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HYPOTHALAMIC CONTRIBUTION TO SLEEP–WAKE CYCLE DEVELOPMENT

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Abstract—Infant mammals cycle rapidly between sleep and wakefulness and only gradually does a more consolidated sleep pattern develop. The neural substrates responsible for this consolidation are unknown. To establish a reliable measure of sleep-wake cyclicity in infant rats, nuchal muscle tone was measured in 2-, 5-, and 8-day-old rats, as were motor behaviors associated with sleep (i.e. myoclonic twitching) and wakefulness (e.g. kicking, stretching). Sleep–wake cycles of 2-day-old rats were characterized by short periods of muscle atonia followed by equally short periods of high tone. In 8-day-olds, sleep periods lengthened significantly and disproportionately in relation to awake periods. Next, locus coeruleus (LC) lesions in 8-day-olds resulted in rapid sleep–wake cycling similar to that exhibited by 2-day-olds; in addition, LC lesions had no effect on the duration of awake periods. Finally, transections caudal, but not rostral, to the anterior hypothalamus also reinstated rapid cycling in 8-day-olds, again without affecting the duration of awake periods. This last finding implicates neural structures within the anterior hypothalamus (e.g. ventrolateral preoptic area) in the modulation of sleep–wake cyclicity. The temporal coherence of atonia and myoclonic twitching was not disrupted by any of the manipulations.

These results suggest the presence of a bistable mesopontine circuit governing rapid sleep–wake cycling that does not include the LC and that comes increasingly under hypothalamic control during the first postnatal week. This circuit may represent a basic building block with which other sleep components become integrated during ontogeny. **© 2003 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: REM sleep, active sleep, atonia, myoclonic twitching, locus coeruleus, ventrolateral preoptic area.

Every parent is acutely aware of the newborn's rapid cycling between sleep and wakefulness during the day and night and eagerly awaits the time when a more stable pattern develops. Indeed, rapid sleep–wake cycling is a ubiquitous feature of mammalian sleep in altricial infants (Kleitman and Engelmann, 1953; Meier and Berger, 1965; Shimizu and Himwich, 1968; Gramsbergen et al., 1970). What are the changes in neural circuitry that underlie the transition from rapid cyclicity to the more stable circadian sleep states of adults? Clues emerge from other instances of rapid cycling. For example, human adults with narcolepsy, a condition characterized by "sleep attacks" that include the sudden loss of muscle tone during the day or night, accumulate normal daily amounts of sleep in small, rather than large, chunks (Taheri et al., 2002). Recent evidence suggests that these and other instances of rapid cycling result from disrupted balance between sleep- and wake-promoting circuits in the anterior hypothalamus and mesopontine region (Saper et al., 2001), including such nuclei as the ventrolateral preoptic area (VLPO) and locus coeruleus (LC). Are these same circuits involved in sleep development in infants?

Sleep and wakefulness in rats younger than 10 days of age (postnatal [P]10) have typically been characterized using behavioral indices alone (Gramsbergen et al., 1970; Jouvet-Mounier et al., 1970; Hilakivi and Hilakivi, 1986). This is due in part to the fact that neocortical EEG does not exhibit state dependency in these younger infants (Gramsbergen, 1976; Corner and Mirmiran, 1990; Frank and Heller, 1997). Interestingly, recent evidence suggests that the hippocampal electroencephalogram exhibits state-dependent activity in pups as young as P2, although its usefulness for defining sleep–wake states appears limited (Lahtinen et al., 2001; Karlsson and Blumberg, 2003). In contrast, we have found that nuchal muscle tone provides a highly reliable and reproducible measure of sleep and wakefulness in rats as young as P2, especially in conjunction with measures of behavior (Karlsson and Blumberg, 2002).

The sleep–wake cycle of infant rats evolves as follows: behavioral arousal begins with a rapid increase in muscle tone accompanied by high-amplitude motor behaviors (e.g. kicking, stretching); these behaviors subside within seconds as muscle tone remains elevated; soon thereafter, muscle tone decreases and, within seconds of the onset of atonia, myoclonic twitching begins and persists with few pauses throughout the period of atonia; the cycle is completed by a rapid increase in muscle tone accompanied by high-amplitude motor behavior. Separate labels are sometimes applied to each of these stages of sleep–wake cycle (e.g. active wakefulness, quiet wakefulness, quiet sleep [QS], active sleep [AS]), but there is still a lack of consensus as to which labels are most valid for describing behavioral states in infants (Blumberg and Lucas, 1996; Frank and Heller, 2000; Vogel and Feng, 2000; Karlsson and Blumberg, 2002; Frank and Heller, 2003).

