Behavioral Neuroscience
2004, Vol. 118, No. 6, 1253–1261

Copyright 2004 by the American Psychological Association
0735-7044/04/$12.00 DOI: 10.1037/0735-7044.118.6.1253

Sleep Homeostasis in Infant Rats

Mark S. Blumberg, Jessica E. Middlemis-Brown, and Eric D. Johnson
University of Iowa

Homeostatic regulation is a defining characteristic of sleep but has rarely been examined in infants. This study presents an automated method of sleep deprivation in which 5-day-old rats were shocked whenever the nuchal muscle became atonic. The intensity of shock was always set at the minimal level required to maintain arousal. Deprived pups exhibited rapid increases in sleep pressure, as evidenced by increased attempts to enter sleep and subsequent increases in sensory threshold; this increased sensory threshold was not due to sensory adaptation of peripheral receptors. In addition, myoclonic twitching was suppressed during the 30-min deprivation period, leading to rebound twitching during recovery sleep. These results provide the earliest demonstration of the homeostatic regulation of sleep in an altricial mammal.

Sleep during infancy in rats exhibits many of the defining characteristics of sleep as defined in adults (Campbell & Tobler, 1984; Hendricks, Sehgal, & Pack, 2000). For example, infant rats housed in a thermoneutral humidified environment enter and exit sleep spontaneously, as defined by rapid cycling between periods of high muscle tone and atonia (Karlsson, Kreider, & Blumberg, 2004). Motor activity mirrors these cycles, with high-amplitude coordinated movements accompanying periods of high muscle tone and myoclonic twitching of the limbs and tail occurring only against a background of atonia (Karlsson & Blumberg, 2002). Sensory threshold also increases during sleep in infants, as was demonstrated recently by presenting an olfactory stimulus, dimethyl disulfide, to infants during periods of sleep and wakefulness (Seelke & Blumberg, 2004). Finally, there is evidence of state-related neural activity in the reticular formation and hippocampus (Corner & Bour, 1984; Karlsson & Blumberg, 2003, 2004; Lahtinen et al., 2001; Tamásy, Korányi, & Lissák, 1980). Thus, on the basis of the absence of high-amplitude behaviors, spontaneity, reversibility, increased sensory threshold, and state-dependent neural activity, infant rats exhibit many of the standard hallmarks of sleep.

Missing from the infant sleep characteristics outlined above is homeostatic regulation, widely considered a central feature of sleep. Sleep homeostasis is typically assessed by depriving a subject of sleep and monitoring the corrective responses (Bonnet, 2000; Rechtschaffen, 1979; Rechtschaffen, Bergmann, Gilliland, & Bauer, 1999). Specifically, sleep deprivation is thought to evoke two homeostatic responses: sleep pressure, which occurs during a 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. These two responses are thought to be separable; for example, in adult cats, sleep rebound, but not sleep pressure, is at least partially regulated by the hypothalamus (de Andres, Garzon, & Villablanca, 2003).

Although there have been numerous sleep deprivation studies in adult rats, only a few deprivation studies have been conducted in infants. Specifically, pharmacological agents have been used to chronically suppress REM sleep beginning at 8 days of age (P8) with subsequent assessment of affects on brain development and adult behavior (Mirmiran, Scholtens, et al., 1983; Mirmiran, van de Poll, Corner, Van Oyen, & Bour, 1981). Two acute sleep deprivation studies have also been conducted in infant rats, each of them reporting similar results despite using different deprivation procedures (Feng, Ma, & Vogel, 2001; Frank, Morrissette, & Heller, 1998). For example, Frank et al. (1998) used a forced locomotion or gentle handling procedure to deprive P12–P24 rats of sleep. They reported that sleep need, as measured by the number of attempts to enter sleep, accumulated rapidly during deprivation; in addition, REM sleep rebound developed only around P20, at least 1 week later than non-REM (NREM) sleep rebound.

The focus of these two previous acute sleep deprivation studies on pups P12 and older reflects the belief that sleep is most reliably studied at ages where the neocortical electroencephalogram exhibits state-dependent differentiation. In rats, such differentiation appears to be absent until P12 (Frank & Heller, 1997; Gramsbergen, 1976; Mirmiran & Corner, 1982). Thus, if sleep homeostasis is to be investigated at younger ages, then it is necessary to rely on other measures of sleep. Given recent evidence that nuchal atonia and myoclonic twitching are valid indicators of sleep in infant rats, we hypothesized that these measures would also be sufficient for demonstrating sleep homeostasis.

Thus, a new method was developed for depriving infant rats of sleep that entails the delivery of electric shock to the skin whenever the nuchal muscle becomes atonic; consequently, myoclonic twitching is also disrupted. Shock was chosen over other methods

Mark S. Blumberg, Jessica E. Middlemis-Brown, and Eric D. Johnson, Program in Behavioral and Cognitive Neuroscience, Department of Psychology, University of Iowa.

This work was supported by National Institute of Mental Health Grants MH50701 and MH66424 and National Institute of Child Health and Human Development Grant HD38708. We thank Karl Karlsson for technical assistance and Adele Seelke and Karl Karlsson for many helpful comments and suggestions.

Correspondence concerning this article should be addressed to Mark S. Blumberg, Department of Psychology, E11 Seashore Hall, University of Iowa, Iowa City, IA 52242. E-mail: mark-blumberg@uiowa.edu

(e.g., manual stroking, handling, vibrotactile stimuli) because it is a relatively discrete stimulus that is easily delivered and controlled; moreover, the intensity of shock is easily quantifiable and can be gradually and minimally increased so as to maintain arousal under conditions that allow for the continuous monitoring of nuchal electromyogram and behavior. Pups are tested in a supine position to allow for unobstructed views of the limbs for scoring of myoclonic twitching.

We show here that Postnatal Day 5 (P5) rats deprived of sleep accumulate sleep pressure within minutes and, when given the opportunity to sleep after only 30 min of deprivation, exhibit rebound twitching characterized by intense and rapid movements of the limbs. In a follow-up experiment, we show that the increase in sensory threshold that occurs during shock-induced sleep deprivation is not attributable to adaptation of peripheral receptors. All together, the present results provide the earliest demonstration of sleep homeostasis in an altricial mammal.

Method

All experiments were performed under National Institutes of Health guidelines for the care of animals in research and were approved by the Institutional Animal Care and Use Committee of the University of Iowa.

Experiment 1: Demonstrating Sleep Homeostasis in Infant Rats

Subjects

A total of 24 P5 male ($n = 12$) and female ($n = 12$) rats from 21 litters were used. Males and females were equally distributed among the three experimental groups; moreover, when littermates were used, they were always assigned to different experimental groups. Body weights ranged from 9.4 to 16.4 g. All pups were born to Harlan Sprague–Dawley rats housed in the animal colony at the University of Iowa. The pups were raised in litters that were culled to 8 pups within 3 days of birth (day of birth = Day 0). Litters and mothers were housed in standard laboratory cages (48 cm × 20 cm × 26 cm) in which food and water were available ad libitum. All animals were maintained on a 12-hr light–dark schedule, with lights on at 7 a.m.

