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POTENTIAL CANDIDATES FOR TREATING DEFICITS ASSOCIATED WITH DEVELOPMENTAL ETHANOL EXPOSURE IN A RODENT MODEL: SOLIDAGO NEMORALIS & DIMETHOXYBENZYLIDENE-ANABASINE

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DISSERTATION

A dissertation submitted in partial fulfillment of the

requirements for the degree of Doctor of Philosophy in the

College of Arts and Sciences

at the University of Kentucky

By

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ABSTRACT OF DISSERTATION

POTENTIAL CANDIDATES FOR TREATING DEFICITS ASSOCIATED WITH DEVELOPMENTAL ETHANOL EXPOSURE IN A RODENT MODEL: SOLIDAGO NEMORALIS & DIMETHOXYBENZYLIDENE-ANABASINE

Prenatal alcohol exposure (Fetal Alcohol Syndrome [FAS] and Fetal Alcohol Spectrum Disorders [FASD's]) represents the leading preventable cause of intellectual disabilities in the western world, with FASDs estimated to affect approximately 2-5% of live births in the United States at an approximate annual cost of $3.6 billion (CDC, 2015; May et al., 2009). Ethanol (ETOH) exposure during development can lead to a variety of long-term behavioral impairments including problems with executive functioning, motor coordination, spatial learning, attention, and hyperactivity (Jones, 2011; Mattson & Riley, 1998). Much research has been conducted to develop pharmacological and/or environmental interventions to reduce these deficits, however, there are currently no clinically approved medications to treat the deficits related to fetal ETOH exposure. The current study used a developmental "3rd trimester" ETOH exposure model in neonatal rats to test the hypothesis that compounds targeting the nicotinic system will reduce deficits associated with ETOH exposure. Both compounds demonstrated promise in reducing some of the effects of developmental ethanol exposure, with DMXB-A treatment after ethanol exposure reducing balance deficits in females and spatial memory deficits in males. *Solidago nemoralis* treatment after ETOH exposure reduced learning and memory deficits in males and balance and executive functioning deficits in both sexes. With these results and previous work in this lab and others there appears to be ample evidence for their usefulness in reducing various forms of neurotoxicity. The long-term goal of this research is to evaluate the usefulness of both DMXB-A & *Solidago nemoralis* (SN) in treating deficits related to developmental ETOH exposure in humans and hopefully develop a treatment for these disorders.

KEYWORDS: DMXB-A, SOLIDAGO NEMORALIS (SN), FAS/FASD

Logan James Fields
July 25th, 2018

POTENTIAL CANDIDATES FOR TREATING DEFICITS ASSOCIATED WITH DEVELOPMENTAL ETHANOL EXPOSURE IN A RODENT MODEL: SOLIDAGO NEMORALIS & DIMETHOXYBENZYLIDENE-ANABASINE

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Chapter 1:

Overall Introduction

Ethanol Use, Abuse, and Outcomes

Excessive alcohol consumption remains an issue in western civilization with approximately 25% of the population (18 years or older) reporting a binge drinking episode in the last month (NIAAA, 2017). Alcohol use among pregnant women in the United States was estimated to be at 15% while binge drinking during this same period was around 3% (Popova, et al. 2017). Consuming ethanol (ETOH) during pregnancy can have numerous negative consequences for the developing fetus including Fetal Alcohol Syndrome (FAS) or Fetal Alcohol Spectrum Disorder (FASD). The syndrome itself was first clinically described in France in 1968 by Lemoine and colleagues (Lemoine et al., 1968) and then later (1973) in America by Jones & Smith (Jones & Smith, 1973). FAS is diagnosed using a characteristic set of facial anomalies including: short palpebral fissures, abnormalities in the premaxillary zone (including a flat upper lip, flattened philtrum, and flat midface), evidence of pre/post-natal growth retardation (low birth weight, failure to thrive), and finally evidence of central nervous system (CNS) damage (microcephaly, intellectual disability, or neurobehavioral deficits) (Stratton, 1996). As understanding of the disorder increased researchers eventually described a spectrum of disorders – Fetal Alcohol Spectrum Disorders (FASD's) caused by ethanol exposure during fetal development with FAS being the most severe. Importantly, the guidelines for identifying FASD is that children do not have to demonstrate all of the characteristic symptoms found in FAS to be identified as being affected by prenatal ethanol exposure

(Jones, 2011). Broadening the category of those individuals affected by prenatal ethanol exposure may help families and children that may not have all of the facial abnormalities affected by the disorder receive the potential help they need and lead to the development of pharmacotherapies aimed at treating or eliminating these disorders before or after they occur. As of 2016 the clinical guidelines for diagnosing FAS and other disorders relating to developmental ETOH exposure were revised in an effort to increase sensitivity to the disorder (Hoyme, et al. 2016). Within these new guidelines children believed to be affected by prenatal ETOH exposure must demonstrate evidence of neurobehavioral impairment including deficits in global intellectual ability (full-scale, verbal, performance or spatial IQ), cognition (executive functioning, learning, memory, visual-spatial skills), behavior and self-regulation (mood, behavioral regulation, attention, and impulse control), and adaptive skills (Hoyme, et al., 2016). In response to these new guidelines, prevalence of the disorder has now been conservatively estimated to be around 1-5% of first-grade children (May, et al. 2018). Interestingly, this study also suggests that underdiagnosis or missed diagnosis is incredibly high, of the over 6000 children they investigated 222 were diagnosed with prenatal ethanol effects by their clinicians, however, only 2 of them were previously diagnosed (May, et al. 2018). Individuals living with FAS/FASD typically express some behavioral/learning deficits and often experience social/economic difficulties later in life (Bishop et al., 2007). Common behavioral deficits associated with developmental ETOH exposure include issues with executive functioning, cognitive flexibility, motor coordination, working memory, spatial learning, attention, and hyperactivity (Jones, 2011; Mattson et al., 2010; Mattson & Riley, 1998). FAS/FASD remains the leading preventable cause of intellectual disabilities in the

Western World and healthcare and other indirect costs associated with the disorder are estimated to be as high as $4 billion annually in the US alone (Olson, et al. 2009; Hoyme, et al. 2016).

ETOH's effects on the brain:

The developing fetus is in a constant state of growth which encompasses the development of the central and peripheral nervous systems. These systems are highly susceptible to the neurotoxic effects of ethanol during their development. Some of ethanol's effects on the CNS include: disruption in cellular metabolism, an increase in hypoxic events, the creation of free radicals, neuroinflammation, excitotoxicity, an inhibition of DNA methylation, and membrane fluidization (For review see: Alfonso-Loeches & Guerri 2011; Berman & Hannigan 2000; Goodlett & Horn, 2001 & 2005; Jones 2011, Olney, J. 2004; West et al., 1994). These various mechanisms of damage lead to disruption of brain development and subsequent cognitive, motor, and behavioral functions in almost every region of the brain (for review see Moore et al., 2014). In a human study conducted by Chen and colleagues (2011), they reported that absolute volume of nearly every region of the brain was significantly reduced in young adults who were exposed prenatally to ethanol and that certain regions appeared to be more affected by higher levels of ethanol including the corpus callosum, cerebellum, and frontal areas. This loss is seen in both grey and white matter when compared to control individuals (Lebel et al., 2011; Archibald et al., 2001). The corpus callosum, a structure crucial to interhemispheric communication, appears to be one of the most highly affected structures in the CNS with complete agenesis of this structure being reported in a number of severe cases of FAS (Moore et al., 2014). Additionally, imaging studies using young children

diagnosed with FAS have shown dramatic changes in volume to the hippocampus, a structure critical to learning and memory (Archibald et al., 2001; Riley & McGee, 2005). Moore (2014) said this loss in volume and change of shape in the hippocampus has been highly correlated with deficits in spatial recall and learning. In animal models of developmental ETOH exposure, studies using *in-vitro* hippocampal cultures have shown that ETOH exposure followed by withdrawal in the hippocampus drastically reduces survival of the cells in several regions of the hippocampus (Barron et al., 2008; de Fiebre, 2003 & 2005, Li et al., 2000 & 2002; Prendergast et al., 2000 & 2004; Wilkins et al., 2006). Much of the basal ganglia, important for a variety of functions including procedural and habit learning, movement, emotion, and cognition, is significantly reduced in volume after developmental ETOH exposure when compared to control individuals (Mattson et al., 1996; Archibald et al., 2001; Lebel et al., 2011). This is particularly true with the caudate nucleus and putamen (Chen et al., 2011). Within the frontal lobe, an area that controls executive function and control, there is a loss in white matter, grey matter, and total lobe volume when compared to controls (Lebel et al., 2011). The prefrontal cortex appears to be particularly sensitive to developmental ethanol exposure resulting in decreased cell volume as a result of apoptosis that persist into adulthood (Ikonomidou et al., 2001). This decrease in cell volume in the prefrontal cortex could lead to reductions in connectivity with other subcortical structures leading to behavioral deficits, particularly cognitive function (Gorsky & Klinstova 2017). Within the sub-regions of the frontal cortex, a review by Coles and colleagues (2011) using fMRI data showed that activity is decreased compared to controls in individuals with FASD as executive functioning tasks become more difficult. Another structure that

undergoes significant cell loss is the cerebellum (Coles et al., 2011). Within the cerebellum, a structure critical for its role in movement and coordination, researchers have found significant reduction in total volume (Archibald et al., 2001). Interestingly, reductions of volume in these structures often correlate to the behavioral deficits most often found in FAS/FASD, this has also been found to be true of rodent models as well (Schneider, et al., 2011; Jones, et al., 2011; Mattson et al., 2010; Mattson & Riley, 1998).

