Norepinephrine-Deficient Mice Exhibit Normal Sleep-Wake States but have Shorter Sleep Latency after Mild Stress and Low Doses of Amphetamine

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Study Objectives: Mice lacking the ability to make norepinephrine (NE) were used to investigate how NE may be involved in regulating sleep and sleep latency under normal conditions and as a response to mild stress or varying doses of amphetamine.

Design: Sleep latency was measured in NE-deficient and control mice after behavioral interventions and after 3 low doses of amphetamine. Sleep-wake states were measured using electroencephalography and electromyography for the first 6 hours after lights-on under baseline conditions and after an injection of saline. The first 6 hours after lights-off were also measured under baseline conditions.

Setting: N/A

Patients or Participants: Mice lacking the dopamine β-hydroxylase gene (Dbh -/-), which is required for NE synthesis, and their littermate controls were used.

Interventions: N/A

Measurements and Results: As measured behaviorally and with electroencephalography, sleep latency was significantly shorter in the NE-deficient mice after cage changing, saline injection, and 3 different doses of amphetamine. There were no differences between the 2 groups in any sleep parameters under baseline conditions or after saline injection during the day or night.

Conclusions: The NE-deficient mice showed a significantly shorter latency to sleep under many different conditions, measured both behaviorally and with electroencephalography. These data suggest that NE is wake promoting during the period of time between a mildly stressful event or a low dose of amphetamine and sleep onset. The NE-deficient mice did not show deficits in wake or increases in rapid eye movement sleep, as predicted from current models of the involvement of NE in the regulation of these 2 states.

Key Words: Norepinephrine; sleep; mice; knockout; sleep latency; amphetamine.

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INTRODUCTION

NOREPINEPHRINE (NE) IS TRADITIONALLY THOUGHT OF AS "WAKE PROMOTING," AS WELL AS BEING NECESSARY FOR NORMAL AMOUNTS OF THE WAKING STATE. There is also evidence that NE has a reciprocal relationship to rapid eye movement (REM) sleep, and the reciprocity of noradrenergic and cholinergic neurons in the brainstem is a prominent feature of models that attempt to explain transitions from non-REM (NREM) to REM sleep.[1,2] Over the years, these models have been refined to include the discovery of new neurotransmitters as well as new roles for existing ones (i.e., orexin/hypocretin, GABA, histamine, etc.).

Neurons in the rat pontine locus coeruleus (LC), the major source of NE in the central nervous system, exhibit high firing rates during wake, exhibit intermediate firing rates during NREM sleep, and are quiescent during REM sleep.[3] A similar pattern of neuronal activity has been recorded in the cat.[4] In an in vivo study using microdialysis in the cat, Shouse et al[5] found that in the LC and the amygdala, NE concentrations were high during wake, low during NREM sleep, and very low during REM sleep.

Because of the decrease in noradrenergic signaling as an animal progresses from wake to NREM to REM sleep, it has been suggested that inhibition of the LC is necessary for REM sleep to occur and that NE plays a critical role in its regulation. An extension of this notion is the model of "REM sleep gating," which causally links the "turning on" of REM–sleep-generating cholinergic neurons in the pons with the "turning off" of NE neurons in the same area.[1,6] This model is supported by studies showing that application of NE into the LC of cats decreased the amount of REM sleep,[7] and continuous low-intensity electrical stimulation of the rat LC decreased the number of REM episodes.[8] In addition, Monti et al[9] found that destroying the LC neurons in rats with the selective neurotoxin DSP-4 significantly increased REM sleep. Thus, we predicted that the chronic lack of NE in the central nervous system of NE-deficient mice should have a similar effect of increasing REM sleep, perhaps at the expense of either NREM sleep or wake.

In rats, mild stressors can mobilize physiologic measures of stress. For example, a single injection of saline or acute handling increases corticotropin-releasing factor (CRF) immunoreactivity in the hypothalamus[10] and plasma corticosterone levels[11] and causes a transient rise in core temperature.[12] Additionally, more moderate forms of stress such as physical restraint and electric footshock affect sleep in both rats and mice.[13-15] Since NE and epinephrine (EPI) are the primary neurotransmitters participating in the hypothalamic-pituitary-adrenal axis during periods of stress, and stress affects sleep, it was logical to hypothesize that mice deficient in both NE and EPI could exhibit differences in their sleep following mild stressors such as a cage change and saline injection. We also chose these particular "mild stressors" because they are common laboratory procedures, and any differences between the genotypes would be relevant to future studies that include placebo injections as well as more severe methods of inducing stress.

Amphetamine increases arousal and locomotion in nocturnal rodents.[16-18] These effects are believed to be the result of increased monoamine release (see reference 18 for review), although increased waking activity has been shown to be attributed primarily to increased dopamine.[19-21] In the present study, we wanted to perturb the balance of arousal-regulating neurotransmitters in the knockout mice without inducing long periods of wake or locomotion. Therefore, we used low doses of amphetamine and predicted that they would have differential effects on the sleep immediately following the injection between the control and NE-deficient mice.