Surgery

Under isoflurane anesthesia, bipolar stainless steel hook electrodes (50 μm diameter; California Fine Wire, Grover Beach, CA) were inserted bilaterally into the nuchal muscle, as described previously (Karlsson et al., 2004). In addition, two shock electrodes were implanted subcutaneously on either side of the thorax and secured using collodion as an adhesive. The pup was placed on a felt pad in a supine position (to gain visual access to the limbs for scoring of myoclonic twitching), lightly restrained using soft pipe cleaners, and transferred to a humidified incubator maintained at thermoneutrality (35 °C) to recover for at least 1 hr. Toward the end of recovery, the pup was intubated with 0.2 ml of warm cream (half-and-half) and then transferred to the testing chamber. The experiment began at least 30 min after transfer.

Apparatus

Pups were tested inside an electrically shielded, double-walled glass chamber (height = 17 cm; i.d. = 12.5 cm) with a Plexiglas lid. Air temperature inside the chamber was maintained at 35 °C using a temperature-controlled water circulator. Access holes in the side and lid of the chamber allowed for the passage of air (300 ml/min) as well as the passage of shock and nuchal EMG electrodes.

EMG signals were amplified (×10,000) and filtered (300–5000 Hz) using a differential amplifier (Model 1700, A-M Systems, Carlsborg, WA). The electrode wires attached to the pup were connected to a shock controller (Coulbourn Instruments, Allentown, PA) connected to a custom-built device that, when triggered, turned on the shock controller for 1 s. This device, in turn, was connected to a computer-controlled digital interface (Model MP150, BioPac Systems Inc., Santa Barbara, CA). Dedicated acquisition software (Acknowledge, BioPac Systems Inc., Santa Barbara, CA) was used to trigger the delivery of shock for 1 s whenever the pup's nuchal muscle became atonic. Nuchal EMG, shock data, and video data were recorded using a digital recording system (Model DV8, WinTron Technologies, Rebersburg, PA).

Procedure

Each test consisted of three contiguous 30-min periods (see Figure 1). During the first and third periods (hereafter designated as the baseline and recovery periods, respectively), all pups were allowed to cycle undisturbed between sleep and wakefulness while EMG and video data were collected. During the middle period (hereafter referred to as the deprivation period), pups were divided into three groups ($n = 8$ pups per group). First, in the deprived group, shock was delivered to a pup whenever its nuchal muscle became atonic. Shock intensity was set initially to 0.1 mA and was increased to 0.2 mA when five shocks were delivered during a 15-s period (a high number of shocks in a short period of time indicates an increase in the number of attempts to enter sleep and, therefore, an increase in sleep pressure). Using this same criterion of five shocks per 15 s, shock intensity was increased if necessary to 0.3 mA and 0.4 mA. Second, in the no-shock control group, pups were instrumented identically to pups in the other groups but were never shocked.

Finally, a shock control group was used to control for the delivery of shocks to the pups. Ideally, a yoked control would be used to control for all aspects of the procedure experienced by the deprived pups (Rechtschaffen, Gilliland, Bergmann, & Winter, 1983). A yoking procedure was not possible here, however, because of the disproportionately long atonia periods and the rapid sleep–wake cycling characteristic of infant sleep (Karlsson et al., 2004); in other words, yoked pups would unavoidably experience nearly as much sleep deprivation as would deprived pups. Therefore, a compromise control procedure was adopted in which shocks

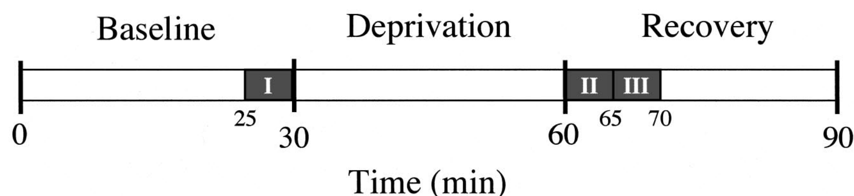

Figure 1. Schematic of the design for Experiment 1. In addition to the 30-min baseline, deprivation, and recovery periods, the three 5-min phases (I, II, and III) at Minutes 25–30, 60–65, and 65–70 are indicated.

were delivered at a fixed intensity of 0.1 mA throughout the 30-min deprivation period.

Data Analysis

A variety of analyses was used to assess the accumulation of sleep pressure, the effectiveness of the shock protocol for depriving pups of sleep, and the expression of sleep rebound.

Sleep pressure. The number of shocks delivered to the deprived and shock control pups was assessed for each 5-min segment of the 30-min deprivation period. Also, for the deprived pups, the percentage of shocks within each 5-min segment at each shock intensity level was determined. Finally, a shock index was computed by multiplying the number of shocks at a given intensity level (i.e., 1–4) by that intensity level and then taking the sum: 4 shocks at an intensity of 0.1 mA, 3 shocks at an intensity of 0.2 mA, and 2 shocks at an intensity of 0.3 mA yields a total number of shocks of 9 but a shock index of $(4 \times 1) + (3 \times 2) + (2 \times 3) = 16$. In effect, the shock index quantifies the relative amount of electric current delivered to each pup.

Effectiveness of the sleep deprivation procedure. The delivery of electric shocks to the deprived and shock control pups during the deprivation period created large artifacts in the EMG record, thus precluding the assessment of sleep using this measure. Therefore, myoclonic twitching was used instead as a measure of sleep during the deprivation period. Myoclonic twitching was defined as phasic, rapid, and independent movements of the limbs and tail (Blumberg & Stolba, 1996; Gramsbergen, Schwartze, & Prechtl, 1970). An experienced observer, naive to experimental condition, pressed the key of an event recorder when a twitching movement of any of the limbs or tail was detected. We and others have used similar scoring procedures in the past and have found them to be highly reliable, with inter- and intrarater reliability coefficients typically exceeding .85 (Blumberg & Lucas, 1994; Smotherman & Robinson, 1991). Scoring of each 30-min segment was accomplished in a single pass through the video record.

Sleep rebound. Two independent measures were used to assess sleep rebound. First, EMG signals were digitized at 2 kHz using a data acquisition system (BioPac Systems Inc., Santa Barbara, CA). Digitized signals were integrated and full-wave rectified. Then, the EMG signal was dichotomized into bouts of sleep and wakefulness using the following method, as described previously (Karlsson et al., 2004): 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 rat were determined for the 30-min baseline and recovery periods.

