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Developmental Ethanol-Induced Sleep Fragmentation, Behavioral Hyperactivity, Cognitive Impairment and Parvalbumin Cell Loss are Prevented by Lithium Co-treatment

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Abstract—Developmental ethanol exposure is a well-known cause of lifelong cognitive deficits, behavioral hyperactivity, emotional dysregulation, and more. In healthy adults, sleep is thought to have a critical involvement in each of these processes. Our previous work has demonstrated that some aspects of cognitive impairment in adult mice exposed at postnatal day 7 (P7) to ethanol (EtOH) correlate with slow-wave sleep (SWS) fragmentation (Wilson et al., 2016). We and others have also previously demonstrated that co-treatment with LiCl on the day of EtOH exposure prevents many of the anatomical and physiological impairments observed in adults. Here we explored cognitive function, diurnal rhythms (activity, temperature), SWS, and parvalbumin (PV) and perineuronal net (PNN)-positive cell densities in adult mice that had received a single day of EtOH exposure on P7 and saline-treated littermate controls. Half of the animals also received a LiCl injection on P7. The results suggest that developmental EtOH resulted in adult behavioral hyperactivity, cognitive impairment, and reduced SWS compared to saline controls. Both of these effects were reduced by LiCl treatment on the day of EtOH exposure. Finally, developmental EtOH resulted in decreased PV/PNN-expressing cells in retrosplenial (RS) cortex and dorsal CA3 hippocampus at P90. As with sleep and behavioral activity, LiCl treatment reduced this decrease in PV expression. Together, these results further clarify the long-lasting effects of developmental EtOH on adult behavior, physiology, and anatomy. Furthermore, they demonstrate the neuroprotective effects of LiCl co-treatment on this wide range of developmental EtOH's long-lasting consequences.

Key words: fetal alcohol syndrome, sleep fragmentation, slow-wave sleep, lithium chloride, insomnia, diurnal rhythm, parvalbumin, perineuronal nets.

INTRODUCTION

Sleep plays a vital role in memory, perception, cognition, emotional regulation, as well as a variety of physiological processes (Stickgold et al., 2001; Yoo et al., 2007; Diekelmann and Born, 2010; Killgore, 2010; Harvey, 2011; Abel et al., 2013; Talamini et al., 2013). Disruptions in sleep can impact any or all of these processes. These sleep effects are bidirectional; for example, reduced sleep can impair memory consolidation (Killgore, 2010; Havekes and Abel, 2017; Krause et al., 2017), while enhanced sleep duration or quality can facilitate memory consolidation (Huber et al., 2004; Marshall et al., 2006; Barnes and Wilson, 2014). Impaired or fragmented sleep (i.e., short sleep bouts, frequent sleep/wake state transitions) is associated with a variety of disorders (Wulff et al., 2010; Krause et al., 2017), and is increasingly seen as a contributing factor in some psychopathologies, rather than just a consequence or side-effect of the psychopathology. For example, treatment of insomnia that is co-morbid with depression can reduce depressive symptoms (Manber et al., 2008).

Among a variety of other consequences (Abel and Sokol, 1986; Riley and McGee, 2005; Mattson et al., 2010), developmental exposure to ethanol disrupts subsequent sleep structure in maturing humans (D'Angiulli

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Abbreviations: AOI, area of interest; LFPs, Local field potentials; LiCl, lithium chloride; P7, postnatal day 7; PBS, phosphate-buffered saline; PNN, perineuronal net; PV, parvalbumin; PV+, PV-positive; RS, retrosplenial; s.c., subcutaneous; SWS, slow-wave sleep; WFA, wisteria floribunda agglutinin.

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et al., 2006; Pesonen et al., 2009; Jan et al., 2010; Wengel et al., 2011; Chen et al., 2012) and other animals (Stone et al., 1996; Veatch, 2006; Criado et al., 2008; Ehlers and Criado, 2010; Wilson et al., 2016), resulting in severe sleep fragmentation. Importantly, our recent work in a mouse model of developmental ethanol exposure suggests that the extent of sleep impairment in adulthood predicts cognitive function as assessed by contextual fear conditioning (Wilson et al., 2016). This leads to the hypothesis that repair or prevention of developmental ethanol exposure effects on sleep could be a potential treatment for cognitive and/or emotional outcomes.

The effects of developmental ethanol on adult sleep could be related to the hyper-excitability of cortical/limbic circuits (D'Angiulli et al., 2006; Criado et al., 2008; Wilson et al., 2011) and/or the loss of GABAergic interneurons (Coleman et al., 2012; Sadrian et al., 2014; Smiley et al., 2015), given the role of GABAergic circuits in sleep–wake cycles (Manfridi et al., 2001; Saper et al., 2010; Xu et al., 2015; Zucca et al., 2017), and other sleep-dependent processes. In particular, parvalbumin (PV)-expressing GABAergic interneurons in hippocampus are also important for sleep-dependent memory consolidation of contextual fear memory (Ognjanovski et al., 2017). Thus, the deficits in contextual fear memory consolidation produced by EtOH may be related to its effects on the GABAergic interneuron populations involved in this and other sleep-related functions, if not sleep structure itself.