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Abbreviations: AS, active sleep; BAT, brown adipose tissue; DMH, dorsomedial hypothalamus; EMG, electromyogram; LC, locus coeruleus; P, postnatal; PPT, pedunculopontine tegmental nucleus; QS, quiet sleep; REM, rapid eye movement; SCN, suprachiasmatic nucleus; SubCA, subcoeruleus nucleus; TMN, tuberomammilary nucleus; VLPO, ventrolateral preoptic area.

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Therefore, because the nuchal electromyogram (EMG) exhibits two distinct levels of activation in infants before P10, we focus exclusively on this measure in the present study.

To demonstrate developmental changes in sleep–wake cyclicity, nuchal EMG was monitored during undisturbed sleep–wake periods in infant rats. We report that P2 rats exhibit rapid cycling that slows over the next 6 days. Furthermore, P8 rats with LC lesions or transections caudal to the anterior hypothalamus exhibit sleep patterns that revert to those exhibited by P2s. These results suggest the presence of a bistable mesopontine circuit that governs rapid sleep–wake cycling and that comes increasingly under hypothalamic control during the first postnatal week.

EXPERIMENTAL PROCEDURES

All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) and were approved by the Institutional Animal Care and Use Committee of the University of Iowa. All efforts were made to minimize the number of animals used and their suffering.

Subjects

Forty-nine P2, P5, and P8 male and female Sprague–Dawley Norway rats (*Rattus norvegicus*) from 37 litters were used. Body weights ranged from 6.54–9.69 g at P2, 8.16–15.73 g at P5, and 16.77–21.28 g at P8. Litters were culled to eight pups within 3 days after birth (day of birth=day 0). Mothers and their litters were housed in standard laboratory cages (48×20×26 cm) in the animal colony at the University of Iowa where food and water were available *ad libitum*. All animals were maintained on a 12-h light/dark schedule with lights on at 07:00 h and all tests, with one exception, were conducted during the lights-on phase.

Development of sleep/wake cyclicity in P2, P5, and P8 rats

A subset of these data have been published previously (Karlsson and Blumberg, 2002). Briefly, a total of 18 pups were used at P2, P5, and P8 (n=6 at each age). Under isoflurane anesthesia, bipolar stainless steel hook electrodes (50 μm diameter; California Fine Wire, Grover Beach, CA, USA) were inserted bilaterally into the nuchal muscle. The pup was placed on a felt pad in a supine position (to enable observation of myoclonic twitches of individual limbs), lightly restrained using soft pipe cleaners, and transferred to a humidified chamber maintained at thermoneutrality (35.25 °C). A microcamera was located above the Plexiglas lid. After 1 h of acclimation, EMG and behavioral data were recorded for 1 h.

EMG signals were amplified (×10,000) and filtered (300–5000 Hz) using a differential amplifier (Model 1700; A-M Systems, Carlsborg, WA, USA). EMG and video signals were recorded using a digital recording system (Model DV8; WinTron Technologies, Rebersburg, PA, USA).

LC lesions

A pup was immersed for 3–4 min in an ice bath to induce anesthesia (Phifer and Terry, 1986) and was then transferred to a platform maintained at 5–7 °C; this platform was mounted on a stereotax (David Kopf Instruments, Tujunga, CA, USA). A stainless steel electrode (00 insect pin) was lowered under stereotaxic guidance (coordinates: AP −1.2 mm from lambda, ML ±1.0 mm, and −5.0 mm ventral to the skull surface). Bilateral lesions (n=6) were made with 0.5 mA of DC current for 12 s. Sham controls (n=6) experienced the same procedure except that current was not applied. When littermates were used, they were always assigned to different experimental groups. After implantation of nuchal EMG electrodes, the pup was secured in a supine position and recovered inside an incubator maintained at 34–35 °C for 3 h. Because of the added length of the surgery and recovery time, the pup was intubated with warm milk (commercial half-and-half) using a tracheal cannula; the volume in milliliters was 2–4% of body weight. The pup was then transferred to the test chamber where it acclimated for 1 h before data were recorded for at least 15 min.

Although excitotoxic lesions have the benefit of sparing fibers of passage, our previous attempts using ibotenic acid in week-old rats typically resulted in death shortly after surgery, consistent with the observations of others in adults (Coyle and Schwarcz, 1983). For this reason, electrolytic lesions were used here.

