the SCN and thereby entrain SCN activity (Allen & Earnest, 2005; Liang, Allen, & Earnest, 2000; Tanaka, Iijima, Amaya, Tamada, & Ibata, 1999). Such upregulation of gene activity—seen during everyday entrainment to light in adults—could also play an inductive, organizational role during a sensitive period when the RHT is forming functional connections with the SCN.

On the Similarly Fragmented Sleep–Wake Patterns of Infants and Narcoleptics

The development of sensitive indicators of infant sleep and wakefulness, as discussed above, may provide additional insights into the development and treatment of sleep disorders in infants and adult humans. For example, narcolepsy is a sleep disorder characterized in humans by excessive daytime sleepiness, the sudden loss of muscle tone (i.e., cataplexy), sleep-onset hallucinations, and paralysis at sleep transitions (Taheri, Zeitzer, & Mignot, 2002). Its prevalence has been reported to be 20–60 incidences per 100,000 persons, similar to multiple sclerosis and Parkinson's disease (Overeem, Mignot, Gert, van Dijk, & Lammers, 2001).

Narcolepsy has recently been recognized as a neurodegenerative disorder (Siegel, Moore, Thannickal, & Nienhuis, 2001; Taheri et al., 2002; van den Pol, 2000). Central to this reclassification has been the recent discovery of a neurotransmitter, orexin (or hypocretin) (de Lecea et al., 1998; Sakurai et al., 1998), which is produced by a distinct set of neurons within the caudal hypothalamus that project to the locus coeruleus and other nuclei implicated in the regulation of sleep and wakefulness (Peyron et al., 1998). Degeneration or deficient functioning of the orexinergic system has been linked to narcolepsy in humans (Peyron et al., 2000; Thannickal et al., 2000), dogs (Lin

et al., 1999), and mice (Chemelli et al., 1999). Moreover, adult orexin knockout mice exhibit patterns of sleep and wakefulness that mirror those seen in narcoleptic humans (Chemelli et al., 1999; Mochizuki et al., 2004; Willie et al., 2003).

As with narcolepsy and as discussed above, the sleep and wake bouts of infant humans (Kleitman & Engelmann, 1953) and rats (Blumberg et al., 2005b; Gramsbergen et al., 1970) are highly fragmented, characterized by rapid transitions between short-duration states. We wondered whether the sleep–wake fragmentation observed in narcoleptics and infants result from a common neural mechanism. Specifically, we hypothesized that orexin knockout mice would retain the more fragmented sleep and wake bout durations that characterize normal infancy. Such an observation would indicate that narcolepsy in orexin knockout mice, though characterized in part by the novel expression of pathological symptoms such as cataplexy, is also characterized by retention of the infantile pattern of sleep–wake fragmentation. By extension, adult-onset narcolepsy in humans might entail reversion back toward that infantile pattern.

To test this hypothesis, we assessed sleep and wakefulness in orexin knockout and wild-type mice at P4, P12, and P21 (Blumberg, Coleman, Johnson, & Shaw, 2007). As shown in Figure 20.13, we found little difference between the two strains at P4 and P12, although both exhibited age-related consolidation of sleep and wake bouts. By P21, further consolidation occurred in both strains, along with the emergence of power-law wake behavior. But now, the knockouts were lagging behind their same-age wild-type counterparts, retaining the more fragmented bouts characteristic of earlier ages. Thus, it appears that the orexinergic system is not necessary for consolidation of sleep and wake bouts during the first 2 postnatal weeks, nor is it necessary for the developmental emergence of power-law wake behavior. Orexin does appear, however, to further consolidate bouts beyond the values attained in early infancy.

Thus, the infant's sleep–wake system operates, like a narcoleptic's, without a fully functioning orexinergic system and that the result—for both infant and narcoleptic—is fragmentation of sleep and wake bouts. Moreover, if the normally fragmented sleep of infants and the abnormally fragmented sleep of narcoleptic adults arise through the action of a common neural mechanism, then infants may provide a useful model for understanding the etiology of narcolepsy and for developing effective treatments.

