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Effect of gabapentin on sleep and delta and theta EEG power in adult rats exposed to chronic intermittent ethanol vapor and protracted withdrawal during adolescence

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Abstract

Rationale—Adolescents and young adults with alcohol problems may also have sleep difficulties. However, whether these sleep problems are a result of a history of drinking or arise due to other comorbid disorders is difficult to disentangle in human studies. Additionally, the mechanisms underlying adolescent alcohol-induced sleep disturbances and potential targets for therapy also remain under-investigated. Recent clinical trials have demonstrated that the anticonvulsant and analgesic drug gabapentin may have therapeutic value in normalizing sleep quality in adult recovering alcoholics, yet its potential for the treatment of adolescent sleep disturbances has not been investigated.

Objectives—This study sought to evaluate the effects of a history of 5 weeks of chronic intermittent ethanol vapor exposure, administered during adolescence (AIE), on EEG sleep, in young adult rats ($n = 29$). The ability of two doses of gabapentin (30, 120 mg/kg) to modify sleep and slow wave activity were also investigated in these young adult rats exposed to alcohol vapor during adolescence.

Results—Adolescent vapor exposure in the rat was found to result in deficits in delta (1–4 Hz) and theta (4–8 Hz) power during slow wave sleep. Administration of gabapentin caused a “normalization” of the delta power deficits but did not affect theta power.

Conclusions—This report suggests that the potential mechanisms and therapeutic targets for sleep disturbance associated with adolescent alcohol exposure can be studied in preclinical models and that gabapentin may show partial efficacy in ameliorating these sleep deficits.

Keywords

Adolescence; Alcohol; Sleep; Slow wave sleep; Gabapentin

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Compliance with ethical standards

The experiments comply with the current laws of the country in which they were performed.

Conflict of interest The authors declare that they have no conflict of interests.

Introduction

Frequent binge drinking has been associated with health risk behaviors in teens (Miller et al. 2007), as well as risk for alcohol use disorders in adolescents and adults (Dawson et al. 2008; Ehlers et al. 2006). Problems in sleep regulation are one of the health risks associated with adolescent alcohol drinking. Adolescence is a time when both sleep problems and drug-seeking behaviors begin to increase; and adolescents may be particularly sensitive to the ability of substances of abuse to impair sleep (Bartel et al. 2015; Hasler and Clark 2013; Hasler et al. 2013, 2014a, b, 2015).

Despite the clear importance of sleep disturbance in adults and adolescents associated with alcohol use, use disorders, and the maintenance of sobriety (Brooks and Wallen 2014; Brower 2015; Chakravorty et al. 2016; Conroy et al. 2006), the complex mechanisms that underlie alcohol-induced insomnia and suitable therapeutic approaches to its management remain to be fully developed. A recent review and meta-analysis of treatments for insomnia in the context of AUD stressed that few studies have been conducted and the need for more research is clear (Miller et al. 2017). The development of animal models of alcohol-induced insomnia allows for the experimental control necessary to study the effects of ethanol on sleep independent of many factors that confound human studies, such as differences in genetic risk, psychiatric comorbidity, and other substance use. Studies from our laboratory and others, in rats, have demonstrated that chronic high-dose ethanol exposure can produce long-term and persistent changes in sleep, similar to what is seen in human alcoholics. For instance, adult rats treated with chronic ethanol show increases in sleep latency and reductions in the mean duration of slow wave sleep (SWS) episodes and the total amount of time spent in SWS (Ehlers and Criado 2010; Ehlers et al. 2013a; Ehlers and Slawecki 2000; Thakkar et al. 2015). We have also demonstrated that adolescent alcohol exposure also leads to sleep disturbances characterized by significantly reduced duration of slow wave sleep (SWS) episodes and the total amount of time spent in SWS (Criado et al. 2008). Despite the development of these animal models, their use to identify mechanisms underlying alcohol-induced insomnia and to identify therapeutic targets has been limited.

