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PSPH-D-18-00526: Effect of a Dual Orexin Receptor Antagonist (DORA-12) on Sleep and Event-related oscillations in rats exposed to ethanol vapor during adolescence

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Abstract

Rationale—Sleep difficulties are one of the problems associated with adolescent binge drinking. However, the mechanisms underlying adolescent alcohol-associated sleep disturbances and potential targets for therapy remain under investigated. Orexin receptor antagonists may have therapeutic value in the treatment of insomnia, yet the use of this class of drugs in the treatment of sleep disturbances following adolescent alcohol exposure has not been studied.

Objectives—This study employed a model whereby ethanol vapor exposure occurred for 5 weeks during adolescence (AIE), and waking event-related oscillations (EROs) and EEG sleep were subsequently evaluated in young adult rats. The ability of 2 doses (10, 30 mg/kg PO) of a Dual Orexin Receptor Antagonist (DORA-12) to modify sleep, EEG and EROs was investigated in AIE rats and controls.

Results—Adolescent vapor exposure was found to produce a fragmentation of sleep, in young adults, that was partially ameliorated by DORA-12. DORA-12 also produced increases in delta and theta power in waking EROs recorded before sleep, and deeper sleep as indexed by increases in delta and theta power in the sleep EEG in both ethanol and control rats. Rats given DORA-12 also fell asleep faster than vehicle treated rats as measured by a dose dependent reduction in the latency to both the first slow wave and REM sleep episodes.

Conclusions—This study showed that DORA-12 can affect the sleep disturbance that is associated with a history of adolescent ethanol exposure and also has several other sleep-promoting effects that are equivalent in both ethanol and control rats.

Keywords

adolescence; alcohol; sleep; EROs; DORA

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CONFLICTS OF INTEREST

The authors have no conflicts of interest. The experiments comply with the current laws of the country in which they were performed.

INTRODUCTION

Disturbance in sleep regulation is one of the health risks associated with adolescent alcohol and drug use. Adolescence is a time when both drug and alcohol seeking behaviors and disturbances in sleep emerge; and adolescents may be particularly vulnerable to sleep disturbance associated with substance use (Bartel et al. 2015; Hasler and Clark 2013; Hasler et al. 2014a; Hasler et al. 2013b; Hasler et al. 2014b; 2015).

Recently, animal models of alcohol-induced insomnia have been developed that allow for study of the effects of ethanol on sleep independent of factors that may confound human studies, such as premorbid conditions and other substance use. Investigations conducted in our laboratory as well as others, in rodents, have shown that chronic ethanol exposure can produce persistent and long-term changes in sleep, similar to what has been reported in humans with alcohol use disorders (AUD) (Criado and Ehlers 2010; Ehlers et al. 2013a; Ehlers and Slawecki 2000; Sanchez-Alavez et al. 2018; Thakkar et al. 2015). More recently an animal model of sleep disturbance, resulting from alcohol administration during adolescence, has been developed (see (Criado et al. 2008b; Ehlers et al. 2011; Ehlers et al. 2018)). We have shown that alcohol exposure during adolescence, in this rat model, can result in persistent electrophysiological, behavioral and neuroanatomical deficits in young adulthood (see (Ehlers et al. 2011; Ehlers et al. 2014; Ehlers et al. 2013b; Ehlers et al. 2013c)) that parallel findings seen in human adolescent binge drinkers (Ehlers et al. 2019; Hanson et al. 2011; McQueeny et al. 2009; Schweinsburg et al. 2011; Squeglia et al. 2009). Although these animal models have been developed, they have been little used to study therapeutic targets.

A recent body of literature supports a prominent role of the hypothalamic peptide hypocretin/orexin (Hct/OX) in homeostatic control of a number of regulatory processes (see (de Lecea 2012; Li et al. 2014)). The Hct/OX system has not only been suggested to be an important regulator of the sleep wake cycle (Hoyer and Jacobson 2013; Inutsuka and Yamanaka 2013; Krystal et al. 2013; Mieda et al. 2013), but has also been shown to influence a range of other physiological processes including: feeding and energy metabolism, reward/motivated behavior, the modulation of stress responses (Baldo et al. 2003; Cason et al. 2010; DiLeone et al. 2003; Martin-Fardon et al. 2010; Xu et al. 2013), as well as ethanol-seeking and drinking behaviors (Anderson et al. 2014; Brown et al. 2013; Brown et al. 2016; Jupp et al. 2011a; Jupp et al. 2011b; Kastman et al. 2016; Kim et al. 2012; Lawrence 2010; Lawrence et al. 2006; Martin-Fardon and Weiss 2012; Moorman 2018; Moorman and Aston-Jones 2009; Moorman et al. 2017; Srinivasan et al. 2012; Ubaldi et al. 2016; Walker and Lawrence 2017). However, few studies have investigated the ability of the Hct/OX system to modify alcohol-related sleep pathology (Sanchez-Alavez et al. 2019).