After testing, all pups were overdosed with sodium pentobarbital and perfused with saline and formalin. Heads were post-fixed in a sucrose–formalin solution for 24 h, whereupon the brain was removed and post-fixed for an additional 24 h. Brains were sliced in the coronal plane into 50 μm sections, mounted, and stained with Cresyl Violet. The extent of each lesion was evaluated under a microscope and drawn onto an archival set of digitized microphotographs using image software (Adobe Photoshop 5.5, Adobe, San Jose, CA, USA).

Brain transections

Under isoflurane anesthesia, transections were made in P8 rats by manually inserting a blunted 25 g needle to the base of the brain and gently rotating the needle using a side-to-side motion. For anterior transections (n=6) the insertion point was immediately rostral to bregma and for posterior transections (n=6) the insertion point was between lambda and bregma. Sham controls (n=7) experienced the same procedure except that the needle was not inserted into the brain. After surgery, EMG electrodes were implanted and the pup was secured in a supine position. After recovery in an incubator for 1.5 h at thermoneutrality, the pup was intubated with warm milk (as described above) and moved to the testing chamber. After 45 min of acclimation, EMG and behavioral data were recorded for 1 h. Because transections can disinhibit heat production by brown adipose tissue (BAT; Blumberg et al., 1995), skin temperature above the interscapular BAT pad was measured at the end of the test.

Transected brains were prepared for histology as described above for the LC-lesioned pups. After gross inspection, brains were sliced in 180 μm sagittal sections, mounted, and stained.

Data analysis

EMG signals were digitized at 2 kHz using a data acquisition system (BioPac Systems Inc., Santa Barbara, CA, USA). Digitized signals were summed (when there were two usable EMG signals), integrated, and full-wave rectified. Then, the EMG signal was dichotomized into bouts of sleep and awake using the following method: The amplitude of five 1-s segments of noise-free, uninterrupted atonia and high-tone periods was measured for each pup, averaged, and the midpoint between the two was calculated. A bout of atonia (indicative of sleep) and high tone (indicative of wakefulness) was defined as a period in which muscle tone was below or above, respectively, the midpoint value for at least 1 s. Mean sleep and awake durations for each subject were determined from 19 to 50 cycles, depending on the experiment; each cycle was defined as a sleep period and its succeeding awake period. Analysis of variance or unpaired t-tests were used to test for group effects. For post hoc tests, Fisher's PLSD was used. Paired t-tests were used to test for within-subject differences in sleep and awake duration. α was set at 0.05. Means are presented with their S.E.

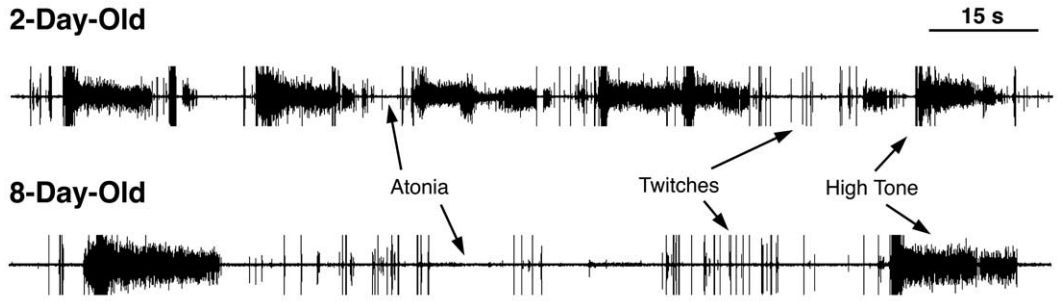

Fig. 1. Nuchal EMG data from representative P2 (top) and P8 (bottom) rats. Each segment is 2.4 min long. Atonia (i.e. sleep) and high-tone (i.e. awake) periods are indicated by arrows. The P2 rat cycles between the two EMG states more rapidly than the P8 rat. Instances of myoclonic twitching against a background of atonia are also indicated.

A more complete picture of the distribution of temporal data (such as sleep and awake durations) can be gained by constructing log-survivor plots (Fagen and Young, 1978). The durations of randomly occurring events form a negative exponential distribution that, on a log-survivor plot, falls along a straight line with a slope that is proportional to the rate at which events occur. For the analyses performed here, sleep durations for subjects within a group were pooled, as were awake durations.

