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Developmental Emergence of Power-Law Wake Behavior Depends Upon the Functional Integrity of the Locus Coeruleus

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Study Objectives: Daily amounts of sleep and wakefulness are accumulated in discrete bouts that exhibit distinct statistical properties. In adult mammals, sleep bout durations follow an exponential distribution whereas wake bout durations follow a power-law distribution. In infant Norway rats, however, wake bouts initially follow an exponential distribution and only transition to a power-law distribution beginning around postnatal day 15 (P15). Here we test the hypothesis that the locus coeruleus (LC), one of several wake-active nuclei in the brainstem, contributes to this developmental transition.

Design: At P7, rats were injected subcutaneously with saline or DSP-4, a neurotoxin that targets noradrenergic (NA) LC terminals. Then, at P21, sleep and wakefulness during the day and night were monitored. The effectiveness of DSP-4 treatment was verified by measuring NA, dopamine (DA), and serotonin (5-HT) concentration in cortical and non-cortical tissue using high performance liquid chromatography.

Results: In relation to controls, subjects treated with DSP-4 exhibited significant reductions only in cortical and non-cortical NA concentration.

Consistent with our hypothesis, the wake bout durations of DSP-4 subjects more closely followed an exponential distribution, whereas those of control subjects followed the expected power-law distribution. Sleep bout distributions were unaffected by DSP-4.

Conclusions: These results suggest that the fundamental developmental transition in the statistical structure of wake bout durations is effected in part by changes in noradrenergic LC functioning. Considered within the domain of network theory, the hub-like connectivity of the LC may have important implications for the maintenance of network function in the face of random or targeted neural degeneration.

Keywords: Wakefulness, DSP-4, power-law, noradrenaline, locus coeruleus, rat, development, circadian rhythms

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IN HUMANS,¹ RATS,² AND MICE,³ NEWBORNS CYCLE RAPIDLY BETWEEN SLEEP AND WAKEFULNESS. ONE OF THE DEFINING FEATURES OF EARLY SLEEP-WAKE development is the consolidation of individual sleep and wake bouts, as well as the circadian entrainment of the sleep-wake rhythm.⁴ In addition, analyses of the statistical distributions of sleep and wake bouts have indicated dramatic changes in the dynamics of sleep-wake activity. Specifically, whereas sleep bout durations distribute exponentially in infant⁵ and adult^{6,7} mammals, wake bout durations distribute exponentially during the early postnatal period and then transition to a power-law distribution that, in rats, occurs around the time of eye opening at postnatal day (P)15.⁸

Several factors may account for the developmental transition from exponential to power-law wake behavior. For example, because the transition occurs around the time of eye opening, it is possible that some aspect of visual stimulation is involved. However, bilateral enucleation at P3 or P11 does not prevent the normal emergence of power-law wake behavior.⁴ Alternatively, it is possible that orexinergic neurons, localized in the lateral hypothalamic nucleus (LHA) and dorsomedial hypothalamic nucleus (DMH), contribute to the emergence of power-law wake behavior by activating wake-active monoaminergic

and cholinergic neurons in the brainstem.⁹⁻¹¹ However, power-law wake behavior emerges in orexin knockout mice just as it does in wild-types.³ A third possibility, examined here, is that functional changes in locus coeruleus (LC) activity contribute to the development of power-law wake behavior.

The LC is a major source of noradrenaline (NA) in the central nervous system and plays an important role in arousal. In adult rats, activity levels of LC neurons increase during wakefulness, decrease their firing rate during quiet sleep, and are nearly silent during active sleep^{12,13}; state-related changes in LC activity are detectable as early as P8 in rats.¹⁴ Increased discharge rates of LC neurons are associated with spontaneous or sensory-evoked interruptions of sleep.^{12,15} Finally, stimulation of LC activity promotes wakefulness,^{16,17} whereas inhibition of LC activity promotes sleep.¹⁸

To test the hypothesis that the LC contributes to the developmental emergence of power-law wake behavior, we administered N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4), a neurotoxin that selectively destroys noradrenergic LC terminals in infant¹⁹ and adult²⁰⁻²³ rats. In the present study, DSP-4 was administered to P7 rats and we examined their sleep-wake behavior 2 weeks later, at P21. We predicted that interfering with LC function in early infancy would prevent or dampen the developmental transition from exponential to power-law wake behavior.

METHODS

All experiments were performed in accordance with National Institutes of Health guidelines for the care of animals in

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research 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.

Subjects

Thirty-two Sprague-Dawley rats from 8 litters were used. Males and females were equally represented and littermates were always assigned to different experimental groups. Litters were culled to 8 pups within 3 days of birth (day of birth = P0). Mothers and their litters were housed and raised in standard laboratory cages (48 × 20 × 26 cm) in the animal colony at the University of Iowa. Food and water were available *ad libitum*. All animals were maintained on a 12-h light-dark schedule with lights on at 07:00.