As a consequence of structural alterations produced by developmental ETOH exposure there are also many deficits in cognition and behavior. One of the more common behavioral deficits found in human populations exposed to ETOH during development is hyperactivity and inattention (Jones et al., 2011). Often, individuals with prenatal ethanol effects are diagnosed with ADHD (Mattson et al., 2011). Another deficit commonly found in those individuals with FAS/FASD's is executive function or higher cognitive processes (Riley et al. 2011; Jones et al., 2011). Executive function involves the frontal lobe which sends projections to the basal ganglia, amygdala, hippocampus, and thalamus (Vertes, 2006; Quirk et al., 2003). These structures and networks are responsible for integration and regulation of many complex signals (Miller & Cohen, 2001). The amygdala, for example, helps regulate attention to fear and anxiety and other stressors (Yin et al., 2006), while the basal ganglia regulates motivated learning and goal-oriented behaviors (Pourtois et al., 2013), with the hippocampus being responsible for spatial processing and memory formation (Bannerman et al., 2004). Together, these integrated structures allow an organism to attend to specific stimuli, problem-solve, and maintain goal-oriented behavior, which is a large part of many complex (like planning, response inhibition, and working memory) and basic behaviors (attention, memory,

sensation, perception, and motor activation) affected by prenatal ETOH exposure (Mattson, et al., 2011). One aspect of executive function that is of interest in the current project is concept formation and set-shifting ability. In children exposed to ETOH during development their ability to complete the Wisconsin Card Sorting Test (WCST, a test designed to test concept formation and set-shifting ability) was significantly lower than control children (McGee et al., 2008). It also appeared that individuals with a diagnosis of FAS or those exposed to developmental ETOH but not FAS showed similar levels of deficits relative to controls (McGee et al., 2008). Along with deficits in set-shifting ability, many children exposed to prenatal ETOH also had trouble with inhibitory control, as measured on the Stroop Test (Mattson et al., 1999). For working memory, a function largely controlled by the hippocampus as well as other structures including the basal ganglia and frontal lobe, children exposed to ETOH were deficient at a Digit Span test when compared to control children (Burden et al., 2009). Additionally, spatial working memory was impaired in children and adults exposed to prenatal ETOH (Green et al., 2009). Regarding motor function, controlled largely by the cerebellum, it appears that children with FASD or FAS have deficits in postural stability, gait, motor reaction time, fine motor speed and coordination, timing, hand eye coordination, goal directed movements, and sensory processing and performance (see Mattson et al., 2011 for review). As reviewed by Schneider and colleagues (2011) and discussed later, animal correlates of these behaviors have been demonstrated using many different models. Within these models, animals exposed to developmental ETOH have shown similar deficits as the human population in all of the above-mentioned behaviors including

attention, learning, memory, executive function, and motor function (Riley et al., 2005; Mattson, et al., 2011; Thomas et al., 2004; Waddell et al., 2017).

Modeling FAS/FASD

Although the exact pattern, timing, and dose of ETOH exposure required for adverse outcomes is still relatively unknown, it appears that these factors do play a critical role in the development of FAS/FASD. First, alcohol readily crosses the placental barrier which causes the unborn fetus's blood alcohol content (BAC) to rapidly elevate to that of the mothers (Painter, 2012). During development, the fetus does not possess the necessary enzymes (alcohol dehydrogenase [ADH] and cytochrome P450 2E1 [CYP2E1]) of alcohol until later in pregnancy (CYP2E1 at week 16 on and ADH at week 26 on) and even after these metabolites are present, they are not as effective as that of adults which cause extended ethanol exposure (Ehrhart et al., 2018). Research in rodents related to dose has shown that moderate to high levels of ETOH exposure (ranging from 100-400mg/dl) can reliably produce neurotoxicity and adverse behavioral effects, but much of this is often dependent on pattern of drinking as well (for review see Patten et al., 2014). Binge exposure followed by a period of withdrawal led to greater elevations in peak BAC's and greater neurotoxicity than other patterns of drinking (Livy et al., 2003). In clinical populations this pattern of binge drinking followed by periods of withdrawal has been linked to more severe deficits in children with FASD's (Painter et al., 2012). This pattern of drinking (short periods of both binge and withdrawal) is very common in individuals with an alcohol use disorder (AUD) (Painter et al., 2012).

The timing of ETOH exposure is also critical when considering the consequences of developmental ETOH exposure. Because mammals have a strikingly similar CNS developmental trajectory it makes comparison between species easier for investigating the effects of developmental ETOH exposure (Patten et al., 2014). They go through massive amounts of neural cell generation, migration, proliferation, and cell death during critical periods of development (Goodlett et al., 2005). A significant period of brain growth referred to as the "brain growth spurt" occurs in humans during the 3rd trimester and extending shortly after birth while in rodents the equivalent period is the first weeks after birth (Dobbing & Sands, 1979). Because this growth spurt occurs postnatally in rodents, it presents a great opportunity to manipulate and investigate this sensitive period with a high level of control that might be impossible *in utero*. Though many deficits both physiological and psychological have been reported in models focusing on the rodent prenatal ETOH exposure model (1st and 2nd trimester human equivalent), the rodent postnatal ETOH exposure model (3rd trimester human equivalent) which overlaps with the brain growth spurt seems to be particularly sensitive to the teratogenic effects of ETOH exposure (Barron et al., 2016; Goodlett et al., 1997; Riley et al., 1993). Indeed, model's focusing on this period of CNS development have reliably found deficits in motor coordination (Idrus et al., 2011; Lewis et al., 2007; Thomas et al., 2004), attention/activity (Smith et al., 2012; Thomas et al., 2007; Lewis et al., 2012), learning and memory (Hunt et al., 2009; Thomas et al., 2009; Tiwari et al., 2012), and executive function (Thomas et al., 2000). These deficits are not surprising however because the structural deficits caused by ETOH exposure during the third trimester appear to have significant effects on the cerebellum, hippocampus, and prefrontal cortex; all structures

that undergo significant growth during the first weeks after birth and are involved in these behaviors (for review see Dobbing & Sands, 1979; Riley & McGee, 2005 & Patten et al., 2014). Though drinking during the third trimester is low (as low as 2.5% of women who drink during pregnancy reported drinking during the third trimester [CDC, 2016]), targeting this period in animals is beneficial to further our understanding of ETOH's effects on the developing brain and how treatment with various compounds may prevent this damage.

ETOH's Mechanism of Damage & Potential Targets for Treatment

Ethanol's effects on the brain are diverse and so too are the mechanisms in which it damages the brain (disscused in depth by Alfonso-Loeches & Guerri 2011). ETOH has an overall inhibitory effect on CNS function by increasing binding capacity and potentiating the action of the inhibitory neurotransmitter GABA at the type-A GABA receptor subunit and also type 1 glycine receptors (Reviwed in Proctor et al., 2006). Exposure to ETOH also inhibits function of glutamatergic N-methyl-D-Aspartate (NMDA) and influences synaptic clustering of these receptors (Kalluri, et al., 1998; Ticku 1980). With prolonged exposure the brain adapts to restore homeostasis by upregulating NMDA receptor density and/or function. When alcohol is removed from the system withdrawal is initiated and the system goes into a state of neuronal hyperexcitability due to the increased expression/function of the NMDA receptors along with a release of inhibition by the GABA'ergic receptors (reviewed in Hoffman et al., 1990 & Hunt, W. 1983). In addition to ETOH's general effects on GABA and glutamate, it appears ETOH can potentiate serotonin type-3 (5-HT3) receptor function, inhibits or stimulates 5'-triphosphate-gated purinergic receptors, and also decrease agonist binding

affinity (Hendricson et al., 2007; Proctor et al., 2006). Exposure to ETOH also appears to enhance ACh-evoked currents specifically from nicotinic receptors (Covernton & Connolly 1997). Calcium flux through voltage-gated ion channels declines with inhibition by ETOH exposure which can lead to downstream effects discussed later (Proctor et al., 2006). Some of the specific effects of ETOH, as mentioned above, depend on the stage of development and when ethanol is on board. ETOH can have varying effects on cell proliferation, migration, growth, and differentiation in various regions of the brain. In early development (embryonic stage) for instance, the reduction of retinoic acid (a critical regulator of the Hox gene which directs bodily growth) caused by ethanol's competitive binding on ADH, appears to play a role in neural crest death and defects throughout the brain (Alfonso-Loeches et al., 2011). Also, ethanol exposure during early and later brain development can disrupt the production of critical neurotrophic factors (which support cell survival and maturation). Indeed, brain-derived neurotrophic factor (BDNF, responsible for neural survival and synaptic plasticity) and its receptor TrkB (tyrosine kinase receptor, which activates PI-3-K/Akt and extracellular signal-regulated kinases [ERK] pathways which stimulate cell survival transcription factors) are lowered by ethanol exposure leading to cell death (for review see Climent et al., 2002). Ethanol also appears to disrupt growth factors related to cell-cycle events (specifically cyclin-dependent kinases) and insulin-like growth factor (IGF) involved in cell differentiation and proliferation respectively (Alfonso-Loeches et al., 2011). Additional effects of ethanol on the developing brain include reduction of neural cell adhesion molecules (NCAM's) critical for cell-cell interaction and brain organization by participating in cell migration, morphogenesis, synaptogenesis and synaptic plasticity in

the brain (Shapiro et al., 2007; Alfonso-Loeches et al., 2011). Ethanol also appears to promote caspase-3 activation (a protease involved in apoptosis) through inhibition of N-methyl-D-aspartate receptors (NMDAr's) and GABA'ergic activation which can lead to cell death in the developing brain (Olney et al. 2002). Glial formation and development, which plays an integral part in migration of young neurons in early development, formation of healthy synapses, regulation of neurotransmitter and energy levels, plasticity, cell to cell communication (through oligodendrocytes and myelin formation), and neuroimmune function also appear to be altered by ethanol exposure during development (see Alfonso-Loeches et al., 2011 for review).