RESULTS

Sleep bouts consolidate during the first postnatal week

Representative 2.4-min segments of EMG data for P2 and P8 rats are presented in Fig. 1. The relatively rapid cycling of the P2 subject is evident. In addition, it can be seen that myoclonic twitching of the nuchal muscle occurs against a background of atonia, as is also true for twitching of the limbs and tail (Karlsson and Blumberg, 2002).

Age had a significant effect on mean sleep ($P<0.01$; $F=7.3$; df=2,15) and awake durations ($P<0.05$; $F=3.7$; df=2,15); in Fig. 2A, it can be seen that sleep duration at P8 was significantly greater than at P2 or P5 (awake duration differs only from the value at P5, not shown). Moreover, at P5 and P8 (but not P2), sleep duration was significantly longer than awake duration. The consolidation of sleep periods between P2 and P8 is more clearly seen in the log-survivor plots (Fig. 2B). In contrast, the developmental trend is less pronounced for the awake durations.

The percentage of time that pups slept at each age was assessed by dividing mean atonia duration by mean cycle length. The resulting mean values were 63.2±8.3% at P2, 74.2±5.8% at P5, and 72.1±5.9% at P8 (NS; $F=0.7$; df=2,15).

It is possible that restraining pups in a supine position significantly modified sleep behavior. To ensure that this was not the case, 12 additional P2 and P8 subjects ($n=6$ at each age) were implanted with EMG electrodes and tested unrestrained for 1 h. As shown in Fig. 3, unrestrained pups exhibited virtually identical developmental changes in sleep cyclicity as did the restrained pups (see Fig. 2). In addition, no significant differences were found on any sleep measure at either age between restrained and unrestrained groups.

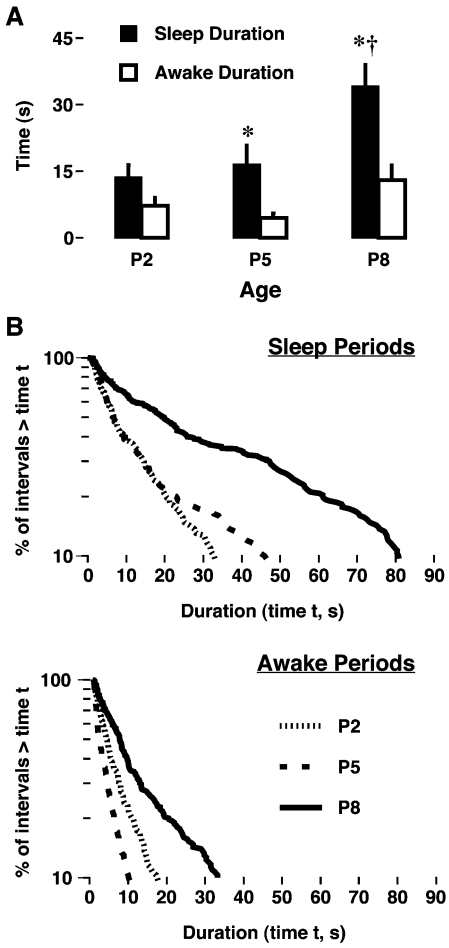

Fig. 2. Development of nuchal EMG cyclicity during the first postnatal week in restrained subjects. (A) Mean sleep and awake durations for P2, P5, and P8 rats. * Significant difference from Awake Duration. † Significant difference from Sleep Duration at P2 and P5. (B) Log-survivor plots of sleep periods (top) and awake periods (bottom) for the same subjects as in (A). Each plot is constructed from 300 data points (50 per subject).