In the present study, we used an animal model of adolescent alcohol exposure to study the effects of two doses of a newly developed Dual Orexin Receptor Antagonist (DORA-12) on waking and sleep physiology (Gotter et al. 2014; Ramirez et al. 2013). For the evaluation of DORA-12 on waking electrophysiology, we used measures of event-related oscillations

(EROs). EROs are oscillatory changes within the dynamics of ongoing EEG rhythms that are enhanced or synchronized by a time locked sensory and/or cognitive stimulus (see (Anokhin 2014; Basar et al. 2000; Klimesch et al. 2007; Roach and Mathalon 2008)). EROs have been demonstrated to be sensitive measures of normal (Basar et al. 1999; Schack and Klimesch 2002) and abnormal cognitive functioning, as well as more importantly, endophenotypes for alcohol use disorders (Ehlers et al. 2015; Pandey et al. 2012; Rangaswamy and Porjesz 2014). We also studied the effects of this drug on sleep measures and the spectral content of the sleep EEG during slow wave (SW) and rapid eye movement (REM) sleep in young adult rats who experienced alcohol vapor or control conditions during their adolescence.

MATERIALS AND METHODS

Animal subjects

Forty-four adolescent male Wistar rats (Charles River (USA) arrived with their dams on postnatal day (PD) 21. They were triple-housed under a 12h light/dark cycle (lights on 08:00), with water and food available *ad libitum*. The study was approved by The Scripps Research Institute's Animal Care and Use Committee and adheres to the guidelines outlined in the NIH *Guide for the Care and Use of Laboratory Animals* (NIH publication No. 80–23, revised 1996).

Ethanol vapor exposure

The methodology for ethanol vapor inhalation during adolescence have been previously described (see (Ehlers et al. 2011; Ehlers et al. 2013a; Ehlers et al. 2013c)). The chambers used to expose rats to ethanol were titrated to produce constants levels of high to moderate BECs (Blood Ethanol Concentrations) between 175–225 mg/dL, which are levels that are similar to binge drinking seen in adolescence in some studies (Patrick et al. 2013). Rats in the ethanol group were exposed to vaporized 95% ethanol from 8 p.m. to 10 a.m. daily for a 5-week period (P22–57). Tail blood samples were collected during this time every 3–4 days to assess BECs (5-week average= 215.53 ± 6.45 mg/dL). Rats in the control group were handled identically including tail blood collection. An Analox micro-statAM1 (Analox Instr. Ltd., Lunenberg, MA) was used to estimate BECs. After the 5-week ethanol or control exposure, rats were housed in standard cages for the rest of the experiment.

Surgical procedure

The surgical procedures performed in this study have been previously described elsewhere (Ehlers et al. 2013b; Ehlers et al. 2018; Ehlers and Slawecki 2000). Briefly, rats were implanted (PD 55–71) with 2 screw electrodes in the calvarium, one overlying the frontal cortex (FCTX, AP: 1.5 mm, ML: ± 3.0 mm, FR1), and another over the parietal cortex (PCTX, AP: −4.5mm, ML: ± 4.5 mm) with a reference implanted over lambda, guided by the (Paxinos and Watson 1986) atlas. A multi-pin Plastics One® connector was used to make electrical connections.

Electrophysiological recordings

Two weeks after recovery from surgery, rats were habituated to the electrophysiological recording conditions. All ERO/EEG sessions began at 08:00. The EEG was recorded from

the 2 leads (frontal cortex and parietal cortex) that were referenced to the lambda ground using a Sensorium preamplifier/amplifier unit (Shelburne, VT). Signals were digitized at a rate of 256 Hz using software described previously.

ERO collection and a 5h EEG sleep recording were obtained for each session. EROs were elicited by auditory stimuli that were presented through a small speaker centered approximately 70 cm above the rat's head. EROs were elicited by an acoustic "oddball" plus noise paradigm. The acoustic parameters were three square wave tones (rise/fall times,1 ms): a frequent tone (50 ms, 2 KHz, 70 dB SPL) presented on 83% of the trials (n=259), an infrequent tone (50 ms, 2 KHz, 80 dB SPL) presented on 10.3% of the trials (n=32), and a noise burst (50 ms, noise, 100 dB SPL) presented on 6.7% of the trials (n=21). Signals were digitized at a rate of 256 Hz and transferred to a PC for offline analyses. Immediately following the ERO session the 5-hour sleep EEG was obtained.

Acute DORA-12 administration

At PD 92 the pharmacology experiments were begun. Rats were randomized for the order of the dose: vehicle or DORA-12 (Merck): low dose (10 mg/kg) or high dose (30 mg/kg) using a Latin square design. The vehicle for this compound was a Vitamin E TPGS (D-a-Tocopherol polyethylene glycol 1000 succinate) 20% solution, in sterile culture grade water that was sonicated for 30 minutes prior to administration. To maximize bioavailability of the compound, vehicle or DORA-12 was administered by oral gavage. Intragastric administration was performed by gently inserting a ball-tipped stainless steel curved needle into the esophagus. Approximately 60 min post-gavage the 5-hour sleep recordings were begun. To avoid carry over effects, at least a week elapsed between drug doses. The vehicle was given in an equivalent volume to the DORA-12 doses.

Event Related Oscillation (ERO) analyses

ERO energy (peak magnitude of the S transform output, squared, in a time frequency region of interest) was obtained from the auditory paradigm for each stimulus. Methods for these analyses have been described in detail elsewhere (Ehlers and Criado 2009; Ehlers et al. 2012). The ERO trials were digitized at a rate of 256 Hz. Trials containing excessive artifact were eliminated prior to averaging (<5% of the trials). An artifact rejection program was utilized to eliminate individual trials in which the EEG exceeded ± 400 μV. Data from single trials generated by the stimuli were entered into the time frequency analyses algorithm. The *S*-transform (ST), a generalization of the Gabor transform (Gabor 1946) was used.