The present set of studies addresses several issues raised by the current literature on sleep and alcohol use in adolescence, through the use of animal models. Adolescent development has been suggested to occur over the entire second decade of life (10–20 years) (Spear 2000). During this developmental period, changes in behavior and neurobiological organization are observed with a number of similarities seen across mammalian species. In the rat, it has been suggested that the periadolescent period may span postnatal days (PD) 28–42 (Ojeda and Skinner 2006; Spear and Brake 1983; Varlinskaya et al. 1999). However, depending on gender and the measures used to define adolescence, early harbingers of adolescence may be seen as early as PD 22 in females, and it may last until PD 55 or so in males (Spear 2000). Thus, while adolescence in the human lasts 10 years, in the rat, it only lasts a little over a month. Although human adolescents typically do not drink daily, the fact that adolescence in the rat is only a matter of weeks means daily alcohol administration is necessary in order to model exposure over the entire equivalent rat adolescent period. Thus, we use an animal model of adolescent alcohol exposure where alcohol vapor is administered to the animal daily over the entire period of adolescence (see Ehlers et al. 2011). Using this

model, we have previously demonstrated that behavioral and neuroanatomical deficits can be produced (see Ehlers et al. 2011, 2013b, c, 2014) that mimic what has been reported in some human adolescent drinkers (Hanson et al. 2011; McQueeney et al. 2009; Schweinsburg et al. 2011; Squeglia et al. 2009). In the present study, we used this model to investigate the effects of adolescent alcohol exposure on the sleep EEG of young adult rats. In addition, we evaluated the effects of two doses of gabapentin on sleep and slow wave activity in these young adult rats who were exposed to alcohol vapor during their adolescence.

Materials and methods

Animal subjects

Thirty-two adolescent male Wistar rats were obtained from Charles River (USA) and arrived on postnatal day (PD) 21. Adolescent rats were triple-housed in standard plastic cages in a temperature-controlled room with a 12-h light/dark cycle (lights on 08:00). Food and water were available ad libitum. The work described herein adheres to the guidelines stipulated in the NIH *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 80-23, revised 1996) and was reviewed and approved by The Scripps Research Institute's Animal Care and Use Committee.

Ethanol vapor exposure

Ethanol vapor exposure has been shown to reliably allow for the titration of blood ethanol concentrations (BECs) that are sufficient for inducing physical ethanol dependence. The ethanol vapor inhalation procedure and the chambers used in this study have been previously described (see Ehlers et al. 2011, 2013b, c). Ethanol vapor chambers were calibrated to produce high to moderate BECs between 175 and 225 mg/dL, levels that mimic extreme binge drinking seen in adolescence (Patrick et al. 2013). Rats were randomly divided into two groups: ethanol or control group. Ethanol-exposed rats were housed in sealed chambers, infused with vaporized 95% ethanol from 8 p.m. to 10 a.m. For the remaining 10 h of the day, ethanol vapor was not infused into the chambers. Rats were exposed to this daily vapor cycle for 5 weeks (PD 22–57). Blood samples were collected from the tip of the tail every 3–4 days during the 5-week exposure period to assess BECs (5-week average = 204.6 ± 4.24 mg/dL). Control animals were handled identically to ethanol rats. BECs were determined using the Analox micro-statAM1 (Analox Instr. Ltd., Lunenburg, MA). Following the 5-week exposure, animals were transferred to standard polycarbonate cages for the duration of the experiment.

Surgical procedure

Surgical and electrophysiological recording procedures performed in this study have been previously described (Ehlers et al. 2013a; Ehlers and Slawecki 2000). Following withdrawal from vapor exposure or control conditions, rats were surgically implanted (PD 55–71) with screw electrodes in the skull overlying the frontal cortex (FCTX, AP 1.5 mm, ML $\pm$ 3.0 mm, FR1) and parietal cortex (AP – 4.5 mm, ML $\pm$ 4.5 mm) with a corresponding reference electrode posterior to lambda overlying the cerebellum. Surgical coordinates were obtained from the Paxinos and Watson (Paxinos and Watson 1986) atlas. EEG electrodes were connected to a multi-pin PlasticsOne® connector and the assembly was anchored to the

skull with dental acrylic and anchor screws. Rats were given at least 2 weeks of recovery before EEG recordings were made.