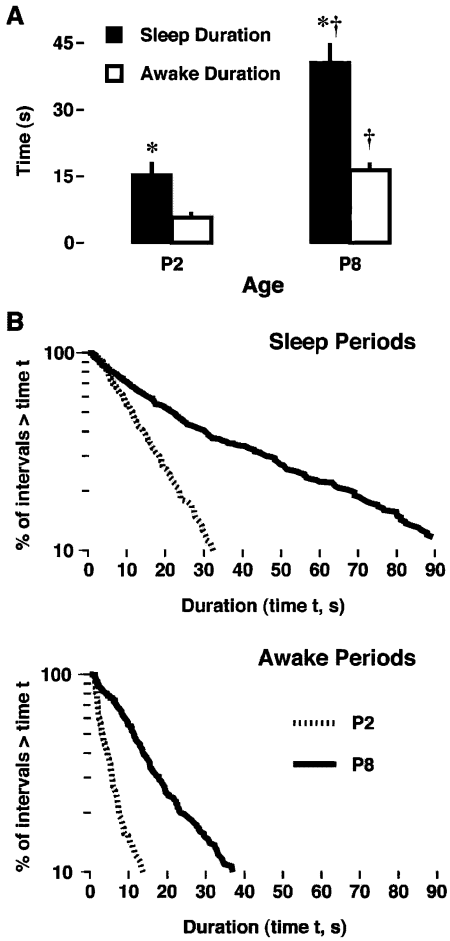

Fig. 3. Development of nuchal EMG cyclicity during the first postnatal week in unrestrained subjects. (A) Mean sleep and awake durations for P2 and P8 rats. * Significant difference from Awake Duration. † Significant difference from Sleep Duration. (B) Log-survivor plots of sleep periods (top) and awake periods (bottom) for the same subjects as in (A). Each plot is constructed from 300 data points (50 per subject).

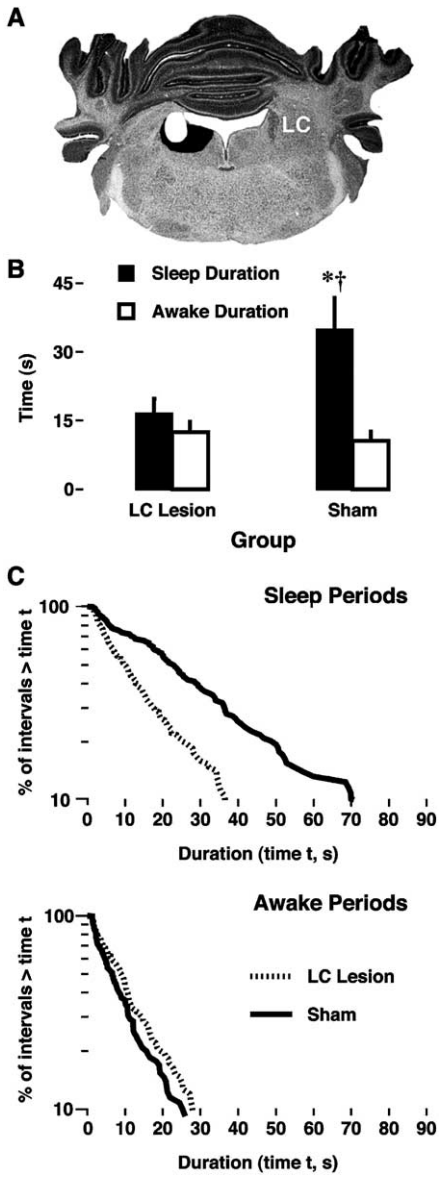

Fig. 4. Effect of bilateral LC lesions on nuchal EMG cyclicity in P8 rats. (A) Coronal section at the level of the LC in a P8 rat. The smallest lesion (white blob) and the largest lesion (black blob) are indicated. Lesions on the contralateral side (which are nearly identical) are not shown so that the LC, characterized by the densely staining vertical column of cells, can be visualized. (B) Mean sleep and awake durations for the pups with LC lesions and sham controls. * Significant difference from Awake Duration. † Significant difference from Sleep Duration in the lesion group. (C) Log-survivor plots of sleep periods (top) and awake periods (bottom) for the same subjects as in (B). Each plot is constructed from 114 data points (19 per subject).

LC lesions reinstate rapid cycling in P8 rats

The extent of the neural damage for the six subjects with bilateral LC lesions is shown on the left side of the coronal section in Fig. 4A (contralateral lesions were nearly identical). Even the smallest lesions were large enough to destroy the LC at this coronal level and throughout the entire rostrocaudal extent of this nucleus. In addition, there was damage to surrounding structures, including the subcoeruleus nucleus (SubCA), the medial part of the vestibular nucleus, and the caudal part of the lateral parabrachial nucleus.

Fig. 4B shows that LC lesions significantly reduced mean sleep duration ($P<0.05$; $t=2.4$, df$=10$) but not awake duration ($t=0.6$; df$=10$), resulting in P8 rats with cycles that resemble those of normal P2s (see Fig. 2). Log-survivor plots (Fig. 4C) illustrate clearly the selective effect of LC lesions on sleep durations.

Percentage of muscle atonia per sleep cycle was reduced from 74.8±5.3% in the shams to 55.6±7.5% in the lesioned pups, although this difference was not significant ($P=0.06$; $t=2.1$; df$=10$). This reduction was due to a disproportionate decrease in sleep duration.