Beyond narcolepsy, this analytical approach may provide useful information concerning normal and pathological human development. Because sleep disturbances are associated with many aspects of disease and psychopathology (Kryger, Roth, &

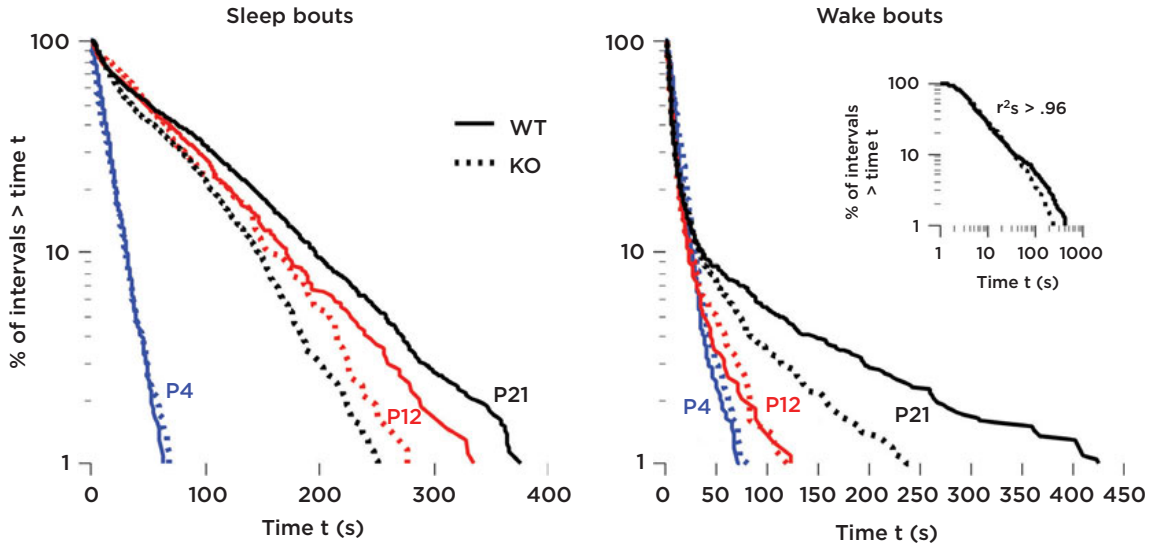

Figure 20.13 Survivor plots of sleep and wake bout durations for wild-type (WT; solid lines) and orexin knockout (KO; dashed lines) mice at P4 (blue), P12 (red), and P21 (black). Straight lines on these plots indicate that the data follow an exponential distribution. The inset is a replotting of the P21 wake data using log–log coordinates; straight lines on these plots indicate that the data follow a power-law distribution. Individual data points were pooled across all subjects. (From Blumberg et al., 2007.)

Dement, 2000; Nishino, Taheri, Black, Nofzinger, & Mignot, 2004), any method that provides greater sensitivity for tracking developmental milestones, detecting the onset of sleep disturbances, and assessing responses to treatment could be of use to clinicians. Accordingly, the analyses of sleep and wake bout durations described here may prove superior to gross measures of total sleep and wake time because they reveal more about the fine structure of sleep–wake organization and they more closely reflect the neural processes that govern transitions between states.

Functional Aspects of Infant Sleep

How we conceptualize the phenomenology of infant sleep and its relation to adult sleep can have a profound influence on the kinds of functional theories that will be entertained and tested. For example, if the "presleep hypothesis" had been correct, then we might understand the tendency among most sleep researchers to disregard infant sleep in the formulation of sleep function hypotheses. However, as reviewed in this chapter, that hypothesis is not supported by the available evidence, leading us to argue, once again (Blumberg & Lucas, 1996), that theories of sleep function should strive for applicability to infants as well as adults.

Although there is no dearth of theories of sleep function, emphasis continues to be placed on theories that posit a role in learning and memory, especially memory consolidation in humans (Stickgold, 2005). Some investigators are highly critical of such theories (Siegel, 2001; Vertes, 2004) and highlight instead the usefulness of comparative data as a source of valuable information concerning the phylogenetic history of sleep and, therefore, its functional importance (Siegel, 2005a).