To quantify *S*-transform magnitudes, a region of interest (ROI) was identified by specifying the band of frequencies and the time interval contained in the rectangular ROI. The time-frequency points saved from each *S* transformation are from 100 ms before to 900 ms after the onset of the stimulus, and from 1 Hz through 50 Hz at intervals of 0.5 Hz. Energy is the square of the magnitude of the *S*-transform output in a time frequency region of interest. The *S*-transform magnitude squared for a time/frequency interval is proportional to volts squared.

Rectangular regions of interest (ROIs) were defined by specifying, for each ROI, a time interval relative to the stimulus onset time over a specific frequency band. The ROI

frequencies were: delta (1–4 Hz), theta (4–7 Hz), and beta (13–30 Hz). The ROI time intervals were delta (200–500 ms), theta (10–400 ms), and beta (0–300 ms). ROI time intervals were selected based on ERO energy in specific Event Related Potential (ERP) component locations (N1, P3) in previous ERP studies (Ehlers et al. 1998). Using mean values over trials, the maximum values were calculated for each ROI, for the two electrode locations.

Sleep EEG analyses

Slow-wave sleep (SWS) (1–4 Hz) was scored for the 5-h EEG recording sessions for the: vehicle (control), DORA-12 10 mg/kg and DORA-12 30 mg/kg P.O. SWS episodes were defined as an increase in EEG power that exceeded twice the amplitude of waking baseline EEG power lasting longer than 8 s. Rapid eye movement (REM) sleep was visually identified as synchronized theta activity (4–8 Hz) that was preceded by an episode of SWS in the absence of muscle activity. Sleep patterns were identified and analyzed for SWS and REM states. Measures included: 1) latency to the onset of the first episode of SW and REM sleep, 2) the mean duration of all SW and REM sleep episodes, and 3) the total number of SW and REM sleep episodes.

Spectral characteristics of the EEG were also quantitated for the 5-hour recording period as described previously (Ehlers and Havstad 1982; Ehlers et al. 2018). EEG signals were band-pass filtered (0.53– 70 Hz), digitized, artifact removed, and Fourier transformed to generate the power spectrum. EEG analyses focused on 2 frequency bands: delta (1–4 Hz) and theta (4–8 Hz) activity. Spectral power was calculated separately for the first slow wave sleep epoch and for the average of all the SWS epochs over the entire 5-h recording session.

Statistical analyses

Data analyses were based on the two aims of the study which were to test the effects of vehicle and two doses of DORA-12 on: 1) waking electrophysiology as indexed by event-related oscillations (EROs), and 2) sleep physiology as indexed by REM and SW sleep parameters, and EEG sleep spectral characteristics, in the ethanol and control treatment groups. For the ERO analyses, energy in the 3 time-frequency regions of interest (delta, theta, beta), were compared in response to the infrequent tone, in leads frontal cortex (FCTX) and parietal cortex (PCTX) in the alcohol vapor and control animals for the three drug conditions using a group (ethanol, control) X 3 condition (vehicle, DORA-12 10 mg/kg, DORA-12 30mg/kg) ANOVA. Due to non-normal distributions of the data, alcohol vapor and control animals were compared on the REM and SW sleep measures (latency to onset, mean duration of episodes, number of episodes) for the treatment condition (vehicle, DORA-12 10mg/kg, DORA-12 30 mg/kg) using Friedman’s test and between the two alcohol exposure groups using Mann-Whitney U (MWU). Spectral power in the sleep EEG in two frequency bands (delta, theta) over all the SW and REM sleep episodes in leads FCTX and PCTX were also evaluated for the three drug conditions using a 2 group (ethanol, control) X 3 condition (vehicle, DORA-12 10mg/kg, DORA-12 30 mg/kg) ANOVA. Five animals were not used in the analysis due to decreasing function of electrodes leads over the duration of testing. Post hoc analyses were used when significant main effects were found. Significance was set at $p<0.05$.

RESULTS

Effects of DORA-12 on waking EROs

Our first aim evaluated the response to vehicle and 2 doses of the DORA-12 as indexed by waking ERO energy, in the control and EtOH-exposed groups in frontal (FCTX) and parietal (PCTX) cortex. Repeated measure ANOVA revealed an effect of condition (Vehicle (VEH), DORA-12 10 mg/kg, 30 mg/kg) on ERO energy in delta frequency band, to the infrequent tone, in frontal cortex (condition: $F(2,38)= 34.0$, $p<0.001$) and parietal cortex (condition: $F(2,38)=47.6$, $p<0.001$). Post hocs revealed that these results were significant when vehicle was compared to the 10 mg/kg dose ($F=39.5$, $p<0.0001$) or the 30 mg/kg dose ($F=45.2$, $p<0.0001$) and when the low dose was compared to the high dose ($F=8.5$, $p<0.006$) in frontal cortex as well as in parietal cortex (VEH vs 10: $F=69.2$, $p<0.0001$; VEH vs. 30: $F=73.1$, $p<0.0001$; 10 vs 30: 12.0, $p<0.001$) (see figure 1). In addition, repeated measures ANOVA revealed condition effects on ERO energy in the theta frequency band, in both frontal cortex (condition: $F(2,38)=$, 34.6, $p=0.001$) and parietal cortex (condition: $F(2,38)=25.5$, $p<0.001$). Post hocs revealed that these results were significant when vehicle was compared to the 10 mg/kg dose ($F=47.8$, $p<0.0001$) or the 30 mg/kg dose ($F=52.0$, $p<0.0001$) and when the low dose was compared to the high dose ($F=4.9$, $p<0.03$) in frontal cortex as well as in parietal cortex (VEH vs 10: $F=20.7$, $p<0.0001$; VEH vs. 30: $F=56.4$, $p<0.0001$; 10 vs 30: 5.2, $p<0.03$) (see figure 1).