DSP-4 Treatment

At P7, 4 same-sex litter mates were chosen as experimental subjects. Two of these pups were injected subcutaneously with 50 mg/kg of DSP-4 (Sigma, St. Louis, MO), and 2 control pups were injected with saline. Pups were then tattooed and placed back in the litter for testing 2 weeks later. The same procedure was used for the remaining 7 litters.

Surgery

On the day of testing at P21-22 (hereafter referred to as P21), 2 same-sex littermates, 1 previously injected with DSP-4 and 1 with saline, were implanted with nuchal EMG electrodes. Under isoflurane anesthesia, 2 bipolar stainless steel electrodes (50 µm diameter, California Fine Wire, Grover Beach, CA) were inserted bilaterally into the nuchal muscles and secured with flexible collodion.²⁴ After surgery, pups recovered in a humidified test chamber maintained at thermoneutrality. All surgeries took place at least 2 h before testing. For testing at night, surgery was performed under dim red light illumination, with care being taken to ensure that infants were not exposed to white light. After surgery, each pup was transferred to an individual test chamber maintained at thermoneutrality (i.e., 32°C) where it recovered and acclimated for at least 2 h before testing.

Test Procedure

DSP-4 and saline control subjects were tested simultaneously but separately in two electrically shielded double-walled glass chambers (height, 18 cm; i.d., 12 cm). Air temperature inside each chamber was maintained at thermoneutrality by regulating the temperature of the water that circulated through the walls of the chamber. Compressed, humidified air passed through the sealed chamber at the rate of 300 mL/min. A round platform constructed of polyethylene mesh was fitted inside each chamber, which allowed the pup to move freely on the platform's surface.

The nuchal electrodes were connected to differential amplifiers (A-M systems, Carlsborg, WA) and their signals were amplified (×10 k) and filtered (300–5000 Hz). EMG data were acquired (1000 samples/s) and stored using a data acquisition system (Biopac Systems, Santa Barbara, CA).

During the day, data were acquired from 11:00 to 16:00. At night, the 2 remaining same-sex littermates were implanted with EMG electrodes (again, under red light illumination) and tested identically to those during the day, with data being acquired from 23:00 to 04:00. The order of daytime and nighttime tests was counterbalanced across litters.

HPLC Analysis of Noradrenaline (NA), Dopamine (DA), and Serotonin (5-HT) Concentrations

No more than 2 days after testing, each pup was anesthetized with isoflurane and decapitated. The brain was removed rapidly and placed on ice. The cerebral cortex was quickly dissected out, placed in 15 mL Sarstedt polypropylene tubes, and frozen in liquid nitrogen. Samples were stored at –80°C until homogenization. The cerebral cortex of each rat was homogenized in 0.1 Normal (N) perchloric acid. Homogenate was centrifuged at 17,300 g for 10 min at 4°C. The clear supernatant was filtered through a 0.22 µm Millex-GP filter unit (Millipore, Bedford, MA) and analyzed for levels of NA. Analysis was performed using a Waters 2690 HPLC (Milford, MA) equipped with an ESA Coulochem III (Cherford, MA) with E1 and E2 potentials set to –150 mV and 200 mV, respectively. Eight µL of sample diluted 1:4 with water were injected into a Synergi Hydro 4 µm reversed phase column (Phenomenex, Torrance, CA) with a mobile phase containing 75 mM phosphoric acid, 25 mM citric acid, 1.8 mM sodium dodecyl sulfate, and 2% acetonitrile adjusted to pH 3.0.

A follow-up experiment was performed using 12 additional subjects from 6 litters. From each litter, 2 P7 littermates were injected with DSP-4 or saline as described earlier. At P21, the brain was removed, placed on ice, and the cortex was dissected out for analysis. In addition, the remaining brain tissue (including medulla, midbrain, and diencephalon, minus the cerebellum) was also saved. These cortical and non-cortical samples were prepared for HPLC analysis of NA, DA, and 5-HT concentrations. (These animals were not tested for sleep and wakefulness.) Samples were analyzed using an Agilent 1100 HPLC system (Santa Clara, CA) and an ESA Coulochem III electrochemical detector (settings as previously described). Separation was achieved by injecting 20 µL of undiluted sample onto a Thermo Aquasil C18 5 µm 150 × 2.1 mm column. DA and 5-HT were analyzed by an isocratic method using mobile phase containing 0.1% trifluoroacetic acid (TFA), 50 mM citric acid, 1% methanol, and 1% acetonitrile adjusted to pH 3.0. NA analysis was carried out using a gradient program; the aqueous phase contained 0.2% trifluoroacetic acid, 50 mM citric acid, and 2 mM sodium heptane sulfonate pH 3.0, and the organic phase contained acetonitrile 3% to 18% (3% at 0–8 minutes, linearly increasing to 18% by 19 minutes, and returning to 3% at 20 minutes).