Comparative analysis has long been used to test hypotheses concerning the evolution, function, and mechanistic control of sleep (Campbell & Tobler, 1984; Dave & Margoliash, 2000; Flanigan, 1973; Flanigan, Wilcox, & Rechtschaffen, 1973; Hendricks & Sehgal, 2004; Hendricks et al., 2000; Huntley & Cohen, 1980; Rattenborg, Amlaner, & Lima, 2000; Rattenborg et al., 2004; Siegel, 1999, 2005a; Siegel, Manger, Nienhuis, Fahringer, & Pettigrew, 1998; Tobler, 1995; Tobler & Deboer, 2001; Zepelin, 2000; Zepelin & Rechtschaffen, 1974). Relatively few studies, however, have combined comparative with developmental analysis. We believe, however, that systematic examination of the development of sleep and wakefulness in carefully chosen nontraditional species will help to answer a variety of interesting and important questions.

As already discussed, a recent comparison of data from adult mice, rats, cats, and humans yielded useful insights into the temporal structure of sleep and wakefulness (Lo et al., 2004). A similar comparative analysis of sleep and wake development would be valuable. For example, Figure 20.14 compares the log-survivor plots of infant Norway rats (Blumberg et al., 2005b) and mice (Blumberg et al., 2007). The similar patterns exhibited by these two species are striking: sleep and wake bouts during the early postnatal period follow an exponential function, whereas wake bouts exhibit a power-law function several weeks later. This developmental correspondence attests to an underlying conservation of sleep processes in these two rodent species. But what can we really conclude from comparison of these two species alone? After all, rats and mice are both muroid rodents, both are nocturnal, both are omnivorous, and both are altricial. Therefore, to determine whether the developmental trajectories illustrated in Figure 20.14 represent a widely shared feature among mammals, it is necessary to examine additional species that differ from rats and mice on critical dimensions. Such comparisons may then inspire novel hypotheses concerning the mechanisms and functions of sleep.

Historically, the single most influential developmental hypothesis regarding the function of active sleep remains that of Roffwarg et al. (1966) who, noting the developmental relation between sleep and brain development in newborns, suggested that the two processes are related. Later, this hypothesis was elaborated further by considering all that we have learned in recent decades regarding the developmental significance of activity-dependent neural processes during fetal and postnatal development (Blumberg & Lucas, 1996). For example, we now know that spontaneous activity by retinal ganglion cells, even in the rat fetus, contributes significantly to the development of topographic relations in the visual system (Galli & Maffei, 1988; Shatz, 1990). Indeed, researchers continue to identify effects of sleep processes on neural plasticity in the developing brain, especially within the visual system (Frank, Issa, & Stryker, 2001; Shaffery, Sinton, Bissette, Roffwarg, & Marks, 2002). More broadly, recent evidence supports a role for myoclonic twitching in the developmental of somatotopic maps in the spinal cord (Petersson, Waldenström, Fåhraeus, & Schouenborg, 2003; Schouenborg, 2003; see Chapter 12).