The sleep behavior of the lesioned pups appeared normal. Specifically, twitching of the limbs and tail began soon after the onset of muscle atonia and persisted until sleep was disrupted by a sudden increase in muscle tone accompanied by high-amplitude, awake behaviors.

Transections caudal to the anterior hypothalamus reinstate rapid cycling in P8 rats

The activity of the LC and adjacent nuclei in adults is modulated by adjacent structures in the mesopontine region as well as by nuclei in the caudal and rostral hypothalamus (Aston-Jones et al., 2001; Saper et al., 2001; Pace-Schott and Hobson, 2002). We hypothesized that the consolidation of sleep during the first postnatal week results from the maturation of descending hypothalamic circuits. To test this hypothesis and to narrow down the range of possible nuclei that might modulate LC activity in P8 rats, transections ranging from the caudal hypothalamus to basal forebrain were performed.

Gross inspection of the transected brains indicated that most were completely separated into two parts; for the remainder, only the dorsal or lateral cerebral cortex or dorsal midbrain remained intact. Fig. 5A presents a mid-sagittal section of a P8 rat and illustrates the range of the transections in each group. It is apparent that neither anterior nor posterior transections destroyed tissue within the region of the hypothalamus immediately ventral to the anterior commissure.

Mean durations of sleep and wakefulness for the three experimental groups are presented in Fig. 5B. There was a significant effect of group on mean sleep ($P<0.05$; $F=5.6$; df=2,16) but not awake duration ($F=1.8$; df=2,16). There were no differences between the sham and anterior transection groups, with both groups showing significantly longer sleep durations than awake durations. Both of these groups had significantly higher sleep durations than did the pups with posterior transections, which exhibited equivalent sleep and awake durations. Log-survivor plots indicate that posterior transections reduced sleep durations but that anterior transections had no effect in relation to the shams (Fig. 5C).

None of the transections affected the percentage of the sleep–wake cycle occupied by sleep. Specifically, mean values were 73.6±4.4% for the pups with anterior transections, 62.7±5.6% for the pups with posterior transections, and 70.5±4.8% for the shams (NS; $F=1.2$; df=2,16). Moreover, as expected from a previous study in which transections within the caudal hypothalamus in P8 rats were shown to have no effect on rates of myoclonic twitching (Kreider and Blumberg, 2000), the transected pups in this experiment exhibited the same temporal coherence of twitching and muscle atonia seen in unmanipulated pups.

Finally, measurements of interscapular temperature at the end of the tests indicated that BAT heat production was disinhibited in some pups in both groups. Specifically, whereas normal interscapular temperature under these conditions is approximately 37.5 °C, two pups with anterior transections and three pups with posterior transections had interscapular temperatures of 40–40.5 °C. There was no relationship, however, between interscapular temperature and mean sleep or awake durations.