Data Analysis

For all subjects, EMG data were analyzed off-line using AcqKnowledge software (Biopac Systems, Santa Barbara, CA). The EMG signal was integrated and full-wave rectified, and then dichotomized into periods of high muscle tone and atonia (or wakefulness and sleep, respectively), as described earlier.²

Table 1—Sleep and Wake Bout Durations, Percentage of Time Awake, and Number of Sleep-Wake Cycles per Hour in P21 Rats Treated on P7 with DSP-4 or Saline

Group	Sleep bout duration (s)		Wake bout duration (s)		Mean % of time awake	Mean no. of sleep-wake cycles per h
	Mean	Median	Mean	Median		
DSP-4						
Day	82.5 (8.2)	53.6 (7.4) ^a	40.9 (5.6) ^b	17.3 (2.7) ^b	33.3 (4.3) ^{a,b}	30.4 (2.4)
Night	73.4 (8.6)	32.9 (6.2)	55.2 (10.7) ^b	25.5 (7.2) ^b	41.4 (4.1) ^b	31.6 (4.5)
Saline						
Day	89.2 (7.0)	62.3 (6.9) ^a	25.4 (3.2)	6.5 (0.7)	21.9 (1.6) ^a	33.2 (3.8)
Night	79.8 (6.0)	48.0 (6.3)	35.7 (3.3)	7.1 (0.5)	30.8 (1.9)	32.1 (2.3)

Standard Errors are in Parentheses. ^asignificant main effect of test time (i.e., day vs. night). ^bsignificant main effect of group (i.e., DSP-4 vs. saline)

An experienced individual blind to experimental condition scored the data. It should be stressed that EMG measures alone allow for accurate measurement of sleep and wake durations in infant rats before, during, and after the emergence of delta activity.²⁵ Moreover, the statistical structures of sleep and wake bouts derived from EMG measures at P21 in rats⁸ and mice³ resemble those derived in adults using additional electrographic measures.⁷

Sleep and wake bout durations were imported into Statview 5.0 (SAS Institute, Cary, NC) for analysis. Mean and median bout durations were determined for each subject and mean values were calculated across subjects. Mean percentage of time awake was calculated by dividing the mean wake bout duration by the sum of the mean sleep and wake bout durations, and multiplying by 100. The mean number of sleep-wake cycles per hour was also calculated. Two-factor analysis of variance (ANOVA) was used to test for differences across groups (i.e., DSP-4 vs. saline control) and test times (i.e., day vs. night).

NA, DA, and 5-HT concentrations were expressed as ng/mL tissue homogenate and reported as percentage change in DSP-4 subjects in relation to paired saline controls. Paired *t* tests were used to test group differences.

As described elsewhere,⁸ log-survivor distributions of sleep and wake bouts were produced from individual and pooled data. For each subject, least-squares estimates (r^2) were calculated to assess the degree of fit to exponential and power-law distributions. Two-factor ANOVAs, performed separately for the day and night, were used to test for differences across group (i.e., DSP-4 vs. saline control) and distribution (i.e., power-law vs. exponential). Paired *t* tests were used for planned comparisons to assess within-group differences in r^2 .

For these tests, α was set at 0.05. Means are presented with their standard errors.

We used a second analytic technique to test whether bout duration data provided more evidence for a power-law or exponential model. Specifically, using pooled data, log-likelihood functions were calculated and maximized for each model.²⁶⁻²⁸ The comparison was quantified by calculating the Akaike weights, w_i ($i = 1, 2$), for each model: $w_i = \exp(-\Delta_i/2) / (\exp(-\Delta_1/2) + \exp(-\Delta_2/2))$. Here, Δ_i is a measure of each model relative to the better model; $\Delta_i = 0$ for the model with larger log-likelihood value. For the other model, $\Delta_i > 0$ is calculated as the difference of Akaike Information Criterion (AIC) values, $AIC_i = -2(\log\text{-likelihood}_i) + 2k_i$, where k_i is the number of parameters in model *i*. (Note that the sum of the Akaike weights equals 1). The Akai-

ke weight for a model estimates the probability that the model is the better of the 2 candidate models.^{26,29} The Akaike weights are useful for model comparison. They give the probability of a certain model being a better fit among all the chosen candidate models. Therefore, the model with a weight of 1 is a better fit than the model with a weight of 0. It should be emphasized, however, that this criterion cannot tell us if the best-fit model is indeed a good fit. For goodness of fit, we use the more traditional approach.

RESULTS

Table 1 shows that mean sleep bout durations were not significantly affected by DSP-4 treatment during the day or night (group: $F_{1,28} = 0.7$; test time: $F_{1,28} = 1.5$; group \times time interaction: $F_{1,28} = 0.0$); median sleep bout durations were significantly longer during the day ($F_{1,28} = 6.9$, $P < 0.05$); there was no main effect of group ($F_{1,28} = 3.2$) and no group \times time interaction ($F_{1,28} = 0.2$). Both mean and median wake bout durations were significantly longer in DSP-4 subjects ($F_{1,28} > 7.3$, $P < 0.05$); there were no main effects of time ($F_{1,28} < 3.6$) and no group \times time interactions ($F_{1,28} < 1.0$). Similarly, mean percentage time awake was significantly longer for DSP-4 subjects than saline controls ($F_{1,28} = 11.8$, $P < 0.005$), and was significantly greater at night than during the day ($F_{1,28} = 7.0$, $P < 0.05$); the interaction was not significant ($F_{1,28} = 0.0$). Finally, the number of sleep-wake cycles per hour was not affected by DSP-4 treatment (group: $F_{1,28} = 0.2$; time: $F_{1,28} = 0.0$; interaction: $F_{1,28} = 0.1$).