Lithium, a common treatment for bipolar disorder, has been shown to have neuroprotective effects in several neuropathological conditions including traumatic brain injury (Yu et al., 2012), intracerebral hemorrhage (Kang et al., 2012), and stroke (Doeppner et al., 2017). Lithium has been demonstrated to affect a variety of molecular cascades related to neural plasticity, neurogenesis, neural migration, and cell survival (Chuang, 2004; Luo, 2009; Luo, 2010; Yu et al., 2012; Doeppner et al., 2017). Lithium treatment near the time of developmental ethanol exposure in mouse models has also been demonstrated to ameliorate many of ethanol's immediate and long-lasting consequences (Zhong et al., 2006; Chakraborty et al., 2008; Young et al., 2008; Luo, 2010; Sadrian et al., 2012), though lithium itself can be a teratogen (Sharma and Rawat, 1986).

Here, as a beginning to our investigation of sleep as a target for treatment of developmental ethanol's behavioral effects, we explored whether lithium chloride (LiCl) co-treatment with postnatal day 7 (P7) ethanol could prevent adult sleep fragmentation that co-occurs with diverse other behavioral and neuroanatomical outcomes. These results significantly extend our previous work by assessing whether the neuroprotective effects of LiCl extend to the sleep, behavioral, and neuroanatomical effects of developmental EtOH. It additionally addresses more detailed neuroanatomical questions by assessing the effects of developmental EtOH on PV cell number and perineuronal nets (PNNs) which frequently surround PV neurons, in specific areas of hippocampus and cortex, and the ability of LiCl to repair these effects. Although LiCl might itself be a teratogen (Sharma and Rawat, 1986), this work begins investigation into whether preventative treatments which may involve the mechanisms of EtOH's developmental action may be a viable target for future investigation.

EXPERIMENTAL PROCEDURES

Subjects

A total of 124 C57BL/6By mice, bred and housed at the Nathan Kline Institute animal facility, were maintained on *ad lib* food and water at all times. All procedures involving animals were approved by the Nathan Kline Institute IACUC and were in accordance with NIH regulations for the proper treatment of animals. Dams and litters were housed in standard mouse cages. Subcutaneous (s.c.) injection of ethanol into P7 mice is a well-established model of developmental ethanol neuropathology (Olney et al., 2002b; Wozniak et al., 2004; Izumi et al., 2005; Gil-Mohapel et al., 2010). While the effects of the frequency of alcohol consumption, potency consumed, and developmental timing of alcohol exposure are factors which produce a range of fatal alcohol-induced developmental deficits (May and Gossage, 2011), this model focuses insult during the rodent brain growth spurt period that is developmentally equivalent to third trimester of human gestation (Schlessinger et al., 1975). P7 pups were injected with ethanol (2.5 g/kg; s.c.) twice at 0 h and 2 h as originally described for C57BL/6 mice (Olney et al., 2002a; Olney et al., 2002b). This model induces a peak truncal blood alcohol level of ~0.5 g/dL at 0.5, 1, 3, and 6 h following the second ethanol injection, as assessed with the Alcohol Reagent Set (Pointe Scientific, Canton, MI, USA) (Saito et al., 2007). This alcohol level is similar to previous reports by others (Wozniak et al., 2004; Young and Olney, 2006; Saito et al., 2007). Lithium chloride (0.6 M LiCl in saline, 10 μl/g, 6 mEq/kg body weight) or saline was injected intraperitoneally 15 min after the first ethanol injection as described in (Zhong et al., 2006; Chakraborty et al., 2008; Sadrian et al., 2012). Pups were returned to the litter after treatment, and typically gain weight normally in the following days (Coleman et al., 2012), though this was not assessed in the current study to limit postnatal handling. Weaning occurred at P25–30 and mice were tested as young adults at 3 months old. Sex differences in the effects of P7 EtOH have not been previously observed (Wilson et al., 2011; Sadrian et al., 2012; Sadrian et al., 2014), nor were any significant differences observed between sexes here (with the exception of one neuroanatomical analysis described below), thus, data from males and females were combined.

Telemetry recordings and slow-wave analyses

Mice (postnatal age 85–100) were anesthetized with isoflurane and surgically implanted with a single stainless steel (125 μ diameter) electrode in the frontal cortex. The electrode and reference were connected to a telemetry transmitter (DSI, model ETA-F10), which was implanted subcutaneously at the back. The

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