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DISTINCT NEUROBEHAVIORAL DYSFUNCTION BASED ON THE TIMING OF DEVELOPMENTAL BINGE-LIKE ALCOHOL EXPOSURE

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Abstract—Gestational exposure to alcohol can result in long-lasting behavioral deficiencies generally described as fetal alcohol spectrum disorder (FASD). FASD-modeled rodent studies of acute ethanol exposure typically select one developmental window to simulate a specific context equivalent of human embryogenesis, and study consequences of ethanol exposure within that particular developmental epoch. Exposure timing is likely a large determinant in the neurobehavioral consequence of early ethanol exposure, as each brain region is variably susceptible to ethanol cytotoxicity and has unique sensitive periods in their development. We made a parallel comparison of the long-term effects of single-day binge ethanol at either embryonic day 8 (E8) or postnatal day 7 (P7) in male and female mice, and here demonstrate the differential long-term impacts on neuroanatomy, behavior and *in vivo* electrophysiology of two systems with very different developmental trajectories. The significant long-term differences in odor-evoked activity, local circuit inhibition, and spontaneous coherence between brain regions in the olfacto-hippocampal pathway that were found as a result of developmental ethanol exposure, varied based on insult timing. Long-term effects on cell proliferation and interneuron cell density were also found to vary by insult timing as well as by region. Finally, spatial memory performance and object exploration were affected in P7-exposed mice, but not E8-exposed mice. Our physiology and behavioral results are conceptually coherent with the neuroanatomical data attained from these same mice. Our results recognize both variable and shared effects of ethanol exposure timing on long-term circuit function and their supported behavior.

Key words: FASD, neural circuit development, piriform, hippocampus, parvalbumin.

INTRODUCTION

Fetal alcohol spectrum disorder (FASD) describes the range of clinically observed developmental deficits resulting from gestational alcohol exposure. Despite widespread acceptance and awareness of the teratogenic effects of alcohol consumption during pregnancy, it persists as one of the greatest causes of mental deficits, impacting an estimated 1 in 100 children born in the U.S. (Abel, 1998; May and Gossage, 2001), with reports of much greater incidence in certain global regions (Harris and Bucens, 2003; Viljoen et al., 2005; May et al., 2011). There are numerous and multi-modal risk factors for FASD that potentiate gestational alcohol exposure. For instance, a woman's age, extent and frequency of consumption, as well as the form of alcohol consumed are all implicated in variable FASD risk (Abel, 1998; May and Gossage, 2011). In addition to these factors, the developmental timing of binge drinking is particularly influential toward the type of neurobehavioral pathology expressed in both human FASD (O'Leary et al., 2010) and animal-modeled FASD (Maier et al., 1997). This suggests that specific brain regions have critical sensitive periods to alcohol toxicity, which can influence pathological outcome.

Temporally confined ethanol exposure (binge or extended binge/semi-chronic) in rodents causes multiple deficits in brain anatomy, physiology and behavior, shown both immediately following exposure (Ikonomidou et al., 2000; Galindo et al., 2005), and in the long-term (Wozniak et al., 2004; Izumi et al., 2005; Wilson et al., 2011; Sadrian et al., 2012). The resulting profiles of pathology in each of these studies originate from a specific epoch of ethanol exposure (as brief as a single day), and therefore limit developmental correlates to a snapshot. Furthermore, the comparative impacts on long-term *in vivo* circuit function from different developmental points of exposure have not been examined. It is of particular importance to examine circuit function outcome in fully developed adults, so that the long-term impacts of developmental ethanol exposure may be further investigated in a coordinated way that has clinical relevance for those affected with FASD.

We and others have previously shown that single day binge ethanol at postnatal day 7 (P7) creates an immediate wave of neurodegeneration in specific brain regions that include the hippocampus and specific

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Abbreviations: ANOVA, analysis of variance; AOI, area of interest; aPCX, anterior piriform cortex; BAL, blood alcohol level; BrdU, Bromodeoxyuridine; E8, embryonic day 8; FASD, fetal alcohol spectrum disorder; IPI, inter-pulse intervals; ITI, inter-trial interval; LFP, local field potential; LOT, lateral olfactory tract; OB, olfactory bulb; P7, postnatal day 7; PCX, piriform cortex; PV, parvalbumin; PV+, PV-positive.

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cortical regions (Ikonomidou et al., 2000; Saito et al., 2010; Wilson et al., 2011). In the same mice we also observed long-term differences in local and regional neuronal circuit function within the olfacto-hippocampal pathway, thereby establishing a model of long-term ethanol-induced neuronal circuit dysfunction. These effects were prevented when the neuroprotective agent lithium was given on the same day as ethanol (Sadrian et al., 2012). We hypothesize that the immediate wave of neurodegeneration caused by binge ethanol toxicity initiates disruptive cascades in circuit maturation, the effects of which are sustained long after the ethanol has been metabolized. The susceptibility to ethanol-induced cell loss has been shown to vary based on developmental stage of exposure (Ikonomidou et al., 2000) as well as by cell type (Tran and Kelly, 2003). The specific timing of ethanol-derived cytotoxicity is likely of crucial importance with regard to the specific long-term functional outcomes.