Figure 1 presents pooled log-survivor distributions for P21 subjects treated at P7 with DSP-4 or saline. Sleep bout durations for both the DSP-4 and saline control P21 subjects during the day and night fall along a straight line, indicative of an exponential distribution (Fig. 1A). As predicted, wake bout durations for DSP-4 subjects more closely followed an exponential distribution during the day and night (Fig. 1B); in contrast, the wake bout durations for the saline control subjects followed a power-law distribution.

As shown in Figure 2A, sleep bout durations were always better fit by an exponential than a power-law distribution. ANOVAs performed separately for the day and night revealed significant main effects of distribution ($F_{1,28} > 139.5$, $P_s < 0.0001$), but there were no main effects of group ($F_{1,28} < 2.1$). Also, the group \times distribution interaction was significant at night ($F_{1,28} = 6.8$, $P < 0.05$) but not during the day ($F_{1,28} = 2.6$).

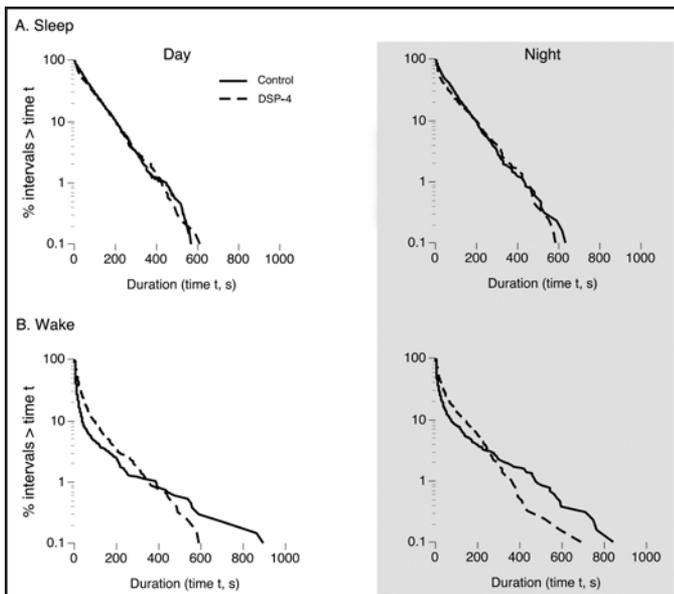


Figure 1—Log-survivor plots of sleep (A) and wake (B) bout durations in P21 rats treated with DSP-4 or saline. Each semi-log plot was constructed using pooled data (677-1180 data points). Straight lines on these plots indicate that the data follow an exponential distribution.

Table 2—Mean Percentage Change in Cortical and Non-Cortical Noradrenaline (NA), Dopamine (DA), and Serotonin (5-HT) in P21 Rats Treated on P7 with DSP-4 in Relation to Saline Controls

	NA	DA	5-HT
Cortical tissue	-80.7 (4.2)*	-13.3 (23.0)	+4.5 (16.0)
Non-cortical tissue (excluding cerebellum)	-73.9 (7.8)*	-4.7 (37.1)	+23.6 (25.6)

Standard errors are in parentheses. *significant difference from 0%

Figure 2B shows, as expected,⁸ that wake bout durations for saline control subjects during the day and night were better fit by a power-law distribution. In contrast, for DSP-4 subjects at night, the better fit was to an exponential distribution. For DSP-4 subjects during the day, the fit to a power-law distribution was clearly reduced, although the fit to an exponential distribution was not increased. ANOVAs revealed a significant main effect of distribution during the day ($F_{1,28} = 16.4$, $P < 0.0005$) but not at night ($F_{1,28} = 1.4$). There were no main effects of group during the day or night ($F_{1,28} < 3.2$), but the group \times distribution interactions were significant ($F_{1,28} > 11.6$, $P_s < 0.005$).

As expected, analyses of pooled and individual Akaike weights indicated that sleep bout durations were better fit by an exponential model in both saline control and DSP-4 subjects during the day and night (Akaike weights equal to 1). Also as expected, wake bout durations for saline control subjects were better fit by a power-law model during both the day and night (Akaike weights equal to 1). In contrast, wake bout durations for DSP-4 subjects were better fit by an exponential model during both the day and night (Akaike weights equal to 1), thus providing even stronger support for the finding, presented in