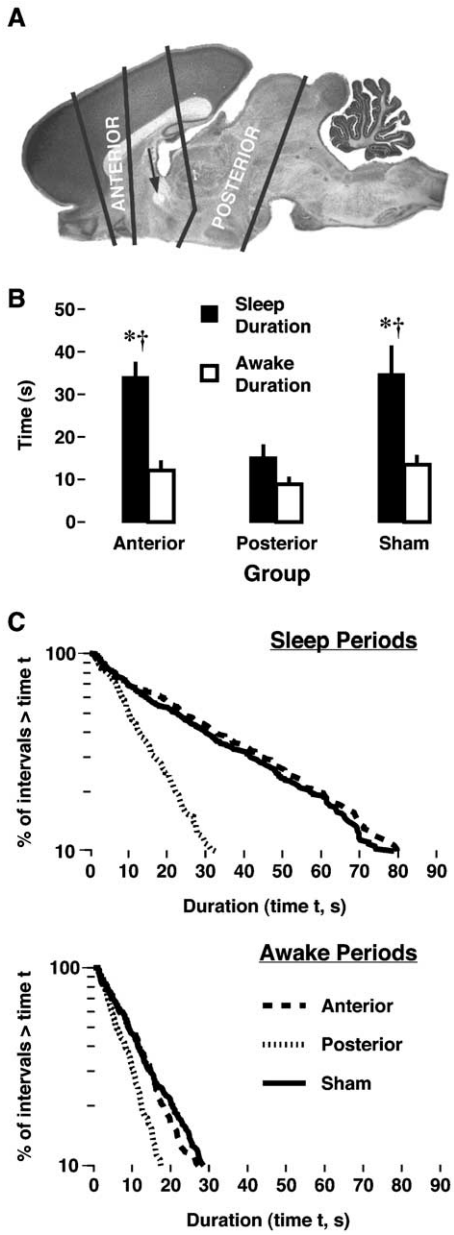

Fig. 5. Effect of anterior and posterior transections on nuchal EMG cyclicity in P8 rats. (A) Mid-sagittal section in a P8 rat, with the rostral–caudal range of anterior and posterior transections depicted. Arrow indicates anterior commissure. (B) Mean sleep and awake durations for pups with anterior and posterior transections and for sham controls. * Significant difference from Awake Duration. † Significant difference from posterior transection group. (C) Log-survivor plots of sleep periods (top) and awake periods (bottom) for the same subjects as in (B). Each plot is constructed from 300 to 341 data points (41–50 per subject).

DISCUSSION

We have shown here in infant rats that sleep–wake cycles, as determined by alternations in nuchal muscle tone, begin to consolidate in the first postnatal week. This finding, which is consistent with previous investigations of sleep–wake cyclicity in infant rats (Gramsbergen et al., 1970),

was consistently expressed by pups regardless of whether they were tested restrained or unrestrained. The rapid cycling typical of P2s was reinstated in P8s by LC lesions as well as by transections that spared the anterior hypothalamus. These reversions to rapid cycling were attributable primarily to reductions in sleep durations. Moreover, the tight coupling between muscle atonia and myoclonic twitching was not affected by either LC lesions or brain transections, attesting to the usefulness of these two components for defining sleep in infants (Karlsson and Blumberg, 2002).

It is significant that LC lesions at P8 did not abolish sleep–wake cyclicity but only altered its patterning. This finding, in conjunction with the results of the transection experiments, suggests that sleep–wake cyclicity in P2s is produced by a mesopontine circuit in which the LC plays little, if any, role. Whether the LC begins to contribute by P8 is less clear, however, because we cannot be sure that destruction of the LC was responsible for the changes in sleep–wake cyclicity depicted in Fig. 4. Although these lesions were small, other structures were affected. For example, it is possible that the effects seen here were mediated by destruction of the SubCA, a nucleus that is associated with the modulation of muscle tone (Mileykovskiy et al., 2000; Kiyashchenko et al., 2001) and that also receives inputs from many of the same structures as the LC (Pace-Schott and Hobson, 2002).

As shown in Fig. 5, a wedge of tissue at the level of the anterior commissure was spared by both the anterior and posterior transections. The anterior commissure is a reliable marker of the location of several anterior hypothalamic nuclei that have been implicated in sleep–wake cyclicity in adults, including the suprachiasmatic nucleus (SCN; Ibuka and Kawamura, 1975; Stephan and Nunez, 1977), VLPO, and the extended VLPO (Lu et al., 2000, 2002). Thus, we can be confident that these nuclei and their descending fibers were not damaged in any of the pups with anterior transections. It should be noted, however, that the anterior transections spared descending connections from the parts of other forebrain structures, including neocortex and hippocampus; on the other hand, there is no basis for implicating these structures in the modulation of sleep–wake cyclicity.

The SCN, VLPO, and extended VLPO project caudally to hypothalamic and mesopontine structures that modulate sleep and wakefulness. For example, the SCN forms a circuit with the dorsomedial hypothalamus (DMH), a primary site of orexin production in the rat brain that sends its most dense projections to the LC (Peyron et al., 1998), forming an SCN–DMH–LC circuit that is thought to modulate sleep–wake patterns (Aston-Jones et al., 2001). Interestingly, in adult rats, both the LC and SubCA respond to orexin stimulation with increases and decreases in muscle tone, respectively (Kiyashchenko et al., 2001). In newborn rats, orexin is already expressed by hypothalamic neurons and is able to increase the firing rate of LC neurons (Van Den Pol et al., 2001, 2002).

