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Postnatal Ethanol Exposure and Attention: Implications for Sustained Attention Performance and Cholinergic System Integrity in Adult Rats

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POSTNATAL ETHANOL EXPOSURE AND ATTENTION

Implications for sustained attention performance and cholinergic system integrity in adult rats

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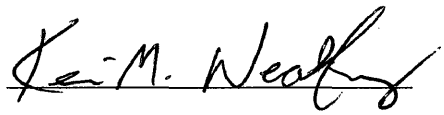

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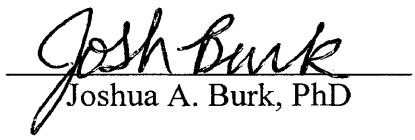

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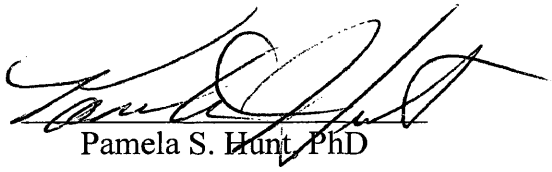

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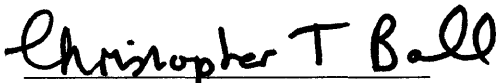

Christopher T. Ball, PhD

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ABSTRACT

Fetal alcohol exposure is known to have deleterious effects on sustained attention in humans, a deficit that may be due to altered development of the cholinergic system. This experiment was designed to model these ethanol-induced attentional deficits in rats using a task that has been shown to be sensitive to alterations in the activity of the basal forebrain cholinergic system. Male Long-Evans rats were randomly assigned to one of three groups: ethanol (5.25 g/kg/day) administration via intragastric intubation on postnatal days (PD) 4-9, sham-intubation, or no treatment. Beginning on PD 90, all rats were shaped to perform a two-lever sustained attention task that required discrimination of brief and variable visual signals (500, 100, and 25msec illumination of the central panel light) and of non-signals. The ethanol exposed rats did not exhibit a delay in reaching criterion during early, less attention-demanding stages of training. In the final version of the task, ethanol-exposed rats showed a deficit relative to the other groups in accurately detecting signals of all durations that was stable over additional training sessions. Further task manipulations including a distracter task, and short and long intertrial intervals did not differentially affect the ethanol and control conditions. Acetylcholinesterase staining revealed minor reductions in EtOH exposed animal anterior cingulate and posterior parietal cortex fiber density. The implications of these findings for future work on the interaction between ethanol and the cholinergic system are also discussed.

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Introduction

Fetal Alcohol Exposure in Humans

The consumption of alcohol by pregnant women has been shown to have devastating effects on fetal development. Intrauterine alcohol exposure has been linked to a number of birth defects including structural abnormalities and neurocognitive deficits. In its most severe form, prenatal alcohol exposure can result in Fetal Alcohol Syndrome (FAS), a condition characterized by a) growth deficiency (pre and/or postnatal), b) a distinctive pattern of craniofacial dysmorphology, and c) central nervous system dysfunction (Streissguth, Landesman-Dwyer, Martin, & Smith, 1980; Jones & Smith, 1973). Behaviors exhibited by FAS patients include hyperactivity, reduced ability to habituate to irrelevant stimuli, irritability, poor attention span, and very low I.Q. scores (Streissguth et al., 1980). Estimates indicate that FAS is one of the leading causes of mental retardation in newborns (Abel & Sokol, 1987) and that FAS and other prenatal alcohol exposure-related disorders are present in 1 of every 100 live births (Sampson et al., 1997).

Overwhelming clinical and experimental evidence indicates that damage by prenatal alcohol exposure is dose-dependant and can be conceptualized as a continuum of impairment (Stratton, Howe, & Battaglia, 1996; Smith, Coles, Lancaster, Fernhoff, & Falek, 1986). Many individuals prenatally exposed to alcohol do not present all of the symptoms required for a formal FAS diagnosis. A broader term that describes any alcohol related adverse birth outcome including sub-FAS threshold symptoms is Fetal Alcohol Effects (Clarren and Smith, 1978). Recently, a more refined diagnostic category has been devised for classifying humans suffering from non-FAS prenatal alcohol

exposure impairment. An alcohol-related neurodevelopmental disorder (ARND) designation indicates that a person has some level of central nervous system abnormality as evidenced by brain structural anomalies, various neurological hard and soft signs (e.g impaired fine motor skills, neurosensory hearing loss, poor eye-hand coordination), and a complex pattern of behavioral and cognitive abnormalities (Stratton, Howe, & Battaglia, 1996).

FAS/ARND symptoms are complex and can vary over time. Structural symptoms such as growth deficiency are most profound during infancy but are partially attenuated as the child matures (Streissguth, Barr, & Martin, 1983; Streissguth, Martin, Barr & Sandman, 1984; Streissguth, Barr, Kogan & Bookstein, 1997). Though some of the craniofacial abnormalities become less pronounced during childhood and disappear after adolescence, the neurobehavioral effects of prenatal alcohol exposure remain prominent. The Seattle Longitudinal Perspective Study is currently tracking the development of individuals with FAS/ARND and has revealed deficits in attention, memory and auditory processing in afflicted 14 year olds (Striessguth, et al., 1994). However, much is still unknown about adult patterns of FAS/ARND symptoms. Correlational studies indicate that adults with FAS/ARND are at much greater risk for experiencing mental health problems, trouble with the law/incarceration, and inappropriate sexual behavior (Streissguth, Barr, Kogan, & Bookstein, 1996).

Research on fetal alcohol exposure is challenging due to the global effects of alcohol on the brain and the vast array of potentially influential developmental factors involved. For example, the severity of FAS/ARND symptoms has been associated with both the timing and amount of alcohol consumed by the mother. Consuming large

quantities of alcohol over short periods of time (binge-like drinking) is more neurobehaviorally detrimental to the fetus than equal amounts diffused over a greater time interval (West, Goodlett, Bonthius, & Pierce, 1989). Also important is the fetus's stage of development during ethanol exposure. Three stages of development have been highlighted that result in differential outcomes with ethanol insult. The predifferentiation period refers to the time between fertilization and implantation into the endometrium. Binge-drinking during this stage seems to result in either the death of the blastocyte or little effect at all. The second stage begins with major cell differentiation and migration and ends with completion of organogenesis. It is at this stage when the embryo is most vulnerable to ethanol-induced structural abnormalities such as craniofacial dysmorphology, and microencephaly (Cartwright & Smith, 1995). The interval from the end of organogenesis until birth, known as the period of the fetus, is the stage in which the fetal brain tissue is susceptible to more subtle damage from abnormal migration, synaptogenesis, and continued pathological cell loss (Goodlett & Johnson, 1999). Such damage is often manifest in behavioral disorders with the absence of gross structural deformities.

Fetal alcohol exposure: Implications for attention

Among the most persistent and potentially debilitating effects of prenatal ethanol exposure are deficits in attention. However, a general consensus on the exact nature of attention has not been reached and the literature is rife with disparate definitions of the attentional construct. Though this complicates the interpretation of some research results, there is still a substantial amount of evidence indicating a correlation between prenatal ethanol exposure and attentional impairment. It is useful to review results reported from

a variety of research perspectives including cognitive, behavioral and clinical studies before returning to the issue of defining attention.

As infant research avoids many potential environmental and experiential confounds, it is a useful tool in evaluating the effects of prenatal alcohol exposure on attention. It has been demonstrated that the cardiac-orienting response, the dramatic reduction in heart rate observed upon the introduction of a novel stimulus, is related to infant attentional capacity (Richards, 1989). Through analyzing heart rate fluctuation in response to novel stimuli, high-risk 6 month olds (as identified by maternal drinking reports) showed attentional deficits in latency to detect visual & auditory stimuli as determined by delayed heart rate depression (Kable & Coles, 2004). This delayed orienting response was interpreted by the authors as a reduction in the high-risk children's speed of processing. It was noted that the orienting response occurs through an inhibitory signal that prevents what would otherwise be a stimulus-related increase in arousal/heart rate. A lack of inhibition of this initial acceleratory heart rate response was identified as a possible cause of the diminished processing speed. Interestingly, older infants are better able to attenuate the initial acceleratory response, suggesting that ethanol-exposed children may be developmentally delayed in this respect. This possibility is an intriguing one and is partially supported by neurological findings that will be addressed below.

To assess attentional capacity in children and adults, the Continuous Performance Test (CPT), a task originally developed to assess brain damage in clinical populations (Rosvold, Mirsky, Sarason, Bransome, & Beck, 1956), is often utilized. These tests are computer controlled and require the participant to respond to unpredictable, rarely

occurring stimuli for an extended period of time. The CPT attempts to evaluate the vigilance or sustained attention aspect of attention and is also capable of reporting reaction times. Assessment of 4-year-old fetal alcohol exposed (FAE) children using a CPT demonstrated poorer performance and extended reaction time relative to age matched controls (Streissguth, et al., 1984). Specifically, these children made more errors of commission (indicating the presence of a stimulus when it was absent) and omission (failing to respond to a stimulus when it was present). These results remained significant even after adjusting for a number of potentially confounding variables such as maternal education level and prenatal nicotine/caffeine exposure.

A longitudinal study by the same authors has followed the children in the original Streissguth et al. (1984) study into adolescence, reporting continued impairment at 7 and 14 years of age (Streissguth, Bookstein, Sampson & Barr, 1995). This study sought to characterize the stability of fetal alcohol exposure-related attentional deficits over time and to determine the correlation between maternal alcohol consumption and outcome. A partial least squares design was incorporated to determine which aspects of maternal drinking were most salient (i.e. most highly correlated with the composite vigilance measure) for child outcome and conversely, which outcome measure was most salient for maternal drinking. Both total ounces of alcohol consumed and degree of binge consumption were highly salient to outcome. The most salient outcome measure was the standard deviation of reaction time (SDRT) in which alcohol exposed children exhibited greater variability in their latency to respond to task stimuli. Interestingly, children scoring with the lowest composite vigilance score (a combination of omissions, false alarms, reaction time, etc.) at age 14 also had low scores at age 7. Furthermore, these

composite vigilance scores were capable of predicting scores on teacher-generated attention reports in both second, fourth and fifth grade. Though cross-sectional studies provide evidence for the persistence of FAE-related attentional deficits through adolescence (e.g. Brown et al. 1991) and into adulthood (Connor, Streissguth, Sampson, Bookstein, & Barr, 1999), results from longitudinal studies such as the Seattle longitudinal prospective study on alcohol and pregnancy are necessary to demonstrate the consistency of this dysfunction over time.