FASD-modeled rodent studies examining acute early embryonic exposure (around embryonic day 8 – E8) have been used to simulate alcohol toxicity during human brain development in the first trimester. During this period gastrulation and neurulation occur, and acute ethanol exposure induces excessive apoptotic cell death in many regions, especially in the developing CNS (Dunty et al., 2001). Acute exposure at P7 in rodents on the other hand represents exposure during a sensitive midpoint in the human embryonic third trimester equivalent (Cudd, 2005), which is considered the brain growth spurt period of rampant synaptogenesis and refinement in behaviorally relevant circuits (Dobbing and Sands, 1979; Bonthius and West, 1991). Alcohol insult during different stages of embryogenesis will affect different populations of cells, disrupt different developmental processes, and make different long-term functional impacts, which have not been fully described previously.

Here, we took advantage of an extended functional circuit – the olfacto-hippocampal pathway which includes regions expressing very different neurobehavioral developmental trajectories (Webster et al., 1983; Seress, 2007; Sarma et al., 2011; Burd et al., 2012). For example, the primary olfactory system (including the olfactory bulb [OB] and piriform cortex [PCX]) is functional at birth in rodents (Brunjes, 1994; Miller and Spear, 2009; Sarma et al., 2011), and in fact is necessary for infant survival (Moriceau and Sullivan, 2004). In contrast, the hippocampal formation which receives robust olfactory input via the entorhinal cortex, is a relatively late developing system with hippocampal-dependent behaviors not emerging until near weaning in rodents (Freeman et al., 1994; Rudy, 1994; Raineki et al., 2010). Despite these differences in functional emergence, it is interesting that both the OB and hippocampal formation show continued neurogenesis throughout life (Lledo et al., 2006). Given the differences in timing of development between these two components of the olfacto-hippocampal pathway, we hypothesized different temporal sensitivities to developmental ethanol exposure. We utilized the single day acute (binge-like) exposure model in mice, delivering ethanol or saline either intraperitoneally to pregnant mothers at E8 or via direct subcutaneous injection to P7 pups. The binge model was specifically chosen to allow a developmental dissection of when different cell types and circuits are particularly vulnerable to ethanol exposure (Sadrian et al., 2013). Each group was tested as adults at 15 weeks of age for long-term outcomes in cell proliferation, interneuron cell count, spontaneous and odor-evoked *in vivo* physiology, and spatial memory performance (please see schema in Fig. 1).

EXPERIMENTAL PROCEDURES

Subjects

C57BL/6By mice were bred at the Nathan Kline Institute animal facility, and maintained on *ad lib* food and water at all times. All procedures were approved by the Nathan Kline Institute IACUC and were in accordance with NIH guidelines for the proper treatment of animals. E8 embryos were exposed to saline or ethanol via intraperitoneal injection to the mother, twice at a four hour interval with 2.8 g/kg (each dose) ethanol in saline for the ethanol group and saline only for the control group as previously described (Parnell et al., 2009). P7 pups were directly injected subcutaneously with saline or ethanol as described (Olney et al., 2002; Saito et al., 2007). Each mouse in a litter was assigned to the saline or ethanol group at an equivalent proportion of the total number of mice with a distributed gender ratio. Ethanol treatment (2.5 g/kg) was delivered twice in the same day at a 2-h interval as originally described for C57BL/6 mice (Olney et al., 2002). After injections, pups were returned

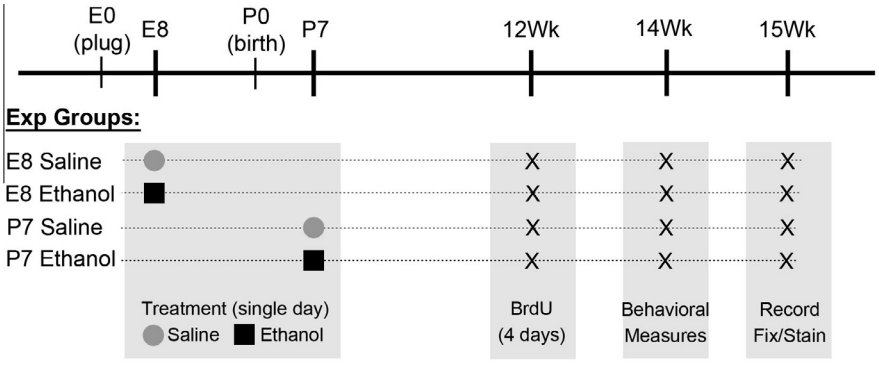

Fig. 1. Schematic of ethanol and control saline treatment timing for each experimental group, including points for each measure taken. Acute anesthetized recordings were made immediately prior to tissue fixation for subsequent immunohistochemical analysis.