Mice deficient in dopamine β-hydroxylase (*Dbh* -/-), the enzyme nec-

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essary to convert dopamine to norepinephrine, were generated by Thomas et al.[22] These mice require pharmacologic rescue with synthetic NE precursors and noradrenergic agonists during the latter part of gestation, but after birth they do not require any special care for survival under laboratory conditions. Thus, these mice provide an opportunity to examine the consequences of chronic NE and EPI deficiency on sleep under both normal and stressful conditions.

METHODS

Animals

Mice lacking NE and EPI (*Dbh* -/-) were created[22] and maintained on a mixed C57BL/6J and 129SvCPJ hybrid background. Heterozygous mice (*Dbh* +/-) were used as controls because they have been shown to have normal NE levels.[23] Control and knockout mice were bred under specific pathogen-free conditions, and adult mice were housed in a separate animal room appropriate for sleep studies. The mice were maintained on a 12:12 light:dark cycle, with food, water, and nesting material available at all times. All the mice were group housed, with 2 knockouts and 2 controls per cage.

All procedures adhered to the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences Press, Washington, DC, 1996).

Behavioral Observations of Sleep Latency

Eleven *Dbh* -/- and 9 *Dbh* +/- female mice were used for the behavioral observations of sleep latency, and they were not implanted for electroencephalographic (EEG) recordings. On each morning of the experiment, 2 knockout and 2 control mice were each placed in a new cage with clean bedding and a piece of food and transported to an observation room on a different floor. An experimenter trained in behavioral sleep observations (MH) watched the mice and recorded how many minutes passed before each animal fell asleep. The same procedure was performed for a subset of the same mice 2 to 3 weeks later (N=8) except that an intraperitoneal injection of saline was given 30 minutes after the animals arrived in the observation room. For this group, if a mouse did not fall asleep within 30 minutes after the injection, it was given a score of 30 minutes.