As previously mentioned, past research has been largely inconsistent in the definition of attention as a cognitive construct. Though many studies have reported attentional deficits in response to alcohol and other neurotoxic substances, caution must be exercised in making comparisons between the results of these studies. Attentional measures have been taken using a variety of methodologies such as qualitative descriptions of behavior, heart rate depression, and computerized continuous performance tasks. Frequently, animal models are also employed in the study of attention, adding another level of complexity to the interpretation of reported attentional deficits.

In response to this confusion, some have sought to systematize the process of attentional assessment. Mirsky and colleagues reviewed the neuropsychological literature and, recognizing that attention is likely a multifaceted construct, described three primary components that likely comprise attention: *focus*, *sustain*, and *shift* (Mirsky, Anthony, Duncan, Ahearn, & Kellam, 1991). The focus component represents the ability to select a single stimulus from an array for further processing. The sustain component (i.e. vigilance) is the ability to maintain focus on a target stimulus over time. The shift

component represents the ability to maintain flexibility in attentional focus. To provide support for this model, a principal components analysis of a variety of attention test scores from 203 adult neuropsychiatric patients was conducted. Such analyses are designed to organize and simplify the relationships among the test scores and reveal any underlying attentional components that may be characterized by the collective battery of tests. The analysis revealed a 4 factor structure, indicating 4 distinct attentional components. Based on the tasks that loaded (i.e. were correlated with) highly on 3 of the factors, the focus, sustain and shift labels could be confidently assigned to these factors. Also identified was a new factor termed *encode* that is thought to represent a memorial aspect of attention. A second principal components analysis of data collected from 435 elementary school children yielded a similar factor structure, further supporting the Mirksy et al. model.

The need for a clearly defined attention construct is made apparent by work comparing children with FAS and children with attention deficit, hyperactivity disorder (ADHD). Traditional methods of assessing attention (e.g. parental report, Wechsler Intelligence Scale for Children) were not able to differentiate between FAS and ADHD behavioral manifestations of attention and led researchers to conclude that FAS and ADHD children have similar patterns of impairment and even to suggest that treatments for ADHD may also be effective for FAS (Nanson & Hiscock, 1990). In contrast, researchers employing Mirksy's multicomponent model of attention found distinct patterns of deficits for FAS and ADHD children (Coles, Platzman, Raskind-Hood, Brown, Falek, & Smith, 1997). Specifically, FAS children exhibited poorer performance on encoding and shift measures relative to controls whereas ADHD children were most

impaired in the focus and sustain domains. Discriminant function analysis demonstrated that FAS group membership was most accurately predicted using the Mirsky et al. paradigm-related tasks as opposed to conventional ADHD diagnostic methods. These results accentuate the importance of a precisely defined attentional component when characterizing the behavioral deficits of a clinical disorder.

Surprisingly, the Coles et al. (1997) study did not report a deficit in sustained attention in FAS children, but rather *better* performance on a CPT task relative to controls. There are a number of explanations for these results such as the high attrition rate (52%) or sample-specific demographic factors, but more importantly, this line of inquiry raises questions about what parameters should be assessed in a vigilance test. Though many tasks purportedly assess vigilance, these tasks usually have only weak correlations with one another as is evidenced by the disparity in the Coles et al. (1997) study and the Streissguth et al. (1997) study. To address this issue, Parasuraman and colleagues have worked to develop a task classification system to aid in the interpretation of vigilance task performance results and guide future task development (Parasuraman & Davies, 1976; Parasuraman, Warm, & Davies, 1987). Importantly, this taxonomy is not derived from factor analysis of human abilities or vague concepts not conducive to empirical assessment (e.g. task induced boredom, level of arousal) but rather processes that occur specifically during vigilant behavior such as referencing recent memory and reassessment of response criterion.

Parasuraman et al. (1987) note that a finding consistent in most vigilance paradigms is the presence of a vigilance decrement, or reduction in the speed and accuracy of signal detection over time and have highlighted a number of task

characteristics that affect the vigilance decrement. The first task attribute is the target stimulus signal type, which may be either simultaneous or successive. Simultaneous presentation of target and non-target stimuli is less demanding, as the participant is able to make direct comparisons between the stimuli. Conversely, successive presentation of stimuli requires the individual to access recent memory and make comparisons between the current stimulus and the stimulus previously detected. A second taxonomic category is the stimulus event rate. Higher event rates are associated with poorer accuracy and should quicken the onset of the vigilance decrement. The third category is the sensory modality used to detect signals (auditory/visual). Source complexity (i.e. multiple vs. single points of signal origin) is the fourth taxonomic category. It is possible that the conflicting results observed by Coles et al. (1997) and Streissguth et al. (1995) could be accounted for by carefully classifying the vigilance tasks used in both experiments. Though Parasuraman et al. taxonomy was designed for human vigilance tasks, it has been successfully incorporated in animal tests of sustained attention (e.g. McGaughy & Sarter, 1995). This important development has aided in the characterization of brain structures and neurotransmitter systems essential to sustained attention. A brief review of this literature is warranted.

Neurobiology of Sustained Attention

Two influential models of the neural correlates of attention have been posited by Posner & Peterson (1990) and Mesulam (1981). Though some discrepancies exist between the models, both include an anterior and a posterior attention system that are subserved by an arousal-modulating component. The anterior system is thought to be involved in target signal detection and may consist of the anterior cingulate and other

prefrontal cortical regions mediating motor responses. The posterior system is responsible for spatial orientation and is thought to arise from parietal cortex, the superior colliculus and the thalamic pulvinar nucleus. These models also emphasize the role of noradrenergic neurons in the locus coeruleus in maintaining a sufficient level of arousal for attentional processing to persist. A variety of electrophysiological, imaging, and pharmacological studies have supported the basic principles of the Posner and Mesulam models (see Coull (1998) for a review).

Much of the evidence identifying macroanatomical structures important in sustained attention arises from studies conducted on neuropsychiatric populations and brain imaging studies (Sarter, Givens & Bruno, 2001). Patients with damage to right frontal and posterior cortical regions exhibited performance deficits on a CPT and a lengthy, multi-trial reaction time task (Rueckert & Grafman, 1996; 1998). Positron emission tomography studies have also implicated a right prefrontal system as a neural substrate essential to sustained attention. For example, healthy subjects completing vigilance tasks displayed increased blood flow in right prefrontal and superior parietal cortex, regardless of task sensory modality (Pardo, Fox, & Raichle, 1991). Though these studies highlight the role of gross brain structures in vigilant performance, additional work is required to fully characterize the neural circuits underlying sustained attention.

In order to accurately assess the components of putative neural circuits, it is useful to analyze the performance of the entire system after compromising a specific element. Animal studies provide an effective means of accomplishing this goal and have been used extensively for this purpose. This process has been aided by the creation of animal attention tasks that are analogous to those that evaluate human sustained attention

performance. For instance, a spatial monitoring task has been developed that requires animals to respond to stimuli presented in an array of 5 potential sources (Robbins, Everitt, Marston, Wilkinson, Jones, & Page, 1989). An operant task of sustained attention has also been developed in which animals are required to detect and respond to brief and rare visual signals (McGaughy & Sarter, 1995). Studies incorporating these tasks have attempted to investigate various nuclei that project to cortical regions purported to mediate sustained attention.

A region that has been demonstrated to be essential to sustained attention is the basal forebrain. This structure features a number of sub-regions and a heterogeneous cellular makeup with both cholinergic and non-cholinergic neurons present in varying concentrations. To simplify the complex organization of the basal forebrain, Mesulam (1995) has proposed a system for identifying BF cholinergic neurons based on the regions to which they project. Cholinergic neurons of the medial septum and the vertical limb of the diagonal band of Broca innervate the hippocampus and are labeled Ch1 and Ch2, respectively. The olfactory bulb receives projections from cholinergic cells in the horizontal nucleus of the diagonal band of Broca that are designated Ch3. The final component of the BF is the nucleus basalis of Meynert (nbM) or Ch4. This large collection of cholinergic neurons is found in the substantia innominata and projects to widespread cortical regions.

Both lesion and pharmacological studies have strongly implicated the basal forebrain cholinergic system in sustained attention (Sarter et al., 2001). Excitoxic lesions of the substantia innominata in rats have resulted in performance deficits on the 5-choice serial reaction time task (Robbins et al, 1989). Non-human primate studies have also

shown attentional deficits in ibotenic acid lesioned subjects as assessed by a variation of Posner's (1987) covert orientation task (Voytko, 1994). A pharmacological study using rats has revealed that drugs that alter cholinergic signaling in the cortex (e.g. scopolamine and pilocarpine) have deleterious effects on sustained attention (Bushnell, Oshiro, & Padnos, 1997). Similarly, pharmacological manipulations of the cholinergic system in healthy adult humans using nicotine (a nicotinic receptor agonist) and scopolamine (a muscarinic receptor antagonist) have altered performance on vigilance tasks (Wesnes & Revell, 1984). Nicotine improved signal detection and response speed whereas scopolamine reduced accuracy.

Rats with highly specific lesions to cholinergic cells in the nbM have also been shown to exhibit performance decrements in McGaughy & Sarter's (1995) operant task (McGaughy, Kaiser, & Sarter, 1996). Immunotoxic lesions using 192 IgG-saporin provide robust lesioning of corticopetal cholinergic neurons without significant damage to neighboring cells that project to the amygdala. Such lesions resulted in deficits in signal detection but not in the correct rejection of non-signal trials, suggesting that the lesions did not interfere with the rats' mnemonic representation of the task rules or the motor skills required for response. Additionally, the extent of the NBM lesion was highly correlated with the degree of behavioral impairment.