With this evidence there are many potential targets for treating the effects of developmental ETOH exposure. One particularly promising direction for potentially reducing or eliminating the development of FAS/FASD could be treating the toxicity associated with ETOH withdrawal (EWD). ETOH has an overall inhibitory effect on CNS function by increasing binding capacity and potentiating the action of the inhibitory neurotransmitter GABA at the type-A GABA receptor subunit. With prolonged exposure the brain adapts to restore homeostasis by upregulating NMDA receptor density and/or function. When alcohol is removed from the system withdrawal is initiated and the system goes into a state of neuronal hyperexcitability (reviewed in Hoffman et al., 1990 & Hunt, W. 1983). Withdrawal from ETOH in the neonate is severely damaging to the CNS and produces many deficits demonstrated both in cellular (de Fiebre et al., 2003; Li et al., 2004; Prendergast et al., 2000 & 2004) and *in-vivo* models (Barron et al., 2008, Goodlett & Horn 2001; Thomas et al., 1997). This ETOH withdrawal induced damage is thought largely to be caused by neuroinflammation (Cantacorps et al., 2017),

excitotoxicity (Guizzetti et al., 2014), and oxidative stress (Bondy, 1992). ETOH exposure appears to be linked to the release of pro inflammatory lipopolysaccharide (LPS) which activates neuroimmune cells and microglia that can lead to neuroinflammation and cell death (Lutz, 2015). This process can also cause the excess buildup of reactive oxygen species (ROS) leading to oxidative stress (Lutz, 2015). Oxidative stress can also occur in cells in response to a buildup of ROS as a consequence of ETOH metabolism, which is particularly true when excessive amounts of ETOH are present (Ehrhart et al., 2018; Bondy, 1992). ROS oxidizes lipids, proteins, and other metabolites and can lead to DNA damage which can trigger apoptosis. These apoptotic events triggered by ROS are correlated to brain volume loss and abnormalities of cortical structures which lead to deficits in cognition and behavior (Ehrhart et al., 2018). The adult and especially developing brain is particularly sensitive to ROS mediated damage because of its high concentrations of unsaturated fatty acids, high oxygen consumption, reduced anti-oxidant concentration, and presence of metals catalyzing free radical formation (Alfonso-Loeches et al., 2011). Excitotoxicity is believed to occur because of the upregulation of the glutamatergic NMDAR's (a magnesium blocked ion channel that regulates calcium entry into the cell) and changes to their subunit composition in response to elevated levels of ETOH exposure that makes the receptors hypersensitive to excitatory signaling (Smothers et al., 1997). In a normally developing brain NMDAR's are involved in neuronal differentiation, cell survival, neuronal migration, synaptogenesis, structural remodeling, long-lasting forms of synaptic plasticity and higher cognitive functions (for review see Costa et al., 2000). ETOH withdrawal induces glutamate release which then leads to overactivation of the upregulated NMDAR's and

eventually to excitotoxicity (Rossetti et al., 1999; Prendergast et al., 2004). Additionally, this process leads to a buildup of excess calcium intracellularly which can also lead to the buildup of ROS which can cause cell death (Lutz, 2015).

Many compounds have been investigated using *in-vitro* models for their ability to reduce one or several of the damaging mechanisms discussed above with varying levels of success. Many studies have focused on reducing glutamate activity to try to reduce the effects of excitotoxicity as described above. One of the first studies investigating toxicity related to the glutamatergic system discovered that when antagonized, the NMDA receptor (with MK-801 [dizocilpine], a noncompetitive antagonist which binds inside the magnesium channel preventing calcium flow) during EWD, there is a reduction in cell death and behavioral deficits (Thomas et al., 1997). Interestingly, it also appeared that this neuroprotection produced by MK-801 resulting in NMDAR blockade only occurred during EWD (Thomas et al., 2001). This may mean that NMDAR blockade is only effective during periods of withdrawal. Additional studies looking at the NMDAR showed that MK-801 and ifenprodil (NMDA antagonist at the NR2B subunit which prevents receptor activation) reduce neurotoxicity and calcium entry produced by EWD in organotypic hippocampal slices (Mayer et al., 2002). Memantine (a noncompetitive antagonist to the NMDAR and partial agonist to the α7-nAChR) was also able to reduce EWD induced neurotoxicity in a similar organotypic hippocampal slice culture model (Stepanyan, et al., 2008). Another route for treatment is targeting polyamines (spermine and spermidine), which appear to be complex modulators of the NMDAr's. Polyamines, whose levels are elevated during EWD, lead to increased activation of the NMDAr's and so are correlated with EWD induced neurotoxicity. Reduction of polyamine activity by

ifenprodil reduced the damaging effects of EWD both *in vitro* and *in vivo* models (Barron et al., 2008; Barron et al., 2016). Other treatments have focused on the negative effects of oxidative stress caused by ETOH exposure. As mentioned above, ETOH damaging effects are thought to be produced by several methods including oxidative stress and the buildup of ROS. Antioxidants are believed to be able to reduce the effects of developmental ETOH exposure (for review see Cohen-Kerem & Koren 2003). Supplements like ascorbic acid (vitamin C) or Vitamin C in combination with Vitamin E can reduce some of the effects of prenatal ETOH exposure in a number of *in vivo* and *in vitro* models (Peng et al., 2005). Even with this research there is still not a clinically approved medication for treatment of development ETOH exposure. Because of this, it is clear that further work needs to be completed. Development of a compound aimed at reducing the damage associated with EWD is appealing because it is potentially a one-time treatment that would protect the fetus or newborn after ETOH has left the system.

Cholinergic System

One neurotransmitter system that has shown great potential for possibly treating the toxic effects of developmental ETOH exposure is the cholinergic system. The cholinergic system is one of the most commonly studied systems in the nervous system and responds endogenously to the neurotransmitter acetylcholine (ACh). Acetylcholine is synthesized by choline acetyltransferase (ChAT) using both choline and acetyl CoA by (Abreu-Villaca et al., 2011). The expression of ChAT during brain development is commonly used to indicate the location and development of the cholinergic system (Lauder & Schambra, 1999). In rats ChAT immunoreactive cells appear 1-2 days following generation of neurons from dividing progenitor cells, however the rat brain

does not reach adult levels of ChAT reactivity until over a week after birth, depending on the structure (cortex & hippocampus is 10 days post-natal development (PND) and PND 25 for most other structures) (Abreu-Villaca et al., 2011). During early development ACh is first detected in the medial septum, diagonal band, and magnocellular preoptic regions but as development continues, ACh can be found in target locations like the hippocampus and cortex. Along with this it appears that early ACh signaling along with nerve growth factor (NGF) may help growing cholinergic neurons find their way to their target destination (Abreu-Villaca et al., 2011). In adult brains, the primary source of ACh is from projection neurons (found in the pedunculopontine nucleus, laterodorsal tegmental areas, medial habenula, and basal forebrain) and interneurons (found throughout the striatum, nucleus accumbens, and to a lesser extent the neocortex) (Picciotto et al., 2012). In the CNS, the cholinergic system is thought to change neuronal excitability, alter presynaptic release of neurotransmitters, and coordinate firing of groups of neurons (for review see Picciotto et al., 2012). Along these lines, ACh is thought to be a facilitator and or modulator of many behaviors including processes related to wakefulness and attention and is also thought to play a critical role in both learning and memory (Hurst et al., 2013).

ACh acts on two distinct types of receptors, the nicotinic ACh receptors (nAChR's) and muscarinic ACh receptors (mAChR's). These ACh receptors play roles in the modulation of cell proliferation and survival, neuronal differentiation, regulation of gene expression, synapse formation and maturation, axonal pathfinding, and neurotransmitter release (For review see Abreu-Villaca et al., 2011). For purposes of the current dissertation, the focus will be on the role of the nicotinic system.