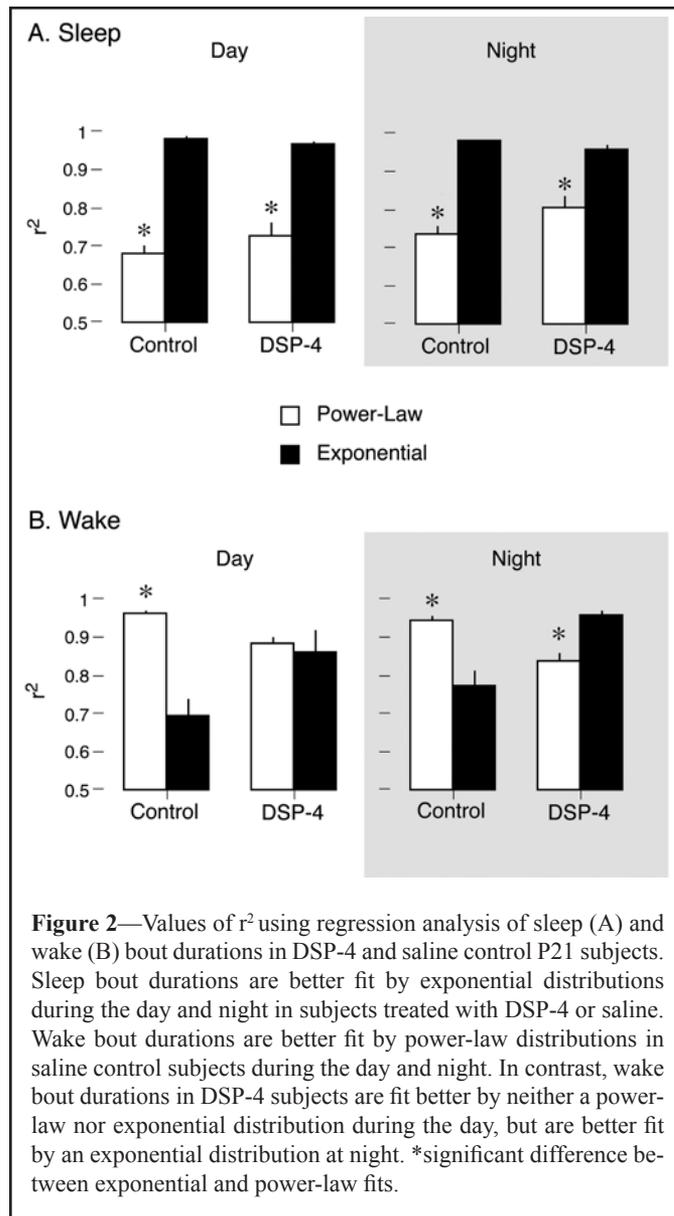


Figure 2—Values of r^2 using regression analysis of sleep (A) and wake (B) bout durations in DSP-4 and saline control P21 subjects. Sleep bout durations are better fit by exponential distributions during the day and night in subjects treated with DSP-4 or saline. Wake bout durations are better fit by power-law distributions in saline control subjects during the day and night. In contrast, wake bout durations in DSP-4 subjects are fit better by neither a power-law nor exponential distribution during the day, but are better fit by an exponential distribution at night. *significant difference between exponential and power-law fits.

Figure 2B, that DSP-4 treatment suppresses the expression of power-law wake behavior at P21.

A follow-up analysis of individual data indicated that 2 DSP-4 subjects exhibited differences from the pooled results. Specifically, one DSP-4 subject's wake bout durations were better fit by a power-law model (Akaike weight equal to 1), and the other DSP-4 subject's wake bout durations distributed poorly to both exponential and power-law models.

Finally, to provide additional information regarding the effect of DSP-4 treatment on NA, DA, and 5-HT concentrations in cortical and non-cortical tissue, 12 additional subjects from 6 litters were tested identically to the initial set of subjects (with the exception that sleep-wake activity was not monitored). As shown in Table 2, animals treated with DSP-4 at P7 exhibited significant reductions at P21 in both cortical ($t_{21} = 19.2$, $P < 0.0001$) and non-cortical ($t_5 = 9.5$, $P < 0.0005$) NA concentration in relation to saline controls. Importantly, and as expected,¹⁹ DSP-4 subjects did not exhibit significant changes in cortical or non-cortical DA or 5-HT concentration in relation to saline controls ($t_5 > 0.1$, NS).

DISCUSSION

The locus coeruleus is a major source of noradrenaline in the central nervous system that mediates spontaneous and evoked arousals.^{12,15} Here we used the neurotoxin DSP-4 to destroy NA terminals from the LC in order to assess the contributions of this structure to sleep and wake bout durations in P21 rats. We found no significant effect of DSP-4 on sleep bout durations, but wake bout durations were significantly increased, as was the percentage of time awake.

As expected, sleep bout durations uniformly followed exponential distributions that were unaffected by treatment with DSP-4. In contrast, whereas wake bout durations in saline control subjects were better fit by a power-law distribution, wake bout durations of DSP-4 subjects were better fit by an exponential distribution, as is the case in untreated younger rats.^{4,8} These conclusions were supported using 2 analytic procedures: least-squares (r^2) and maximum likelihood estimation.^{26,28,29} Thus, it appears that developmental changes in LC function contribute to the development of power-law wake behavior during the third postnatal week.