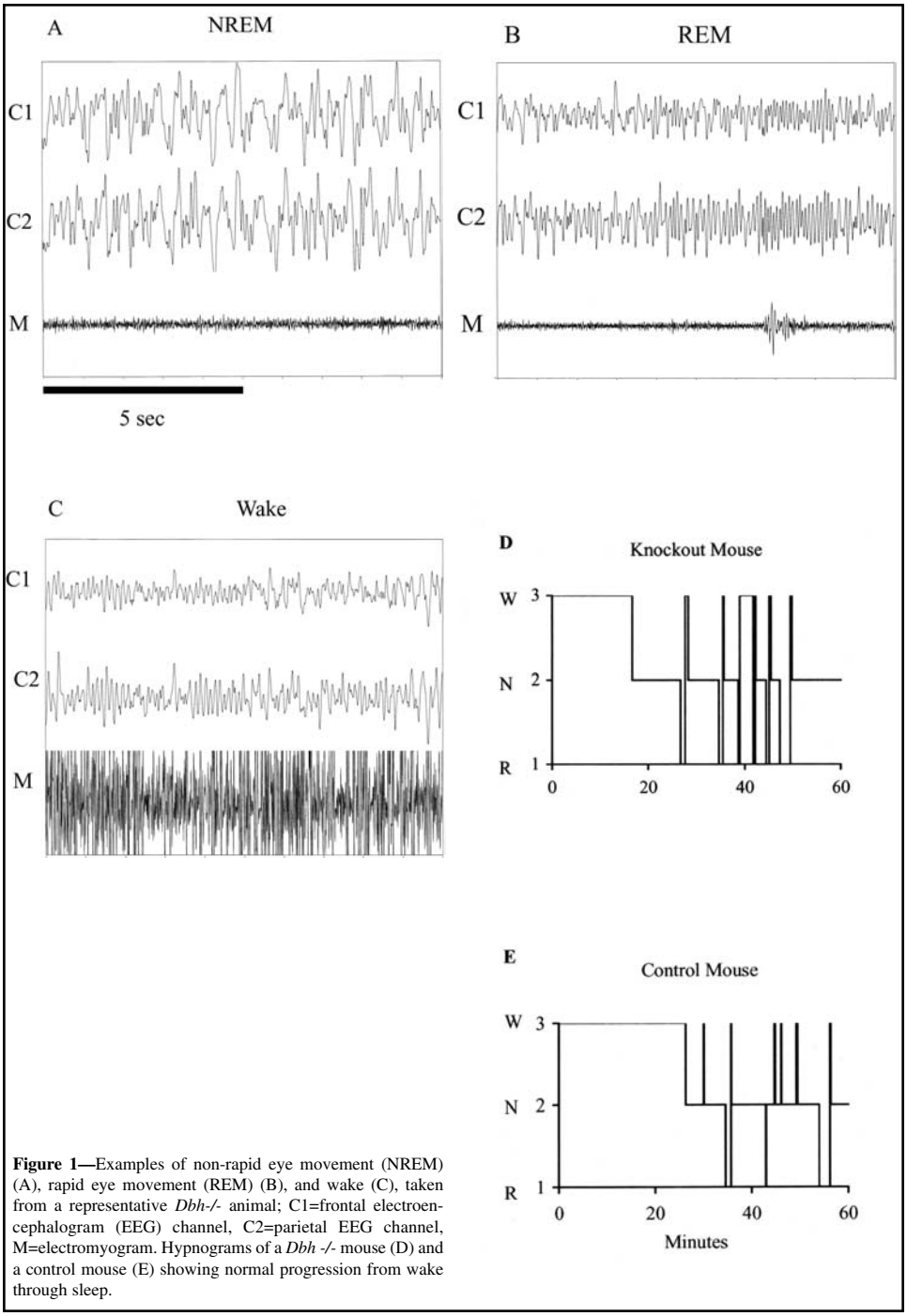

Figure 1—Examples of non-rapid eye movement (NREM) (A), rapid eye movement (REM) (B), and wake (C), taken from a representative *Dbh-/-* animal; C1=frontal electroencephalogram (EEG) channel, C2=parietal EEG channel, M=electromyogram. Hypnograms of a *Dbh -/-* mouse (D) and a control mouse (E) showing normal progression from wake through sleep.

Surgery

Female *Dbh -/-* and *Dbh +/-* littermates were implanted with EEG and electromyogram (EMG) electrodes between 4 and 7 months of age. Animals were anesthetized with a mixture of ketamine and xylazine (13 mg/mL and 0.88 mg/mL, respectively, at a volume of 10 μL/g), and 3 miniature stainless steel screws (0.9 mm in diameter) attached to 36-gauge solid silver wires were inserted into the skull. These electrodes were placed over the right frontal cortex (~0.5 mm anterior to bregma, ~0.5 mm lateral to the central suture), right parietal cortex (~1 mm posterior to bregma, ~2-3 mm lateral to the central suture), and cerebellum (~0.5 mm posterior to lambda, in line with the central suture). The wires were crimped to a small 6-channel headpiece (Microtech Inc.) that was attached to the skull with dental acrylic. Two of the same silver wires were sewn through the nuchal muscles and also crimped to the headpiece, serving as the EMG electrodes. After the surgery, mice were singly housed and allowed to recover with their cage placed halfway on a heating pad (35-40°C). After 24 hours of recovery, the mice were housed with 2 to 4 littermates. At least 3 weeks of postsurgical recovery was allowed before beginning the sleep recordings.

Recording Procedure

One week prior to sleep recordings, mice were singly housed. At least 24 hours before the recording, 1 knockout and 1 control mouse were each placed in a high-walled, open-topped Plexiglas mouse cage containing bedding and nesting material, inside a sound-attenuated chamber. Food and water were available ad lib. A cable (20-cm long) was attached to the headpiece on the mouse and then to a commutator/swivel (Plastics One, SL6C/SB lowest torque), which was connected to a model 15

Grass polygraph that digitally recorded EEG and EMG activity at a rate of 200 samples per second. The cable was constructed from six, 38-gauge, stainless steel, Teflon-coated wires plus 1 wire (1 mm in diameter) that was stiffer and provided support. Cyanoacrylate glue was used to cement the wires at both ends of the cable (~1 cm at each end). Because the cable weighed less than 3 grams (~1 gram perceived by mouse), the mice were able to move, groom, eat, and drink freely, and they habituated to the recording chamber and cable within a few hours. The EEG and EMG data were recorded and processed using the Grass-Telefactor PolyviewPro data acquisition software for Windows 98.

Baseline and Mild-Stressor Recordings

Six mice of each genotype were used for this study. The baseline recording (Day 1) began shortly after lights-on after the habituation day. Mice were not disturbed for this recording. Simultaneous with the EEG and EMG recordings, a video camera recorded behavior for the first 4 hours. On the mild-stressor day (Day 2), shortly after lights-on, each mouse was picked up (cable still attached) and given an intraperitoneal injection (0.25 mL) of phosphate-buffered saline.