Effects of DORA-12 on REM and SW sleep parameters

We evaluated whether DORA-12 had an effect on sleep parameters using non-parametric tests. An overall effect of group was found for DORA-12 for both the mean duration of slow wave sleep episodes (MWU= 1263, $p<0.017$) and for the total number of slow wave sleep episodes (MWU=1279, $p<0.02$). As seen in figure 2 the adolescent ethanol exposure group had both shorter duration of their SWS episodes and a larger number of episodes overall as compared to the air control group resulting in a fragmentation of sleep. Post-hoc analyses revealed that this effect was only significant for the duration of SWS episodes in the VEH condition (MWU= 120, $p<0.05$). Significant effects of condition were found, using the Friedman test, for 2 of the SWS variables as seen in figure 2 (Latency to first SWS: Chi Square: 41.2, $p<0.001$, total number of SWS episodes: Chi Square: 18.5, $p<0.001$). Post hoc analyses using the Wilcoxon test showed that both doses of DORA decreased the latency to the onset of the first SWS episode (VEH vs 10: $Z=-4.6$, $p<0.0001$; VEH vs 30: $Z=-5.1$, $p<0.0001$) in a dose dependent fashion (10 vs 30: $Z=-2.0$, $p<0.04$), as seen in figure 2. Both doses of DORA-12 were also found to increase the number of slow wave sleep episodes (VEH vs 10: $Z=-4.0$, $p<0.0001$; VEH vs 30: $Z=-2.4$, $p<0.016$, 10 vs. 30: $Z=-2.26$, $p<0.024$).

REM parameters were also found to significantly differ both as a function of group and condition. AIE animals were found to have significantly more REM episodes overall (MWU: 1275, $p<0.02$), although the post hocs were not significant between the groups within condition levels. Significant effects of condition were found, using the Friedman test, for all 3 REM variables as seen in figure 3 (Latency to first REM: Chi Square: 40.2, $p<0.001$, mean length of REM episodes: Chi Square= 6.8, $p<0.03$, total number of REM

episodes: Chi Square: 6.7, p<0.04). Post hoc analyses using the Wilcoxon test showed that both doses of DORA-12 decreased the latency to the onset of the first REM episode (VEH vs 10: Z=−4.9, p<0.0001; VEH vs 30: Z=−5.1, p<0.0001) in a dose dependent fashion (10 vs 30: Z=−2.0, p<0.04), as seen in figure 3. The high dose of DORA-12 was also found to reduce the mean duration of REM episodes when compared to VEH (Z=−2.6, p<0.01).

Effects of DORA-12 on sleep EEG spectra

An evaluation of the EEG sleep spectra during the first SWS episode demonstrated that DORA-12 produced increases in EEG delta power in frontal (F=25.6, p<0.0001) and parietal (F=24.5, p<0.0001) cortex, as seen in figure 4. Post hoc analyses showed that VEH was significantly different from the low and high dose (FCTX: VEH vs 10: F=35.1, p<0.001; VEH vs. 30: F= 42.6, p<0.0001; PCTX: VEH vs 10: F=33.5, p<0.001; VEH vs. 30: F= 36.6, p<0.0001), in delta power during the first slow wave sleep episode, but low and high dose were not different from each other in either cortical region. However, when the amount of power in the delta frequencies was assessed over all SW sleep episodes DORA-12 was found to produce a slight decrease in frontal cortex only (F=4.4, p<0.018), and post hocs revealed that it was only significant when comparing VEH to the lowest dose (F=10.8, p<0.002) (data not shown).

An evaluation of the EEG sleep spectra during the first SWS episode demonstrated that DORA-12 also produced increases in EEG theta power in frontal (F=8.9, p<0.001) and parietal cortex (F=9.0, p<0.001), as seen in figure 4. Post hoc analyses showed that VEH was significantly different from the low dose (FCTX: F=13.9, p<0.001; PCTX: 14.6, p<0.001), high dose (FCTX: F=7.62, p<0.009; PCTX: 4.8, p<0.04). The parietal cortex was also significantly different between the low dose and high dose (6.9, p<0.01). DORA-12 did not produce changes in EEG theta power when the mean of all SWS episodes were evaluated, in either lead (data not shown).

Spectral changes in REM sleep as a function of dose of DORA-12 were also determined and the only findings were a decrease in mean delta power for all REM episodes, over the entire recording period, in both leads (FCTX F=6.6, p<0.002; PCTX: F=4.1, p<0.023). Post hoc analyses found that it was significant when VEH was compared to low dose (FCTX: F=11.2, p<002; PCTX: F=7.6, p<0.009) and high dose (FCTX: F=7.8, p<008; PCTX: F=4.4, p<0.04), but not when the two doses were compared (data not shown).