Given the pattern of NBM cholinergic efferents found throughout the cortex, it is not surprising that these neurons play an important role in regulating higher attention centers. Cholinergic fibers of NBM origin have been detected in an extensive number of brain regions and appear to be uniformly distributed over cortical regions and layers (Quirion, Richard, & Wilson, 1987). Also interesting is the finding that acetylcholine

release appears to be homogenous, with comparable amounts of ACh being recorded at diverse cortical sites following excitation of corticopetal cholinergic afferents (Phillis & Chong, 1965). Collectively, these data indicate that cortical cholinergic innervation is best conceptualized as exerting system-level control rather than influencing specific populations of cells (Sarter & Bruno, 1997).

Though cholinergic innervation of the cortex was previously suspected to play a role in cortical activation, its specific function remained unknown until Krnjevic, Pumain & Renaud (1971) demonstrated the ability of ACh to amplify other cortical input. Studies have reported ACh-amplified input signals in visual, auditory, and somatosensory cortex, as well as prefrontal association areas (See Sarter & Bruno, 1997 for a review). Such evidence indicates that cortical ACh release functionally enables frontal and parietal cortices to detect and process sensory input more efficiently. Collectively, findings from basal forebrain cholinergic manipulation studies indicate that acetylcholine release in the cortex may be capable of facilitating "bottom-up" or sensory driven attentional mechanisms (Sarter, Givens, & Bruno, 2001).

Individuals prenatally exposed to ethanol demonstrate impaired sustained attention and strong evidence exists implicating the cholinergic system in sustained attention. Therefore, it is worthwhile to review the literature concerning the effects of ethanol on cholinergic system integrity.

The Effects of Perinatal Alcohol Exposure on the Cholinergic System

Though the behavioral outcomes of fetal alcohol exposure have been well characterized, the underlying teratogenic neurochemistry is still poorly understood. Recent work has indicated that ethanol is capable of inducing widespread apoptotic

activity in the fetal cortex (Ikonomidou et al., 2000). It is known that blocking NMDA receptors during synaptogenesis causes apoptotic neural cell death (Ikonomidou et al., 1999). Ethanol, an NMDA receptor antagonist, produced apoptotic effects similar to but more extensive than other NMDA receptor antagonists such as MK-801. Drugs that elevate GABA activity during synaptogenesis were shown to create patterns of neurodegeneration that matched those of ethanol but could not be accounted for by NMDA blockade (Ikonomidou et al., 2000). This dual NMDA/GABA apoptotic pathway results in dramatic reductions in brain mass and likely contributes to the microencephaly and neurobehavioral deficits commonly observed in developing animals exposed to alcohol.

Another putative mechanism for the effects of fetal alcohol exposure is the disruption of normative cholinergic functioning (Costa & Guizzetti,1999). Ethanol has been shown to alter functioning in a variety of second messenger systems, including acetylcholine-binding muscarinic receptors. Recent studies have indicated that muscarinic receptors are instrumental in neuroprotection and cell proliferation for both neurons and glial cells during rat development (Guizzetti, Costa, Peters, & Costa, 1996; Mount, Dreyfus & Black, 1994). Muscarinic inhibition by ethanol may contribute to cell loss and cognitive impairment observed in ethanol-exposed children.

A substantial body of evidence indicates that ACh plays a morphogenic role during ontogeny in addition to its mature function as a moderator of sustained attention (Hohmann & Berger-Sweeney, 1998). Studies have demonstrated that basal forebrain cholinergic projections are capable of regulating the development of cortical networks. Animals given electrolytic and 192 IgG-saporin basal forebrain lesions on PD 1 and 3

exhibited alterations in cortical organization in adulthood (Hohmann & Berger-Sweeney, 1998; Ricceri, Hohmann, & Berger-Sweeney, 2002). This disorganization is characterized by alterations in cortical layer thickness and is expressed differentially in males and females. Lesion induced cortical alteration has also been linked to behavioral deficits in Morris water maze performance (Arters, Hohmann, Mills, Olaghere &Berger-Sweeney, 1998).

The timing of cholinergic intervention also appears crucial in determining the extent and nature of subsequent cortical irregularity. A study examining the effects of the acetylcholinesterase inhibitor chlorpyrifos identified gestational days 17-21 as particularly vulnerable to chemical insult (Qiao, Seidler, Padilla & Slotkin 2002). This period overlaps with an intense period of neurogenesis in the basal forebrain (Semba & Fibiger, 1988). It has also been hypothesized that the cholinergic system formation may experience a second critical period of vulnerability that occurs postnatally in rats (Berger-Sweeney, 2003). During the first postnatal week, cholinergic projections enter the cortex and undergo synaptogenesis with their eventual target cells (Hohmann & Ebner, 1985). In the following week, there is a steep rise in cortical concentrations of acetylcholine (Coyle, & Yamamura, 1976). It has also been demonstrated that basal forebrain cholinergic neurons undergo a period of somatic and dendritic reorganization postnatally that peaks on PD 18 (Gould, Farris, & Butcher, 1989). Interference with the morphogenic role of ACh during this period may have direct repercussions for cortical organization.

A histological and immunocytochemical study of the mouse brain yields further support for the hypothesis that prenatal ethanol affects the cholinergic system (Schambra,

Lauder, Petrusz & Sulik, 1990). Pregnant mice were administered a high dose of ethanol (2.9 g/kg maternal body weight) on gestational day 7. Immunocytochemical analysis revealed a substantial reduction in ChAT activity as well as structural abnormalities in the ventricular zone where cholinergic target cells normally reside. Catecholaminergic and monaminergic systems originating in more rhombencephalic structures were not similarly affected. This finding is consistent with earlier evidence indicating a reduction in cortical acetylcholinesterase levels in response to prenatal and early postnatal ethanol exposure (Rudeen & Guerri, 1985). In this study, animals received alcohol in one of three regimens: 1) GD 14-PD-14, 2) entire gestational period, 3) entire gestational period, continuing through PD-14). Interestingly, deficits in telenchephalic enzyme levels for the late gestation/early postnatal exposure group were not detected until PD 25. Light, Serbus & Santiago (1989) also reported striatal AChE level abnormalities in response to postnatal ethanol exposure on PD 4-8. This effect persisted through day 20.

Additional evidence from animal studies indicates that disturbances in cholinergic function may persist into adulthood. Three month old rats exposed to ethanol prenatally exhibited differential memory deficits in response to cholinergic drugs in a spontaneous alternation task (Nagahara & Handa, 1999). Low doses of scopolamine (0.1 mg/kg), a muscarinic antagonist, were capable of impairing memory in prenatal ethanol-exposed rats but not control rats. Given that ethanol's potential teratogenic mechanism may feature cholinergic compromise, the effects of early postnatal choline diet supplementation on cognitive performance have been explored (Thomas, La Fiette, Quinn & Riley, 2000). Rats prenatally exposed to ethanol were intubated with a choline-rich solution on PD 2-21. These rats performed significantly better than their non-

supplemented peers and did not differ from control animals on a visual discrimination task administered on PD 45. Taken together, these findings indicate that ethanol-induced cholinergic alterations are robust and are not attenuated over time.

Examining the effects of prenatal ethanol exposure on sustained attention in adult rats

In reviewing the relevant literature, three recurrent themes emerge; 1) Prenatal ethanol exposure is strongly related to profound deficits in human attention, 2) Prenatal ethanol exposure is capable of producing abnormalities in cholinergic system functioning, and 3) the effects of prenatal ethanol exposure persist at least through early adolescence. It is the goal of the current study to build on these findings through exploring the effects of early postnatal ethanol exposure on sustained attention using an animal model of fetal alcohol exposure and a test of sustained attention that has been shown to be sensitive to cholinergic system compromise.

The importance of timing and dosage has considerable implications for using animal models to characterize the effects of human prenatal ethanol exposure. Rodents are often used as animal models, but have very different developmental timetables from humans. For example, the period of time most analogous to the human third trimester (late period of the fetus) occurs postnatally in rats (Dobbing & Sands, 1979). During this interval is the critical period known as the brain growth spurt. The brain growth spurt is characterized by accelerated neuroproliferation and dendritic elaboration throughout the brain and is thought to be a period of increased susceptibility to environmental insult. As mentioned above, it also represents a time crucial to cholinergic system development. Traditional protocols that provide an alcohol-supplemented diet for the pregnant dam do not expose the rat brain to ethanol during this critical period. Prenatal ethanol

administration therefore does not represent a complete model of maternal drinking throughout pregnancy in humans.

To model the effects of postnatal ethanol exposure in the current experiment, an acute intragastric intubation strategy was employed using rats. This methodology allows for a reasonably normative environment for the pups during treatment while still delivering precise quantities of ethanol (Serbus, Young, & Light, 1986). This process involves binge-like exposures to alcohol over a period of days that roughly approximates the appropriate critical period in humans. Subsequent intragastrically delivered nutritive feedings allow the ethanol-exposed animals to maintain weights similar to controls during treatment, mitigating, at least in part, possible dietary confounds. Such a methodology has some advantages over traditional artificial rearing processes involving gastronomy tube ethanol administration (e.g. West, et al 1989) in which the pup is raised in a completely synthetic environment, devoid of any social interaction with the dam or littermates (Goodlett & Johnson, 1997).

To investigate the often subtle effects on cognition exhibited by perinatal ethanol exposure, it is necessary to utilize an attention task that is sufficiently demanding. The current study utilized a sustained attention task characterized by McGaughy & Sarter (1995) that has been validated in accordance with the Parasuraman et al. vigilance taxonomy as a highly demanding task. This operant task requires rats to respond to differentially respond to brief, unpredictable visual signals and non-signals. Aspects of this task that increase attentional demand include 1) a successive signal discrimination type, 2) a high event rate, 3) multiple signal intensities (i.e. signals that differ in salience), and 4) event asynchrony. In keeping with evidence from human and animal studies, the

current experiment was designed to test the general hypothesis that perinatal ethanol exposure has deleterious effects on both attentional performance and cholinergic functioning. The effects of further attentional demand manipulation by incorporating a flashing houselight distracter stimulus and varying the length of the intertrial interval were also tested. Finally, acetylcholinesterase staining was conducted in order to assess the effect of early postnatal ethanol on basal forebrain cholinergic integrity.

Method

Subjects

Male Long-Evans rats from six litters housed and bred in the College of William and Mary vivarium were used as subjects (N = 30). Alcohol administration began on post-natal day 4 (i.e. five days after birth) or when the animals' weight had reached a 12 g minimum. Litters were standardized to 10 animals. Rats were weaned on post-natal day 21 and housed in separate cages with same-sex littermates. The animals were singly housed on day 60. The vivarium ran on a 14:10 hr light/dark cycle and food and water were available ad lib, excluding periods of water deprivation. Rats were treated in accordance with a protocol passed by the Institutional Animal Care and Use Committee at the College of William & Mary.