Electrophysiological recordings

After recovery from surgery, rats were habituated to the EEG testing chambers prior to the electrophysiological testing. A 5-h EEG sleep recording was conducted to obtain a baseline (no injections) (PD 78–86) and also following saline and two doses of gabapentin (PD 92–114). Sessions began at 08:00 and all rats were awake at the start of these EEG sessions. The EEG was recorded from two monopolar leads referenced to cerebellum ground (i.e., frontal cortex and parietal cortex) on a Sensorium preamplifier/amplifier unit (Shelburne, VT). Signals were transferred to a PC and digitized at a rate of 256 Hz. The EEG amplifier input range corresponding to the full range of the 12-bit analog-to-digital converter was about $\pm$ 250 microvolts. Periodic calibration results were used to scale the digitized EEG to microvolts.

Acute gabapentin administration

At PD 92, the pharmacology experiments were begun. Rats were randomized for the order of the dose: saline or gabapentin (Tokyo Chemical Industry Co–TCI): low dose (30 mg/kg) or high dose (120 mg/kg) using a Latin square design. Gabapentin was administered intraperitoneally (i.p.). The 5-h sleep recordings began approximately 20 min post-injection. At least a week was given between doses to avoid carry-over effects from the drug. The gabapentin concentrations were prepared fresh for each day of testing for groups containing three to six rats each, and the saline doses were given in equal frequency and at equivalent volumes to the gabapentin doses.

Rat sleep and EEG analyses

Data analyses were based on the two aims of the study which were to (1) quantify the changes in EEG sleep in those exposed to ethanol during adolescence compared to controls as adults at baseline following ethanol exposure and (2) test the effects of two doses of gabapentin on sleep and slow wave parameters in the ethanol and control treatment groups. Slow wave sleep (SWS) was visually identified as synchronized slow wave activity (1–4 Hz) during the 5-h EEG recording sessions that included baseline, saline injection control, gabapentin 30 mg/kg, and gabapentin 120 mg/kg. Increases in EEG power of at least twice the amplitude of waking baseline EEG power lasting longer than 8 s were counted as episodes of SWS. Sleep patterns were analyzed by (1) determining the onset latency of the first episode of SWS, (2) calculating the mean duration of all episodes of SWS, and (3) counting the total number of instances of SWS. The onset of the first SWS episode was identified from the raw EEG as the first transition from low-amplitude high-frequency EEG to SWS (high-amplitude low-frequency EEG) and lasting at least 8 s. Alcohol vapor and control animals were compared on the three sleep measures at baseline following ethanol exposure using a one-way ANOVA and following gabapentin using a 2 (alcohol vs. control) X 3 (saline, gabapentin 30, gabapentin 120) ANOVA. Significance was set at $p < 0.05$.

The 5-h EEG was also analyzed for spectral characteristics. Raw EEG signals were amplified (50% gain), band-pass filtered (0.53–70 Hz), digitized at a rate of 256 Hz, and

then transferred to an IBM-compatible PC. A Fourier transform of 4 s epochs was used to generate the power spectrum. Mean power density was quantified in micro V²/octave and peak frequency was calculated in hertz. Individual spectral epochs were averaged and compressed into two frequency bands: to assess power in delta (1–4 Hz) and theta (4–8 Hz) frequency ranges. EEG spectra were identified as containing artifact when average cortical power was > 2000 micro V²/octave. Artifact epochs were excluded only after visual analysis of the raw EEG and spectral distributions. Mean spectral power within each band was calculated from the first slow wave sleep epoch and an average calculated for all SWS epochs over the entire 5-h recording session. These analysis procedures have been described previously (Ehlers and Havstad 1982). Alcohol vapor and control animals were compared at baseline following ethanol exposure for the two frequency bands using a one-way ANOVA and following gabapentin using a 2 (alcohol vs. control) X 3 (saline, gabapentin 30 mg/kg, gabapentin 120 mg/kg) ANOVA. Significance was set at $p < 0.05$.