Amphetamine Recordings

There were 7 to 8 mice in each group for the injections of amphetamine. After 36 hours of habituation to the recording cage and cable, mice were given an intraperitoneal injection of either saline or 0.25, 0.5, or 1.0 mg/kg of body weight of d-amphetamine sulfate (Sigma) shortly after lights on. One group of 8 knockouts and 8 controls received both saline injection and 0.25 mg/kg amphetamine. A different group of 8 knockouts and 8 controls received saline, 0.5 mg/kg and 1.0 mg/kg of amphetamine (these 2 doses of amphetamine were given on consecutive days). The purpose of the amphetamine injections was to provide a mild perturbation to the arousal system, not to greatly increase locomotion. In addition, the knockout mice are more sensitive to the effects of amphetamine so we chose low doses of the drug, the highest of which (1.0 mg/kg) has been shown to affect locomotion equally in both genotypes.[24]

Data Analysis

Sleep-wake data were visually scored for the states of wake, NREM sleep, and REM sleep. Scoring was done with the Grass Rodent Sleep Stager program, which facilitates manually scored 10-second epochs (see Figure 1 for representative EEG and EMG signals). During the scoring procedure, the high-pass digital filters were set at 1 Hz and 30 Hz for EEG and EMG, respectively, and the low-pass filters were set at 15 Hz and 100 Hz for EEG and EMG. Wake was considered to be higher frequency (>10 Hz), lower amplitude, with medium to high muscle activity. Non-REM sleep was considered to be lower frequency (1-4 Hz), higher amplitude, and low to medium muscle activity. Rapid eye movement sleep consisted mostly of theta waves (5-9 Hz) at a low amplitude, with low muscle activity. Each epoch had to contain at least 50% of wake, NREM or REM for it to be labeled that state. While scoring the digital EEG and EMG signals for the baseline day, the behavior of the mice was also monitored from the video recording.

Baseline and Mild-Stressor Recordings

For the baseline day, the first 6 hours of the light portion of the light:dark cycle and the first 6 hours of the dark portion were analyzed. Total number of epochs of each state (converted into minutes), the number of bouts of each state, and the average length of each bout were determined for each 6-hour period. On the mild-stressor day, the first 6 hours after the injection (equivalent to the first 6 hours of the light cycle) were analyzed. The same sleep-wake state variables were analyzed for this day.

Sleep Latency after Amphetamine Recordings

Latency (in minutes) to the first 20 seconds of sleep was measured for each mouse after the amphetamine injection, and the sleep states for the first hour after the injection were scored for the .25 mg/kg dose.

Statistics

All of these variables were compared between genotypes using analysis of variance (ANOVA) statistics, and the analyses between days were performed using correlated *t*-tests.

RESULTS

Behavioral Observations of Sleep Latency Showed a Difference Between the Genotypes

After placing mice in a new cage with clean bedding and then transporting them to a different room, the *Dbh* -/- knockout animals fell asleep quicker than did the controls, (27.7±3.1 minutes [N=11] vs 43.3±3.4 minutes [N=9], F[1,18]=11.6, $P<.01$, respectively). The same pattern occurred after transport between rooms followed by an injection of saline (knockout mean = 10.5±3.3 minutes [N=8], control mean=21.75 ±2.9 minutes [N=8], F[1,14]=6.6, $P<.05$). The difference between the means of the 2 groups can be attributed to the fact that the latter group's scores were capped at 30 minutes (see Methods). One-hour sleep hypnograms from representative knockout and control mice are presented in Figure 1. Due to the tremendous variability in behavior during a 1-hour period, these short sleep hypnograms are presented primarily to show that the progression from wake to NREM to REM sleep is normal.

Table 1—Sleep-state distributions for the first 3 hours and the second 3 hours of the day and night in minutes. No significant differences were found in any of the sleep-wake state measures between the genotypes.

		DAY Wake			NIGHT Wake		
	Time	Total Minutes	# Bouts	Average Bout Length	Total Minutes	# Bouts	Average Bout Length
Control	0-3 hr	45.4 ± 4.9	25.8 ± 4.0	2.0 ± .4	133.8 ± 9.5	15.7 ± 4.9	13.0 ± 3.6
	3-6 hr	33.8 ± 4.0	26.3 ± 2.9	1.4 ± .2	119.8 ± 15.0	17.5 ± 4.5	19.9 ± 14.0
Knockout	0-3 hr	53.1 ± 7.0	28.8 ± 3.1	1.9 ± .2	140.2 ± 10.9	20.7 ± 4.3	34.7 ± 29.1
	3-6 hr	39.4 ± 6.8	25.2 ± 2.8	1.5 ± .2	123.6 ± 8.2	20.2 ± 3.0	6.9 ± 1.2
		NREM			**NREM**		
	Time	**Total Minutes**	**# Bouts**	**Average Bout Length**	**Total Minutes**	**# Bouts**	**Average Bout Length**
Control	0-3 hr	115.6 ± 4.9	31.5 ± 3.2	3.8 ± .3	41.5 ± 8.1	16.2 ± 5.7	3.2 ± .7
	3-6 hr	124.2 ± 3.3	33.8 ± 2.3	3.8 ± .3	53.3 ± 13.8	18.2 ± 4.6	2.6 ± .7
Knockout	0-3 hr	108.7 ± 7.1	33.5 ± 3.6	3.4 ± .4	37.5 ± 10.4	21.0 ± 4.6	1.5 ± .4
	3-6 hr	117.4 ± 7.2	33.3 ± 2.5	3.7 ± .5	49.7 ± 7.1	21.5 ± 3.3	2.5 ± .3
		REM			**REM**		
	Time	**Total Minutes**	**# Bouts**	**Average Bout Length**	**Total Minutes**	**# Bouts**	**Average Bout Length**
Control	0-3 hr	18.4 ± .7	16.0 ± 1.4	1.2 ± .1	4.6 ± 1.5	5.2 ± 2.1	.8 ± .2
	3-6 hr	21.1 ± .7	20.0 ± 1.4	1.1 ± .1	6.8 ± 2.8	6.0 ± 2.3	.8 ± .2
Knockout	0-3 hr	17.5 ± .7	17.0 ± 1.8	1.1 ± .1	2.3 ± .8	3.5 ± 1.1	.5 ± .1
	3-6 hr	22.3 ± 1.4	22.7 ± 2.4	1.0 ± .2	6.5 ± 1.8	6.5 ± 1.7	1.1 ± .2