Apparatus

Ethanol Exposure

The ethanol solution created daily consisted of dry similac baby formula w/iron, water and 95% ethanol. Ethanol intubations were accomplished using Intramedic PE-10 polyethylene tubing (Clay Adams, Sparks, MD) lubricated with corn oil and 1 ml

syringes with 27 ½ gauge needles. While separated from the dam, pups were kept warm with heat pads (34°C).

Operant Chambers

Adult behavioral testing was conducted in 8 Med Associates (Georgia, VT) operant chambers featuring two retractable levers, a central panel light (2.8 W), a houselight (2.8 W) and a water dispenser equipped with a pair of photocells to detect reward retrievals. The water dispenser was centrally located between the two levers and under the central panel light. The illuminance of the central panel and house lights was measured using an EasyView Digital Light Meter, Model EA31 (Extech Instruments, Waltham, MA). The light meter's sensor was placed 12.7 cm from the central panel light, 7.6 cm above the grid floor and 12.7 cm from the chamber door. Illuminance measurements were houselight alone: 0.05+/-0.002 fc; houselight + 500 ms panel light: 1.36+/-0.13 fc; houselight + 100 ms panel light: 0.34+/-0.07 fc; houselight + 25 ms panel light: 0.078+/-0.003 fc. A Gateway PC running Med-PC software (v. IV) was used to control and record stimuli presentation, lever operation and reward delivery.

Procedure

Design and Intubation Procedure

Male and female animals were randomly assigned to one of three groups: ethanol-intubated (etoh), sham-intubated (sham), or non-intubated (naïve). Group identity was maintained through the use of non-toxic markers until post-natal day 9, when an ear clip identification scheme was implemented. Weight was assessed and recorded daily using an Ohaus GT 8000 (Ohaus Scale Corp., Pine Brook, NJ). Each litter was removed from the dam as a group and placed on a heating pad for the duration of the intubation

procedure. Intubations for ethanol-intubated and sham-intubated animals occurred two hours apart, three times each day. All intubations were delivered via corn oil-lubricated polyethelene tubing through the animal's mouth, esophagus and into the stomach. The ethanol-exposed group was administered alcohol during the first two daily administrations from PD 4 through PD 9, and a third formula-only administration for nutritive purposes. Alcohol delivery was accomplished by intragastric administration of a mixture comprised of dry similac baby formula, water, and 95% ethanol (5.25 g/kg/day), closely paralleling the protocol first outlined by Serbus, Young, & Light (1986). The sham control group received polyethelene intubations, but were not administered any formula. Non-exposed rats in the second control group accompanied their littermates to the feedings but were not intubated.

Behavioral Training

Behavioral training began on PD 90. Animals were initially trained to lever press for water using a fixed-ratio 1 reinforcement schedule. More than five consecutive same-side bar presses were not rewarded to avoid the development of side-biases. Once the animals reached criterion (120 rewards per day for 3 consecutive days), the rules of the behavioral task were introduced.

The first stage of the task trained rats to discriminate between signal (1 s illumination of the central panel light) and non-signal events (no illumination of the central panel light). The animals were placed in the house-light illuminated operant chambers for five minutes prior to training. Each session consisted of 162 trials with signal and non-signal events occurring in random order. The inter-trial interval (ITI) was 12 ± 3 s. Immediately following a signal event, both levers would extend into the

chamber for 3 seconds or until a lever press occurred. For signal event trials, pressing the left lever was considered correct, scored as a hit and then reinforced via the water dispenser (0.1 ml tap water). For non-signal events, pressing the right lever was considered correct, scored as a correct rejection and rewarded. Incorrect responses were scored as misses (signal trials) or false alarms (non-signal trials) and were not reinforced. Failure to press a lever within 3 s of extension was scored as an omission. All incorrect responses were followed by a trial identical to the previous trial in signal type and ITI. After 3 consecutive incorrect responses, a forced-choice trial was introduced in which only the proper lever was extended for a 90 s period. On signal trials, the central panel light also remained illuminated during the period that the lever was extended. Once the rats achieved 70 % performance on both hits and correct rejections for 3 consecutive sessions, they entered the second phase of training.

The second training phase featured shorter and variable signal event durations (500, 100, & 25 ms as opposed to 1 s). Sessions featured 27 trials at each of the three signal durations as well as 81 non-signal trials, for a total of 162 trials. Sessions were also divided into three blocks (54 trials each) with 9 trials at each of the three signal durations and 27 non-signal trials arranged in pseudo-random order. ITIs were reduced to 9 ± 3 seconds and the correction and forced-choice trials were eliminated. Animals were trained until they reached 70 % accuracy on both 500 ms signal duration trials and non-signal trials for three consecutive sessions. After reaching criterion, the rats were then trained for five sessions or until asymptotic performance was reached. Asymptotic performance was defined as three consecutive sessions in which accuracy on 500 msec signal duration hits did not vary more than 15% and correct rejection accuracy did not

vary more than 10%. Training then continued for an additional three sessions to establish baseline performance.

After baseline performance was reached, a visual distracter stimulus in the form of a flashing houselight (½ sec on/off) was introduced for three sessions. Following the distracter sessions, standard training resumed until performance returned to pre-distracter levels. Baseline performance was again determined by three consecutive sessions. After baseline was reestablished, the ITI was reduced to 4.5 ± 3 seconds for three sessions. Once again, baseline was restablished following the short ITI sessions. Finally, three long ITI (18 ± 3 s) sessions were introduced.

Behavioral Measures

The total number of hits (h), misses (m), correct rejections (cr), and false alarms were collected for each signal duration (where appropriate) and across each 54 trial block. Accuracy on signal trials was calculated as h/(h + m) and cr/(cr + fa) on non-signal trials. Trials in which the rats failed to respond were recorded as omissions. The latency to press levers after extension was also collected for each trial. Finally, the latency to reward retrieval was collected as the amount of time between an appropriate lever press (h or cr) and the breaking of the water dispenser photocell.

Histological Procedures

Rats were transcardially perfused with a 10% sucrose solution followed by 4.0 % paraformaldehyde. Following removal, brains were stored in 4.0 % paraformaldehyde overnight. The brains were then cryoprotected in 30 % sucrose in phosphate buffer for 2-3 days. Frozen brains were sliced to 40 μM using a microtome (AO Instrument Co., Buffalo, NY).

Acetylcholinesterase (AChE) staining was accomplished using a modified version of the protocol outlined by Tago, Kimura, & Maeda (1986). Sections were washed in 0.1 M phosphate buffer, then immersed for 10 minutes in a 0.125% hydrogen peroxide solution. Sections were then rinsed in 0.1 M maleate buffer and incubated in a solution containing 0.0147 g of sodium citrate, 0.00165 g of potassium ferricyanide, 0.00749 g of copper sulfate, and 10mg of acetylthiocholine iodide in 200 ml of 0.1 maleate buffer. After rinsing with 50 mM Tris Buffer, sections were incubated in a solution containing 6 ml of liquid 3, 3'- diaminobenzidine tetrahydrochloride (DAB) and 0.180 g nickel ammonium sulfate in 54 ml of DAB buffer/peroxide solution (Sigma-Aldrich, St. Louis, MO). Eight drops of 30% peroxide solution were added and the sections remained in the DAB solution until cortical layering was observable. Sections were mounted on gelatin-coated slides, dehydrated through a series of alcohol baths and cleaned in a xylene wash before coverslipping.

Quantification of cholinergic fibers was accomplished by photographing anterior cingulate (AC) and posterior parietal cortical (PPC) sections as identified by the Lysakowski, Wainer, Bruce, & Hersh, (1989) brain atlas. AChE-stained sections from 12 animals (5 naïve, 3 sham, and 4 etoh) were used to obtain these cortical samples. An Olympus BX-51 microscope (Olympus America Inc., Melville, NY) with camera connected to a dell pc running ImagePro Discovery, v. 4.5 imaging software (Media Cybernetics, Silver Spring, MD) was used to take 40X photographs of AC and PPC regions. A modified grid counting method was utilized to assess AChE + fiber density (Stichel & Singer, 1987). Two intersecting, orthogonal lines were then overlaid on each micrograph. Fibers crossing these lines were counted.

Statistical Analysis

For behavioral data analyses, percentage data were tested using mixed-model analyses of variances (ANOVAs), including condition (Naïve, Sham & EtOH), signal duration (500, 100 & 25 ms), block, and session type (baseline, distracter, short ITI & long ITI). All *p*-values for within-subjects main effects and interactions were adjusted using the Huynh-Feldt correction. An α level of 0.05 was adopted. All data analyses were conducted using SPSS 11.5 software (SPSS, Chicago, IL).

Results

Attention test training

The total number of sessions each rat required to reach criterion on the training phases was compiled. Condition means were compared using a series of ANOVAs. No condition differences were found in sessions to criterion for the water shaping (Naïve: 4.90±0.99; Sham: 5.00±0.47; EtOH: 5.2±1.14), attention correction (Naïve: 12.78±3.38; Sham: 15.10±4.01; EtOH: 16.33±4.30), and attention test trials (Naïve: 7.67±4.47; Sham: 6.10±2.92; EtOH: 7.10±2.18). The condition means are depicted in Figure 1.

Baseline task performance

After reaching asymptotic performance for three consecutive sessions, signal detection accuracy was assessed by a 3 (condition) X 3 (block) X 3 (signal duration) mixed model ANOVA. The results showed significant main effects for group, $F(2, 27) = 5.05$, $p = 0.014$, signal duration, $F(2, 54) = 356.20$, $p < .001$, and block, $F(2, 54) = 3.18$, $p = 0.049$. Post hoc analysis revealed that the relative number of hits for the EtOH group was decreased compared to the Naïve and Sham control conditions; Naïve: 0.70±0.02, Sham: 0.71±0.02, EtOH: 0.62±0.02. As expected, Bonferonni-corrected paired *t*-tests

showed significantly improved performance as signal duration length increased (25 ms < 100 ms < 500 ms). The effect of signal duration is very robust and was present in all subsequent accuracy analyses. However, post hoc analysis was unable to differentiate between blocks; block 1: 0.62±0.02, block 2, 0.59±0.021, block 3: 0.61±0.018. Correct rejections of non-signal events were assessed by a 3 (condition) X 3 (block) mixed model ANOVA. In contrast to the signal trial accuracy, no significant condition differences or interactions with block were found for the correct rejection rate. The percent accuracy for hits and correct rejections are summarized in Figure 2. It is important to note that rats in the EtOH condition display signal duration-dependant performance despite their impairment in overall accuracy.