Previous studies have examined the role of DSP-4 on sleep and wakefulness in adults using conventional measures (e.g., mean, median, total sleep and wake durations); these studies have yielded variable and inconsistent results.^{20,30-33} In these studies, sleep and wake bout distributions were not analyzed, thus making it difficult to make direct comparisons between the results found in these earlier studies and those found here. Importantly, the current study is unique in that DSP-4 was injected in early infancy—in particular, *before* the development of power-law wake behavior. Thus, the timing of DSP-4 administration may critically determine its effects on wake bout distributions. It is also possible that analyses of sleep and wake bout distributions, rather than mere assessments of cumulative sleep and wake durations, provide a more reliable and sensitive indicator of sleep-wake dynamics and their underlying neural control.^{8,34}

It has been suggested that arousal is maintained, at least in part, by an SCN-DMH-LC wake-active circuit.³⁵ Orexinergic neurons within the DMH have been hypothesized to be critically involved in the maintenance of arousal³⁵ and provide dense projections to the LC.¹¹ However, power-law wake behavior develops normally in orexin knockout mice³ and is expressed in adult mice.³⁶ Thus, to the extent that the SCN-DMH-LC circuit is critically involved in arousal, it is possible that *non*-orexinergic afferents to the LC, possibly from the DMH or elsewhere,³⁵ contribute to the development of power-law wake behavior in infant rats and mice. In addition, because the LC exerts inhibitory influences on the sleep-promoting ventrolateral preoptic area (VLPO),³⁷ it is possible that DSP-4 diminished this inhibitory influence, thereby altering the reciprocal interactions among LC, VLPO, and other nuclei involved in the regulation of sleep and wakefulness.³⁸

The effects of DSP-4 treatment on NA in specific brain areas depend upon the age at which DSP-4 is administered and the interval between DSP-4 administration and NA measurement.¹⁹ Using similar methods to those used here, DSP-4 treatment (50 mg/kg) in week-old rats selectively and rapidly (i.e., within 1 day) depletes NA in cerebral cortex, brainstem, and spinal cord,

and these effects persist into adulthood.¹⁹ Thus, we chose to inject rats with DSP-4 at P7 and test them 2 weeks later with the expectation that NA would be depleted in any brain region to which the LC projects. In other words, NA concentration was used as a bioassay of the efficacy of the DSP-4 treatment. Our findings that DA and 5-HT concentrations were unaffected by DSP-4 treatment support previous findings that DSP-4 selectively reduces NA concentration in the brain.¹⁹

The expression of sleep and wakefulness throughout the day and night reflect the variable influences of circadian and homeostatic mechanisms.³⁹ However, sleep and wake durations accumulate one bout at a time, and we now know that these individual bouts follow discernible statistical rules.^{4,6-8} These rules, in turn, reflect the dynamic interactions among hypothalamic and brainstem nuclei involved in the expression of ultradian sleep-wake cycles.

Wake bouts follow a power-law distribution in humans, cats, and rats,^{7,8,40} and wild-type and orexin knockout mice.^{3,7,36} This power-law structure (in which a small percentage wake bouts is considerably longer than most other wake bouts) may contribute to the efficient partitioning and budgeting of wake-related activities. Specifically, this power-law property may reflect the value for an animal of continuous and lengthy periods of wakefulness.

Among mathematical biologists, the significance of power-law distributions and the mechanisms that give rise to them are topics of vigorous discussion.^{41,42} In the present context, it is worth considering that a power-law distribution is the signature of so-called scale-free networks comprising centralized hubs from which a disproportionate number of connections to other nodes in the network is made.⁴³ In this regard, the neural system that produces wakefulness may be viewed as a complex network comprising a collection of hubs with varying degrees of connectivity.^{41,44} Interestingly, LC neurons differentiate very early in development⁴⁵ and soon give rise to a complex network of dense connections that project widely throughout the central nervous system, including the cerebral cortex, hippocampus, thalamus, midbrain, brainstem, cerebellum, and spinal cord.⁴⁶⁻⁴⁹ The LC also receives dense projections from glutamatergic, GABAergic, orexinergic, serotonergic, and noradrenergic neurons, among others.⁴⁷ Accordingly, the LC, with its dense efferent and afferent projections, qualifies as a highly connected hub.

An interesting property of scale-free networks is that they are more robust (i.e., more resistant to failure) than are random networks⁵⁰ in the face of random damage to the network. On the other hand, scale-free networks are more vulnerable to failure when damage comes in the form of an attack targeted at a hub. Thus, we hypothesize that the neural circuitry underlying the power-law structure of wake bouts has evolved in part because of its enhanced robustness in the face of random attack and that the use here of DSP-4 can be considered a “targeted” (i.e., non-random) attack on the LC. If so, then it follows that the sleep system may be more sensitive than the wake system to random neuronal loss (e.g., as appears to occur during aging^{51,52}), perhaps providing an explanation for the relative prevalence of sleep-related disorders such as insomnia.⁵³ We caution, however, that the convincing application of network theory to sleep-wake organization will depend in part on the development of a

process model of network behavior that can produce phenomena akin to sleep and wake bouts.⁴¹

The present results highlight how a structure like the LC exerts a subtle and complex influence that can be overlooked if sufficiently sensitive measures of sleep-wake cyclicity are not used. Thus, while we agree that the LC is likely more than simply a wake-active nucleus that modulates arousal,⁵⁴ even our understanding of the role of the LC in the regulation of arousal is incomplete. We suggest that the LC comprises one component of a system that serves to modify the temporal structure of wake bouts such that prolonged periods of spontaneous wakefulness are possible. Given that this modification occurs in rats at the beginning of the third postnatal week, the LC and its associated structures may make it possible for the developing infant to engage more effectively with its environment at a time of increasing independence from its mother and littermates.