Nicotinic Receptor System

The nicotinic receptor system is named so because of its high affinity for nicotine (the major psychoactive compound commonly found in tobacco). These receptors were the first membrane receptor proteins identified in the 1970s (Dani, 2001). Nicotinic receptors are among the first proteins to appear in the developing brain, and some of the receptors subunits can be found as early as embryonic day 7 (Atluri et al., 2001). Deficits in nicotinic receptors, have been implicated in several neurological disorders including Alzheimer's disease, Parkinson's diseases, and Down's syndrome (Dineley et al., 2015; Levin, 2002). They fall into a class of ligand-gated ion channels that respond endogenously to acetylcholine or exogenously to nicotine (Dani, 2001; Pauly et al., 2004). The function and location of the receptor in either the CNS or peripheral nervous system (PNS) is dependent upon the type and arrangement of its subunits. The nicotinic receptor is made of five membrane-spanning regions; each of the five constituent regions is composed of different or similar subunits (Wu & Lukas, 2011). The subunits that form nicotinic receptors in neural tissue include alpha (2-10) and beta (2-4); these subunits can be arranged in many different conformations that are either heteromeric (alpha2-alpha6 and beta2-beta4), or homomeric (alpha7-alpha9) (Alfonso-loeches et al., 2011; Dani, 2001). The major subtypes found in the CNS are the alpha4-beta2 heteromeric nAChR's and the alpha7 homomeric nAChR's (Dani, 2001). On neurons, the role the nAChR's play in the CNS are thought to be largely modulatory because they are often dispersed and non-centralized along neuronal membranes of the cell body, presynaptic terminals, and even axons (Picciotto et al., 2012). They modulate the release of glutamate, GABA, DA, ACh, norepinephrine, and serotonin depending on their location and the subunits

they are composed of (for review see Dineley et al., 2015). Recent evidence has also demonstrated some nAChR's are also found on glial cells (astrocytes, macrophages, microglia) throughout the nervous system and play a role in modulation of immune response which can be protective of events like neuroinflammation, oxidative stress, and plaque formation (Suzuki, et al. 2006), discussed further below. These receptors are found throughout the brain, including the location of ACh cell bodies as discussed above, and sites of their projections including the areas that are most affected by developmental ETOH exposure including the frontal cortex, hippocampus, cerebellum (For review see Hurst et al., 2013). Activation of neuronal nicotinic receptor system has demonstrated efficacy in protection of cells from a variety of toxic challenges, including brain injury, oxygen-glucose deprivation, oxidative stress, β-amyloid toxicity, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) kainite and glutamate toxicity, ethanol exposure, and nerve growth factor (NGF) deprivation (reviewed extensively in Benchreif et al., 2014).

α7 nAChR

Of particular interest to the current dissertation is the α7 nAChR; this receptor is of interest to the current study because of its high concentration in areas associated with FAS/FASD's including the hippocampus, prefrontal cortex, and cerebellum (Dani, 2001; Hurst et al., 2013). The α7 nAChR is highly permeable to calcium, and is responsible for fast postsynaptic transmission, the modulation of release of numerous transmitters, and long-term potentiation in various areas throughout the brain (Hurst et al., 2013; Dani, 2001). During development, the α7 nAChR is thought to be involved in regulating neural growth, differentiation and synapse formation through second messenger systems activated by calcium entry into the cell (Abreu-Villaca, et al., 2011). This receptor has

been given considerable attention recently and is being investigated for its potential activation/modulation to treat the multifaceted causes/effects of many neurological diseases and disorders (for review see Bencherif et al., 2014).

In neurons, these receptors are located on the cell at the axon terminal, the pre-terminal (region just before the terminal that can affect several terminals at once), and the postsynaptic dendritic membrane (Albuquerque et al., 2009). This receptor has also been found in many non-neuronal cells as well including the macrophage, astrocyte, and microglia, which seem to play a part in reducing neuroinflammation (Suzuki et al., 2006; Wang et al., 2003; Xiu et al., 2005; Shytle et al., 2004). Previous studies have shown that activation of the α7 nAChR has shown considerable promise in reducing excitotoxicity, neuroinflammation, and oxidative stress (Benchrief et al., 2014; de Jonge & Ulloa, 2007; Prendergast et al., 2000 & 2001; Li et al., 1999). α7 nAChR's role on microglia, when activated, reduce microglial activation and reduce LPS-induced TNF-alpha release (Suzuki et al., 2006; Shytle et al., 2004). Activation of the α7 nAChR has also been shown to reduced LPS-induced TNF-alpha release in an *in-vitro* model of EWD in the hippocampus of neonatal rodents (Lutz et al., 2015 a & b). The internal mechanism for the α7 nAChR modulation of inflammatory response in these cells remains unclear. However, several studies have postulated that activation of the receptor creates a neuroprotective state in the microglia and in turn reduces transcription and phosphorylation of critical components required for activation of an inflammatory response like nuclear factor kappa-b, and mitogen-activated protein kinase (see Lutz, 2015 & Suzuki et al., 2006 for review).

Nicotinic agonists that target the α7 nAChR have also been shown to reduce glutamatergic excitotoxicity as well as EWD induced excitotoxicity. Dajas-Bailador and colleagues (2000) demonstrated that reduction in NMDA-induced toxicity was modulated by the α7 nAChR because co-exposure with MLA (an α7 nAChR antagonist) blocked the protective effects of nicotine. Activation of the α7 nAChR has also been directly linked to reducing EWD related toxicity in PC12 cell lines, primary hippocampal cultures and in organotypic hippocampal cultures (Prendergast et al., 2000 & 2001; de Fiebre et al., 2003 & 2005; Yangxin et al., 2002). Again, the exact mechanism with which activation of the α7 nAChR reduces excitotoxicity is not clear. One theory proposes that activation of the α7 nAChR increases calbindin-D28K (a calcium buffer) activity which in turn reduces intercellular calcium and increases cell survival (Mullholland et al., 2003). Another interesting possibility is through cross-desensitization with the NMDAR. Activation of the α7 nAChR by donepezil hydrochloride appeared to mediate phosphorylation and subsequent internalization of specific NMDAR receptor subunits preventing excitotoxicity (Kihara et al., 2010). However, it is believed that these mechanisms do not work in an exclusive manner, instead, it appears that the combination of anti-inflammatory signaling along with modulation of the NMDAR works together to reduce cell death in the CNS (Lutz et al., 2015).

Additional support for the activation of the α7 nAChR reducing the deleterious effects of developmental ETOH exposure stems from choline supplementation. Choline, a chemical precursor to acetylcholine and an essential nutrient that targets the α7 nAChR nicotinic receptor has shown great promise in reducing ETOH's deleterious effects. There are many published reports demonstrating choline's effectiveness in reducing ETOH

related deficits in learning and memory (Thomas et al., 2000), attention (Monk et al., 2012; Thomas et al., 2004), balance (Thomas et al., 2009), and executive function (Mooney & Waddell et al., 2017, Thomas et al., 2004). In humans, when pregnant mothers who had consumed ETOH were given a choline supplementation their babies demonstrated less of the adverse effect of prenatal ETOH exposure including reduced postnatal growth and cognitive deficits when compared to the babies of women given a placebo during pregnancy (Jacobson et al., 2018). These results are promising, however, to better understand the role of the *α7 nAChR* in developmental ETOH exposure this dissertation used compounds that have much higher affinity for this receptor. Because of this and the vast amounts of data supporting the receptors role in reducing neuroinflammation, excitotoxicity, and behavioral deficits, it could be very useful to assess agents that have a higher specificity for the α7 nAChR during critical periods of CNS development including during the third trimester brain growth spurt.

Two Compounds Targeting the α7 nAChR

This dissertation investigated the potential of two compounds that target the α7 nAChR to investigate the importance of this receptor in reducing the deleterious effects of developmental ETOH exposure. As stated previously, the damage caused by ETOH exposure and EWD is multifaceted and in-turn requires a multi-functional pharmacotherapy to treat this damage. Multi-target compounds either hit numerous targets simultaneously or are specific to a single target that is capable of interfering with several pathological mechanisms. Interestingly, both compounds investigated (discussed at length below) appear to reduce excitotoxicity, oxidative stress, and neuroinflammation through selective activation of the α7 nAChR. One of the compounds, DMXB-A is

derived from marine worms and has gone through several stages of clinical testing for various neurological and neurodegenerative diseases (Kem, 2007) but has not been tested in developmental ETOH exposure. Though it's safety has been tested in adults, it would clearly need further testing before it could be evaluated in pregnant women and children before it could be considered a clinically viable treatment. The flavonoid enriched extract *Solidago nemoralis* on the other hand contains active flavonoids that can be found in common crop plants like apples and corn which would classify this compound as a "nutraceutical" or "functional food" and make regulatory approval easier to obtain. This dissertation aims to compare these two compounds to determine if either *Solidago nemoralis* or DMXB-A is more or less efficacious in reducing behavioral deficits following developmental ETOH exposure. Also, neither compound has been investigated in our model of ETOH exposure before, and so positive results for either compound would further our understanding of the α7 nAChR's role in reducing developmental ETOH exposure as well as potentially lead the way for one or both compounds to be investigated clinically to treat developmental ETOH exposure in human populations.