Trial omissions were examined by a 3 (condition) X 3 (block) X 2 (signal type) mixed model ANOVA. This analysis revealed a significant effect of block, F (2, 54) = 9.83, $p < .001$; block 1: 1.23±0.29, block 2: 0.80±0.18, block 3: 2.27±0.44. Post hoc t-tests indicated that significantly more omissions occurred during the third block than the first two blocks. The increase in omissions over trials is a reflection of a temporally mediated vigilance decrement. There was no significant effect of condition nor significant interactions between condition and any other factor.

The latency for the rats to respond following lever extension was analyzed using a 3 (condition) X 4 (outcome, i.e. hit, miss, correct rejection, false alarm) X 3 (block) ANOVA. Significantly different latencies were detected in outcome, $F(3, 81) = 8.87$, $p < .001$ and block, $F(2, 54) = 3.40$, $p = 0.041$. Post hoc analysis showed that correct responses (hits, correct rejections) featured shorter latencies than incorrect responses (misses, false alarms) and are depicted in Figure 3. Additional post hoc t-tests indicated

that bar press latencies in the third block were significantly longer than during the second block; block 1: 466.2±20.6, block 2: 435.4±18.6, block 3: 481.5±19.2. Following correct responses, the latency to retrieve the water reward was also measured. The reward retrieval latencies were analyzed using a 3 (condition) X 3 (block) ANOVA. No significant effects for condition or block were detected.

Immediately preceding each task manipulation (short and long inter-trial intervals (ITI)), asymptotic performance over three consecutive sessions was reestablished. Comparisons among these periods were made to explore the consistency of the effects present in the initial baseline analysis. The average hit detection across each three session period (original baseline, pre-short ITI and pre-long ITI) was assessed using a 3 (condition) X 3 (period) X 3 (session) X 3 (signal duration) ANOVA. The effect of condition remained significant, $F(2, 26) = 5.58$, $p = .01$ with post hoc analysis showing poorer performance by the EtOH condition relative to controls, Naïve: 65.7±2.3%, Sham: 66.1±2.4%, EtOH: 56.4±2.3%. Importantly, condition did not interact with period, indicating that the effects of ethanol treatment on the hit rate persisted with subsequent training. The stability of the effect is depicted in Figure 4. An analysis of non-signal trial accuracy by a 3 (condition) X 3 (period) X 3 (block) ANOVA continued to indicate no effect of condition. For omissions, a block effect was evident, $F(2, 52) = 5.77$, $p = .017$, reflecting increased omissions in the third block relative to the second block. Bar press latencies were also analyzed by a 3 (condition) X 3 (period) X 3 (block) X 4 (outcome) ANOVA. The main effects of outcome, $F(3, 78) = 14.30$, $p < .001$ and block, $F(2, 52) = 6.50$, $p = .004$ remained significant; correct responses featured shorter latencies relative to incorrect responses and third block response latencies were longer

than first block response latencies. A significant effect of period was also indicated, $F(2, 52) = 5.96$, $p = .006$. Interestingly, the third asymptotic performance period featured longer latencies than the first period; period 1: 461.7±17.3, period 2: 503.7±18.8, period 3: 525.2±22.0. Overall, these analyses indicate that the performance of the performance of the treatment groups in the standard task did not differentially vary with subsequent training.

Distracter Task

The effect of the flashing houselight on signal detection accuracy was assessed using a 3 (group) x 4 (session) X 3 (block) x 3 (signal duration) ANOVA. A baseline average and three consecutive sessions of the distracter task comprised the four levels of "session" in the analysis. For hits, the effect of condition remained significant, $F(1, 27) = 4.21$, $p = 0.026$; Naïve: 56.2±2.7%, Sham: 55.2±2.9%, EtOH: 45.1±3.8%, but did not interact with any other factors. Greater accuracy was observed in the baseline average session relative to the three distracter sessions, reflecting the increased attentional demands presented by the distracter stimulus, $F(3, 81) = 19.13$, $p < .001$, baseline: 61±1.9%, day 1: 49±2.5%, day 2: 49±2.3%, day 3: 49±2.2%. There was no main effect of condition and condition did not interact with any factors for analyses of correct rejections, omissions, lever press latencies or reward retrieval latencies. On nonsignal trials, accuracy was reduced on distracter session 1 relative to baseline levels, but recovered to by session 3, $F(3, 81) = 12.65$, $p < .001$; baseline: 88±0.9%, day 1: 78±2.0%, day 2: 83±1.5%, day 3: 85±1.5%.

An analysis of reward retrieval latencies revealed an increase in latency during the first distracter session, with latencies returning to baseline levels during subsequent

distracter sessions, $F(3, 75) = 4.94$, $p = .016$; baseline: 563.7±41.6, day 1: 825.5±128.1, day 2: 532.9±60.9, day 3: 492.8±37.2. The distracter did not have a main effect on omissions or lever press latencies.

Short Intertrial Interval

Rats were trained on three consecutive sessions featuring 4.5±3 sec ITIs. Reducing the average ITI by half resulted in poorer signal detection accuracy relative to baseline performance as revealed by a 3 (condition) X 4 (session) X 3 (block) X 3 (signal duration) ANOVA, $F(3, 75) = 4.20$, $p = .008$; baseline: 69.5±1.2%, short ITI session 1:64.2±1.5%, short ITI session 2: 64.3±1.7%, short ITI session 3: 65.6%±1.5%. For hits, there was no condition main effect, as the control animals tended to exhibit a greater performance decrement in response to the short ITI than the ethanol animals. However, condition did not interact with any other factor, indicating that the EtOH exposed rats were not differentially affected by the ITI reduction. No group differences were evident for correct rejections, omissions, bar presses or reward retrieval latencies.

Long Intertrial Interval

The final stage of training employed three consecutive 18±3 sec ITI sessions. A signal detection accuracy ANOVA failed to reveal a main effect of condition, but did show an interaction between condition and block, $F(4, 52) = 2.74$, $p = .047$. Though it appears that sham animals exhibited a decrement in performance over blocks that was not present for Naïve and EtOH animals, post hoc analyses were unable to detect differences in the response patterns of each condition (Figure 5). Accuracy on nonsignal trials was not affected by extending ITIs. Condition did not interact with any other factor, nor were

there condition main effects on correct rejections, omissions, bar press latency or reward retrieval latencies.

Histological Analysis

Cortical AChE-postive fiber staining was marginally reduced in both the anterior cingulate and posterior parietal cortex in ethanol exposed animals relative to controls. This can be qualitatively observed in posterior parietal cortex as slightly less dense fiber presence in the ethanol animal in Figure 6. Fiber counts using standard grid overlays at 40X showed a 5.2% reduction in fibers in the AC relative to the average of the control group and a 10.8% reduction in the PPC.

Discussion

The present study sought to investigate the effects of early postnatal ethanol exposure on adult rat vigilance performance and cholinergic system integrity. The vigilance task used in this experiment has been validated as a test of sustained attention using the taxonomy of Parasuruman et al. (1987). Further, it has been previously shown to be sensitive to lesioning and pharmacological manipulations of the basal forebrain cholinergic system (e.g. McGaughy, Kaiser & Sarter, 1996).

Behavioral Assessment

Early postnatal ethanol exposure did not have an effect on the number of sessions required to reach criterion on the water shaping task, the attention correction trials and the sustained attention task. Ethanol exposed animals were able to learn to press levers for reward and learn the complex rules of the attention task over a period of time comparable to controls. This indicates that ethanol exposure did not grossly impair motor

control, disrupt motivation or otherwise compromise the ability of the rats to respond to the rules of the task.

After reaching criterion, the rats continued training on the attention task until they reached asymptotic performance. It was at this stage of training that the ethanol group displayed a deficit in the ability to detect signals, as their relative number of hits were reduced compared to control animals. However, their number of correct rejections did not differ from controls. These data suggest that the ethanol group was less proficient at maintaining attention on the target stimulus and therefore missed the cue more often than the control group. Importantly, this pattern of results is similar to those obtained in prior work on the behavioral effects of basal forebrain cholinergic compromise. McGaughy et al (1996) reported a substantial reduction in hit detection following selective lesioning of corticopetal nbM afferents. Additionally, administering chlordiazepoxide, a benzodiazepine receptor agonist that functionally blocks increased attention-related ACh cortical release, was also found to reduce the number of hits while sparing correct rejections (Holley, Turchi, Apple, & Sarter, 1995). Following these studies, the reduced hits/unaffected correct rejection result is presently interpreted as supporting the hypothesis that postnatal ethanol disrupts the ability to maintain vigilance. However, alternative explanations for the data require comment.

It can be argued that ethanol exposure might make animals more prone to developing a side bias and that this bias might only emerge after extensive training. This is not a compelling argument as the emergence of a side bias is usually paralleled by an increase in the number of correct responses for the favored side. This was not the case in the current experiment, as the ethanol animals did not exhibit a significantly higher

number of correct rejections than controls. Alternatively, it might also be contended that ethanol exposure affected the integrity of the rats' visual system and that the apparent attentional deficits are actually side effects of the animal's inability to detect visual signals of short duration. However, the animals were able to perform the attention task at an above chance level, reaching the 70% response criterion for both hits and correct rejections. Such performance would not be possible with a compromised visual system.

In the literature on humans, it is not uncommon to equate fetal alcohol exposure-related attentional problems with hyperactivity or impulsivity (Coles et al., 1997). Additional measurements of bar press latency and latency to retrieve water rewards were taken during the course of training. The ethanol animals did not display the reduced bar press latencies characteristic of impulsive responding. Further, they did not display reduced reward retrieval latencies that would be indicative of motivational compromise. Neither the data nor general observation of the animals' behavior indicated that the ethanol animals were hyperactive.