Second, three 5-min phases were identified for an in-depth analysis of myoclonic twitching: the final 5 min of the baseline period (Phase I in Figure 1), the first 5 min of the recovery period (Phase II in Figure 1), and the second 5 min of the recovery period (Phase III in Figure 1). At each phase, myoclonic twitching of each of the four individual limbs was scored separately in four passes through the videotape. Then, the four data files were concatenated to produce a single record of twitching for each 5-min segment. Again, the observer was naive to experimental condition.

Statistics. Data were imported into StatView 5.0 for analysis (SAS, Cary, NC). Repeated measures and single-factor analyses of variance (ANOVAs) were used, as were paired and unpaired *t* tests when appropriate. When ANOVAs were significant, Fisher's protected least significant difference was used to test for group differences. Alpha was set at .05. Means are presented with their standard errors.

Data were excluded when a value exceeded the group mean $\pm$ 2.58 standard deviations (1% rejection). Twitching rates for 2 deprived pups during the baseline period were outliers according to this criterion, and the data for these 2 pups were excluded from the analysis of twitching. Exclusion of these data did not significantly alter any of the basic findings.

Experiment 2: Is the Increased Sensory Threshold During Sleep Deprivation Due to Adaptation of Peripheral Receptors?

Subjects, Surgery, and Apparatus

Six P5 male ($n = 3$) and female ($n = 3$) rats from six litters were used. Body weights ranged from 10.9 to 12.1 g. Pups were prepared as in Experiment 1 except for one major change: During surgery, two pairs of electrodes were attached, one pair to the left side of the thorax and one pair to the right side. The two electrodes in each pair were spaced approximately 1 cm apart.

Procedure

A pup was placed in the recording chamber and acclimated as described in Experiment 1. The test began with the delivery of 0.1-mA shocks to one side of the body when the nuchal muscle became atonic. Delivery of shocks continued until a criterion of five shocks within a 15-s period was reached; at this point, and in contrast with Experiment 1, additional time was allotted to ensure that the pup was sleeping through the shocks. Then, shock delivery was switched to the opposite side of the body while recording of data continued. Next, shock intensity was increased to 0.2 mA; again, when the relevant criteria were reached, the shock was switched back to the opposite side of the body. Finally, shock intensity was increased one more time to 0.3 mA. The side of the body that was shocked first was counterbalanced across rats.

Data Analysis

For each pup, four 30-s periods were identified for analysis: (a) at 0.1 mA, when shock delivery was switched from one side of the body to the other; (b) when shock intensity was increased from 0.1 to 0.2 mA; (c) at 0.2 mA, when shock delivery was switched back to the other side of the body; and (d) when shock intensity was increased from 0.2 to 0.3 mA. The change in shock conditions (i.e., side switch or intensity increase) occurred at the midpoint of each 30-s period. In each case, an observer naive to condition scored the incidence of awake behavior and twitching as described in Experiment 1.

Data were imported into StatView 5.0 for analysis (SAS Institute, Cary, NC). The number of 1-s bins in which awake behavior was detected during the 15-s periods before and after the change in shock conditions was determined. Data were excluded when a value exceeded the group mean $\pm$ 2.58 standard deviations (1% rejection). One data point met this criterion. A single-factor repeated measures ANOVA was used to determine the effect of changed shock conditions on awake behavior. Alpha was set at .05. Means are presented with their standard errors.

Results

Experiment 1: Demonstrating Sleep Homeostasis in Infant Rats

Sleep Pressure

Throughout the deprivation period, deprived and shock control rats received increasing numbers of shocks (see Figure 2A). A repeated measures ANOVA did not reveal a significant effect of group, $F(1, 14) = 4.2$, *ns*, but did reveal a significant effect of time, $F(5, 70) = 10.0$, $p < .0001$, as well as a significant Group $\times$ Time interaction, $F(5, 70) = 2.5$, $p < .05$. The increase in the number of shocks delivered was significant in both groups but was especially prominent in the shock controls in which the shock intensity remained constant at 0.1 mA (the increasing number of shocks in the shock controls reflects the fact that these pups

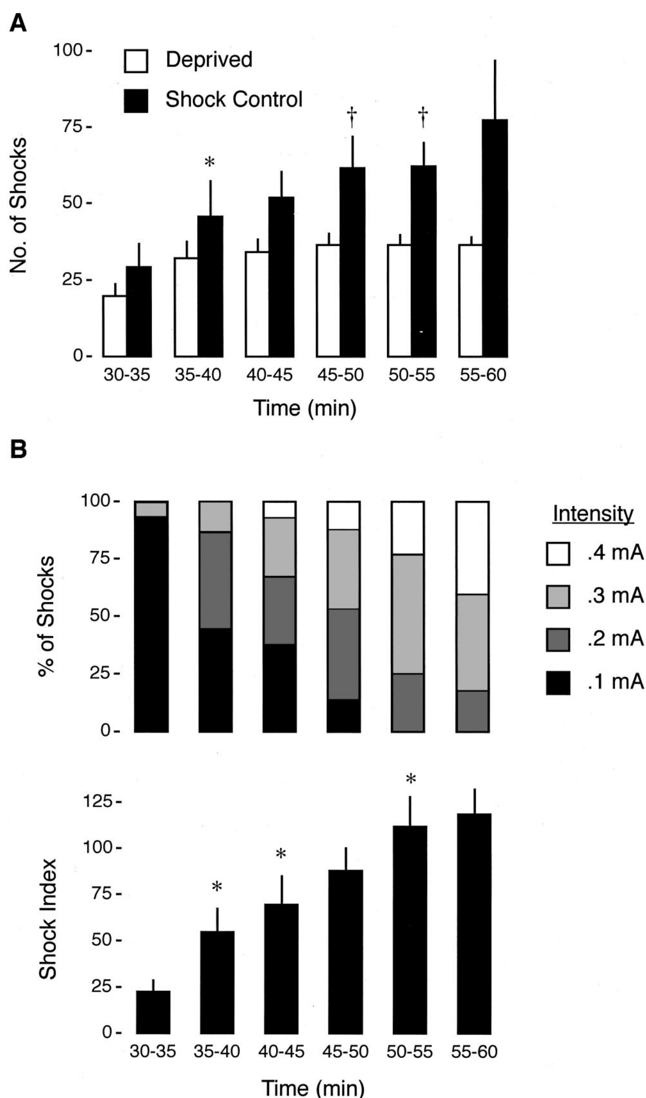

Figure 2. A: Total number of shocks (regardless of intensity) delivered to 5-day-old rats during each 5-min period of the deprivation period in Experiment 1. Pups in the shock control group received more total shocks than pups in the deprived group. B: The top panel shows the percentage of shocks delivered to pups in the deprived group at 0.1, 0.2, 0.3, or 0.4 mA. As the deprivation period progressed, shocks of higher intensity were required to maintain arousal, an indication of increased sleep pressure. The bottom panel shows the increase in shock index (see text for definition) in the deprived pups across the deprivation period. An asterisk indicates within-group significant difference in comparison with the previous time period. A dagger indicates between-group significant difference. $n = 8$ pups per group. Values are means ± standard errors.

aroused quickly to the shocks at the beginning of the period but were sleeping through the shocks by the end of the period).