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DISCLOSURE STATEMENT

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REFERENCES

1. Kleitman N, Engelmann TG. Sleep characteristics of infants. *J Appl Physiol* 1953;6:269-82.
2. Karlsson KÆ, Kreider JC, Blumberg MS. Hypothalamic contribution to sleep-wake cycle development. *Neuroscience* 2004;123:575-82.
3. Blumberg MS, Coleman CM, Johnson ED, Shaw C. Developmental divergence of sleep-wake patterns in orexin knockout and wild-type mice. *Eur J Neurosci* 2007;25:512-8.
4. Gall AJ, Todd WD, Ray B, Coleman CM, Blumberg MS. The development of day-night differences in sleep and wakefulness in Norway rats and the effect of bilateral enucleation. *J Biol Rhythms* 2008;23:232-41.
5. Seelke AMH, Blumberg MS. Thermal and nutritional modulation of sleep in infant rats. *Behav Neurosci* 2005;19:603-11.
6. Lo CC, Amaral LAN, Havlin S, et al. Dynamics of sleep-wake transitions during sleep. *Europhys Lett* 2002;57:625-31.
7. Lo CC, Chou T, Penzel T, et al. Common scale-invariant patterns of sleep-wake transitions across mammalian species. *Proc Natl Acad Sci U S A* 2004;101:17545-8.
8. Blumberg MS, Seelke AM, Lowen SB, Karlsson KA. Dynamics of sleep-wake cyclicity in developing rats. *Proc Natl Acad Sci U S A* 2005;102:14860-4.
9. Nambu T, Sakurai T, Mizukami K, Hosoya Y, Yanagisawa M, Goto K. Distribution of orexin neurons in the adult rat brain. *Brain Res* 1999;827:243-60.
10. Sakurai T. The neural circuit of orexin (hypocretin): maintaining sleep and wakefulness. *Nat Rev Neurosci* 2007;8:171-81.
11. Peyron C, Tighe DK, van den Pol AN, et al. Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J Neurosci* 1998;18:9996-10015.
12. Aston-Jones G, Bloom FE. Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J Neurosci* 1981;1:876-86.
13. Berridge CW, Waterhouse BD. The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res Brain Res Rev* 2003;42:33-84.
14. Karlsson KÆ, Gall AJ, Mohns EJ, Seelke AMH, Blumberg MS. The neural substrates of infant sleep in rats. *PLoS Biology* 2005;3:891-901.
15. Foote SL, Aston-Jones G, Bloom FE. Impulse activity of locus coeruleus neurons in awake rats and monkeys is a function of sensory stimulation and arousal. *Proc Natl Acad Sci U S A* 1980;77:3033-7.
16. Frederickson CJ, Hobson JA. Electrical stimulation of the brain stem and subsequent sleep. *Arch Ital Biol* 1970;108:564-76.
17. Kaitin KI, Bliwise DL, Gleason C, Nino-Murcia G, Dement WC, Libet B. Sleep disturbance produced by electrical stimulation of the locus coeruleus in a human subject. *Biol Psychiatry* 1986;21:710-6.
18. Cespuglio R, Gomez ME, Faradji H, Jouvet M. Alterations in the sleep-waking cycle induced by cooling of the locus coeruleus area. *Electroencephalogr Clin Neurophysiol* 1982;54:570-8.
19. Jonsson G, Hallman H, Sundstrom E. Effects of the noradrenaline neurotoxin DSP4 on the postnatal development of central noradrenaline neurons in the rat. *Neuroscience* 1982;7:2895-907.
20. Cirelli C, Huber R, Gopalakrishnan A, Southard TL, Tononi G. Locus ceruleus control of slow-wave homeostasis. *J Neurosci* 2005;25:4503-11.
21. Cirelli C, Tononi G. Locus ceruleus control of state-dependent gene expression. *J Neurosci* 2004;24:5410-9.
22. Fritschy JM, Grzanna R. Immunohistochemical analysis of the neurotoxic effects of DSP-4 identifies two populations of noradrenergic axon terminals. *Neuroscience* 1989;30:181-97.
23. Fritschy JM, Grzanna R. Restoration of ascending noradrenergic projections by residual locus coeruleus neurons: compensatory response to neurotoxin-induced cell death in the adult rat brain. *J Comp Neurol* 1992;321:421-41.
24. Seelke AM, Karlsson KA, Gall AJ, Blumberg MS. Extraocular muscle activity, rapid eye movements and the development of active and quiet sleep. *Eur J Neurosci* 2005;22:911-20.
25. Seelke AM, Blumberg MS. The microstructure of active and quiet sleep as cortical delta activity emerges in infant rats. *Sleep* 2008;31:691-9.
26. Burnham KP, Anderson DR. Model selection and multimodel interference: a practical information-theoretic approach. 2nd ed. New York: Springer-Verlag, 2002.
27. Clauset A, Shalizi CR, Newman MEJ. Power-law distributions in empirical data. *arXiv* 2007;0706:1062.
28. Myung IJ. Tutorial on maximum likelihood estimation. *J Math Psychol* 2003;47:90-100.
29. Akaike H. A new look at the statistical model identification. *IEEE T Automat Contr* 1974;19:716-23.
30. Gonzalez MM, Aston-Jones G. Circadian regulation of arousal: role of the noradrenergic locus coeruleus system and light exposure. *Sleep* 2006;29:1327-36.
31. Gonzalez MM, Debilly G, Valatx JL. Noradrenaline neurotoxin DSP-4 effects on sleep and brain temperature in the rat. *Neurosci Lett* 1998;248:93-6.
32. Monti JM, D'Angelo L, Jantos H, Barbeito L, Abo V. Effect of DSP-4, a noradrenergic neurotoxin, on sleep and wakefulness and sensitivity to drugs acting on adrenergic receptors in the rat. *Sleep* 1988;11:370-7.
33. Saponjic J, Radulovacki M, Carley DW. Monoaminergic system lesions increase post-sigh respiratory pattern disturbance during sleep in rats. *Physiol Behav* 2007;90:1-10.