Results

Effects of chronic EtOH exposure and withdrawal on sleep and EEG spectra during slow wave activity

In order to assess the effects of chronic adolescent alcohol exposure on baseline sleep, ANOVA was used to determine if the values for the latency to SWS, duration of the first SWS episode, number of SWS episodes, and mean duration of SWS episodes differed between the control and adolescent EtOH vapor-exposed groups. None of these variables were significantly different. However, evaluation of the EEG spectra revealed significant differences between the adolescent alcohol-exposed groups and controls on delta and theta (1–4 Hz, 4–8 Hz) activity during sleep as seen in Fig. 1. A significant decrease in delta (1–4 Hz) ($F = 10.8$, $p < 0.003$) and theta (4–8 Hz) ($F = 5.6$, $p < 0.025$) power in parietal cortex was found in the adolescent ethanol vapor-exposed animals during the first slow wave sleep episode. An evaluation of EEG power over all the episodes of SWS revealed a significant decrease in theta power (4–8 Hz) in the frontal cortex ($F = 7.1$, $p < 0.01$) and a significant decrease in delta (1–4 Hz) ($F = 14.4$, $p < 0.001$) and theta (4–8 Hz) ($F = 8.0$, $p < 0.009$) power in parietal cortex in the adolescent ethanol vapor-exposed animals as compared to controls.

Effects of gabapentin on ethanol-induced alterations in sleep and EEG spectra

We evaluated whether gabapentin had an effect on sleep parameters and whether it could ameliorate the effects on EEG spectra observed following chronic adolescent alcohol exposure. In a 2 (ethanol vs. control) X 3 (saline, gabapentin 30, gabapentin 120 mg/kg) ANOVA, gabapentin was found to produce significant decreases in the latency to the first SWS episode ($F = 12.94$, $p < 0.001$), increases in the average duration of each slow wave episode ($F = 16.53$, $p < 0.001$), and decreases in the number of slow wave sleep episodes ($F = 28.2$, $p < 0.001$) (see Fig. 2). Post hoc analyses revealed that saline was significantly different from low dose and high dose for each measure (p ranges from $p < 0.04$ to $p < 0.001$), but no significant differences were seen in a comparison between low and high doses except for in the number of SWS episodes, which displayed a dose-dependent decrease ($p < 0.05$).

An evaluation of the EEG sleep spectra during the first SWS episode demonstrated that gabapentin produced increases in EEG delta power in the frontal ($F = 7.13$, $p < 0.002$) and parietal ($F = 10.9$, $p < 0.001$) cortex, as seen in Fig. 3. Post hoc analyses showed that saline was significantly different from the low and high doses ($p < 0.01$) in delta power during the first slow wave sleep episode, but low and high doses were not different in either cortical region. Gabapentin also produced dose-dependent increases in EEG delta power over all of the SWS episodes in the frontal ($F = 32.3$, $p < 0.0001$) and parietal ($F = 62.4$, $p < 0.0001$) cortex. Post hoc analyses revealed that saline was different from low dose and high dose gabapentin, in delta power over the entire SWS epochs, and the two doses were also significantly different from each other ($p < 0.01$) in both cortical areas. Additionally, post hoc analyses found no significant group differences between ethanol-exposed and control animals in delta power during the first SWS episode or over the whole sleep time following either gabapentin dose. This suggests that gabapentin, at both doses, may have “normalized” the deficits seen between the groups at baseline. Gabapentin only produced modest effects on theta power in the sleep EEG. An evaluation of the EEG sleep spectra during the first SWS episode demonstrated that gabapentin produced increases in EEG theta power in the frontal ($F = 4.0$, $p < 0.025$) cortex only, as seen in Fig. 4. Post hoc analyses showed that saline was significantly different from the low dose ($p < 0.01$) in theta power during the first SWS episode but other comparisons were not significant. Gabapentin did not produce changes in EEG theta power when all of SWS episodes were evaluated, in either region. Interestingly, like at baseline, overall group differences in theta power were seen between ethanol-exposed and control animals following gabapentin/saline during the first slow wave episode in the parietal cortex ($F = 4.8$, $p < 0.04$) and over all SWS episodes in the frontal cortex ($F = 4.6$, $p < 0.04$) and parietal cortex ($F = 5.0$, $p < 0.03$). Post hoc analyses revealed significant group differences during saline conditions as well as at both doses. Thus, gabapentin did not “normalize” deficits in theta power in the sleep EEG between the groups.