Sleep-Wake States as Measured by EEG do not Differ Between *Dbh -/-* and Control Mice in the Baseline Condition

Using EEG and EMG measures, data analysis for the first 6 hours of Day 1 revealed that there were no significant differences between the genotypes for total number of minutes, number of bouts, or average bout length of wake, NREM, or REM. Figure 2A shows the group means for the total number of minutes. We also divided the 6 hours of recording time into two 3-hour segments to potentially reveal differences between the genotypes as a response to the beginning of the light phase (see Table 1). No significant differences were found in either segment. A subset of the mice was scored for total 24-hour sleep-wake states (4 knockout-control pairs), and there were no significant differences in any variable. Although NREM and REM sleep could not be differentiated on the video recording, visual analysis of the first 4 hours of Day 1 showed agreement between the video and EEG and EMG for periods of sleep and wake.

For the first 6 hours of the nighttime, there were no significant differences between the genotypes on total number of minutes, number of bouts, or average bout length for wake, NREM, or REM (Figure 2B). The first 6 hours of night were also divided into two 3-hour segments, and the same variables were analyzed between genotypes. No significant differences were found for any of the variables (see Table 1).

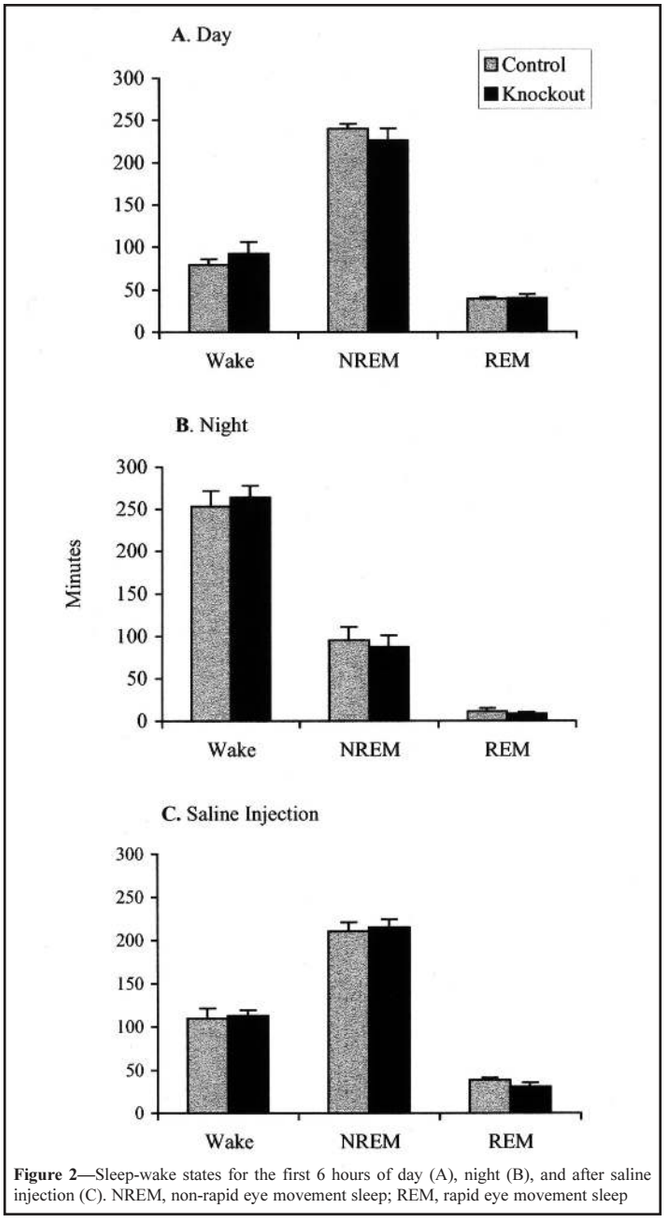

Figure 2—Sleep-wake states for the first 6 hours of day (A), night (B), and after saline injection (C). NREM, non-rapid eye movement sleep; REM, rapid eye movement sleep