Baseline performance levels were reestablished twice over the course of training. The ethanol group deficit in hits remained significant while their correct rejection performance remained at control-like levels. Extensive training did not improve the ethanol group's hit performance, indicating that the impairment is robust and stable over time.

A characteristic aspect of vigilant tasks is the gradual reduction in performance over time within a session. This phenomenon has been labeled the vigilance decrement. In the current experiment, the vigilance decrement was assessed by dividing each session into three blocks of trials. Comparisons between blocks on hit and omission measures

revealed a general trend of poorer performance (i.e. lower hit percentage, more omitted trials) in the third block relative to the preceding blocks. However, condition did not interact with block on any of the baseline trials. Therefore, the ethanol rats were not differentially impaired in their ability to maintain vigilance over the course of the session.

The introduction of a flashing house-light distracter task reduced the average number of hits for both ethanol animals and controls. However, there was not a significant interaction between ethanol condition and the effects of the house-light. This is somewhat surprising given the challenging nature of the distracter manipulation. *In vivo* microdialysis studies have shown that the distracter stimulus elicits an 84% increase in cortical ACh release (Sarter, Bruno, Givens, Moore, McGaughy & McMahon, 1995). In immunotoxin lesion studies, animals with lesions exhibited greater performance decrements in response to the distracter than did control animals (McGaughy et al., 1996). It is possible that the lack of an interaction was due to the less severe nature of the ethanol-induced damage coupled with aspects of the distracter task that reduce its ability to differentiate among groups. The distracter stimulus was presented in a regular, 0.5 sec on/off series. This resulted in half of the signal events occurring in a darkened chamber, thus increasing the salience of the stimuli and reducing the attentional resources required for accurate detection. It is possible that a more demanding distracter stimulus, such as a pattern of periodically increased house-light intensities might differentially affect ethanol-exposed and control animals.

After the animals reestablished baseline performance, the inter-trial interval (ITI was reduced by half for three consecutive sessions. This manipulation had the unexpected result of eliminating the group difference between the ethanol and control

animals on hit detection. The increased rate of stimulus presentation requires greater executive processing speed, but the active portions of the sessions last half as long as the standard task (approximately 15 minutes as opposed to 30 minutes). As it has been noted that it can take up to 35 minutes for the vigilance decrement to fully manifest itself in humans (Parasuraman et al., 1987), it is possible that the ethanol group may have experienced further decline in performance over a longer session. Future experiments should consider adding trials to the short ITI manipulation.

A different pattern of results was observed after doubling the standard task ITI. An interaction emerged between condition and block, with the naïve group remaining steady at a high rate of performance, the sham group showing the normal vigilance decrement over blocks and the ethanol group beginning poorly in the first two blocks, but recovering in the third block. This interaction is being driven by the performance overlap between the sham and ethanol groups in block 3 and is difficult to interpret due to the poor initial performance of the ethanol group. According to the Parasuraman et al. (1987) taxonomy, the long ITI task is less taxing due to its reduced event rate, but more difficult due to its hour-long running time. Future experiments should address the relative importance of event rate and running time by comparing tasks of equal duration with different numbers of trials.

Histological Analysis and Implications

Having supported the hypothesis that early postnatal ethanol exposure adversely affects sustained attention, the second goal of this experiment was to evaluate a possible neural correlate of the observed behavioral deficit. A substantial amount of literature has implicated the basal forebrain corticopetal cholinergic system as a mediator of sustained

attention (e.g. Sarter & Bruno, 1997) and a potential target for ethanol insult (e.g. Rudeen & Guerri, 1985). Assessment of AChE fiber density in the ethanol group revealed a loss of approximately 10% of the cholinergic fibers in posterior parietal cortex and a 5% loss in the anterior cingulate relative to controls. These regions were selected not only for their importance in regulating attentional processes, but because they have been identified as regions particularly susceptible to ethanol-induced apoptotic cell loss (Ikonomidou, 2000). Despite the putative vulnerability of these cell populations to ethanol-mediated deletion, the observed cell loss is well below the threshold that is generally accepted as crucial for behavioral impairment. Results from lesion studies have indicated that a minimum of 50% cell loss is usually required to generate deficits in attentional performance (Sarter & Bruno, 1997).

Given the lack of gross cholinergic cell loss, it is unlikely that ethanol exerts its effects solely by a cell necrotic mechanism. This, however, does not preclude the possibility that cholinergic abnormality is a major factor in the observed attentional deficits. Caution should be exercised in interpreting the current results as AChE staining only indicates the presence or absence of fibers and does not provide information on the functionality of the cholinergic network. Ethanol is a potent teratogen with a variety of mechanisms that can negatively impact the developing brain. Perinatal ethanol exposure is known to not only result in cell loss, but also alter the activity and organization of the cholinergic system (Schambra et al., 1990). Moreover, ACh functions not only as a neurotransmitter in early development, but as a morphogenic agent as well (Berger-Sweeney, 2003). Ethanol interference with normative cholinergic functioning during

development can potentially disrupt cell migration and differentiation and can ultimately result in cholinergic system malformation.

Future research should also incorporate techniques that allow for assessment of cholinergic functioning as well as structural integrity. Staining for choline acetyltransferase (ChAT), the enzyme that catalyzes the synthesis of ACh, is one such method. ChAT staining would reveal any differences in the cholinergic neurons' capacity for manufacturing ACh that might result from formative ethanol exposure. Studies assessing synaptic neurotransmitter levels in response to attention challenges using *in vivo* microdialysis would also be instructive. Techniques such as these could potentially reveal any impairment in cholinergic transmission efficacy caused by early ethanol exposure.

Though ACh plays a very different role in development than in adulthood, the hypothesis that perinatal and adult cholinergic deafferentation will have different outcomes has yet to be explicitly tested (Berger-Sweeney, 2003). To address this issue as it relates to attention, vigilance task performance comparisons could be made between adult rats with either early postnatal or adult immunotoxic nbM lesions. Additional adult comparisons between animals with early postnatal nbM lesions and those exposed to binge-like doses of ethanol would be informative on the extent to which ethanol's effect is mediated by a cholinergic mechanism.

In comparing highly specific lesions with ethanol exposure, it is important recognize ethanol's global effect on the brain and its interaction with a variety of noncholinergic neurotransmitters. For example, early postnatal binge-like exposure to ethanol alters gamma-aminobutyric acid type A receptor (GABARA) development in the

medial septum and diagonal band of Broca (Hsiao., Mahoney, West & Frye, 1998). GABA producing cells are found in abundance in the basal forebrain, leading to investigations of their role in attentional processes. Ibotenic acid-induced lesions of GABAergic neurons in adult animals have been shown to result in a pattern of vigilance task performance that is different from cholinergic lesioned animals (Burk & Sarter, 2001). GABAergic lesioned animals exhibited an inability to reject nonsignal events, but were not impaired in signal detection. From results such as these, it has been inferred that GABA plays an executive role in modulating the recruitment of lower attention circuits (e.g. nbM cholinergic corticopetal projections) (Sarter et al., 2001). Compromising inhibitory GABA functioning can result in excessive ACh release in the cortex and a subsequent increase in false alarms.

The ethanol-exposed animals in the current experiment likely suffered damage to both the GABAergic and the cholinergic pathways. This may explain why an increase in false alarms was not observed. Though less cortical GABA was being released, damage to the cholinergic system may have prevented an over abundance of ACh from accruing. To investigate the interaction of these two systems, future work should also assess the integrity of the GABAergic system using GABA markers such as parvalbumin.

The loss of cholinergic fibers in posterior parietal cortex coupled with the presence of a more general attentional deficit in the ethanol group suggests that the effects of ethanol on other types of attention should also be explored. It has been noted that it is often times difficult to fully dissociate the subtypes of attention, especially in the case of selective and sustained attention (Sarter & Bruno, 1997). Selective attention, or the process in which a subset of sensory information is selected for further processing, is

thought to rely heavily on circuits including the posterior parietal cortex (Behrmann, Geng & Shomstein, 2004). Children suffering from FAS have been noted as having deficits in the ability to selectively attend to important stimuli (Connor et al., 1999). In the animal literature, rats that have a deficit in selective attention have been identified using the 5 choice serial reaction time task (5CSRT) (Barbelivien, Ruotsalainen & Sirviö, 2001). Surprisingly, the 5CSRT performance of animals exposed perinataly to ethanol has not yet been assessed. It is clear that the effects of ethanol on multiple aspects of attention warrants greater research focus.