Discussion

Alcohol and other substance use, especially during early adolescence, has been linked to a number of long-term health risks, including sleep disturbance, that include elevated risk for dependence later in life in a number of populations (Dawson et al. 2008; Ehlers et al. 2006). The development of preclinical models of alcohol-associated sleep disturbance allows for study of the effects of adolescent ethanol exposure on sleep in young adulthood independent of many factors that confound human studies such as differences in genetic risk, and a host of potential cultural and environmental factors. Studies from our laboratory have demonstrated that adolescent alcohol exposure can lead to sleep disturbances, particularly changes in slow wave sleep (SWS) (Criado et al. 2008). In the present study, we extend those findings and report that chronic adolescent vapor exposure produces significant reductions in delta and theta power during slow wave sleep episodes. These data are consistent with our data previously reported in studies of male alcoholics where we found significant reductions in delta EEG power in human alcoholics, as compared to age and race matched controls, which were particularly pronounced during the first NREM period of sleep (Irwin et al. 2000). Deficits in delta power have also been confirmed in a study of male and female alcoholics (Colrain et al. 2009). Additionally, we have demonstrated that

alcoholics also do not respond to partial sleep deprivation with the usually seen rebound in SWS delta power, as compared to controls (Irwin et al. 2002) suggesting a lasting deficit in the mechanisms generating delta sleep.

Despite the development of preclinical models of adolescent alcohol-induced sleep deficits, their use to identify mechanisms underlying insomnia and to identify therapeutic targets is still limited. Current FDA-approved pharmacologic treatments for insomnia, in general, primarily include benzodiazepines and non-benzodiazepine hypnotics and, to a lesser extent, tricyclic antidepressants and several off-label and over the counter agents (for guidelines and reviews, see Coleman et al. 2017; Qaseem et al. 2016; Sateia et al. 2017). More recently, therapeutic drugs that target orexin/hypocretin receptors have been evaluated and shown to have some limited use in insomnia (Keks et al. 2017; Kripke 2015; Patel et al. 2015). However, some agents, in particular hypnotics, may not be suitable for the treatment of sleep disturbance associated with alcohol dependence because of their potential for addiction and potential interactions with alcohol (Mark et al. 2009).

Gabapentin, which binds the $\alpha 2\delta$ auxiliary subunit of the voltage-gated calcium channels, is a novel non-hypnotic compound that has been demonstrated to enhance slow wave sleep in patients with primary insomnia (Foldvary-Schaefer et al. 2002) and improve sleep quality (Bazil et al. 2005; Lo et al. 2010). Additionally, gabapentin has been demonstrated to improve rates of abstinence, measures of mood, sleep, and craving in alcohol-dependent individuals in several clinical trials (Brower 2015; Brower et al. 2008; Mason et al. 2014) and may have a more favorable profile with respect to addiction liability than hypnotics. We have demonstrated that gabapentin can produce dose-dependent increases in slow wave sleep and ameliorate the effects of chronic alcohol on sleep fragmentation, in an adult rat model of alcohol-induced sleep disturbance (Sanchez-Alavez et al. 2018). In the present study, we extend those findings to an adolescent model of alcohol exposure and also demonstrate that gabapentin can produce increases in slow wave power and also ameliorate the deficits seen in slow wave power in adolescent ethanol-exposed animals. The mechanism by which gabapentin produces its effects on sleep is not entirely clear; Cole et al. (2005) have shown that areas involved in slow wave sleep regulation, such as the median preoptic nuclei and the lateral hypothalamic areas, have a high density of the $\alpha 2\delta$ subunits of L-type voltage-gated calcium channels (VGCCs). There is also some data to suggest that alcohol may influence sleep physiology through a mechanism involving voltage-gated calcium channels (Greenberg et al. 1987; Katsura et al. 2006; Whittington et al. 1991; Whittington and Little 1991) that may be impacted by gabapentin administration.