DMXB-A

The first study, discussed in chapter two, investigated 3-dimethoxybenzylidene-anabasine (DMXB-A) also known as GTS-21 ability to reduce behavioral deficits found in our rodent model of developmental ETOH exposure. DMXB-A is a derivative of an anabaseine compound found in marine worms (Olincy & Stevens, 2007). It has a high affinity for the α7 nAChR, showing about 40% of the efficacy of ACh for the receptor and a much lower affinity for other nicotinic receptors like the α4β2 and type 3 5-HT receptors where it acts as an antagonist (Kem et al., 2006; de Feibre, 2003; Olincy &

Stevens 2007). It is lipophilic and readily crosses lipid membranes such as the blood brain barrier (BBB) easily (Kem et al., 2006). In the human brain, DMXB-A is less potent than nicotine (Ki=2000) when displacing α-BTX, however in the rat brain DMXB-A is more potent than nicotine at displacing α-BTX at the α7 nAChR (Ki=650) (Briggs et al., 1997). Because the α7 nAChR desensitizes rapidly after activation some researchers have considered DMXB-A a partial agonist, however, it is believed that lower concentrations of the compound (clinically relevant doses) produce a more prolonged effect (for review see Kem et al., 2006, & Li et al., 1999). In *in-vivo* animal models DMXB-A improved cognitive functioning of eyeblink conditioning in an animal model of Alzheimer's and was neuroprotective against beta-amyloid toxicity (Kem, 2000; Kihara et al., 1997; ll et al., 2010; Woodruff et al., 1994). DMXB-A also rescued cognitive deficits measured by passive avoidance and spatial working memory after mecamylamine exposure in rodents (Meyer et al., 1997). Additionally, it improved object recognition and pre-pulse inhibition (PPI) in male rodents who had been given MK-801 (NMDA receptor antagonist) (Callahan et al., 2014). DMXB-A was also neuroprotective against global ischemia and glutamate-induced excitotoxicity in rodents while also improving cognitive function on a passive avoidance task (Nanri et al., 1998). DMXB-A has also been suggested to have an anti-inflammatory function by activating the α7 nAChR and inhibiting LPS-induced TNF- α release from cultured microglia and splenic monocytes (Thomsen and Mikkelson, 2012; Yang et al., 2017). Additionally, administration of DMXB-A reduced oxidative stress in response to ETOH exposure in cells as measured by 2,7-dichlorofluorescin (which detects ROS in cells)(Li et al., 1999). Data from *in vitro* studies suggest that DMXB-A dose-dependently reduced cell death

following ETOH exposure in E17 (embryonic) cells, PC12 (pheochromocytoma) cells, and in adult male rodents (DMXB-A preexposure 24 h prior to ETOH) (de Fiebre et al., 2003; Li et al., 2002; Shimohama et al., 1997; Tizabi et al., 2004). As mentioned above, the safety of DMXB-A in an adult human population has been verified by its use in clinical trials investigating it's efficacy in the treatment of schizophrenia, Parkinson's disease, and Alzheimer's disease and more (for review see Freedman et al., 2014 or clinicaltrials.gov). In phase II clinical studies of patients with schizophrenia, DMXB-A improved attention and memory and sensory inhibition in an animal model of schizophrenia (Freedman et al., 2014; Olincy et al., 2007). Taken together, it is apparent that DMXB-A can reduce neuroinflammatory response, ETOH-induced neurotoxicity, and various behavioral deficits. However, the present study was the first, to the best of our knowledge, to explore the ability of DMXB-A to reduce the behavioral deficits of third trimester ETOH exposure and withdrawal in male and female rodents.

Solidago Nemoralis

The other compound that was investigated in this dissertation was a flavonoid enriched extract called *Solidago nemoralis (SN)* (derived from the Gray Goldenrod). SN was extracted at the University of Kentucky by Naprogenix Inc while exploring various plant extracts for their potential α7 nAChR binding properties (Littleton et al., 2005). SN had an increased ability to displace methyllycaconitine (MLA, a selective α7 nAChR antagonist) in rodent hippocampal cells which demonstrated its specificity for the receptor (Lutz, 2014). One important characteristic of SN is its high concentration of flavonoids which are highly associated with anti-inflammatory responses in the periphery, CNS, and for their neuroprotective effects in models of neurodegeneration

(Gonzalez, et al., 2011; Spencer et al., 2012; Dajas et al., 2013). Flavonoids have also been studied extensively in models of ETOH exposure. For example, quercetin reversed hepatotoxicity and neurotoxicity induced by 90 days of ETOH administration (Ambadath et al., 2010). One of the flavonoids found in SN, rhamnetin, demonstrated an ability to reduce both neuroinflammation and excitotoxicity in response to EWD in an organotypic hippocampal slice culture (Lutz, et al., 2015 b). Goulart and colleagues (2007) also found that SN was able to reduce neuroinflammation in the periphery of mice. Though previous work with this novel compound is limited, the literature demonstrating that selective activation of the α7 nAChR by various flavonoids has the ability to reduce both neuroinflammation and excitotoxicity provided a strong rationale for using SN in the current study.

Measuring Efficacy of Pharmacotherapies in Behavioral Animal Models

For this dissertation, our lab used various tasks to model behaviors that are commonly affected in human populations with FAS/FASD. The first task we investigated was the open-field, which is an animal test to investigate attention, anxiety, hyperactivity, and impulse control through various measures including distance traveled in the apparatus (for review see Deneberg, 1969). This test was designed to measure anxiety in animals. For rodents, open spaces are aversive because of potential predation and produce anxiety-like behaviors including decreased movement, freezing, and maintaining close proximity to the walls of the apparatus. Hyperactivity in this task (as measured by increased distance traveled) or lack of impulse control (measured by increased entries into the center) are believed to be counterintuitive to rodents and indicative of deficits in this behavior (Deneberg et al., 1969). As previously mentioned, hyperactivity and deficits

in impulse control are common in individuals with FAS/FASD (see Riley et al., 2005). These deficits have been also commonly observed using animal models of developmental ETOH exposure as well (Jones et al., 2011). In our own lab, we have consistently demonstrated increased hyperactivity (as measured by increased distance traveled in the open field) in rodents exposed to ETOH during development (see Lewis et al., 2011). Thomas and colleagues (2004) demonstrated that perinatal choline supplementation reduced hyperactivity in an open-field task in neonatally exposed rodents. This behavior is believed to be mediated in part by the basal forebrain and hippocampus because lesions of this area have produced increases in activity level (for discussion see Thomas et al., 2004). These areas are also heavily innervated by nAChR's, including the α7-nAChR's (Smith et al., 2012; Hurst et al., 2013). So, the hypothesis was that using either of the compounds that target the α7-nAChR will be able to reduce hyperactivity produced in our model of developmental ETOH exposure. We also used a dowel balance paradigm which investigated motor function and coordination. As previously mentioned individuals exposed to prenatal ETOH often have motor and coordination deficits (Riley et al. 2011; Jones et al., 2011). Various animal models have been developed to investigate similar types of motor function as those seen in humans. Dowel rods and roto-rods have been commonly used to investigate motor function because they require the animal to maintain balance and coordinate movement to be successful in the task (for review see Deacon, 2013). This task was chosen because previous studies in this lab using this same task, and other labs using similar tasks have reliably demonstrated deficits in motor function and coordination in rodent models of developmental ETOH exposure (Lewis et al., 2007; Thomas et al., 2004 & 2009). Prenatal choline administration after prenatal ETOH

exposure was able to decrease deficits in balance and coordination (Thomas et al., 2009). In this study, it was believed that this protection against balance deficits was possibly due to choline's effects on the α7-nAChR. This receptor is located in the cerebellum and modulates glutamate release within this structure (Hurst et al., 2013; Koukouli & Maskos 2015). Though this has yet to be explored, it is possible that this modulation of glutamatergic receptors in the cerebellum by α7-nAChR during EWD could reduce cell death and improve future deficits in balance and coordination. An additional test used to investigate the two compounds of interest was a Hebb style water maze developed by Von Euler and colleagues (2006). This maze was developed to investigate spatial working memory in rodent models. Deficits in spatial working memory, as mentioned above, as well as a variety of other deficits regarding memory is very common in both FAS and FASD individuals (Jones et al., 2011). These deficits have also been characterized in rodent models of developmental ETOH exposure in this lab and others (Riley et al., 2005; Lewis et al., 2011; Thomas et al., 2004). The maze we use in our lab is a water based spatial navigation task. Much like other spatial navigation tasks (i.e. the Morris Maze, the T-Maze, and the Y-maze) our task requires the animal to make a series of correct choices over the trial period in order to find the escape platform. One advantage to the maze used in the current studies is that the task can be learned in a single day and retention of the task can be assessed 24 hours later (see Von Euler et al., 2006 for review) in contrast with the more standard "Morris water maze" that's requires multiple days of acquisition and makes interpretation of learning versus retention deficits more difficult. This maze is also advantageous relative to other Hebb-like land mazes because the animals do not need to be put on a restricted diet in order to complete the task, finding

the escape platform is motivated by being in water. As with the previous behaviors, several studies have implicated the α7-nAChR in reducing deficits related to spatial working memory. First, this task is thought to be largely controlled by the hippocampus and prefrontal cortex, both structures that are harmed by developmental ETOH exposure (Lebel et al., 2011), and are innervated by the cholinergic system and contains α7-nAChR's (Hurst et al., 2013). Postnatal choline supplementation was able to rescue deficits in a spatial navigation task (the Morris Water Maze) after ethanol exposure during the third trimester equivalent in rodents (Thomas et al., 2016). DMXB-A was also able to rescue spatial navigation deficits in the Morris task in rodents who had bilateral lesions of the nucleus basalis (a structure rich in cholinergic neurons) (Meyer et al., 1997). Taken together, it appears that as activation of the α7-nAChR can reduce deficits in spatial working memory and we hypothesize that our compounds will be able to do the same. Finally, in order to test executive function in our animals we used an attentional set shifting task (ASST). This task was only assessed in the *SN* study to determine if future use of this paradigm would increase our ability to interpret the efficacy of various compounds to reduce behavioral deficits produced by our model of developmental ETOH exposure in this and future studies. ASST is considered to be an animal correlate to the human Wisconsin card sorting task (see Barnese et al., 2002), where the participant is expected to shift between changing rules for reward. In our model, the animal is required to shift its attention from odor to odor or media to media, while ignoring the other odor and media cues present to receive reward. This task allows assessment of cognitive flexibility and reversal learning (a dimension of executive function) which is demonstrated in the animals ability to "shift" from a previously rewarded cue to a