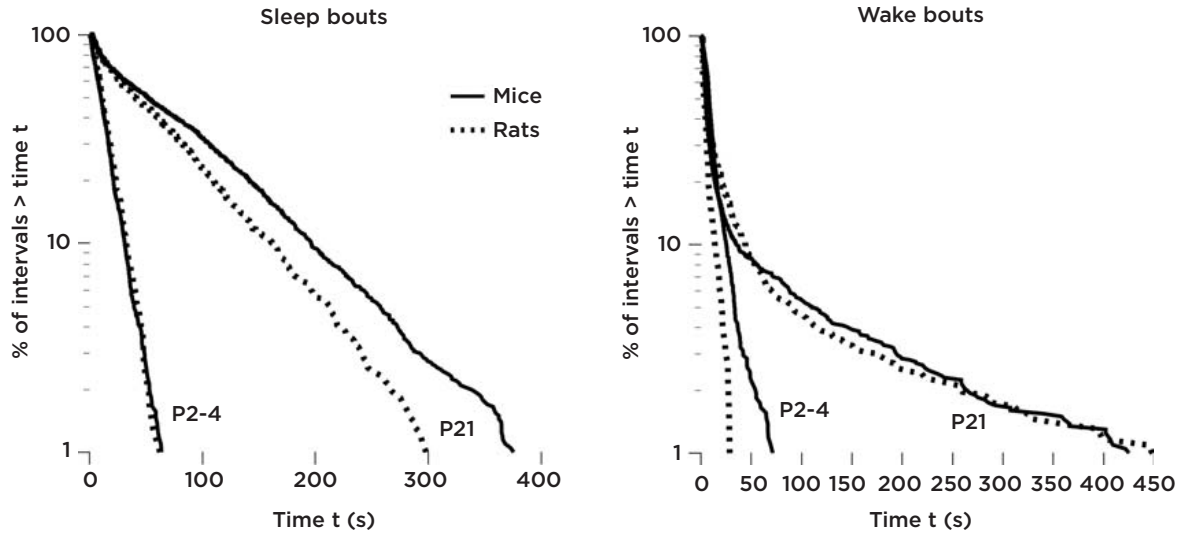

Figure 20.14 Log-survivor plots of sleep and wake bout durations for the P2–4 and P20–22 wild-type mice (solid lines) in Blumberg et al. (2007) and P2 and P21 Norway rats (dotted lines) from Blumberg et al. (2005). All plots were constructed using data pooled from multiple subjects. The gestation length of mice is 3 days shorter than that of rats. Regardless, the distributions are remarkably similar, including the development of wake-related power-law behavior by P21. (From Blumberg et al., 2007.)

Within the context of this chapter, we may move closer to an understanding of the functions of sleep through close examination of the temporal and spatial organization of the various sleep components. Consider Figure 20.15A, which depicts the traditional trio of electrographic measures of sleep in the form of a Venn diagram: nuchal EMG provides a measure of muscle tone; the EOG provides a measure of phasic extraocular muscle activity; and cortical EEG allows for the detection of delta waves. As we have seen, however, conventional methods for measuring EMG and EOG mask the redundant information provided by these two measures of muscle activity. Specifically, phasic activity can be detected in the nuchal EMG at appropriate filter settings and sampling frequencies; similarly, tonic activity can be detected from the eye muscles if their activity is measured directly (Seelke et al., 2005). Of course, as a practical matter, especially when recording in humans, these two measures are treated as separate entities. But as a conceptual matter, the underlying activity in all skeletal muscles provides similar information: oscillations between high and low tone and occasional bursts of phasic activity. Thus, the trio of electrographic measures in Figure 20.15A can be reduced to the two indicators depicted in Figure 20.15B.

Now we can assess the necessity of two electrographic measures. With regard to the EEG, it was recently shown in adult rats that the forebrain exhibits global EEG patterns that are sufficiently distinct to discriminate between AS, QS, and wakefulness (Gervasoni et al., 2004). It was suggested that these EEG patterns provide the basis for the "classification of global states without reference to behavioral or electromyogram data" (p. 11141). Interestingly, the work reviewed in this chapter strongly suggests that the EMG alone is also sufficient for differentiating the behavioral states of infants and, presumably, adults as well. Indeed, EMG data are sufficient for revealing neural mechanisms that have been implicated in adult sleep–wake states using the conventional trio of electrographic measures (Karlsson & Blumberg, 2005; Karlsson et al., 2004, 2005; Seelke et al., 2005). Thus, the reduced Venn diagram in Figure 20.15B can be morphed into the qualitatively distinct arrangement of Figure 20.15C. In that figure, homologous behavioral states defined using EEG or EMG measures alone are linked by their association with common neural sources within the brainstem.

The perspective captured by Figure 20.15C indicates a mechanistic connection between the activational states of the forebrain (EEG) and skeletal muscle (EMG). And through this mechanistic connection it is possible to glimpse the basis for an approach to sleep that transcends description and diagnosis and moves toward explanation. Specifically, the conception depicted in Figure 20.15C presents sleep as a body-wide process that links muscle and brain into a single system that