During the deprivation period, deprived pups increased their attempts to enter sleep, becoming atonic more quickly and triggering the increased delivery of electric shocks. When the number of shocks reached criterion levels (i.e., five shocks within 15 s), shock intensity was increased in increments of 0.1 mA to maintain arousal throughout the deprivation period. As shown in the top panel of Figure 2B, 93% of the shocks during the first 5 min of the deprivation period were delivered at 0.1 mA, and this number decreased to 0% within 20 min. In contrast, by the last 5-min segment of the period, 40% of the shocks were delivered at 0.4 mA. In total, all 8 deprived pups graduated to 0.2 mA at some point during the 30-min deprivation period, 7 out of 8 graduated to 0.3 mA, and 4 out of 8 graduated to 0.4 mA. Finally, the shock index, a combined measure of the number and intensity of shocks, increased steadily throughout the deprivation period (Figure 2B, bottom panel).

Effectiveness of the Sleep Deprivation Procedure

As mentioned in the Method section, EMG artifact produced by the delivery of shock during the deprivation period precluded any sleep assessments using this measure. Thus, the effectiveness of the sleep deprivation procedure was determined using other information.

First, as discussed above and as shown in Figure 2B (bottom panel), the shock index increased incrementally throughout the deprivation period, suggesting that sleep pressure and sensory threshold increased as a result of the deprivation procedure. Second, because myoclonic twitching typically occurs against a background of atonia (Karlsson & Blumberg, 2002), any manipulation that prevents atonia should also prevent twitching. This inference was tested by scoring individual limb twitching during the three 10-min segments of the deprivation period. Repeated measures ANOVA revealed significant effects of group, $F(2, 21) = 50.2$, $p < .0001$, and time, $F(2, 42) = 14.7$, $p < .0001$, as well as a significant Group × Time interaction, $F(4, 42) = 7.4$, $p < .0001$. As shown in Figure 3, pups in the no-shock control group exhibited consistently high rates of twitching throughout the period. In contrast, pups in the deprived group exhibited mean reductions in twitching of nearly 80%. Pups in the shock control group exhibited an intermediate response, that is, low rates of twitching during the first 10 min of the deprivation period (when 0.1 mA shocks elicited strong responses) and high rates of twitching during the final 10 min of the period.

Sleep Rebound

Nuchal EMG. There were no significant group differences for any measure of muscle tone during any period or phase of the experiment. Focusing on the first 5 min of the recovery period (i.e., Phase II), where a rebound effect, if present, should be most obvious, mean atonia durations were 19.0 ± 4.4 s, 18.7 ± 4.2 s, and 19.6 ± 4.5 s for the deprived, shock control, and no-shock control groups, respectively, $F(2, 21) = 0.0$, *ns*. The respective mean high-tone durations were 6.3 ± 1.0 s, 6.6 ± 0.6 s, and 11.5 ± 2.9 s, $F(2, 21) = 2.5$, *ns*. Thus, the nuchal EMG data provide no evidence of sleep rebound.

Myoclonic twitching. Casual observations of twitching by the deprived pups indicated that they twitched vigorously and intensely during the first few minutes of the recovery period. To measure twitching objectively, twitching of all four limbs was scored independently during Phases I, II, and III (see Figure 1). As shown in Figure 4A, deprived pups exhibited significant increases in twitching that occurred only during Phase II, that is, during the first 5 min of the recovery period. Repeated measures ANOVA revealed significant effects of group, $F(2, 19) = 9.4$, $p < .005$, and time, $F(2, 38) = 29.9$, $p < .0001$, as well as a significant Group ×

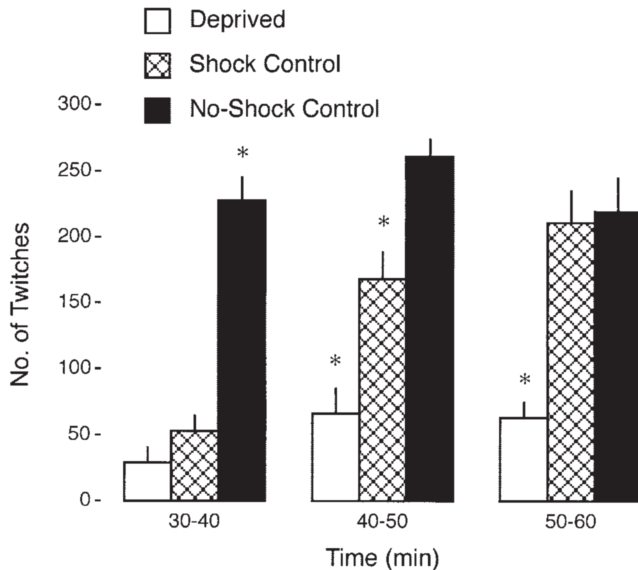

Figure 3. Mean (± *SEM*) number of limb and tail twitches exhibited during the deprivation period by pups in the deprived, shock control, and no-shock control groups in Experiment 1. Only deprived pups exhibited reduced levels of twitching throughout the entire period as shock intensity was increased to maintain arousal. Twitching by pups in the shock control group was affected early in the period, but twitching recovered to normal levels because shock intensity was not increased to maintain arousal in these pups. $n = 8$ pups per group. The asterisks indicate significant difference from the other two groups ($p < .05$).

Time interaction, $F(4, 38) = 11.4$, $p < .0001$. In terms of the percentage increases in twitching during Phase II in relation to Phase I, pups in the deprived group increased twitching 113.9 ± 17.4% during the first 5 min of recovery compared with 28.1 ± 13.5% and 48.8 ± 14.7% for the shock control and no-shock control groups, respectively.

It is interesting to note that 1 shock control pup reacted strongly to the 0.1 mA shocks throughout the deprivation period, exhibiting only 49 twitches during the last 10 min (compared with the group mean of 211 ± 24 twitches). Then, during the first 5 min of the recovery period (i.e., Phase II), this pup exhibited a 112% increase in twitching; the next highest value in this group was just 39%. Therefore, despite the low level of shock intensity, this shock control pup exhibited sleep-deprivation and rebound responses typical of deprived pups.

Because mean atonia durations did not increase during the recovery period, the increases in twitching just described could be due to increases in the distribution of twitching within atonia periods or to increases in the density of twitching. As shown in Figure 4B, the mean number of 1-s bins in which at least one twitch occurred was significantly higher in the deprived group during the first 5 min of the recovery period (i.e., Phase II). Repeated measures ANOVA revealed significant effects of group, $F(2, 19) = 7.2$, $p < .005$, and time, $F(2, 38) = 11.5$, $p < .0001$, as well as a significant Group × Time interaction, $F(4, 38) = 3.5$, $p < .05$. Thus, twitching was distributed more broadly during atonia periods in the deprived group.