different cue that is now the target (Waddell & Mooney 2016). Performance of this task appears to be largely controlled by the prefrontal cortex (Birrell & Brown et al., 2000; Barnese et al., 2002), a structure that contains many α7-nAChR (Hurst et al., 2013) and is particularly sensitive to developmental ETOH exposure (Barron et al., 2017; Goodlett et al., 1997; Riley et al., 1993), especially during the third trimester (Ikonomidou et al., 2000). These deficits are thought to arise from aberrant connectivity from the PFC to other structures critical for the performance of set-shifting like the hippocampus (Gorsky & Klinstova 2017). Previous studies using a similar task have shown that administration of an α7-nAChR agonist can both reduce deficits in an animal model of schizophrenia and improve cognitive flexibility in control animals (Wood, et al., 2016). Recently, choline supplementation after prenatal ETOH exposure improved deficits in a set-shifting task very similar to the task used in the current study (Waddell et al., 2017). Investigating this behavior in our model of ETOH exposure is unique in that it has never been done before and we hypothesize that our compounds will be able to reduce deficits in this task.

Project Overview

This dissertation focuses on the ability of two compounds (DMXB-A and SN) that selectively target the α7 nAChR to reduce behavioral deficits associated with postnatal "third trimester" ETOH exposure in male and female rodents. The primary hypothesis is that one injection of either drug 22 hours after the animal's final exposure to ETOH (during a period of withdrawal) will be able to reduce behavioral deficits caused by ETOH exposure. If both drugs successfully rescue behavior after developmental ETOH exposure in our model this project will lend support to the theory that agonists acting on the nicotinic receptor system, specifically the α7 nAChR, is a

promising route to reducing or eliminating ETOH's adverse effects on developing offspring. It will also provide justification to further explore these compounds in other models of ETOH exposure and CNS disease/disorder as well as possibly lead to clinical development of a pharmacotherapy to treat FAS/FASD. Both drugs are presented separately in their own chapters in a publication-like format.

Chapter 2: DMXB-A

Introduction:

Prenatal ethanol (ETOH) exposure can produce a variety of long-term behavioral, neuroanatomical and physical deficits. Though prevention would be ideal, the use of pharmacotherapies aimed at reducing deficits related to ETOH exposure during fetal development is necessary because of the prevalence of ETOH consumption during pregnancy (CDC, 2015). In fact, alcohol use among pregnant women in the United States was estimated to be at 15% while binge drinking during this same period was around 3% (Popova, et al. 2017). The effects of ETOH on the developing nervous system and studies aimed at reducing these deficits are numerous (for review see Barron et al., 2017), however there is still no medication clinically approved for the treatment of developmental ETOH exposure.

Previous studies have demonstrated that manipulation of the cholinergic system can reduce some of ETOH's effects on the developing fetus in various animal models of developmental ETOH exposure. Within the cholinergic system, activation of central nicotinic receptors has shown considerable promise in reducing behavioral deficits related to developmental ETOH exposure (Barron et al., 2016, Thomas et al., 2009) as well as the ability to reduce alcohol induced cellular toxicity (Hejmadi et al., 2003; Shimohama et al., 2001; Prendergast et al., 2000). The alpha 7 nicotinic acetylcholine receptors (α7-nAChR) activation is believed to provide neuroprotection from alcohol-induced neurotoxicity. The receptors high concentration in brain regions associated with FAS/FASD's make it an ideal target (Hurst et al., 2013). Additionally, previous studies

have shown that activation of the α7-nAChR reduced alcohol induced cell death in both primary neuronal hippocampal cell cultures and PC12 established cell lines (de Fiebre et al., 2003, Li et al., 1999). Supplementation with choline, an essential nutrient which has high affinity for the α7-nAChR, reduced behavioral deficits after neonatal ETOH exposure in rodent models (Ryan et al., 2008, Thomas et al., 2007 & 2009). Though treating deficits related to developmental ETOH exposure using compounds like choline are promising, it is relatively non-specific and to better understand the role of the α7-nAChR in this model it is beneficial to use compounds that specifically target this receptor. If specific activation of the α7-nAChR's do reduce deficits produced by developmental ETOH exposure it is worth pursuing pharmacotherapies that agonize this receptor for treatment of human populations with prenatal ETOH exposure.

Of interest to the current project is 3-(2,4-dimethoxybenzylidene)-anabasine (DMXB-A) or otherwise known as GTS-21. This compound is promising because of its high affinity for the α7-nAChR and the fact that it has been investigated in clinical trials for its ability to reduce the cognitive impairing effects of diseases like Alzheimer's and Schizophrenia (Freedman et al., 2014, Kem et al., 2006, Olincy et al., 2007). *In-vitro* studies suggest that DMXB-A can reduce cell death in response to administration of beta-amyloid in mice (Chen et al., 2010; Kihara et al., 1997), as well as provide neuroprotection against global ischemia in rodents (Nanri et al., 1998). When exposed to ETOH, DMXB-A dose-dependently reduced excitotoxic cell death in E17 (embryonic) cells, PC12 (pheochromocytoma) cells, and in adult male rodents (DMXB-A preexposure 24 h prior to ETOH), and in rodent organotypic hippocampal cells (de Fiebre et al., 2003; Li et al., 1999 & 2002; Shimohama et al., 1997; Tizabi et al., 2004; Fields et al.,

unpublished). DMXB-A has also been suggested to reduce neuroinflammatory response by activating the α7-nAChR and inhibiting LPS-induced TNF- α release from cultured microglia and splenic monocytes (Thomsen and Mikkelson, 2012; Yang et al., 2017). These actions are promising because both excitotoxicity and neuroinflammation are believed to be potent mediators of the damage caused by ETOH exposure which may lead to the development of disorders like FAS/FASD (Barron et al., 2017; Crews & Nixon, 2009). In *in-vivo* animal models DMXB-A improved cognitive functioning through eyeblink conditioning in a rodent model of Alzheimer's (Kihara et al., 1997; Chen et al., 2010; Woodruff et al., 1994). DMXB-A was also able to rescue cognitive deficits measured by passive avoidance and spatial working memory after mecamylamine exposure in rodents (Meyer et al., 1997). It was also able to improve object recognition and pre-pulse inhibition (PPI) in male rodents who had been given MK-801 (NMDA receptor antagonist) (Callahan et al., 2014). Taken together, it is apparent that DMXB-A can reduce neuroinflammatory response, ETOH-induced neurotoxicity, and various behavioral deficits. However, the present study was the first, to the best of my knowledge, to explore the ability of DMXB-A to reduce the behavioral deficits of third trimester ETOH exposure and withdrawal in male and female rodents. We hypothesized that DMXB-A would be able to reduce or eliminate the behavioral deficits found after "third trimester" ETOH exposure in rodents.

Methods:

Subjects & ETOH Exposure Paradigm:

Male and female Sprague Dawley rats were used for all experiments. Offspring were bred in the University of Kentucky Psychology Departments breeding facility. Once born, half of the offspring were exposed to ETOH (6 g/kg/day) administered by oral gavage from postnatal days (PND) 1-7. This model uses a split litter design with 1:1 male/female ratio designated to each of the following treatment groups: alcohol (6 g/kg/day), an intubated control, and a non-treated control. All intubated offspring received .0278 ml/g of body weight. No more than one male and one female were included in any cell of the experimental design to avoid potential litter effects (Abbey & Howard, 1973). Intubations were administered in a milk-based diet designed to mimic the pups mothers milk (West and Harne, 1984). Two daily feeds were administered at 10AM and 2PM and the ETOH exposed offspring received a 3rd intubation at 4PM of milk only to account for decreased bodyweight in the ETOH exposed offspring possibly due to intoxication or decreased feeding while non-ETOH pups received a sham intubation. The intubation schedule was used to achieve a binge-like pattern of exposure which is particularly harmful for the developing fetus (West et al., 1989). Blood ethanol concentrations collected previously from this lab have found that BEC's with this type of exposure regime typically peak at 230 mg/dl which returns to 0 within 10 hours after intubation (Lewis et al., 2007). These levels of alcohol have been shown to reliably produce behavioral deficits in a number of tasks (See Patten et al., 2014) and are clinically relevant to the study of developmental ETOH exposure in offspring because binge drinkers often display this level of BEC (Cherpitel, 2007).