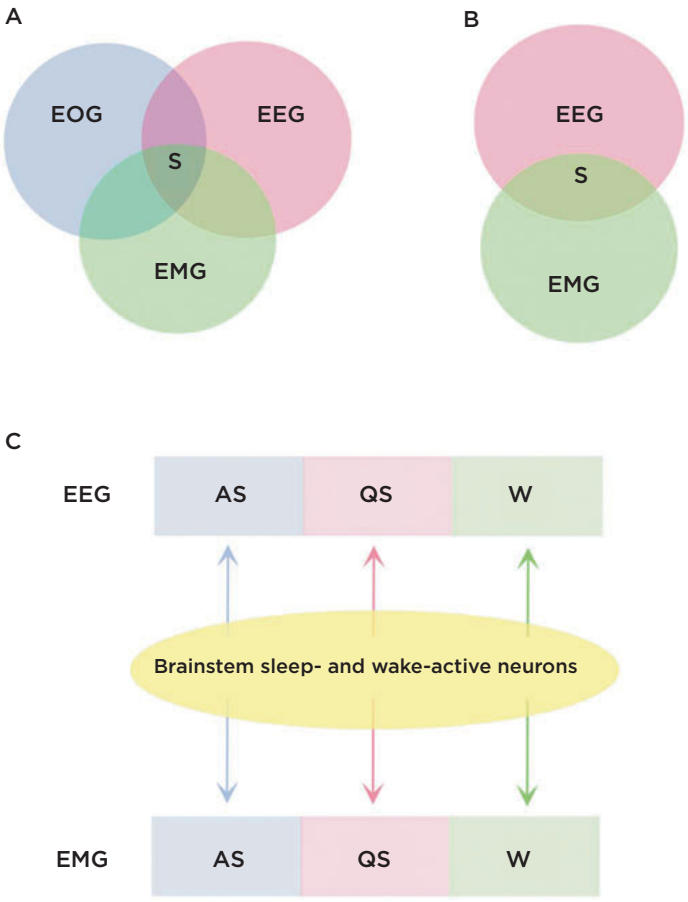

Figure 20.15 Conceptual representations of sleep. (A) Venn diagram depicting conventional diagnostic criteria for assessing sleep–wake states in adults using a trio of electrographic measures: EOG, EMG, and EEG. S denotes the behavioral state (i.e., active or quiet sleep, wakefulness) defined using these three parameters. (B) Reorganization of the Venn diagram in (A) based on the notion that the EOG and EMG provide redundant information. Specifically, REMs (detected from the EOG record) can be viewed as phasic events produced by twitches of the extraocular muscles; in addition, fluctuations in extraocular muscle tone are mirrored by fluctuations in nuchal muscle tone. Now, S denotes the behavioral state defined using only two parameters. (C) Alternative conceptualization that builds on the notion that either EEG (after P11) or EMG (as early as P2) is alone sufficient to define behavioral states in rats. According to this notion, homologous sleep–wake states (AS, QS, W) can be identified in the EEG and EMG records. This homology arises because EEG- and EMG-defined states are generated by common brainstem mechanisms. (From Seelke et al., 2005.)

must develop and maintain topographic relations for proper functioning to occur. The need for integrated relations between muscle and brain forms the basis for the suggestion that infant sleep states, including myoclonic twitching, contribute to neural and neuromuscular development (Blumberg & Lucas, 1996; Corner, van Pelt, Wolters, Baker, & Nuytinck, 2002; Mirmiran, 1995; Roffwarg et al., 1966). The discrete nature of a myoclonic twitch, especially when performed against a background of muscle atonia, provides, we suggest, an enhanced signal-to-noise ratio for accurately processing relationships between outgoing motor signals and sensory feedback. Such conditions may provide the basis by which twitch-related movements of the limbs contribute to the self-organization of topographically organized maps and refinement of neural circuits in spinal cord (Petersson et al., 2003) and somatosensory cortex (Khazipov et al., 2004; Marcano-Reik & Blumberg, 2008), as well as hippocampus (Mohns & Blumberg, 2008).

Conclusions and Future Directions

Our aim in this chapter was to demonstrate how an accurate description of the phenomenology of sleep in early infancy helps us move toward a broader and deeper appreciation of its developmental and evolutionary origins. The work reviewed here builds upon a conceptual framework clearly enunciated by Michael Corner (Corner, 1977),

which in turn was inspired by the embryological research of Viktor Hamburger and his colleagues (Hamburger & Oppenheim, 1967; Narayanan et al., 1971). Their ideas and research challenged subsequent investigators to extend the insights gained from behavioral embryology to later periods of development. Indeed, Corner himself performed important studies in this area, including examination of the development of the brain in relation to sleep (Corner, 1973, 1985).

