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Short communication

Reduced sleep and impaired sleep initiation in adult male rats exposed to alcohol during early postnatal period

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HIGHLIGHTS

- ► Rats received alcohol intragastrically or were sham-intubated on postnatal days 4–9.
- ► 24 h recordings of sleep–wake behavior were obtained when the rats reached adulthood.
- ► Alcohol-exposed rats had longer latency to sleep onset during the rest period.
- ► Alcohol-exposed rats had less rapid eye movement sleep during the active period.
- ► Disrupted sleep may exacerbate disorders associated with prenatal alcohol exposure.

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ABSTRACT

Prenatal alcohol exposure (AE) is associated with cognitive and neurobehavioral abnormalities, such as increased motor activity and elevated anxiety, that may last a lifetime. Persistent sleep disruption may underlie these problems. Using a rat model, we investigated long-term alterations of sleep–wake behavior following AE during a critical early developmental period. Male rats received 2.6 g/kg of alcohol intragastrically twice daily on postnatal days (PD) 4–9, a developmental period equivalent to the third trimester of human pregnancy (AE group), or were sham-intubated (S group). On PD52–80, they were instrumented for tethered electroencephalogram and nuchal electromyogram recording and habituated to the recording procedures. Sleep–wake behavior was then recorded during one 24 h-long session. Wake, slow-wave sleep (SWS) and rapid eye movement sleep (REMS) were scored in 10 s epochs during 6 h of the lights-on (rest) and 6 h of the lights-off (active) periods. During the active period, REMS percentage was significantly lower (4.7 ± 0.9 (SE) *vs.* 8.2 ± 0.9; $p < 0.02$) and the percentage of SWS tended to be lower ($p = 0.07$) in AE than S rats ($N = 6$/group). During the rest period, sleep and wake amounts did not differ between the groups, but AE rats had longer latency to both SWS and REMS onset ($p = 0.02$ and 0.003, respectively). Our data demonstrate that, in a rat model of prenatal AE, impaired sleep–wake behavior persists into the adulthood. Disordered sleep may exacerbate cognitive and behavioral disorders seen in human victims of prenatal AE.

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Prenatal alcohol exposure (AE) is an established cause of disrupted sleep and abnormal sleep electroencephalogram (EEG) in infants. These disruptions correlate with subsequent delays in neurocognitive development [1,2]. Prenatal AE also is a key predictor of disrupted sleep in older children [3] which, in turn, may adversely affect development of cognitive functions and behavior [4]. It is therefore plausible that long-term cognitive and behavioral consequences of prenatal AE may be secondary to, or exacerbated by, sleep deficits. Although results of previous animal studies generally support the concept of long-term consequences of prenatal AE on sleep–wake behavior [5–7], the nature of sleep deficits and the conditions of AE sufficient to produce them are still uncertain, which complicates any systematic investigation of the underlying mechanisms. To date, the effects of early developmental AE on the 24-h sleep pattern were studied with only a short (2 days) and moderate prenatal AE. The result was a reduction of rapid eye movement sleep (REMS) in adult female rats and a similar trend in male rats, with no changes in slow-wave sleep (SWS) [6]. In contrast, in another study with chronic prenatal AE on gestational

Abbreviations: AE, alcohol exposure; ANOVA, analysis of variance; BAC, blood alcohol concentration; EEG, electroencephalogram; EMG, electromyogram; FASD, fetal alcohol spectrum disorders; GABA, γ-aminobutyric acid; GAD, glutamate decarboxylase; HPA, hypothalamic-pituitary-adrenal; PD, postnatal day; PF, perifornical; REMS, rapid eye movement sleep; S, sham-treated; SE, standard error; SWS, slow-wave sleep; VLPO, ventrolateral preoptic.

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days 8 through 20, SWS was significantly increased in adult male rats [7].

Studies in humans and animal models suggest that the nature of different fetal alcohol effects vary with the timing and pattern of AE [1,8–10]. A later gestational exposure appears to have stronger effects on the development of motor activity and cognitive functions than earlier exposures [9,10]. In addition, binge-like AE which produces intermittently high blood alcohol concentrations (BAC) appears to be more damaging to the brain, including the regulation of sleep, than persistently elevated BAC [1,8]. These effects are well reproduced in a neonatal rat model in which AE occurs during a period of brain growth spurt equivalent to the period of human development during the third trimester of pregnancy [8,10–12]. In this model, AE is adjusted daily based on the body weight of each pup, which allows one to precisely control alcohol dosage and timing of administration. We chose this well-established model to better characterize the sleep phenotype in adult male rats exposed early in their development to alcohol and to test the hypothesis that AE during the period of brain development equivalent to the third trimester of human gestation leads to a long-lasting dysregulation of sleep–wake behavior. A preliminary report has been published [13].