Offspring weight was recorded daily. On PND 8, approximately 21 hours after receiving their last intubation, offspring were injected subcutaneously with either a vehicle control of 10mg/kg DMXB-A. Previous studies in animal models have found that doses from 5-15 mg/kg were efficacious in reducing cell loss and cognitive deficits associated with animal models of beta-amyloid toxicity (Callahan et al., 2015; Kem, 2000; Kihara et al., 1997; ll et al., 2010; Woodruff et al., 1994). This design allowed for six treatment groups: milk, milk + drug, ETOH, ETOH + drug, non-treated control (NTC), and NTC +drug. The NTC group contained two pups to avoid investigate potential intubation effects. At PND 21 the rats were weaned from their mother and moved from the nursery room to the colony room of Kastle Hall.

All offspring were kept on a 12 hour light/dark cycle, and were allowed continuous access to food and water. The care of the offspring was carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996), as well as the University of Kentucky's Institutional Animal Care and Use Committee.

Drugs:

For the following experiment DMXB-A was generously provided to the Barron lab by Dr. William Kem of the University of Florida. DMXB-A was administered subcutaneously on PND 8, 22 hours after the final intubation period. This time was chosen because previous research has found that this period of ETOH withdrawal in neonates appears to be particularly sensitive to administration of compounds that may reduce the excitotoxicity associated with ETOH withdrawal (see Thomas et al., 2006 for review). DMXB-A was administered at a dose of 10 mg/kg, a dose that has been

previously found to be efficacious in other rodent models of CNS disease/disorder (see Li. et al., 2001, & Kem et al., 2006). The drug was suspended in a saline solution and offspring that did not receive the drug received an injection of saline alone.

Behavioral Paradigms:

Open Field:

The Open-Field paradigm was used to investigate rodent hyperactivity and impulse control as measured by total distance traveled and distance traveled in the center of the apparatus. During open field testing (PND 20-21), offspring were housed with their mothers and litter-mates until testing began. On PND 20, pups were taken into the testing room alone for 10 min to habituate. The experimental room was illuminated with red lights, and a muffin fan which provided white noise. After habituation, each rat was placed into a round 55 cm open-field chamber for 30 minutes. The rats' activity was recorded using SMART real time video tracking (San Diego Instruments). For analysis, the open field was divided into center and outer zones, comprising 25% and 75% of the total field area respectively. Total distance traveled, and distance traveled in the center of the open field over a 30-minute period was measured. Once testing was completed, bodyweights were recorded, and the subjects were returned to their home cage. Both chambers were then cleaned with a 30% ETOH solution to eliminate any possible odors prior to the next test session.

Dowel Balance Task:

This task was chosen because previous studies in this lab using this same task, and other labs using similar tasks have reliably demonstrated deficits in motor function

and coordination in rodent models of developmental ETOH exposure (Lewis et al., 2007; Thomas et al., 2004). Thomas and colleagues (2009), showed that offspring exposed to ETOH and choline on gestational days (5-20) showed similar levels of performance as controls on a motor function and coordination task, while ETOH alone offspring performed significantly worse. Because of this we hypothesized that DMXB-A would be able to reduce deficits in this task produced by our model of developmental ETOH exposure.

On PND 31-33, offspring were tested on a dowel balance paradigm. The apparatus consisted of one dowel rod (114cm long, 1.85cm diameter) that was elevated (75cm above the ground). Pads were placed under the dowel to avoid injury in case of falling. At the end of the dowel there was a black escape box (21x10x17cm). Before testing, each rat was handled for three minutes, weighed, and individually housed in the colony room. Testing began the following day and continued for three days (PND 31-33). The experimental room for the balance task was illuminated with a red light, a muffin fan helped reduce extraneous environmental noise during testing. Before testing, each rat was brought into the experimental room and habituated to the room for 10 min. On day 1, the subject was placed in the escape box for 1 minute to habituate and then returned to their home cage for 30 sec. After habituation, the subject was placed on the dowel 10 cm away from the escape box and allowed to enter the escape box. Once the subject successfully completed a trial, the distance to the goal box was increased by 13cm. A correct trial was defined as reaching the escape box without slipping or falling. An incorrect trial was defined as a subject slipping (two or more paws falling off the dowel) or falling off the dowel completely. Once the animal reached the escape box it was left in the escape box

for an additional 10 sec. The subject was then returned to their home cage for 30 sec until the next trial began. During the first trial of test days 2 and 3 the animal was placed on the dowel 13 cm further than their last successful distance traveled on the previous testing day. Total distance traveled on the dowel rod was the dependent measure for this task. Once a rat completed the three trials for that day, the dowel was cleaned with 30% ETOH before the next subjects were tested.

Hebb Style Water-Maze

This task was chosen because previous studies in this lab using this same task, and other labs using similar tasks have reliably demonstrated deficits in spatial navigation and working memory in rodent models of developmental ETOH exposure (Lewis et al., 2011; Thomas et al., 2009; Hunt et al., 2009). Lewis and colleagues demonstrated that offspring exposed to ETOH in the same manner as the current study required significantly more trials to complete the task on the second day of testing. Also, Thomas and colleagues (2004) demonstrated in a similar maze, the Morris Water Maze (a paradigm that requires offspring to use spatial navigation and working memory), that prenatal ETOH exposure reduced performance compared to controls and concomitant choline treatment reduced these deficits. Because of this we hypothesized that DMXB-A would be able to reduce deficits in this task produced by our model of developmental ETOH exposure.

The maze was a 130 x 90 x 40 cm black Plexiglass chamber divided into multiple divergent paths, with each path 18 cm wide. The end of the maze was characterized with a submerged platform approximately 1cm below the water. The water was mixed with non-toxic black powder tempura paint to obscure the platform. The temperature of the

water in the maze was kept at 76 degrees +/- 2 degrees F and temperature of the room was 75 degrees +/- 3 degrees F. A plastic sheet surrounded the maze to reduce potential external cues from surroundings and the experimenter. The performance of the offspring was recorded by the experimenter and SMART real time video tracking software (San Diego Instruments). Testing was conducted between PND 40-45 for two days; day 1 was termed acquisition day and day 2 was termed retention day. One day prior to testing, each rat was handled for 3 min and weighed. On day 1, subjects were habituated to the test room for 5 minutes prior to the start of testing. For each trial, the rat was placed at the start of the maze. The rat's swim path was recorded until it reached the platform or after a 1 minute ceiling had passed (resulting in a failed attempt). If the rat failed to reach the platform on a given trial, it was guided to the platform by hand. The rat was allowed to stay on the platform for 10 seconds at which time it was removed from the maze and placed in a holding cage under a heat lamp until the next trial commenced.

This task required thee correct left/right discriminations. A correct trial required reaching the platform with no errors. The learning criterion was defined as two consecutive trials with zero errors with a ceiling of 20 trials. At the conclusion of acquisition training, the rat was left under the heat lamp for 5min to warm up/dry off and then returned to its home cage. The maze was cleaned before the next subject begins testing. For day two, the retention period, the same procedure was used as day one.

Statistical Analysis

The data were analyzed using SPSS analysis of variance (ANOVA) with either a univariate or repeated measure where appropriate. Unless stated otherwise, the milk and NTC groups were compared first for similarity; if they showed no significant variation

they were then collapsed into a single control group. This was done because the NTC group was used to investigate the effect of intubations, if no significant variation was found between the two groups it was assumed that intubation itself had no meaningful effect and these offspring could be considered control. Variables included sex (2), x ETOH (2) x drug (2). Subsequent ANOVAs were based on the interactions from this overall analysis. If there was no main effect or interaction with sex, the data was collapsed across this variable for ease of presentation. Significant main effects were broken down when needed using Least Significant Difference (LSD) post hoc analyses. All tests were investigated for outliers and litter effects before statistical analysis, for this study no offspring were removed from analysis and there were no significant differences between litters regarding performance. Significance for all analyses was set at $p<0.05$.

Results:

Open-Field Performance:

Group performance was investigated using total distance traveled for each day and distance traveled for each 5-minute time block for each day. The first 5 minutes of each day was also investigated as a measure of reactivity to initial placement into the chamber. Entries into the center and percentage of time spent in the center of the chamber was also investigated because this is a common measure for impulse control (lack of inhibition).

Our hypothesis was not supported in that males and females exposed to ETOH did not demonstrate hyperactivity. Males exposed to ETOH alone and those treated with DMXB-A after ETOH exposure had activity levels below that of controls.

<u>Total Distance Traveled:</u>

A 2 x 2 x 2 x 2 (Sex x ETOH x DMXB-A x day) repeated measure ANOVA revealed no significant interactions or main effects between any of the groups. Because of an a-priori hypothesis and previous work in this lab, we predicted that ETOH offspring would be more hyperactive than control offspring and an LSD post-hoc analysis was used to investigate potential group differences. No differences were found between the groups for either day. (Fig 2.1.)

Total Distance Traveled by 5 Minute Time Block:

A 2 x 2 x 2 x 2(6) (Sex x ETOH x DMXB-A x Day[5-minute block]) repeated measures ANOVA revealed that there was a significant main effect of Sex x ETOH x Day (5-minute, $F(1,76)=3.378$, $p<.05$). Because of this interaction involving sex, males and females were investigated separately for these analyses. For males, an overall repeated measures analysis (ETOH x DMXB-A x block [2 x 2 x 2{6}]) revealed there was not a significant interaction between group and time block or a main effect of group on days one or two. Because of an a-priori hypothesis and previous work in this lab, however, we predicted that ETOH offspring would be more hyperactive than control offspring for both sexes. Analysis revealed that in males, both ETOH and ETOH + DMXB-A groups were significantly less active ($p<.05$) than control offspring in the first five minutes of day one. A similar difference between control offspring and ETOH + DMXB offspring occurred during the first five minutes of day two (see Fig. 2.2 & 2.4). For females, no group significantly differed from control animal performance for any time point on either day (see Fig. 2.3 & 2.5).