Figure 4C shows that the density of twitching, defined as the number of twitches that occurred during each 1-s bin, also increased in the deprived group. Specifically, for each 1-s bin in which at least one twitch occurred, the number of twitches within each bin increased an average of approximately one twitch in the deprived pups during the first 5 min of the recovery period (i.e., Phase II). Repeated measures ANOVA revealed significant effects of group, $F(2, 19) = 6.0$, $p < .001$, and time, $F(2, 38) = 12.8$, $p < .0001$, as well as a significant Group × Time interaction, $F(4, 38) = 5.4$, $p < .005$. Because brief bouts of twitching are typically composed of twitches from different limbs (Robinson, Blumberg, Lane, & Kreber, 2000), these data help to explain why deprived pups appeared to twitch more intensely during Phase II.

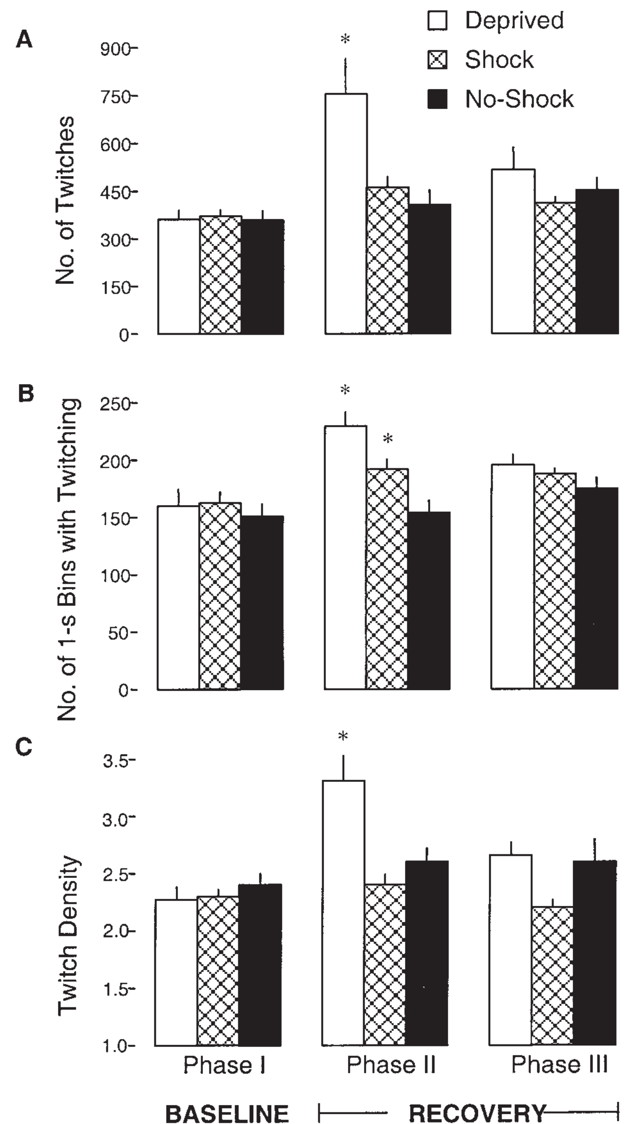

Figure 4. A: Mean (± *SEM*) number of fore and hind limb twitches exhibited by deprived, shock control, and no-shock control pups in Experiment 1. Twitching was assessed during three 5-min periods: before the end of baseline (Phase I; see Figure 1) and at the beginning of recovery (Phases II and III). B: Mean (± *SEM*) number of 1-s bins in which at least one twitch occurred. C: Mean (± *SEM*) density of twitching, defined as the number of twitches divided by the number of 1-s bins in which twitching occurred. $n = 6–8$ pups per group. The asterisks indicate significant difference from the other two groups ($p < .05$).

Experiment 2: Is the Increased Sensory Threshold During Sleep Deprivation Due to Adaptation of Peripheral Receptors?

Two aspects of the results of Experiment 1 indicate that sensory threshold increased over the course of sleep deprivation. First, a shock intensity of 0.1 mA was sufficient to arouse the shock control pups at the beginning of the deprivation period but not at the end (see Figure 3). Second, sleep deprivation could only be sustained in the deprived pups by increasing the shock intensity from 0.1 mA to as high as 0.4 mA during the 30-min deprivation period. One possible explanation for the increase in sensory threshold is that circuits within the central nervous system were modulated as sleep pressure intensified. Alternatively, it is possible that repeated delivery of shocks to the skin resulted in adaptation of peripheral sensory neurons. Experiment 2 was conducted to test this sensory adaptation hypothesis.

In the majority of cases, pups were asleep and twitching during the 15-s period before the change in shock conditions. Because of the short sleep–wake cycles of P5 rats (Karlsson et al., 2004), it was possible that pups would arouse spontaneously—that is, independently of the experimental manipulations—at any time during the test.

On average, pups received 3–4 shocks during the 15-s periods before and after the change in shock conditions. A repeated measures ANOVA did not reveal any significant changes over time or differences between the two experimental conditions.

Visual observations of the pups during the switch from one side of the body to the other indicated few overt changes in behavior; none of the pups responded with substantial increases in awake behavior and, in many instances, pups continued to exhibit myoclonic twitching as the shocks were delivered. In contrast, 4 of the 6 pups responded to the increases in shock intensity with vigorous awake behavior (e.g., kicking).

The observations just described are supported by the statistical comparisons of awake behavior. As shown in the left panel of Figure 5, pups exhibited very low rates of awake behavior both before and after the switch in shock delivery from one side of the body to the other. A repeated measures ANOVA did reveal a significant effect of time, $F(1, 10) = 5.9$, $p < .05$, although the mean increase in awake behavior was slight (from 0.25 to 1.00 1-s bins). The effects of group, $F(1, 10) = 0.5$, and the Group × Time interaction, $F(1, 10) = 0.6$, were not significant. Thus, switching shock delivery to a new region of the body is not sufficient to evoke a marked increase in awake behavior.

In contrast, as shown in the right panel of Figure 5, increasing the intensity of the shock evoked pronounced increases in awake behavior, from a mean of 0.1 to a mean of nearly 6.0. Again, a repeated measures ANOVA revealed a significant effect of time, $F(1, 9) = 22.5$, $p < .005$, but neither the effect of group, $F(1, 9) = 2.0$, nor the Group × Time interaction, $F(1, 9) = 1.8$, was significant.

Discussion

The results of Experiment 1 demonstrate that sleep is homeostatically regulated as early as P5 in rats. Specifically, sleep pressure increased rapidly with the onset of deprivation, as evidenced by the increased attempts to enter sleep and the need to increase shock intensity to maintain arousal throughout the deprivation period. The rapid accumulation of sleep pressure in these infants may reflect the possibility that animals that sleep more accumulate sleep need more rapidly when deprived, as has been argued for adult rats and humans (Rechtschaffen et al., 1999).