DISCUSSION

Alcohol use during early adolescence, has been associated with long-term health consequences, as well as elevated risk for alcohol use disorders in young adulthood (Dawson et al. 2008; Ehlers et al. 2006). Preclinical models allow for study of ethanol exposure during adolescence on sleep independent of factors that may confound human studies such as differences in: genetic risk, psychiatric comorbidity as well as environmental and cultural factors. Studies from our laboratory have shown that adolescent alcohol exposure can lead to a disruption in slow-wave sleep (SWS) (Criado et al. 2008a; Ehlers et al. 2013a; Ehlers et al. 2018). The present study is a replication and confirmation of those studies, in that we found that young adult rats with a history of adolescent alcohol vapor exposure demonstrated a

fragmentation of sleep that consisted of a decrease in the duration and an increase in the number of slow wave sleep episodes. These findings are also similar to what we have reported previously in adult animals exposed to chronic alcohol exposure (Sanchez-Alavez et al. 2018), although the findings appear to be somewhat less pronounced in adolescents as compared to adults.

Although preclinical models of adolescent alcohol exposure allow for the identification of alcohol specific sleep deficits, as opposed to premorbid conditions, they have seldom been utilized to identify therapeutic targets. The treatment of insomnia in adolescents and young adults is particularly problematic since this is a time during development when mental alertness is particularly important and risk for developing addiction is high (see (de Zambotti et al. 2018)). We have demonstrated that Gabapentin, which binds the α2δ auxiliary subunit of the voltage-gated calcium channels, can produce dose dependent increases in slow wave sleep, and ameliorate the effects of chronic alcohol exposure on sleep fragmentation, in both an adult rat model of alcohol-induced sleep disturbance (Sanchez-Alavez et al. 2018), and also ameliorate the deficits seen in slow wave power in adolescent ethanol exposed animals (Ehlers et al. 2018). Gabapentin has been shown to improve sleep and measures of recovery in individuals with AUD in several clinical trials (Brower 2015; Brower et al. 2008; Mason et al. 2014), and may have less potential for addiction liability than benzodiazepine-type hypnotics, however, it may be too sedating for use in teens.

More recently, therapeutic drugs that target orexin/hypocretin receptors have been developed and shown to have some limited use in insomnia (Keks et al. 2017; Kripke 2015; Neubauer et al. 2018; Patel et al. 2015; Winrow and Renger 2014). These drugs may prove to have efficacy in the treatment of adolescent insomnia because they may have less potential for addiction and unacceptable side effects. In one study, in Japan, the tolerability, efficacy, and safety of suvorexant, a dual orexin receptor antagonist, was evaluated in adolescents with insomnia (Kawabe et al. 2017). In that study suvorexant was found to improve the subjective sleep quality, although 40% of those prescribed the drug discontinued its use during the study.

A recent review has proposed that suvorexant may be particularly suited for the treatment of alcohol use disorders (Campbell et al. 2018). However, the effects of suvorexant in patients with AUD are currently unknown and preclinical studies of the treatment of alcohol induced sleep pathology with suvorexant are limited. In one study, adult rats were exposed to chronic ethanol vapor exposure and the effects of suvorexant were evaluated on EEG and sleep parameters. In that study suvorexant was found to hasten the onset of SW and REM sleep but exacerbated the sleep fragmentation observed as a function of alcohol exposure (Sanchez-Alavez et al. 2019). Enhanced fragmentation of sleep has also been shown following almorexant administration in a murine model of narcolepsy as well as cataplexy (Black et al. 2013). In the present study, a newly developed dual orexin receptor antagonist (DORA-12) (Gotter et al. 2014; Ramirez et al. 2013) was administered to adult rats that had been exposed to chronic alcohol exposure during adolescence and their controls. Chronic ethanol vapor exposure during adolescence, in the rat, was found to produce a fragmentation of sleep in young adults that was partially ameliorated by DORA-12. DORA-12 was also found to produce several significant effects on sleep and waking EEG that were equivalent in

the alcohol vapor and control rats. Significant increases in delta and theta power in waking EROs, recorded just before sleep time, as well as deeper sleep as indexed by increases in delta and theta power in the sleep EEG were seen in both alcohol and control rats. Rats given DORA-12 also fell asleep faster than rats given vehicle as measured by a dose dependent reduction in the latency to both the first slow wave and REM episodes. These data suggest that DORA-12 may have some efficacy in the treatment of alcohol associated insomnia and may also demonstrate some overall superiority in its ability to produce deeper sleep, as compared to suvorexant, in preclinical studies in rats.

Adolescent alcohol exposure most likely influences multiple neurotransmitter systems and brain circuits that could ultimately lead to disrupted sleep in young adults, in addition to the hypocretin/orexin system (Veatch 2006). We have shown that persistent reductions in cell counts of ChAT- immunoreactive (ChAT-IR) neurons in the basal forebrain, an area important in sleep regulation, are found in adolescent ethanol vapor–exposed rats (Ehlers et al. 2011). However, whether ChAT-IR reductions are responsible for the fragmentation of sleep found in adolescent ethanol exposed rats is currently unknown. A reasonable hypothesis is that adolescent alcohol exposure influences multiple sleep systems, as has been reported in adult animals exposed to ethanol (see (Ehlers et al. 2013a; Ehlers et al. 2013b; Sanchez-Alavez et al. 2018; Sharma et al. 2010; Sharma et al. 2017; Thakkar et al. 2010)).