34. Blumberg MS, Karlsson KA, Seelke AM. Sleep, development, and human health. *Sleep* 2007;30:549-50.
35. Aston-Jones G, Chen S, Zhu Y, Oshinsky ML. A neural circuit for circadian regulation of arousal. *Nat Neurosci* 2001;4:732-8.
36. Diniz Behn CG, Kopell N, Brown EN, Mochizuki T, Scammell TE. Delayed orexin signaling consolidates wakefulness and sleep: physiology and modeling. *J Neurophys* 2008;99:3090-103.
37. Gallopin T, Fort P, Eggermann E, et al. Identification of sleep-promoting neurons in vitro. *Nature* 2000;404:992-5.
38. Saper CB, Scammell TE, Lu J. Hypothalamic regulation of sleep and circadian rhythms. *Nature* 2005;437:1257-63.
39. Borbely AA, Achermann P. Sleep homeostasis and models of sleep regulation. In: Kryger MH, Roth T, Dement WC, eds. *Principles and practice of sleep medicine*. Philadelphia: WB Saunders; 2000: 377-90.
40. Simasko SM, Mukherjee S. Novel analysis of sleep patterns in rats separates periods of vigilance cycling from long-duration wake events. *Behav Brain Res* 2009;196:228-36.
41. Newman MEJ. The structure and function of complex networks. *SIAM Rev* 2003;45:167-256.
42. Newman MEJ. Power laws, Pareto distributions and Zipf's law. *Contemp Phys* 2006;46:323-51.
43. Barabasi AL, Oltvai ZN. Network biology: understanding the cell's functional organization. *Nat Rev Genet* 2004;5:101-13.
44. Amaral LA, Scala A, Barthelemy M, Stanley HE. Classes of small-world networks. *Proc Natl Acad Sci U S A* 2000;97:11149-52.
45. Lauder JM, Bloom FE. Ontogeny of monoamine neurons in the locus coeruleus, Raphe nuclei and substantia nigra of the rat. I. Cell differentiation. *J Comp Neurol* 1974;155:469-81.
46. Foote SL, Bloom FE, Aston-Jones G. Nucleus locus ceruleus: new evidence of anatomical and physiological specificity. *Physiol Rev* 1983;63:844-914.
47. Aston-Jones G, Shipley MT, Grzanna R. The locus coeruleus, A5 and A7 noradrenergic cell groups. In: Paxinos G, ed. *The rat nervous system*. San Diego: Academic Press; 1995: 183-214.
48. Maubecin VA, Williams JT. Developmental changes that regulate the activity of locus coeruleus neurons. *Tokai J Exp Clin Med* 1999;24:41-51.
49. Marshall KC, Christie MJ, Finlayson PG, Williams JT. Developmental aspects of the locus coeruleus-noradrenaline system. *Prog Brain Res* 1991;88:173-85.
50. Albert R, Jeong H, Barabasi AL. Error and attack tolerance of complex networks. *Nature* 2000;406:378-82.
51. Hof PR, Mobbs, CV. *Functional neurobiology of aging*. San Diego: Academic Press, 2001.
52. Leon-Carrion J, Giannini MJ. *Behavioral neurology in the elderly*. Boca Raton: CRC Press, 2001.
53. Ohayon MM. Epidemiology of insomnia: what we know and what we still need to learn. *Sleep Med Rev* 2002;6:97-111.
54. Aston-Jones G, Cohen JD. Adaptive gain and the role of the locus coeruleus-norepinephrine system in optimal performance. *J Comp Neurol* 2005;493:99-110.