This study introduces a controlled, automated method for depriving rats of sleep in the early postnatal period. A new method was required because standard methods used to sleep-deprive older infants and adults (Feng et al., 2001; Frank et al., 1998; Mendelson, 1974; Rechtschaffen et al., 1983) are not viable in week-old rats in which locomotor skills and thermoregulatory capabilities are limited and when monitoring of myoclonic twitching requires that pups be tested in a supine position. Pharmacological methods of depriving sleep were not adopted because they offer limited experimental control over the onset and offset of deprivation

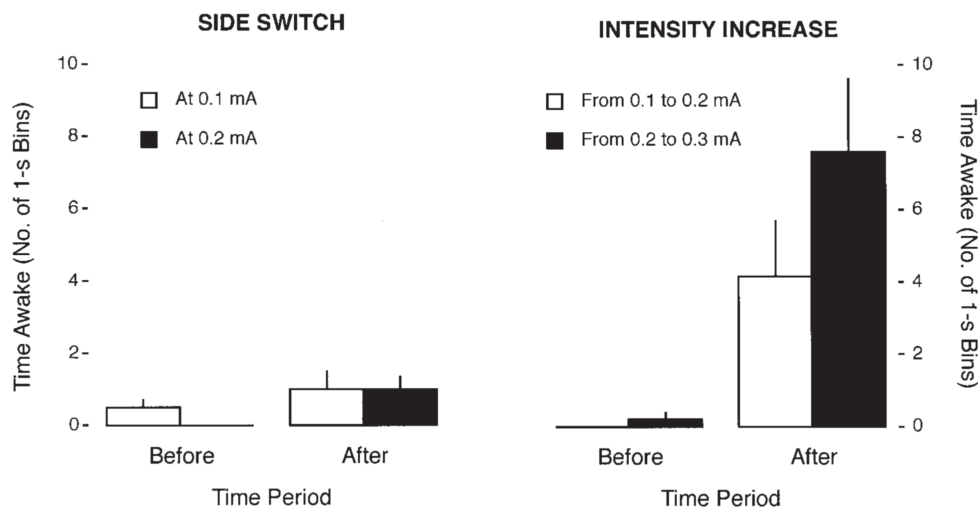

Figure 5. Mean (± *SEM*) number of 1-s bins in which pups exhibited awake behavior during the 15-s periods before and after shock delivery was switched from one side of the body to the other (side switch) or shock intensity was increased (intensity increase) in Experiment 2. $n = 5–6$ pups per group.

(Mirmiran, Uylings, & Corner, 1983; Mirmiran et al., 1981; Saucier & Astic, 1975).

Various methods of depriving animals of sleep differ as to whether control procedures are or are not easily used (Feng et al., 2001; Frank et al., 1998; Rechtschaffen et al., 1983). In Experiment 1, two control methods were used, shock and no shock. (As mentioned earlier, a yoking procedure was not possible here because of the disproportionately long atonia periods and the rapid sleep–wake cycling characteristic of infant sleep.) Of importance, the pups in the shock control group experienced more shocks than did the pups in the deprived group, although their shock index (for the shock controls, the shock index is simply the total number of shocks received) was lower than that for the deprived pups (see Figure 2); in other words, the total amount of current delivered to the deprived pups was slightly higher than the total amount of current delivered to the shock control pups. Nonetheless, the shock control pups received a substantial number of shocks and yet they did not differ from the no-shock control pups on any measure during the recovery period (e.g., see Figure 4). Therefore, it seems reasonable to conclude that experiencing shock per se was not a determining factor for the results presented here.

Deprived pups did not exhibit sleep rebound as measured by the duration of nuchal atonia. In contrast, deprived pups did exhibit significantly increased levels of myoclonic twitching during the recovery period, the first demonstration that deprivation of this phasic component of sleep evokes a compensatory rebound response. The magnitude of this rebound was large but its duration was relatively brief: Specifically, after twitching was reduced 80% over the 30-min deprivation period, rebound twitching consisted of a doubling of twitching but only during the first 5 min of the recovery period. This pattern resembles that seen after REM sleep deprivation in adult rats (Rechtschaffen et al., 1999); for example, after 24 hr of REM sleep deprivation, REM rebound consisted of a doubling of REM sleep time during the first 6 hr of recovery sleep, with rapidly declining values thereafter. At this time, however, parallels between these phenomenologically distinct sleep phenomena are best viewed with caution.

Midthoracic transections reduce rates of twitching by only 50% at P5 (Blumberg & Lucas, 1994), indicating that at least 50% of twitches at this age are generated by structures rostral to the transection. By P8, brain transections caudal, but not rostral, to the mesopontine region reduce twitching by 70% (Kreider & Blumberg, 2000). In addition, descending mechanisms from the mesopontine region, but not spinal mechanisms, evoke rapid limb twitches such as those observed here during rebound twitching (Blumberg & Lucas, 1994; Kreider & Blumberg, 2000). All together, these findings suggest that rebound twitching depends on neural circuits located within the mesopontine region.

The spontaneous muscle twitches that accompany sleep in infant and adult mammals have been considered the overt manifestation of dreams (Romanes, 1883/1977), as by-products of intense brain activation (Chase & Morales, 1990), or as critical components in the development and maintenance of neural and neuromuscular organization (Blumberg & Lucas, 1996). The validity of this last perspective was supported by a recent article showing in young rats how sleep-related twitching can contribute to the organization of the withdrawal reflex system within the spinal cord (Petersson, Waldenström, Fåhraeus, & Schouenborg, 2003). The demonstration here of rebound twitching complements this recent report in that the functional significance and apparent homeostatic regulation of twitching may be related.

Experiment 2 tested the hypothesis that the increase in sensory threshold demonstrated in Experiment 1 was due to sensory adaptation of peripheral receptors. This hypothesis was not supported. Therefore, it appears that sleep deprivation produces an increase in sleep pressure that is subsequently relieved through a centrally modulated decrease in sensory inflow, thereby permitting sleep. Furthermore, it is most parsimonious to suggest that the raised sensory threshold that occurs during sleep in infant rats (Seelke & Blumberg, 2004) and the further raising of sensory threshold that occurs during sleep deprivation are governed by the same central mechanism.

Delivery of shocks to the skin likely produced nonspecific activation of afferent fibers associated with one or more sensory systems. For example, the spinoreticular tract (SRT), which transmits tactile and nociceptive information, comprises ascending axons from spinal neurons that terminate throughout the medullary and pontine reticular formation and ascend further to the thalamus and neocortex (Tracey, 1985). In adult cats, SRT neurons in the spinal cord decrease their activity during REM sleep but not during NREM sleep or wakefulness (Soja, Pang, Taepavarapruk, & McErlane, 2001). Similar findings have been reported for the dorsal spinocerebellar tract (Soja, Fragoso, Cairns, & Jia, 1996), which primarily transmits proprioceptive information. Accordingly, it is hypothesized that sensory inflow is suppressed during sleep deprivation in infants via descending inhibitory connections from the reticular formation directly onto spinal sensory neurons.