Sleep-Wake States do not Differ Between *Dbh -/-* and Control Mice in the Mild-Stressor Condition

Shortly after lights on on Day 2, an intraperitoneal injection of saline was given as a mild stressor. In response to this procedure, there were no significant differences between genotypes in any of the sleep-wake variables for the 6 hours of sleep following the injection (Figure 2C). To reveal potential differences in smaller blocks of time following the injection, this time period was divided into three 2-hour segments, and all sleep-wake variables were analyzed. No significant differences between the genotypes were found (data not shown).

Between Baseline and Stressor Days, the Genotypes do not Differ Significantly from Each Other

To assess whether the sleep-wake states of the groups (knockouts vs controls) changed significantly over time, a repeated measures design was used to assess differences between Day 1 and Day 2. Genotype was used as the independent variable in the ANOVA for repeated measures. The total amount, number of bouts, and average bout lengths of wake and NREM did not differ with respect to genotype between Day 1 and Day 2. There was a marginally significant difference between the knockouts and controls in the amount of REM sleep, with the knockouts having less REM after the saline injection on the second day (Day 1=39.8±4.5 minutes, Day 2=31.3±4.5 minutes for knockouts; day 1=39.5±1.5 minutes, Day 2=38.5±2.8 minutes for controls; $F[1,10]=4.8$, $P=.053$). However, when analyzing the genotypes separately, a correlated *t*-test revealed that the knockouts exhibited significantly less REM sleep on the stressor day than the previous baseline day (Day 1=39.8±4.5 minutes, Day 2=31.3±4.5 minutes; df=5, $t=3.5$). The control mice did not exhibit this difference, and no other differences for this analysis were found for either group.

The Knockouts had Significantly Shorter Sleep Latencies after Saline and Amphetamine Injections

In addition to the sleep latencies measured behaviorally after cage changing, sleep latency was also measured from EEG recordings after the injections of saline and the different doses of amphetamine. The time elapsed from the injection to the first 2 epochs (20 seconds) of NREM sleep was determined for each recording. The amphetamine dose-response curve of NREM sleep latency is presented in Figure 3. There was a significant difference in sleep latency between the knockouts and controls for the injection of saline (knockout mean=18.3±1.8 minutes, control mean=26.7±2.9 minutes, $F[1,30]=5.8$, $P<.05$), for the 0.25 mg/kg dose of amphetamine (knockout=12.1±1.4 minutes, control=36.8 ±6.0 minutes, $F[1,13]=17.9$, $P<.001$), for the 0.5 mg/kg dose of amphetamine (knockout=13.7±1.9 minutes, control=48.5±8.1 minutes, $F[1,14]=17.1$ $P<.01$) and for the 1.0 mg/kg dose of amphetamine (knockouts=20.6±5.1 minutes, control=108.6±11.0 minutes, $F[1,13]=46.9$, $P<.001$). There was no significant difference in the control mice between the saline injection and the 0.25 mg/kg dose; however there was a significant difference between the saline and 0.5 mg/kg ($P<.01$) and the saline and the 1.0 mg/kg dose ($P<.001$). There were no differences between the saline and any dose of amphetamine for the knockout mice.

To depict how the sleep-wake states changed over time in the 0.25 mg/kg-amphetamine groups, the first hour after the initial injection of saline was quantified in 10-minute bins (Figure 3). This figure shows that only by the last 10-minute bin of the hour are most of the control mice asleep. By contrast, 30 minutes after the injection, 40% of the 10-minute bin is spent asleep by the knockouts, and nearly 80% of the fourth 10-minute bin is spent asleep. Because of the difference in sleep latency, the percentages of time spent in NREM and wake were signifi-

cantly different between the groups for this hour, but REM did not differ (data not shown).

DISCUSSION

This study shows that mice lacking NE have a significantly shorter latency to sleep after common laboratory procedures such as cage changing, transport, and injections of saline. These results suggest that NE is directly involved in mechanisms controlling sleep latency after mildly stressful events. The knockouts also fell asleep more quickly than controls after 3 different doses of amphetamine, suggesting that the mutant mice are not as sensitive to low doses of amphetamine as are the mice with normal levels of NE. It is interesting that amphetamine induces equivalent locomotor responses at a dose of 1.0 mg/kg in both control and NE-deficient mice[24] and prolongs wakefulness in control mice to nearly 2 hours, but the sleep latency for the knockouts was unchanged at about 20 minutes. Assuming that locomotion was stimulated similarly in the mice studied here, these results suggest that NE-deficient mice fell asleep during the period of locomotor stimulation. When exposed to escalating doses of amphetamine, the behavioral responses of rats and mice progress from wakefulness to locomotion and then to stereotypy. Weinshenker et al[24] demonstrated that the NE-deficient mice are actually more sensitive than controls to the medium and high doses of amphetamine with regard to locomotion and stereotypy. However, the results from the present study indicate that NE is necessary for low doses of amphetamine to promote wakefulness.

