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Chronic sleep restriction during development can lead to long-lasting behavioral effects

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HIGHLIGHTS

- Study in mice of effects of chronic developmental sleep restriction on behavior
- Effects were sex-dependent and long lasting.
- Sociability, response to social novelty, and repetitive behavior were affected.

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ABSTRACT

Sleep abnormalities are highly correlated with neurodevelopmental disorders, and the severity of behavioral abnormalities correlates with the presence of sleep abnormalities. Given the importance of sleep in developmental plasticity, we sought to determine the effects of chronic sleep-restriction during development on subsequent adult behavior. We sleep-restricted developing wild-type mice from P5–P42 for 3 h per day by means of gentle handling ($n = 30$) and compared behavioral outputs to controls that were handled 10 min daily ($n = 33$). We assayed activity in the open field, social behavior, repetitive behavior, and anxiety immediately following sleep restriction and after four weeks of recovery. At six weeks of age, immediately following chronic sleep-restriction, mice were less active in an open field arena. Sociability was increased, but repetitive behaviors were unchanged in both males and females. After a 4-week period of recovery, some behavioral abnormalities persisted and some became apparent. Sleep-restricted mice had decreased activity in the beginning of an open field test. Female mice continued to have increased sociability and, in addition, increased preference for social novelty. In contrast, male mice demonstrated decreased sociability with medium effect sizes. Repetitive behavior was decreased in sleep-restricted female mice and increased in males. Measures of anxiety were not affected in the sleep-restricted mice. These results indicate that chronic sleep restriction during development can lead to long-lasting behavioral changes that are modulated by sex. Our study may have implications for a role of disrupted sleep in childhood on the unfolding of neurodevelopmental disorders.

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1. Introduction

Sleep has an important role in brain development and synaptic plasticity [1]. Chronic sleep deprivation may result in an allostatic load contributing to cognitive problems [2] and disrupted plasticity [3]. The normal functions of sleep are thought to affect cellular processes important for plasticity, including: myelination, synapse formation/function, cellular detoxification/cell stress reduction, and protein synthesis [3].

Children with disrupted sleep often display an increased prevalence of neurobehavioral issues such as hyperactivity, emotional lability, aggressiveness, and deficits in socialization [4–8]; and disrupted sleep is a prevalent finding in many patients with neurodevelopmental disorders [3].

Given the importance of sleep on plasticity, and the prevalence of disturbed sleep in children with neurodevelopmental disorders, we

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Table 1
Timeline of testing. Sleep restriction began at five days of age, continuing to 42 days. Behavioral testing was conducted following sleep restriction. Following one month of recovery, behavior testing was repeated.

Age (days)	Procedure
5–42	Sleep Restriction
43	Social Behavior Test
44	Open Field Test
45	Marble Burying Test
72	Social Behavior Test
73	Open Field Test
74	Marble Burying Test
75	Rotarod Test
76	Elevated Plus Maze Test
111–122	Sleep Testing Initiation

hypothesized that altered sleep during a critical period in development would result in alterations in plasticity leading to long-lasting behavioral changes. In this study, we examined the behavioral effects, both immediate and long-term, of chronic sleep restriction throughout development and adolescence in otherwise normal mice.

2. Materials and methods

2.1. Animals

Litters of wild-type (WT) mice were produced from harem bred male and female C57Bl/6J mice obtained from Jackson Laboratories (Bar Harbor, ME). Once a female gave birth, the dam and her pups were separated from the other adults in the cage. Litters were randomly assigned to either the sleep-restriction (five litters: 13 male, 18 female offspring) or the control group (eight litters: 11 male, 22 female offspring). Six pups in the control group were cannibalized prior to determination of sex. One female pup in the sleep-restriction group was cannibalized, so the total number of females used for studies in that group was 17. Pups were weaned at P21. All mice were held in a climate-controlled facility with standard alternating 12 h periods of light and darkness (lights on, 6:00 AM–6:00 PM). Food and water were available to mice ad libitum. Animal procedures were carried out in accordance with the National Institutes of Health Guidelines on the Care and Use of Animals and an animal study protocol approved by the National Institute of Mental Health Animal Care and Use Committee.