Investigations of the VLPO and extended VLPO in adults suggest selective roles in non-rapid eye movement (non-REM) and REM sleep, respectively (Lu et al., 2000). Tracing studies indicate that the VLPO and extended VLPO have dense and often reciprocal connections with a number of sleep-related nuclei, including the tuberomammilary nucleus (TMN), laterodorsal tegmental nucleus, pedunculopontine tegmental nucleus (PPT), dorsal raphe, DMH, and LC (Lu et al., 2002). It has been suggested that these nuclei comprise a network of mutual inhibition—a "flip-flop"—that regulates transitions between sleep and arousal (Saper et al., 2001); in this model, the sleep-promoting side of the circuit depends entirely upon the VLPO and extended VLPO in the rostral hypothalamus. The present results, although generally consistent with this model, suggest further that a complete circuit exists in infant rats that is independent of hypothalamic input and is sufficient to support rapid cycling. It seems likely that this elemental circuit is composed of the same mesopontine and medullary structures that are known to modulate muscle tone in adults (Hajnik et al., 2000; Kiyashchenko et al., 2001). Moreover, rapid sleep–wake cycling could reflect the natural oscillation of this circuit when orexin levels are low, as in P2 rats (Van Den Pol et al., 2001) and in human narcolepsy (Thannickal et al., 2000), a condition characterized by "sleep attacks" that include the sudden of loss of muscle tone during the day (Taheri et al., 2002).

Therefore, based on the present results, we hypothesize that a basic sleep–wake cycle exists in P2 rats that does not depend upon input from nuclei in the LC area. We hypothesize further that the changes in sleep–wake cyclicity that occur between P2 and P8 result from development of a circuit connecting the anterior hypothalamus and posterior hypothalamus to mesopontine nuclei, including nuclei in the LC area. Because, however, some of the posterior transections spared the DMH and TMN but nonetheless resulted in rapid cycling (see Fig. 5), these caudal hypothalamic nuclei may be necessary but not sufficient components in this circuit.

We have used nuchal muscle tone as our primary measure of sleep and wakefulness (with additional guidance provided by behavior) because it is a reliable, stable, and easily quantifiable measure of behavioral state at the ages used here (Karlsson and Blumberg, 2002). Moreover, transitions between high-tone and atonia periods are typically abrupt (Fig. 1), thus justifying our division of muscle tone into two mutually exclusive categories. On the other hand, we could have further divided sleep and wakefulness into active and quiet categories. For example, it is possible that the consolidation of sleep between P2 and P8 resulted from an increase in QS (as defined by muscle atonia in the absence of twitching) in relation to AS (as defined by muscle atonia in the presence of twitching). To test this possibility, we determined the proportion of atonia periods occupied by myoclonic twitching and found that AS occupied three-quarters of the sleep periods at both ages (P2: $71.4 \pm 3.3\%$; P8: $74.9 \pm 3.4\%$); similar results were found for the lesioned and transected pups. The stability of the AS:QS ratio between P2 and P8 indicates that the development of sleep consolidation shown here is unaffected by how sleep is partitioned.

Although it has been assumed for many years that AS and REM sleep are developmentally continuous behavioral states (Corner, 1977), this perspective has been challenged recently (Frank and Heller, 1997, 2003). In particular, Frank and Heller refer to AS as "presleep" based on their assessment of the literature and their own work on rats older than 10 days of age. We question, however, the usefulness of introducing yet another name for infant sleep before the developmental relations between infant and adult sleep have been thoroughly explored. For example, Frank and Heller (1997) view AS as a form of fetal motor activity that does not "require intact brain stem sleep structures" (p. R1798). On the contrary, we have found recently that lesions of the PPT significantly reduce myoclonic twitching (J. C. Kreider and M. S. Blumberg, unpublished observations), underscoring a role for the mesopontine region in infant sleep (Kreider and Blumberg, 2000). In addition, as shown here, a nucleus (or nuclei) in the LC area modulates sleep–wake cyclicity in infants and, even more surprising, structures far rostral to the mesopontine region do as well.

Infancy is only one context in which rapid sleep–wake cycles have been observed. Specifically, rapid cycles occur in adults after lesions of the SCN (Ibuka and Kawamura, 1975; Stephan and Nunez, 1977) or VLPO (Lu et al., 2000), as well as in species, such as the blind mole rat, that have adapted to life in dark environments (Tobler and Deboer, 2001). In addition, rapid cycling results from reductions in orexin system function, as seen in humans and canines with narcolepsy and in orexin knockout mice (Taheri et al., 2002). Although there appears to be considerable overlap in the neural substrates linked with rapid cycling, more work is needed to delineate the exact neural circuits involved. Regardless, the present results suggest that rapid sleep–wake cycling is a basic element of sleep regulation upon which developmental and evolutionary modifications are and have been built.

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