All animal procedures followed the guidelines of the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Experiments were performed on male Sprague-Dawley rats. The animals were housed on a 12:12 light/dark schedule with lights on at 7 am and *ad libitum* access to food and water. Pups were obtained from nine timed-pregnant rats with the date of birth designated as postnatal day (PD)0. Male pups were cross-fostered to create four experimental litters with 9–10 pups, with each litter containing pups obtained from different mothers. On PD4, the litters were randomly assigned to either alcohol- (AE) or sham-treated (S) group (non-mixed design [11]). On PD4 through PD9, the AE group was treated with alcohol administered in two intragastric intubations per day (2.625 g/kg per intubation, 11.9% (v/v) in a custom milk formula, 2 h apart), as described previously [14]. An additional intubation with milk only was given to the AE group 2 h after the second alcohol administration. The S group was subjected to the same daily routine of intubations, but no fluid was infused in order to prevent accelerated growth of S animals due to increased caloric intake [12]. The pups were weighed prior to each intubation and were returned to the dams after each intubation. During the treatment, body weight gains remained significantly lower in the AE group, as we described previously [14]. On PD4, trunk blood was collected 2 h after the second intubation from a separate group of 10 AE rats killed by decapitation. Blood alcohol concentration (BAC) was determined using NAD-ADH reagent (Sigma, St. Louis, MO, USA). The mean BAC was 334 ± 19 (SE) mg/dl, which was consistent with previous studies with a similar design [10,14]. The offspring were weaned on PD21–22.

On PD52–80, the rats were instrumented for recording of cortical EEG and nuchal electromyogram (EMG). All surgical procedures were performed under aseptic conditions, as described previously [15]. Briefly, the animals were anesthetized with i.m. ketamine (87 mg/kg) and xylazine (13 mg/kg) followed by isoflurane (1–2.5%). The frontal and parietal bones were exposed, and three stainless steel screws were inserted for EEG recording from the frontal cortex and for electrical reference. A pair of stainless steel, multi-stranded wires was sutured into the dorsal nuchal musculature to record the EMG. Following a recovery period of at least 7 days, the rats were habituated to the handling, hook-up procedure, and recording chamber during 2–6 h each day for two days and then for 10–12 h during an overnight session. The habituation sessions were separated by at least 24 h. Sleep–wake behavior was recorded during habituation sessions to verify the integrity of the connections and quality of the signals. During all recordings, the rat was placed in its home cage and connected with a counter-weighted cable and a swivel (SL6C, Plastics One, Roanoke, VA, USA) to amplifiers (Electroencephalograph model 8–10, Grass, Quincy, MA, USA). The recording cage was placed in a sound-dampened and electromagnetically shielded chamber (1 m^3). EEG and EMG signals were initially filtered (0.3–70 Hz and 5–10,000 Hz, respectively) and then digitized at 100 Hz for the EEG and 1000 Hz for the EMG and stored on a hard disk (Spike-2 software, Cambridge Electronic Design Ltd., Cambridge, UK). Animal movements were counted using infrared beam breaking technique (MicroMax, AccuScan Instruments, Columbus, OH, USA). At the time of final sleep–wake recording, the mean body weight of AE rats did not differ from that of S rats (391 ± 17 g (SE) *vs.* 386 ± 11 g, respectively). Three of nine AE rats and two of eight S rats that were instrumented had signals of unsatisfactory quality and were excluded from the study.

On PD70–97, undisturbed sleep–wake behavior of each rat was recorded during one 24 h-long session that started at 10 AM. Three behavioral states, wake, SWS and REMS, were distinguished in successive 10 s epochs using standard criteria based on the appearance of the cortical EEG and nuchal EMG and the EEG power spectrum simultaneously displayed for each scoring epoch (Somnologica v. 3, Medcare/Embla, Buffalo, NY, USA). The scoring was conducted by a person blind to the treatment and its accuracy was verified as described previously [15].

The following measures were obtained: latency from the hook-up time to the first episode of SWS and REMS, percentage of recording time spent in each behavioral state, and the number and duration of wake, SWS and REMS episodes. For comparison between AE and S rats, all parameters other than sleep onset latency were calculated over a period of last 6 h of recording during the lights-on period (12 PM to 6 PM) and last 6 h of the lights-off period (12 AM to 6 AM). To characterize motor activity, the total activity (total number of beam interruptions) and the number of movement bouts (number of separate periods during which the animal continuously crossed different sets of beams with breaks between such periods longer than 1 s) were quantified using Microlyze software (AccuScan Instruments).

All datasets were tested for normality and equal variance. Statistical significance of differences between the treatment groups and recording periods was then tested using one-way analysis of variance (ANOVA) with Bonferroni's correction. When normality criteria were not met (SWS onset, number of REMS episodes (lights-on period), number of movement bouts (lights-off period) for the AE group, and duration of wake (lights-off period) and REMS episodes (lights-on period) for the S group), Kruskal–Wallis ANOVA with Bonferroni's correction was used. The Pearson's correlation was used to determine the relationship between the percentages of sleep and the total motor activity. Differences were regarded significant at $p < 0.05$ (Analyse-It software, Leeds, UK).

The AE and S rats had similar circadian patterns of sleep–wake behavior and motor activity, with the characteristically higher amounts of sleep during the lights-on period and lower amounts during the lights-off period ($p = 0.048$–0.0001 for day-night differences in the percentage and number of episodes of wake, SWS and REMS, total motor activity and number of movement bouts). In both groups, the percentages of both SWS and REMS were inversely proportional to the total motor activity across the 24 h ($r = -0.75$ to -0.97, $p < 0.0001$). Superimposed on this general pattern were significantly reduced amounts of sleep in AE rats near the beginning and the end of the lights-off period (Fig. 1A). In addition, the latencies to the onset of the first episodes of both SWS and REMS were higher in AE than in S rats ($p = 0.02$ and 0.003, respectively; Fig. 1B).

During the last 6 h of the lights-off periods, AE rats had more wakefulness ($p = 0.029$), lower percentage of REMS ($p = 0.017$) and

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