2.2. Sleep restriction

When pups were five days of age, we began sleep-restriction by gentle handling [9]. Each litter was monitored daily between 11:00 AM and 2:00 PM. Mice showing inactivity or twitching behavior were gently prodded with a paintbrush until a response was elicited. A response was defined as a large movement and if the animal continued moving, we considered it to be awake. If the mother was on top of the pups, blocking them from view, then she was gently prodded away so that pups could be observed and sleep-restricted. Control mice were gently handled in the same manner (regardless of suspected sleep) for 10 min a day, to control for the stress of the prodding.

Sleep restriction occurred through P42. Behavior testing was conducted on three consecutive days beginning the day after cessation of sleep-restriction. Behavior testing was conducted in the light phase.

Table 2
Repeated measures ANOVA results activity/anxiety.

Behavior	Time point	Effect	$F_{(df,\ error)}$ value	P-value	Cohen's F^2
Open field					
Total distance moved	Pre-recovery	Sex × Condition × Epoch	$F_{(5,208)} = 0.530$	0.724	0.011
		Condition × Epoch	$F_{(5,208)} = 1.356$	0.249	0.028‡
		Sex × Epoch	$F_{(5,208)} = 1.395$	0.235	0.029‡
		Sex × Condition	$F_{(1,49)} = 0.718$	0.401	0.014
		Sex	$F_{(1,49)} = 0.397$	0.532	0.008
		Condition	$F_{(1,49)} = 7.774$	0.008*	0.159‡
		Epoch	$F_{(5,208)} = 89.497$	<0.001*	1.825‡‡
Total distance moved	Post-recovery	Sex × Condition × Epoch	$F_{(4,236)} = 1.476$	0.208	0.027‡
		Condition × Epoch	$F_{(4,236)} = 6.440$	<0.001*	0.115‡
		Sex × Epoch	$F_{(4,236)} = 6.476$	<0.001*	0.116‡
		Sex × Condition	$F_{(1,56)} = 0.488$	0.488	0.009
		Sex	$F_{(1,56)} = 0.703$	0.405	0.012
		Condition	$F_{(1,56)} = 1.446$	0.234	0.026‡
		Epoch	$F_{(4,236)} = 100.900$	<0.001*	1.801‡‡
Center/total ratio	Pre-recovery	Sex × Condition × Epoch	$F_{(5,245)} = 1.506$	0.188	0.031‡
		Condition × Epoch	$F_{(5,245)} = 0.605$	0.696	0.012
		Sex × Epoch	$F_{(5,245)} = 0.606$	0.695	0.012
		Sex × Condition	$F_{(1,49)} = 0.497$	0.484	0.010
		Sex	$F_{(1,49)} = 1.902$	0.174	0.038‡
		Condition	$F_{(1,49)} = 0.095$	0.759	0.002
		Epoch	$F_{(5,245)} = 11.62$	<0.001*	0.238‡
Center/total ratio	Post-recovery	Sex × Condition × Epoch	$F_{(5,278)} = 0.895$	0.484	0.016
		Condition × Epoch	$F_{(5,278)} = 3.290$	0.055~	0.058‡
		Sex × Epoch	$F_{(5,278)} = 0.613$	0.689	0.011
		Sex × Condition	$F_{(1,56)} = 0.145$	0.705	0.003
		Sex	$F_{(1,56)} = 1.284$	0.262	0.023‡
		Condition	$F_{(1,56)} = 0.026$	0.872	0.000
		Epoch	$F_{(5,278)} = 4.33$	<0.001*	0.078‡
Elevated plus maze	Post-recovery	Sex × Condition × Arm	$F_{(1,59)} = 0.333$	0.566	0.006
		Condition × Arm	$F_{(1,59)} = 0.286$	0.595	0.005
		Sex × Arm	$F_{(1,59)} = 0.012$	0.914	0.000
		Sex × Condition	$F_{(1,59)} = 0.692$	0.409	0.012
		Sex	$F_{(1,59)} = 0.014$	0.908	0.000
		Condition	$F_{(1,59)} = 0.014$	0.908	0.000
		Arm	$F_{(1,59)} = 1775.114$	<0.001*	30.250‡‡
Rotarod	Post-recovery	Sex × Condition	$F_{(1,58)} = 0.000$	0.989	0.000
		Sex	$F_{(1,58)} = 0.631$	0.430	0.011
		Condition	$F_{(1,58)} = 0.743$	0.392	0.013