To our knowledge, only one previous study examined the effects of sleep deprivation in a mammal so soon after birth (Berger & Meier, 1966). Using the precocial rhesus monkey, a combination of loud tones and tail shock was used to deprive newborns selectively of electrographically defined active or quiet sleep (myoclonic twitching was not monitored). Regarding active sleep deprivation, it was found that sleep pressure increased over the 2 to 7 days of deprivation, and, during recovery, there was no evidence of sleep rebound as conventionally defined. Similar results were found for quiet sleep deprivation. Thus, the present findings of increased sleep pressure without sleep rebound are generally consistent with this earlier report.

The reason that P5 rats did not exhibit sleep rebound in Experiment 1 remains unclear. One possibility is that there is an upper limit to how much pups can sleep and that this ceiling effect is particularly pronounced at the ages used here where sleep durations are already long in relation to awake durations. A similar argument was made by Berger and Meier (1966) to explain the absence of sleep rebound in newborn rhesus monkeys. A second possibility is that sleep rebound depends on neural circuitry that is not yet mature at P5; the gradual emergence of sleep rebound in infant rats beyond the second postnatal week is consistent with this notion (Feng et al., 2001; Frank et al., 1998). It should be stressed, however, that pups were deprived of sleep in Experiment 1 for only 30 min, and perhaps longer deprivation periods would produce more robust sleep rebound effects.

Did the procedure used here result in total or selective sleep deprivation? The answer to this question depends on how one characterizes periods of atonia in infants. Typical periods of atonia can be partitioned into two categories: during the first 25% of the period, pups are behaviorally quiescent, with the remainder of the period being occupied by bouts of myoclonic twitching (Karlsson

et al., 2004). Therefore, on one hand, each period of atonia could be interpreted as a short period of quiet sleep followed by a long period of active sleep. On the other hand, the entire atonia period might represent a single sleep state that consists of a tonic component (e.g., muscle atonia) with the occasional overlay of phasic activation (e.g., myoclonic twitching). Therefore, whether the sleep deprivation protocol used here is most accurately conceptualized as total or selective must await the final resolution of this question. Regardless, at this time we can accurately state that the deprivation protocol used here targets periods of muscle atonia and, as a consequence, also deprives pups of myoclonic twitching.

Is it possible that the shock protocol used here to deprive pups of sleep produced changes in sleep expression via nonspecific stress and/or generalized fatigue (Rechtschaffen & Bergmann, 1995; Rechtschaffen et al., 1999)? This seems unlikely for several reasons. First, sleep pressure increased within only minutes of the start of the deprivation period. Second, the shock control group was designed to control for the effects of nonspecific factors such as stress. Although this control procedure was not perfect, the shock control pups nevertheless received an average of 70 shocks over the final 10 min of the deprivation period and yet were indistinguishable from the no-shock controls on virtually every dimension analyzed. Finally, it is not clear why a nonspecific stressor or fatigue would produce a selective rebound in myoclonic twitching.

Sleep deprivation paradigms, like the one introduced here, can be used effectively to unmask nuclei involved in the regulation of sleep. Thus, recording from sleep-related nuclei during sleep deprivation might be a useful strategy for investigating the neural substrates of infant sleep, including sleep-related modulation of sensory input (Soja et al., 2001). In addition, the development of a reliable and effective sleep deprivation procedure in infants may provide a useful foundation for future studies examining the role of sleep-related processes in neural plasticity during development (Blumberg & Lucas, 1996; Corner et al., 1980; Corner, van Pelt, Wolters, Baker, & Nuytinck, 2002; Frank, Issa, & Stryker, 2001; Marks, Shaffery, Oksenberg, Speciale, & Roffwarg, 1995; Mirmiran, 1995; Petersson et al., 2003; Roffwarg, Muzio, & Dement, 1966).

References

Berger, R. J., & Meier, G. W. (1966). The effects of selective deprivation of states of sleep in the developing monkey. *Psychophysiology, 2,* 354–371.

Blumberg, M. S., & Lucas, D. E. (1994). Dual mechanisms of twitching during sleep in neonatal rats. *Behavioral Neuroscience, 108,* 1196–1202.

Blumberg, M. S., & Lucas, D. E. (1996). A developmental and component analysis of active sleep. *Developmental Psychobiology, 29,* 1–22.

Blumberg, M. S., & Stolba, M. A. (1996). Thermogenesis, myoclonic twitching, and ultrasonic vocalization in neonatal rats during moderate and extreme cold exposure. *Behavioral Neuroscience, 110,* 305–314.

Bonnet, M. H. (2000). Sleep deprivation. In M. H. Kryger, T. Roth, & W. C. Dement (Eds.), *Principles and practice of sleep medicine* (pp. 53–71). Philadelphia: W. B. Saunders.

Campbell, S. S., & Tobler, I. (1984). Animal sleep: A review of sleep duration across phylogeny. *Neuroscience and Biobehavioral Reviews, 8,* 269–300.

Chase, M. H., & Morales, F. R. (1990). The atonia and myoclonia of active (REM) sleep. *Annual Review of Psychology, 41,* 557–584.

Corner, M. A., & Bour, H. L. (1984). Postnatal development of spontaneous neuronal discharges in the pontine reticular formation of free-moving rats during sleep and wakefulness. *Experimental Brain Research, 54,* 66–72.

Corner, M. A., Mirmiran, M., Bour, H. L., Boer, G. J., van de Poll, N. E., van Oyen, H. G., et al. (1980). Does rapid-eye-movement sleep play a role in brain development? *Progress in Brain Research, 53,* 347–356.

Corner, M. A., van Pelt, J., Wolters, P. S., Baker, R. E., & Nuytinck, R. H. (2002). Physiological effects of sustained blockade of excitatory synaptic transmission on spontaneously active developing neuronal networks—An inquiry into the reciprocal linkage between intrinsic biorhythms and neuroplasticity in early ontogeny. *Neuroscience and Biobehavioral Reviews, 26,* 127–185.

de Andres, I., Garzon, M., & Villablanca, J. R. (2003). The disconnected brain stem does not support rapid eye movement sleep rebound following selective deprivation. *Sleep, 26,* 419–425.

Feng, P., Ma, Y., & Vogel, G. W. (2001). Ontogeny of REM rebound in postnatal rats. *Sleep, 24,* 645–653.

Frank, M. G., & Heller, H. C. (1997). Development of REM and slow wave sleep in the rat. *American Journal of Physiology, 272,* R1792–R1799.

Frank, M. G., Issa, N. P., & Stryker, M. P. (2001). Sleep enhances plasticity in the developing visual cortex. *Neuron, 30,* 275–287.