It is likely that adolescent alcohol exposure affects multiple circuits and neurotransmitter systems that could potentially influence sleep. As discussed by Veatch (2006), several brain regions and networks implicated in the regulation of sleep are also sensitive to the effects of EtOH. For instance, neurons from the basal forebrain, the preoptic area, the brainstem, and the diffuse thalamocortical projection relay are all important in the regulation of sleep-wake states (Jones 2005; Pace-Schott and Hobson 2002; Saper et al. 2005) and are also sensitive to the effects of EtOH (Fadda and Rossetti 1998). Electrophysiological recordings indicate that within the basal forebrain, that GABA and glutamate as well as cholinergic neurons may be the functional principal cell types involved in sleep wake activity (Jones 2017). We have

demonstrated, using this adolescent alcohol exposure model, that chronic adolescent vapor exposure produces lasting reductions in cell counts of ChAT-immunoreactive (ChAT-IR) neurons in the Ch1–2 and Ch3–4 regions of the basal forebrain in ethanol vapor-exposed rats. This reduction in cell counts was significantly correlated with more “disinhibitory” behavior in the open field conflict test (Ehlers et al. 2011). However, whether these reductions in ChAT-IR are also responsible for the SWS findings in the present study is not known. It is likely that alterations in the homeostasis of several important sleep modulators such as adenosine and orexin/hypocretin are also impacted by adolescent alcohol exposure, in a similar way to which they are following acute alcohol exposure in adults (see Sharma et al. 2010a, b, 2014; Thakkar et al. 2010). However, evaluation of these important brain systems following adolescent alcohol exposure remains to be fully evaluated.

In conclusion, chronic ethanol vapor exposure during adolescence, in the rat, was found to produce deficits in EEG delta power during slow wave sleep that were “normalized” by administration of gabapentin. While preclinical studies allow the experimental control to investigate the effects of alcohol on adolescent brain development, they do not shed light on the complex motives and risk factors for drinking in adolescence. Additionally, the levels of exposure in this model aim at high drinking levels (> 10 drinks) and thus do not model those adolescents who are drinking at lower levels or on fewer occasions. Despite these limitations, this report represents an important step in an ongoing investigation of sleep disturbance associated with ethanol exposure and to identify mechanisms and potential therapeutic targets in preclinical models.

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Fig. 1.

Delta and theta power during slow wave sleep in the frontal and parietal cortex after adolescent alcohol exposure. Slow wave sleep was assessed in rats approximately 21–28 days after cessation of alcohol vapor exposure or air control. Delta and theta power shown for the first episode of slow wave sleep and across all episodes of slow wave sleep. * indicates $p < 0.05$ group effect. ** indicates $p < 0.01$ group effect. Error bars = S.E.M.

Fig. 2.

Sleep latency and duration measurements following saline and two doses of gabapentin in adolescent alcohol-exposed and control rats. Latency to first slow wave sleep episode and duration and total number and mean duration of all sleep episodes are shown following injections of saline (SAL), 30 mg/kg gabapentin, and 120 mg/kg gabapentin. * indicates $p < 0.05$ group effect. † indicates $p < 0.05$ dose effect. Error bars = S.E.M.

Fig. 3.

Delta power during slow wave sleep in the frontal and parietal cortex following saline and two doses of gabapentin in adolescent alcohol-exposed and control rats. Delta power was indexed following injections of saline (SAL), 30 mg/kg gabapentin, and 120 mg/kg gabapentin. Delta power during first slow wave sleep episode (**a, c**) and the mean of all episodes of slow wave sleep (**b, d**) are shown. * indicates $p < 0.05$ group effect. † indicates $p < 0.05$ dose effect. Error bars = S.E.M.

Fig. 4.

Theta power in slow wave sleep in the frontal and parietal cortex following saline and two doses of gabapentin in adolescent alcohol-exposed and control rats. Theta power was indexed following injections of saline (SAL), 30 mg/kg gabapentin, and 120 mg/kg gabapentin. Theta power during first slow wave sleep episode (**a, c**) and the mean of all episodes of slow wave sleep (**b, d**) are shown. * indicates $p < 0.05$ group effect. † indicates $p < 0.05$ dose effect. Error bars = S.E.M.