Taken together these studies suggest that adolescent alcohol exposure, in the rat, can result in a fragmentation of sleep. This study also showed that DORA-12 can affect the sleep disturbance that is associated with a history of adolescent ethanol exposure but also has several other sleep-promoting effects that are equivalent in both ethanol and control rats. DORA-12 was found to decrease the latency to onset of sleep and produce deeper sleep. While these studies describe potential treatment targets for alcohol-induced sleep pathology, however, they do not mimic the complex environmental and genetic risks for insomnia seen in adolescent and young adult humans (de Zambotti et al. 2018), nor do they model the range of drinking levels observed in adolescent humans (Ehlers et al. 2019).

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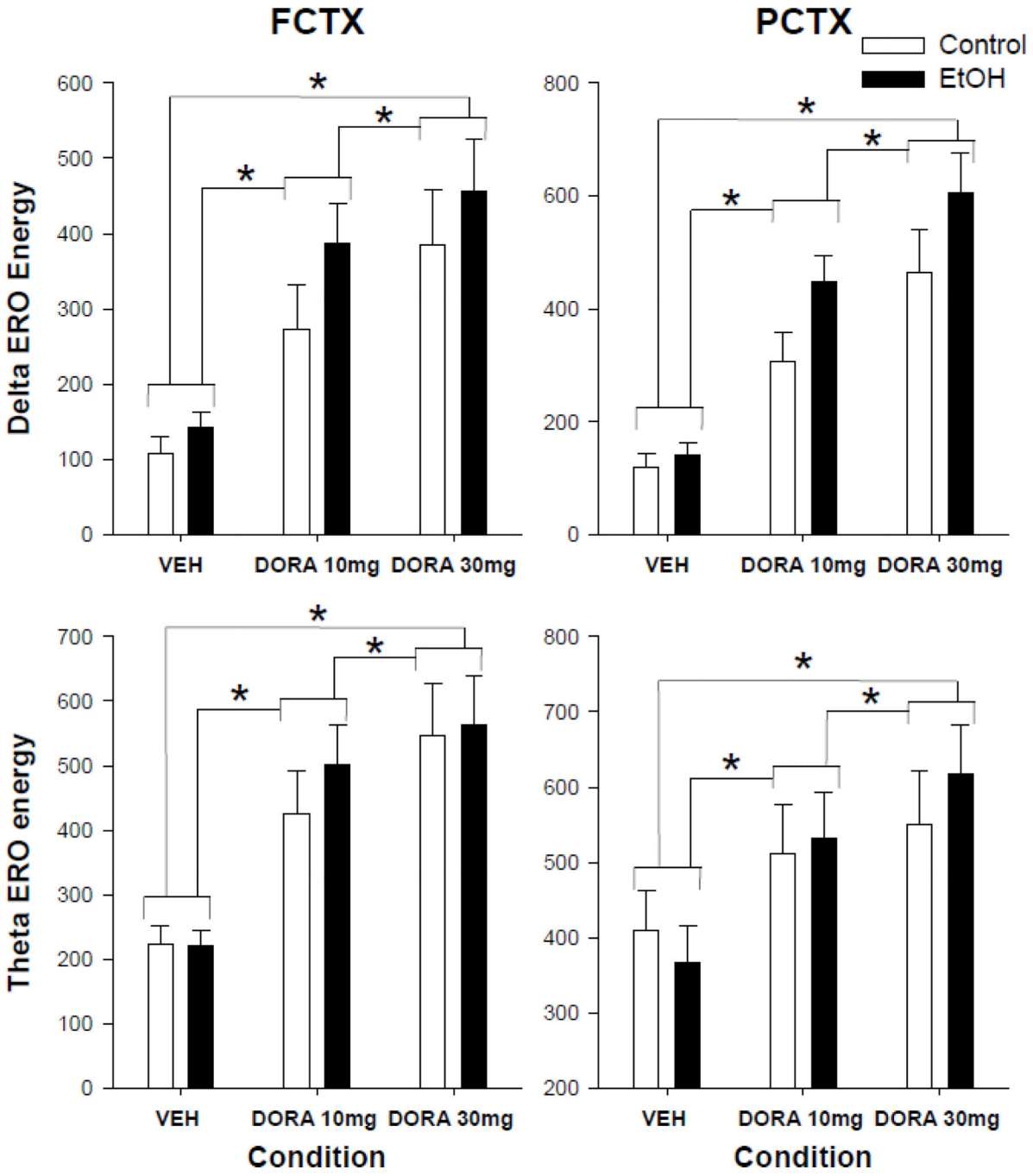

Figure 1.

Event related oscillation (ERO) energy in the delta and theta time-frequency regions of interest (ROI) during waking in frontal and parietal cortex. Delta and theta power at three time points in rats exposed to ethanol vapor during adolescence or air controls: Vehicle (VEH), 10 mg/kg DORA-12(LD), and 30 mg/kg DORA-12(HD). Significant post hoc results shown, * indicates $p \leq 0.05$ dose effect. Error Bars = S.E.M.