In our work, focusing on the early postnatal period in altricial rodents, we have adopted the general approach encouraged by Corner and Hamburger while also keeping an eye on the methods and concepts of traditional sleep research—research performed largely in adults. Straddling these two traditions, we were convinced, would be essential to attaining our goal of building a durable bridge between them. In turn, such a bridge might help to convince clinically oriented sleep researchers that the proper study of development can offer useful insights into the mechanisms and functions of sleep.

Also central to our conceptual approach is a commitment to the epigenetic perspective championed by Gilbert Gottlieb and others (Blumberg, 2005; Gottlieb, 1997; Oyama, Griffiths, & Gray, 2001; see Chapters 2 and 3). Within the context of sleep, the epigenetic perspective places balanced emphasis on genetic and nongenetic factors in the development of ultradian and circadian rhythms. Our work, described earlier, on the possible inductive effects of photic stimulation on the development of the RHT–SCN system provides one example of how epigenetic factors can produce long-term organizational effects on sleep–wake behavior (Gall et al., 2008).

Our task now is to provide comprehensive accounts of the development and evolution of sleep across a diversity of species. As we engage this task, we will benefit from the lessons learned from investigations of sleep in rats and other altricial rodents. Perhaps the most seminal lesson concerns the need to incorporate multiple behavioral and electrographic measures of sleep in our assessments, but never fool ourselves into thinking that any one measure best captures the "essence" of sleep.

Thus, as has been shown many times, behavior alone provides a reliable estimate of wakefulness and sleep, including QS and AS (Corner, 1977; Gramsbergen et al., 1970; Kreider & Blumberg, 2000). With the addition of nuchal EMG (Dugovic & Turek, 2001; Karlsson & Blumberg, 2002), estimates become sharper as, for example, the transition from quiet wakefulness to quiet sleep is now discernible. In addition, the nuchal EMG—when filtered and sampled adequately—also provides a measure of phasic activity (i.e., myoclonic twitching) that complements behavioral assessments. Indeed, this is true of any measure of skeletal muscle activity, including that of the extraocular muscles (Seelke et al., 2005). Finally, the addition of cortical EEG is useful for detecting bouts of QS interposed between bouts of AS in rats older than P11 (Seelke & Blumberg, 2008).

Thus, during the early newborn period in altricial rodents, sleep and wakefulness are constructed developmentally upon a foundation that rests firmly on cyclic changes in skeletal muscle tone. This foundation rests, in turn, on medullary and mesopontine circuits that, with age, are increasingly modulated by forebrain mechanisms (Gall et al., 2007; Karlsson & Blumberg, 2005; Karlsson et al., 2004, 2005; Mohns et al., 2006). It is within the context of this developing circuit that the defining features of sleep, including homeostatic and circadian regulation, emerge. And it is within this context that we can see how sleep and wakefulness are body-wide processes that entail homologous activational states in muscle, spinal cord, brainstem, and forebrain. These homologous states, we believe, provide critical clues regarding the form and function of sleep across the lifespan.

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Notes

1 Startles are distinct from twitches in that they comprise sudden, spontaneous, and simultaneous contraction of multiple skeletal muscle groups, as described in human fetuses (de Vries, Visser, & Prechtl, 1984) and infant rats (Gramsbergen et al., 1970). Thus, although multiple limbs can exhibit myoclonic twitches in rapid succession, such multilimb bouts of twitching are distinct from the simultaneous activation that characterizes startles. In addition, startles exhibit a unique profile of associated hippocampal activity (Karlsson, Mohns, Vianna di Prisco, & Blumberg, 2006; Mohns, Karlsson, & Blumberg, 2007). Also, whereas sleep-related twitching continues into adulthood, startles decline and largely disappear across the postnatal period in rats (Gramsbergen et al., 1970).

2 A multilimb bout is defined as the set of limb movements in which the interval between successive movements does not exceed an established criterion value.

3 For example, in week-old rats, an environmental temperature of 35°C is within the thermoneutral range (Blumberg, 2001) and provides favorable conditions for sleep (Seelke & Blumberg, 2005).

4 The use of electric shock during the sleep deprivation period interfered with the reliable EMG measurement of nuchal muscle activity.