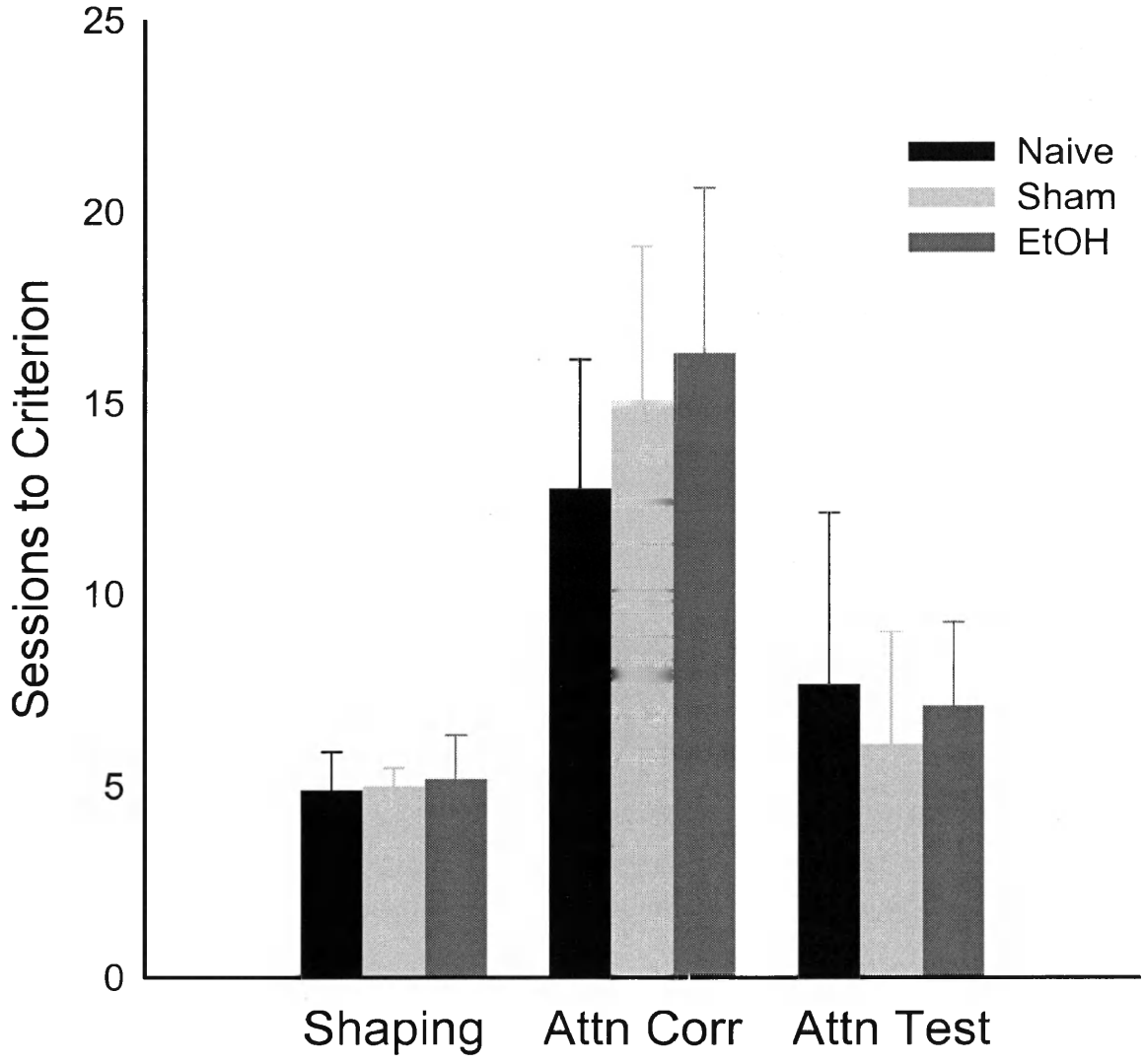
The present experiment focused only on ethanol exposure during the critical period known as the brain growth spurt (Dobbing & Sands, 1979). This approach has advantages in identifying the time frame of crucial developmental events, but it does not have real world validity of other administration regimens. Mothers are not likely to begin binge-drinking in the third trimester after abstaining during the previous two. Moreover, there is evidence suggesting that additional critical periods may be present during early pregnancy (Qiao, et al., 2002) and well after birth (Bayer, Altman, Russo, & Zhang, 1993). Studies that incorporate multiple administration schedules spanning the entire gestational period and including early postnatal life will be instrumental in clarifying both the specific consequences of exposure during critical periods and the cumulative effects of chronic ethanol exposure.

Ultimately, the goal of research on fetal alcohol exposure is to investigate ways in which the effects of this disorder can be mitigated. To the extent that ethanol-induced cholinergic deprivation early in life causes the FAS/FAE developmental abnormalities, drug interventions may prove beneficial. Early postnatal choline supplementation has

been shown to rescue some adult functioning in a rodent visual discrimination task (Thomas, La Fiette, Quinn, & Riley, 2000). Boosting choline levels during certain critical periods, possibly through the maternal diet, may buffer fetuses against the disruption in cholinergic morphogenic functioning caused by ethanol exposure. However, post-critical period choline supplementation may not confer benefits on attention processes, as ACh no longer functions as a morphogen. Artificial introduction of ACh that is not functionally relevant or different in character from endogenous sources may actually be detrimental to performance (Sarter & Bruno, 1997).

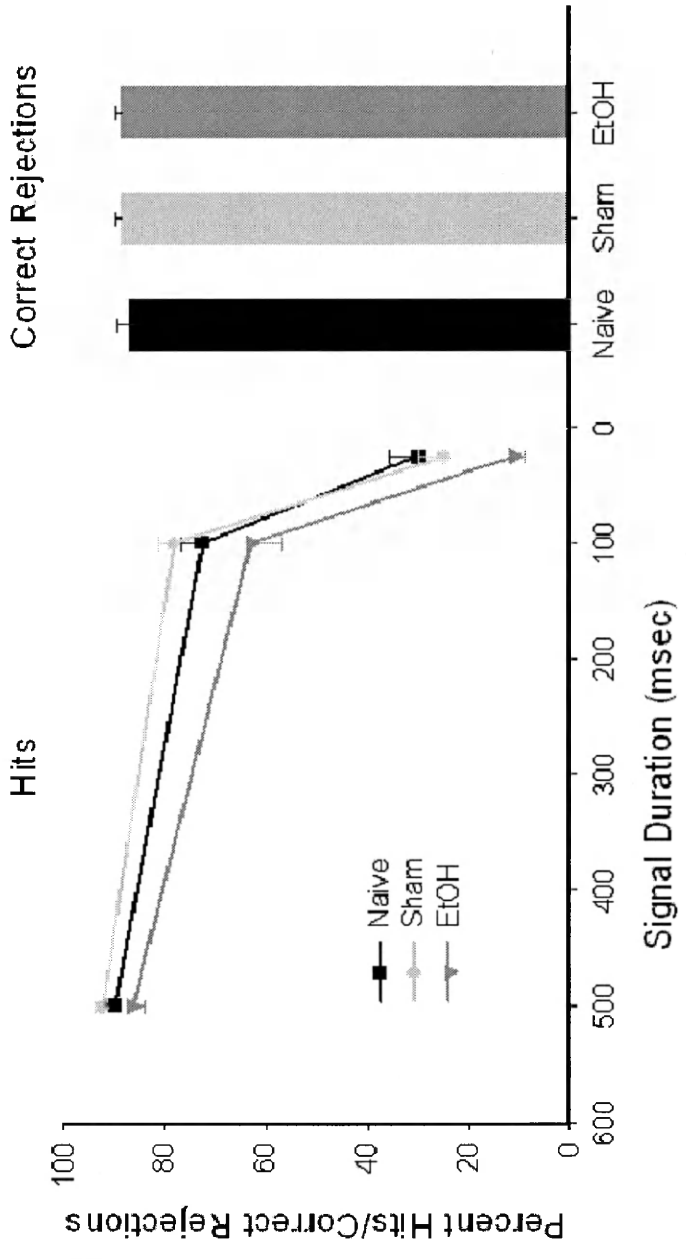
In conclusion, the present work provides further evidence of the harmful and long lasting effects of perinatal alcohol exposure. It is clear that fetal alcohol exposure has adverse consequences for attention that can prevent individuals from reaching their full potential. This experiment is just one of many preliminary steps that are necessary to extend our understanding of ethanol's teratogenic effects. It is hoped that research such as this will contribute to the development of treatment strategies for this debilitating condition.

FIGURE 1.



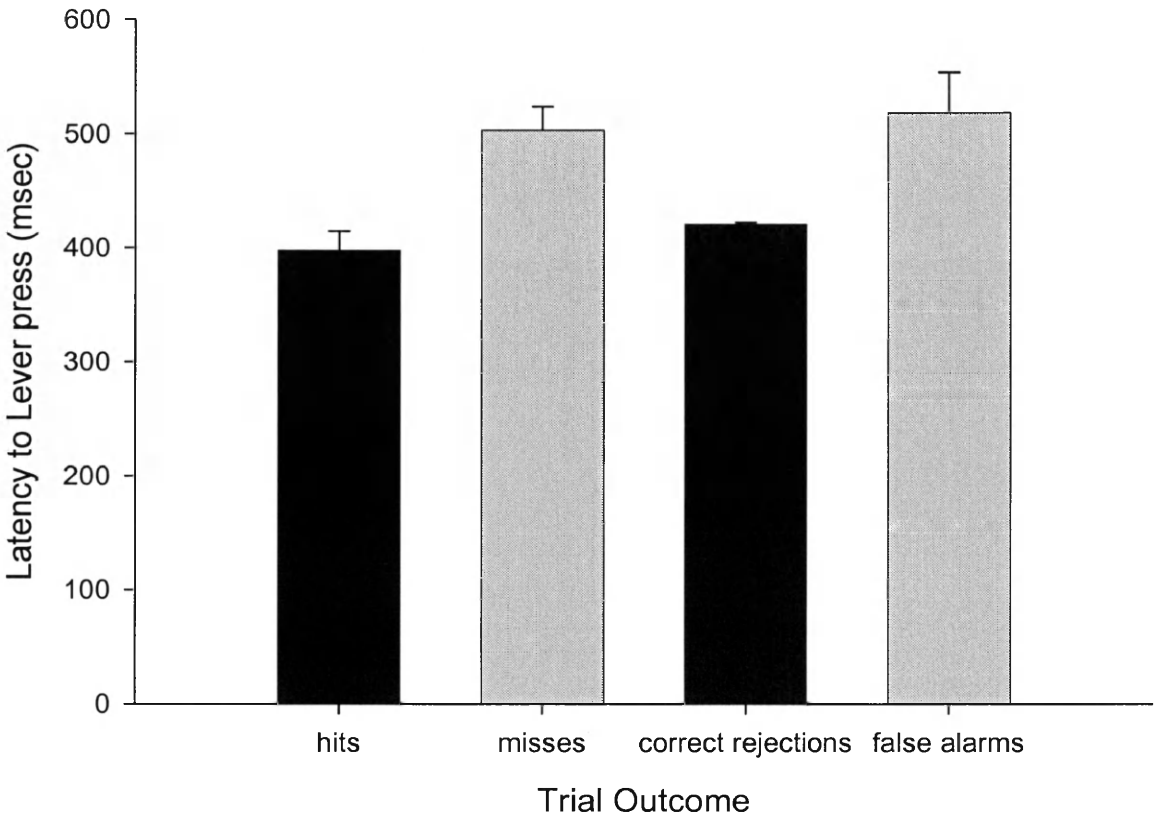
Average sessions to criterion. Prenatal EtOH treatment did not reduce the rate of acquisition for the shaping, attention correction trials, or attention test training phases relative to controls.

FIGURE 2.