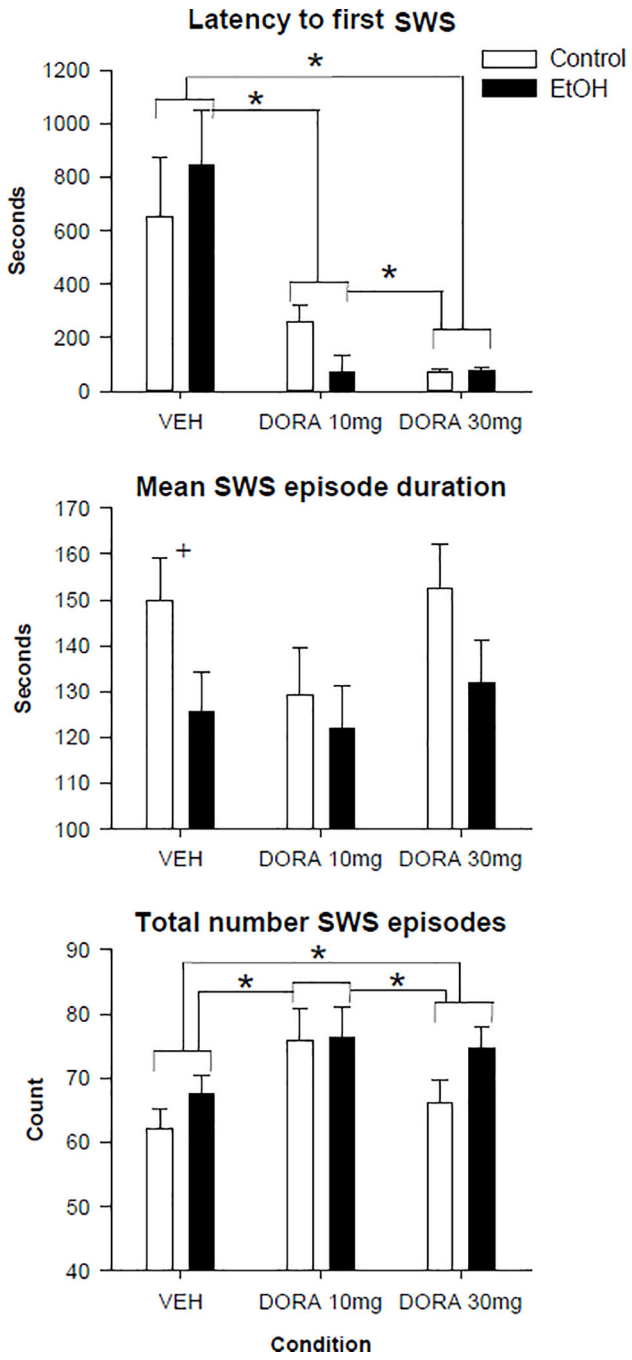

Figure 2.

Slow wave sleep (SWS) measures following vehicle and 2 doses of DORA-12 in control and adolescent alcohol exposed rats. Latency to the first slow wave sleep episode, mean duration and total number of all sleep episodes shown following vehicle (VEH), 10mg/kg DORA-12, and 30 mg/kg, DORA-12. Significant post hoc results shown, + indicates $p \leq 0.05$ group effect. * indicates $p \leq 0.05$ dose effect. Error Bars = S.E.M.

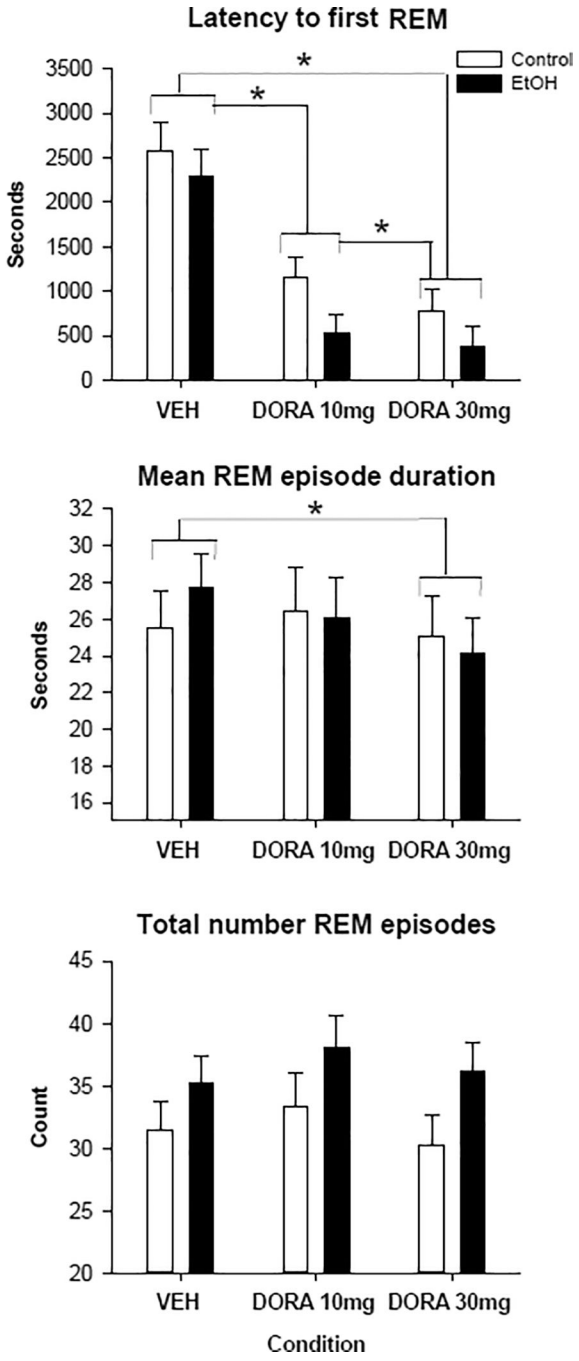

Figure 3.