Frank, M. G., Morrissette, R., & Heller, H. C. (1998). Effects of sleep deprivation in neonatal rats. *American Journal of Physiology, 44,* R148–R157.

Gramsbergen, A. (1976). The development of the EEG in the rat. *Developmental Psychobiology, 9,* 501–515.

Gramsbergen, A., Schwartze, P., & Prechtl, H. F. R. (1970). The postnatal development of behavioral states in the rat. *Developmental Psychobiology, 3,* 267–280.

Hendricks, J. C., Sehgal, A., & Pack, A. (2000). The need for a simple animal model to understand sleep. *Progress in Neurobiology, 61,* 339–351.

Karlsson, K. Æ., & Blumberg, M. S. (2002). The union of the state: Myoclonic twitching is coupled with nuchal muscle atonia in infant rats. *Behavioral Neuroscience, 116,* 912–917.

Karlsson, K. Æ., & Blumberg, M. S. (2003). Hippocampal theta in the newborn rat is revealed under conditions that promote REM sleep. *Journal of Neuroscience, 23,* 1114–1118.

Karlsson, K. Æ., & Blumberg, M. S. (in press). Active medullary control of atonia in week-old rats. *Neuroscience.*

Karlsson, K. Æ., Kreider, J. C., & Blumberg, M. S. (2004). Hypothalamic contribution to sleep-wake cycle development. *Neuroscience, 123,* 575–582.

Kreider, J. C., & Blumberg, M. S. (2000). Mesopontine contribution to the expression of active "twitch" sleep in decerebrate week-old rats. *Brain Research, 872,* 149–159.

Lahtinen, H., Palva, J. M., Sumanen, S., Voipio, J., Kaila, K., & Taira, T. (2001). Postnatal development of rat hippocampal gamma rhythm in vivo. *Journal of Neurophysiology, 88,* 1469–1474.

Marks, G. A., Shaffery, J. P., Oksenberg, A., Speciale, S. G., & Roffwarg, H. P. (1995). A functional role for REM sleep in brain maturation. *Behavioural Brain Research, 69,* 1–11.

Mendelson, W. B. (1974). The flower pot technique of rapid eye movement (REM) sleep deprivation. *Pharmacology Biochemistry and Behavior, 2,* 553–556.

Mirmiran, M. (1995). The function of fetal/neonatal rapid eye movement sleep. *Behavioural Brain Research, 69,* 13–22.

Mirmiran, M., & Corner, M. (1982). Neuronal discharge patterns in the occipital cortex of developing rats during active and quiet sleep. *Brain Research, 255,* 37–48.

Mirmiran, M., Scholtens, J., van de Poll, N. E., Uylings, H. B. M., van der Gugten, J., & Boer, G. J. (1983). Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior in the rat. *Developmental Brain Research, 7,* 277–286.

Mirmiran, M., Uylings, H. B. M., & Corner, M. A. (1983). Pharmacolog-

ical suppression of REM sleep prior to weaning counteracts the effectiveness of subsequent environmental enrichment on cortical growth in rats. *Developmental Brain Research, 7,* 102–105.

Mirmiran, M., van de Poll, N. E., Corner, M. A., Van Oyen, H. G., & Bour, H. L. (1981). Suppression of active sleep by chronic treatment with chlorimipramine during early postnatal development: Effects upon adult sleep and behavior in the rat. *Brain Research, 204,* 129–146.

Petersson, P., Waldenström, A., Fåhraeus, C., & Schouenborg, J. (2003, July 3). Spontaneous muscle twitches during sleep guide spinal self-organization. *Nature, 424,* 72–75.

Rechtschaffen, A. (1979). The function of sleep: Methodological issues. In R. Drucket, M. Shkurovich, & M. B. Sterman (Eds.), *Functions of sleep* (pp. 1–17). New York: Academic Press.

Rechtschaffen, A., & Bergmann, B. M. (1995). Sleep deprivation in the rat by the disk-over-water method. *Behavioural Brain Research, 69,* 55–63.

Rechtschaffen, A., Bergmann, B. M., Gilliland, M. A., & Bauer, K. (1999). Effects of method, duration, and sleep stage on rebounds from sleep deprivation in the rat. *Sleep, 22,* 11–31.

Rechtschaffen, A., Gilliland, M. A., Bergmann, B. M., & Winter, J. B. (1983, July 8). Physiological correlates of prolonged sleep deprivation in rats. *Science, 221,* 182–184.

Robinson, S. R., Blumberg, M. S., Lane, M. S., & Kreber, L. A. (2000). Spontaneous motor activity in fetal and infant rats is organized into discrete multilimb bouts. *Behavioral Neuroscience, 14,* 328–336.

Roffwarg, H. P., Muzio, J. N., & Dement, W. C. (1966, April 29). Ontogenetic development of the human sleep–dream cycle. *Science, 152,* 604–619.

Romanes, G. J. (1977). *Animal intelligence*. Washington, DC: United Publications of America. (Original work published 1883)

Saucier, D., & Astic, L. (1975). Effets de l'alpha-methyl-dopa sur le sommeil du chat nouveaune. Evolution comportementale au cours du ler mois post-natal [Effects of alpha-methyl-dopa on newborn kitten sleep: Behavioral evolution during the first month postbirth]. *Psychopharmacology, 42,* 299–303.

Seelke, A. M. H., & Blumberg, M. S. (2004). Sniffing in infant rats during sleep and wakefulness. *Behavioral Neuroscience, 118,* 267–273.

Smotherman, W. P., & Robinson, S. R. (1991). Accessibility of the rat fetus for psychobiological investigation. In H. N. Shair, M. A. Hofer, & G. Barr (Eds.), *Developmental psychobiology: New methods and changing concepts* (pp. 148–164). New York: Oxford University Press.

Soja, P. J., Fragoso, M. C., Cairns, B. E., & Jia, W. G. (1996). Dorsal spinocerebellar tract neurons in the chronic intact cat during wakefulness and sleep: Analysis of spontaneous spike activity. *Journal of Neuroscience, 16,* 1260–1272.

Soja, P. J., Pang, W., Taepavarapruk, N., & McErlane, S. A. (2001). Spontaneous spike activity of spinoreticular tract neurons during sleep and wakefulness. *Sleep, 24,* 18–25.

Tamásy, V., Korányi, L., & Lissák, K. (1980). Early postnatal development of wakefulness–sleep cycle and neuronal responsiveness: A multiunit activity study on freely moving newborn rat. *Electroencephalography and Clinical Neurophysiology, 49,* 102–111.

Tracey, D. J. (1985). Ascending and descending pathways in the spinal cord. In G. Paxinos (Ed.), *The rat nervous system. Volume 2. Hindbrain and spinal cord* (pp. 311–324). Sydney, Australia: Academic Press.

Received March 22, 2004
Revision received July 13, 2004
Accepted July 13, 2004 ■