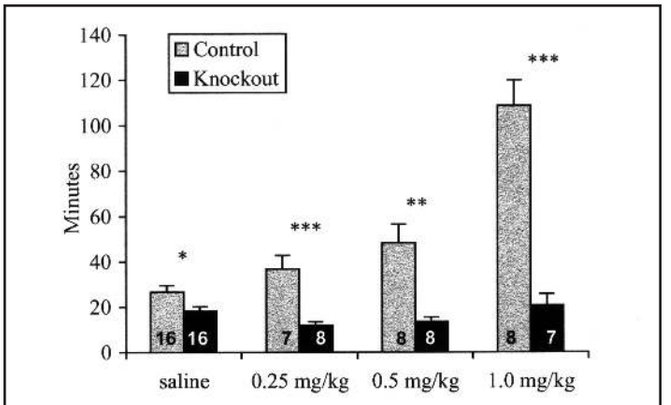

Figure 3—Sleep latency (in minutes) for knockout and control mice after injection of saline and 0.25 mg/kg, 0.5 mg/kg, or 1.0 mg/kg amphetamine. The number of mice per group is shown in each histogram. **P*<.05, ***P*<.01, ****P*<.001.

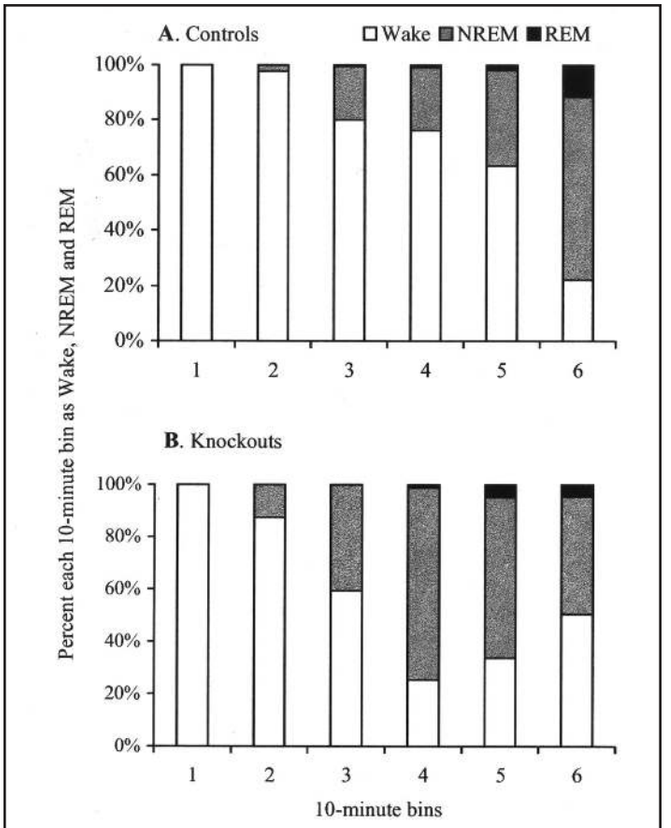

Figure 4—Distribution of sleep and wake in 10-minute bins for the first hour after amphetamine injection for the controls (A) and knockouts (B). An ANOVA revealed that the knockouts had significantly more NREM sleep than the controls during this hour (data not shown); however, this is an artifact due to the sleep-latency difference described in the text. NREM, non-rapid eye movement sleep; REM, rapid eye movement sleep.

At first glance, the difference in sleep latency between the NE-deficient and control mice might indicate that NE is necessary for wake promotion. However, if this were the complete explanation, one might also expect the knockout mice to have more sleep and less wake under normal conditions, which was not the case. These results instead suggest that NE may exert a wake-promoting influence during the period of time immediately prior to sleep onset, perhaps as an adaptive mechanism to increase vigilance and prevent an animal from falling asleep too quickly when predators could be near.