Rapid eye movement (REM) sleep latency and duration measurements following vehicle and 2 doses of DORA-12 in adolescent alcohol exposed and control rats. Latency to first REM episode, mean duration and total number of all REM episodes shown following vehicle (VEH), 10mg/kg DORA-12, and 30 mg/kg, DORA-12. Significant post hoc results shown, * indicates $p \leq 0.05$ dose effect. Error Bars = S.E.M.

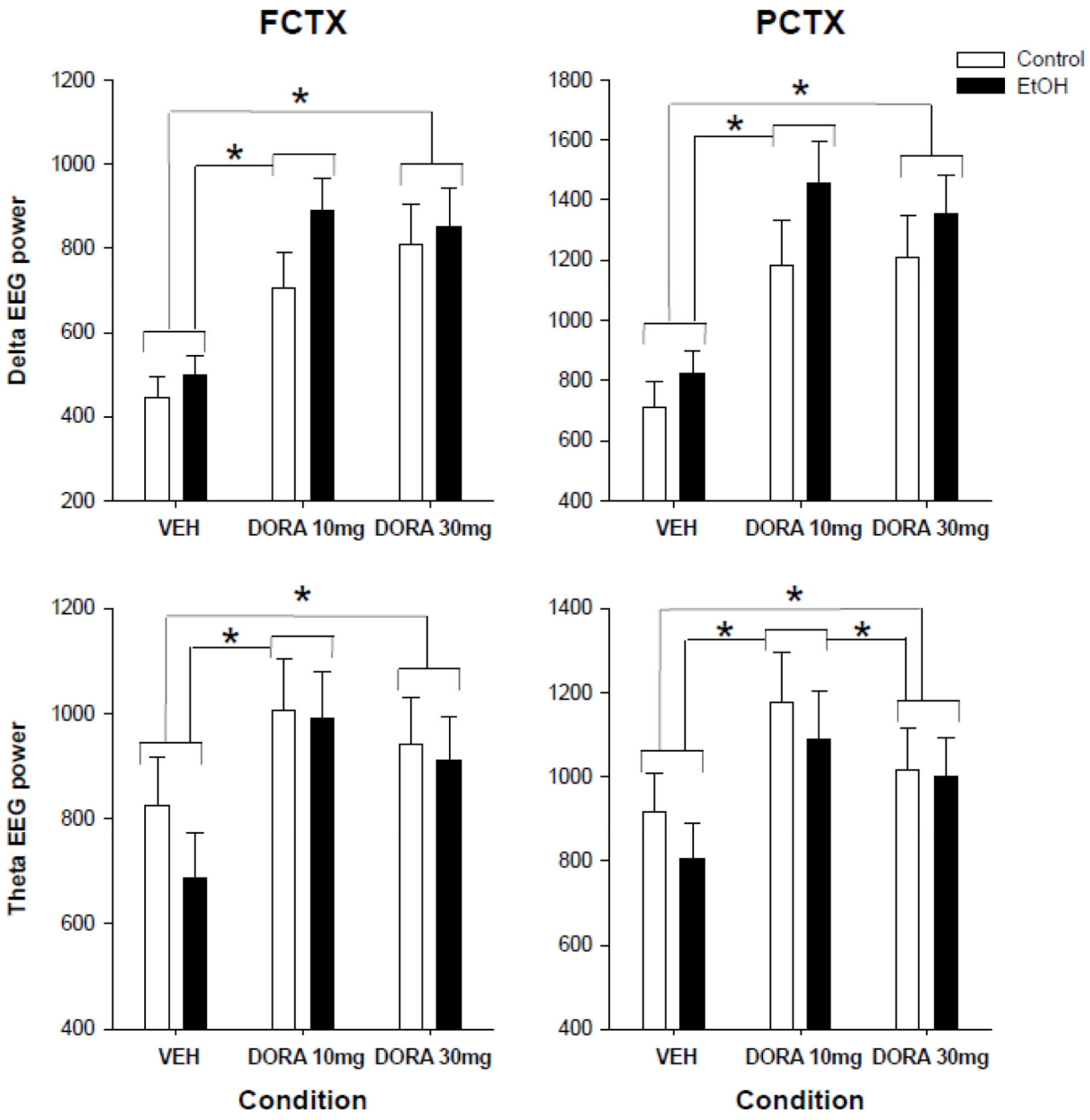

Figure 4.

Delta and Theta power in the EEG during the first slow wave (SWS) sleep episode in frontal and parietal cortex. Delta and theta power during the first slow wave sleep (SWS) at three time points in rats exposed to ethanol vapor during adolescence or air controls following: vehicle (VEH), 10 mg/kg DORA-12(LD), and 30 mg/kg DORA-12(HD). Significant post hoc results shown, * indicates $p \leq 0.05$ dose effect. Error Bars = S.E.M.