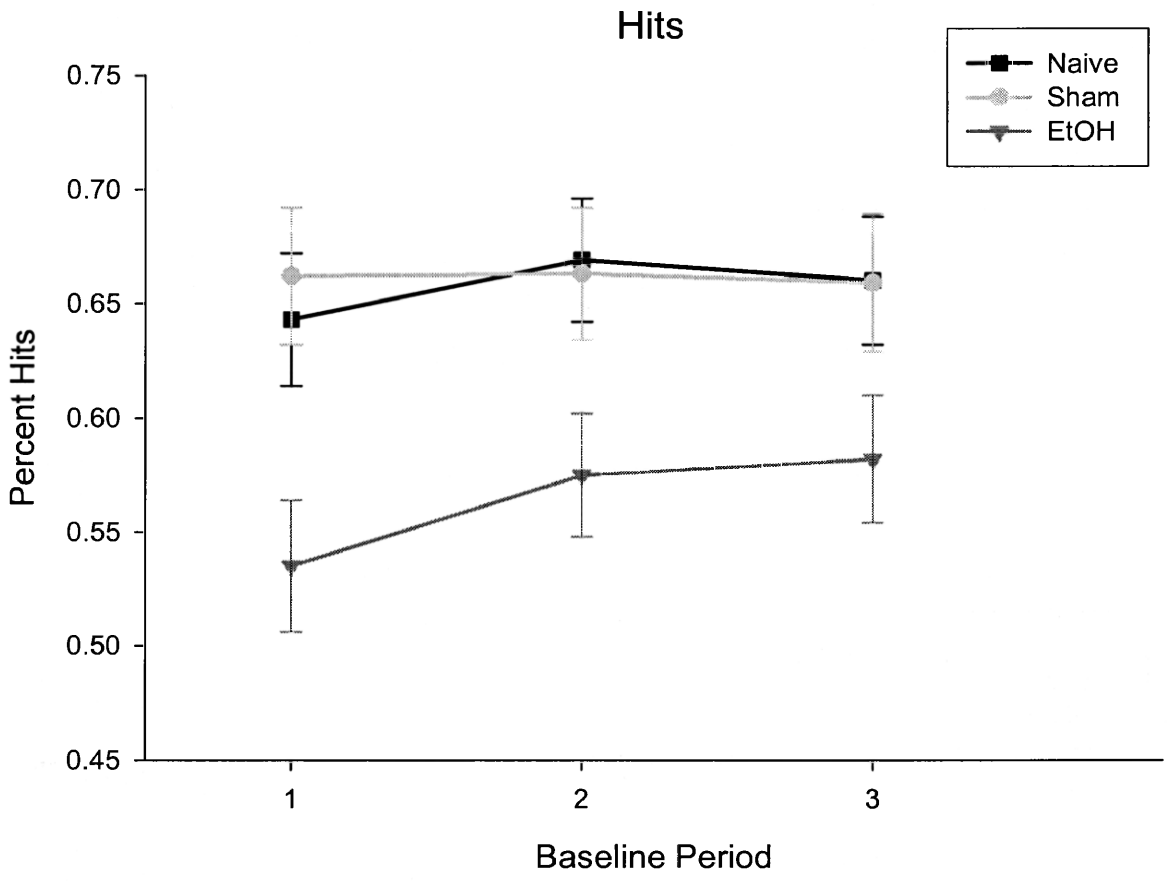
Hit percentage for each signal duration and correct rejection percentage. Animals in the EtOH condition exhibited a reduction in hit detection across all signal durations relative to controls, whereas their ability to correctly reject non-signal events was not affected.

FIGURE 3.



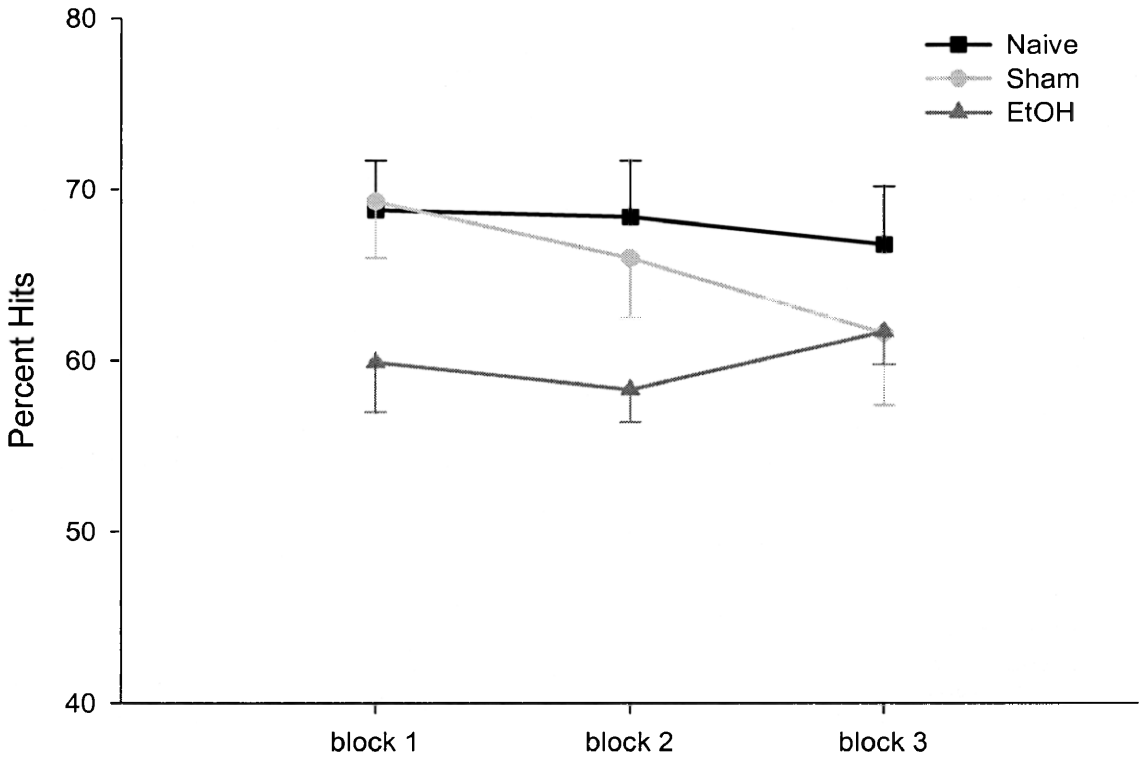
Average bar press latency for each trial outcome. Animals in all conditions displayed reduced response latencies on correct responses (i.e. accurately identifying a stimulus or correctly rejecting the absence of a stimulus).

FIGURE 4.



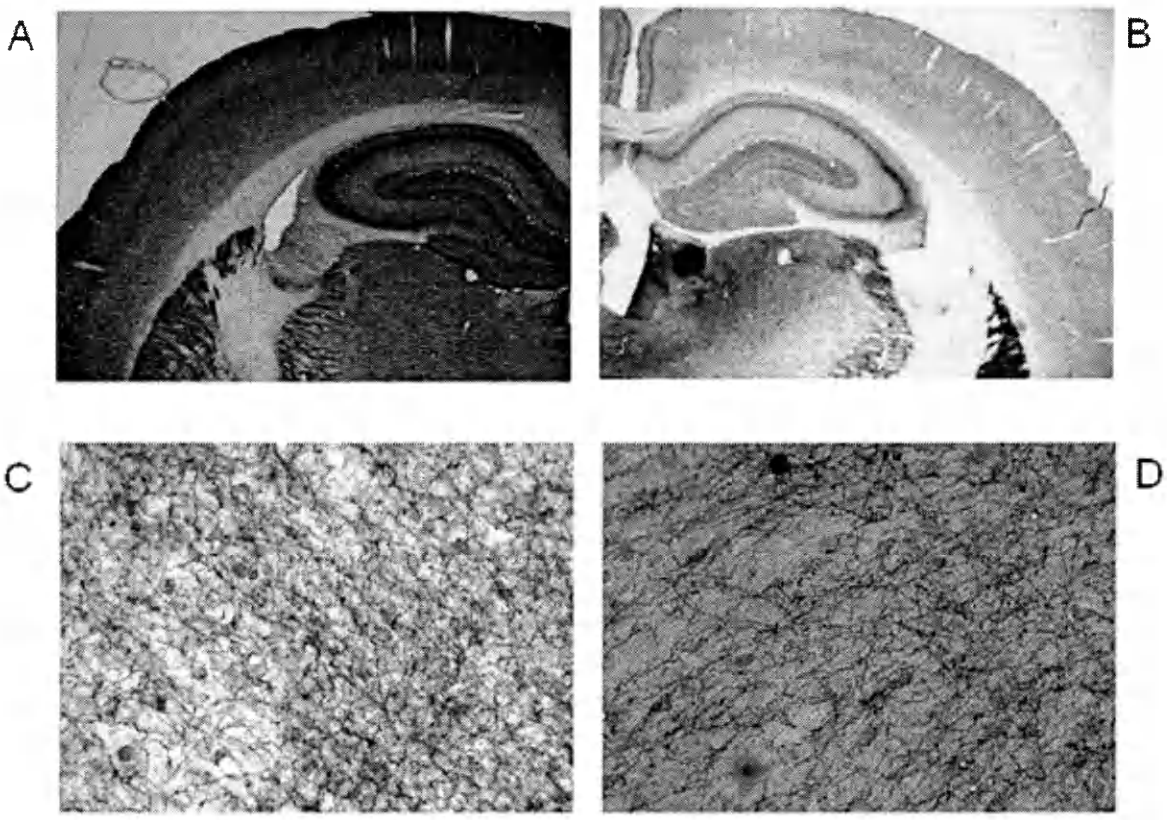
Average hit percentages across baseline periods. Baseline performance was established prior to each task manipulation. Animals in the EtOH condition displayed impaired hit detection relative to controls in each baseline period.

FIGURE 5.



Response accuracy across blocks on trials with long intertrial intervals. For hits, a significant condition X block interaction was noted. However, post hoc analyses were unable to determine the basis for this interaction.

FIGURE 6.



Brain sections stained with acetylcholinesterase from a naïve and an EtOH exposed subject. AChE-stained posterior parietal sections from a naïve (A & C) and an EtOH (B &C) subject reveal the absence of a gross loss of cholinergic fibers in response to early postnatal EtOH exposure. Note. Micrographs on the top row were taken under a 2X objective and micrographs on the bottom row were taken under a 40X objective.

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