The present study also demonstrates that the NE-deficient mice do not exhibit sleep-wake differences during the first half of the night or during the first half of the day. The knockouts showed no differences compared to controls in response to an injection of saline, although they had marginally less REM sleep when the sleep changes were analyzed from the baseline to the injection day in a repeated measures design. The lack of significant differences between the NE-deficient mice and controls in their sleep-wake states was unexpected because NE is generally thought of as a major influence in the regulation of wake and REM sleep. In support of this, lesion studies have shown that destruction or partial destruction of the LC results in changes in sleep.[9,25] However, there are problems interpreting results from animals with neuroanatomic lesions. The destruction of specific areas of the brain may damage other tissue, or the lesion may be incomplete. In addition, many neurons contain more than 1 neurotransmitter, so the destruction of specific cells to deplete 1 neurotransmitter usually has the effect of depleting several. For instance, many LC neurons also contain galanin,[26,27] NPY,[28] CRH, or a combination of these.[29] The present study afforded the opportunity to study the sleep and wake states of NE-deficient mice without the caveats of anatomic or chemical lesioning.

It has been known for more than 20 years that the NE-containing cells of the LC are highly active during waking, less active during NREM sleep, and quiescent during REM sleep.[3,4] This pattern of activity has been the basis for the widely held notion that NE must be actively inhibited for sleep to occur. Indeed, studies have shown that decreasing NE signaling increases sleep,[9,25] and vice-versa.[7,8] However, there is a paucity of data in the literature showing that a decrease in NE signaling results in significantly less wake. This is probably because NE is now recognized to be more critically involved in periods of increased attention,[1,30-33] and perhaps learning,[34] occurring within the waking state rather than the occurrence of wakefulness itself. Results from this study, demonstrating that the NE-deficient mice do not have less waking behavior, support this notion.

The evidence is stronger for a reciprocal relationship between NE transmission and REM sleep. For instance, several investigations have shown that increased noradrenergic signaling decreases REM sleep,[7,8] and decreased signaling increases REM sleep.[9,25] Also, it has been found that GABA actively inhibits the LC during sleep,[35,36] and this inhibition is necessary for cholinergic nuclei in the pons to become active and induce REM sleep (see reference 2 for review). Therefore, we were surprised that the amount of REM sleep was normal in the NE-deficient mice during the first halves of the day and night.

Several reasons for this result are possible. Because the NE-deficient mice were devoid of NE during all of postnatal development, compen-

satory changes may have ensued. Serotonin-producing neurons from the dorsal raphe in the pons show a very similar pattern of activity as the noradrenergic LC and are a likely candidate for compensation. It is also possible that during postnatal brain development in the absence NE, other neurons that normally would respond to NE have changed in such a manner that counteracts the loss of NE. Alternatively, the lack of NE may affect the firing pattern of the pontine "REM-on" cholinergic neurons. These changes could either be part of normal transient feedback loops or consist of permanent changes in neuroanatomy. If these changes are developmental in nature, then restoration of NE in the central nervous system might elicit an alteration in the sleep states. Also, studying sleep in infant *Dbh* -/- mice may uncover sleep differences because the immature central nervous system may not have compensated for the lack of NE. Regardless, because the mutant mice exhibit normal sleep-wake state behavior, we conclude that the presence of NE is not required for the inhibition of REM sleep or the normal regulation of the sleep-wake cycle, at least for mice that develop postnatally without NE.

When analyzing the sleep-wake states from the baseline day to the stressor day, no differences were found for the control mice, and the knockout mice exhibited significantly less REM sleep on the stressor day. The trend for both genotypes was to exhibit less sleep and more wake after the saline injection. By contrast, other investigators have found the opposite effect on sleep in rats and mice[13-15] when using more extreme stressors such as restraint stress and footshock. Using a mild stressor (gentle handling), Meerlo et al[13] found that neither REM nor NREM sleep was affected in mice, except for a slight suppression of REM sleep in 1 of the 2 strains tested. The difference in the direction of the response between the mild and intense stressors could be due to the magnitude of the stressor. Rats generally show small glucocorticoid responses to mild stressors (handling, low-voltage footshock) and large responses to more intense stressors, with not much gradation in between. However, unlike rats, it has been shown that mice are capable of exhibiting intermediate increases of glucocorticoid levels in response to the stress of handling.[37] Since the stressor in this study included both handling and an injection, it is possible that these procedures could have an effect quite different from that commonly seen with more intense stress methods. The significant decrease in REM sleep seen in the knockout mice could be associated with their lack of NE, even though the difference did not reach statistical significance when comparing the 2 groups in a repeated measures ANOVA. However, since 1 of the major neurotransmitters associated with stress is absent in the knockouts, compensation by other neurotransmitters or changes in neural circuits involved in stress could have affected the length of time spent in REM sleep.

The studies presented here indicate that the sleep states of NE-deficient mice are remarkably normal under basal conditions and in response to mild stressors. If there is compensation for NE under basal conditions, then perhaps, under more extreme situations, the consequences of NE deficiency will be more apparent. Hence, this analysis provides a baseline for future studies on the role of NE in regulating sleep in response to more extreme stresses, drugs that promote wakefulness, and other genetic deficiencies that affect sleep.

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