Total Distance Traveled in the Center:

Again, our hypothesis was not supported, in that offspring exposed to ethanol and

treated with DMXB-A were more active in the center than control offspring during day 2 testing, an effect not seen in offspring exposed to only ETOH.

A 2 x 2 x 2 x 2 (Sex x ETOH x DMXB-A x day) repeated measure ANOVA revealed that there was a significant interaction between ETOH and DMXB-A, $F(1,76)=5.413$, $p<.05$. LSD post-hoc analysis revealed on day two, offspring exposed to ETOH and treated with DMXB-A traveled significantly more distance than offspring treated with ETOH ($p<.05$), however, neither of these groups differed significantly from control offspring. (see Fig. 2.6)

Total Distance Traveled in the Center by 5 Minute Time Block:

A 2 x 2 x 2 x 2(6) (Sex x ETOH x DMXB-A x Day[5-minute block]) repeated measures ANOVA revealed that there was a significant interaction between ETOH and DMXB-A, $F(1,76)=5.420$, $p<.05$. However, an LSD post-hoc analysis revealed that there were no significant group differences on either day. However, on day two during the first five minutes the offspring exposed to ETOH and treated with DMXB-A demonstrated a trend toward increased activity that was approaching significant difference from controls. (see Fig. 2.7 and 2.8)

Entries into the Center:

A 2 x 2 x 2 x 2 (Sex x ETOH x DMXB-A x Day) repeated measures ANOVA revealed that there were no significant interactions or main effects for entries into the center of the apparatus. An LSD post-hoc test investigating individual group differences also revealed no significant difference between any of the group for either day. (Fig . 2.9)

Percentage of time spent in the center:

A 2 x 2 x 2 x 2 (Sex x ETOH x DMXB-A x Day) repeated measures ANOVA

revealed that there was a significant interaction between ETOH and DMXB-A for percentage of time spent in the center, $F(1,76)=6.041, p<.05$. An LSD post-hoc analysis revealed that on day two, offspring exposed to ETOH and treated with DMXB-A spent more time ($p<.05$) in the center of the apparatus than control offspring. (Fig. 2.10)

Balance Performance:

Our hypothesis was supported in female offspring because those treated with ETOH performed significantly poorer than control females on the dowel task. Treatment with DMXB-A in ETOH exposed females reduced this deficit to control levels. This effect was not observed in male offspring, instead, treatment with DMXB-A in ETOH exposed males decreased performance on this task. Results presented below.

A 2 x 2 x 2 x 3(3) repeated measures ANOVA (sex x ETOH x DMXB x day [trial]) was conducted and yielded an interaction between sex and DMXB $F(1,109)=6.007, p<.05$. Because of this, further analyses were broken down by sex and investigated separately. A 2 x 2 x 3(3) (ETOH x DMXB x Day[Trial]) repeated measures analysis in males revealed a main effect of DMXB $F(1,48)=4.152$, $p<.05$. After investigating group differences using a post-hoc analysis, it was revealed that ETOH males treated with DMXB achieved significantly less distance on the dowel ($p<.05$) than control offspring during trial 3 of day 2, and trial 1 & 2 of day 3 (see Fig. 2.11 & 2.12). This difference however was not significant in the final trial of testing. For females, there was a main effect of ETOH, $F(1,61)=5.036$, $p<.05$. A post-hoc analysis revealed that during the final trial of testing (day 3 trial 3) that ETOH females traveled significantly less distance on the dowel ($p<.05$) than control females. This difference was not observed in any of the other groups in female offspring (see Fig. 2.13 & 2.14).

Water Maze Performance:

Our hypothesis was again supported in the water-maze task, males treated with DMXB-A after ETOH exposure performed at control levels during acquisition where males exposed to ETOH required more trials to reach criterion. This effect was not observed in females; however, DMXB-A treatment did reduce the amount of trials required to reach criterion to below that of control females during task retention.

A 2 x 2 x 2 x 2 (sex x ETOH x DMXB x day) repeated measures ANOVA revealed that there was a sex x ETOH interaction $F(1,94)=4.884$, $p<.05$, and a main effect of ETOH $F(1,94)=6.288$, $p<.05$. Because of this each sex was investigated separately. In males, a 2 x 2 x 2 (ETOH x DMXB x day) analysis revealed a main effect of ETOH $F(1,38)=14.249$, $p=.001$, but no interaction or main effect of DMXB. LSD post-hoc analysis revealed that during acquisition ETOH exposed males required significantly more ($p=.001$) trials to reach criterion than did control offspring during acquisition and ETOH exposed males treated with DMXB-A performed at control levels (see Fig. 2.15). In females, the initial 2 x 2 x 2 analysis revealed no interactions or main effects of ETOH or DMXB. However, due to the a-prior hypothesis that treatment groups would differ an LSD post-hoc analysis revealed that during retention females treated with DMXB required significantly less ($p<.05$) trials to reach criterion than control offspring did (see Fig. 2.16).

Body Weights:

For all tasks, body weight was recorded after testing. For open-field, weights were recorded on PND 21 and analyzed with neonatal treatment and sex as grouping variables (see Table 2.1). As predicted, a 2 x 4 ANOVA revealed a main effect of both sex

$F(1,76)=5.903$, $p<.01$, where males reliably weighed more than females, and treatment group $F(1,76)=4.780$, $p<.01$ but no interaction. Post-hoc analysis revealed that offspring treated with ETOH weighed less than control offspring ($p<.05$), an effect which was not present in the ETOH offspring treated with DMXB-A. For balance, the ANOVA revealed a main effect of sex $F(1,101)=28.906$, $p<.001$ and ETOH $F(1,101)=4.105$, $p<.05$, where males consistently weighed more than females. Females exposed to ETOH weighed less than control females. This difference was not significant in males. For water maze, the analysis revealed a main effect of sex $F(1,86)=112.495$, $p<.001$ and a main effect of group $F(1,86)=3.305$, $p<.05$, and no interaction. Males, as predicted, were reliably heavier than their female counterparts in all groups, and males treated with ETOH were significantly lighter than any of the offspring from the other remaining groups.

Discussion:

This study was designed to examine whether DMXB-A (at a dose of 10mg/kg) could reduce the behavioral deficits produced by developmental ETOH exposure during the 3rd trimester brain growth spurt in rodents. In short, DMXB-A appeared to reduce some of the deficits produced by developmental ETOH exposure associated with motor coordination in female offspring and memory and spatial navigation in males. However, several of the findings were unexpected and demonstrated that DMXB-A's effects appeared to be sex dependent and that the combination of DMXB-A and ETOH may be deleterious to certain behaviors.

Previous research in this lab and others has demonstrated that developmental ETOH exposure, particularly during the third trimester, can impair behaviors related to attention/hyperactivity (Smith et al., 2012; Thomas et al., 2007; Lewis et al., 2012),

learning/memory (Hunt et al., 2009; Thomas et al., 2009; Tiwari et al., 2012), and coordination/balance (Idrus et al., 2011; Lewis et al., 2007; Thomas et al., 2004). It is believed that these behaviors are compromised because the structures that mediate these behaviors including the cerebellum, hippocampus, and prefrontal cortex are undergoing great growth and development during the third trimester and are more sensitive to ETOH's teratogenic effects (for review see Dobbing & Sands, 1979; Riley & McGee, 2005 & Patten et al., 2014).

The first behavior investigated was activity as measured in an open-field chamber. As mentioned previously, this task was used in the current study because like humans, rodents who are exposed to ETOH during development demonstrate hyperactivity (for review see Mattson et al., 2011 & Patten et al., 2014). Activity is typically assessed by several measures including path length, entries into, or time spent in specific areas of the chamber; normal rodent activity is typically increased in the beginning of a trial, reflecting investigation of novel surroundings, while also avoiding the center or open areas of the chamber, avoiding potential predation (for review see Denenberg, 1969). In offspring who were exposed to ethanol during critical developmental periods, like the third trimester, activity in the open-field is typically increased (hyperactivity as expressed in greater total path length), and greater exploration of the center of the open-field (as measured by path length in the center or number of entries into the center) is common which is characterized as hyperactivity and lack of inhibition (Schneider et al., 2011). These deficits are associated with regions of the brain including the prefrontal cortex (Gorsky & Klinstova 2017) and the basal ganglia (Thomas et al., 2004) that are particularly sensitive to third trimester ETOH exposure (Riley et al., 2011). These

structures are also innervated by the cholinergic system and contain α7-nAChR ‘s (Hurst et al., 2013). Though investigation of compounds that directly activate the α7-nAChR’s effects of this behavior in developmental ETOH exposure models have not been previously investigated, it is believed that activation of this receptor, through choline supplementation, can reduce deficits in this task (Thomas et al., 2004 & 2009). In the current study neither male nor female offspring exposed to ETOH displayed increased activity in the current study. Previous studies in this lab using the same model of ETOH exposure and task for assessing activity levels have reliably found increased activity in offspring exposed to ETOH both in overall activity, activity in the center, and entries into the center (Lewis et al., 2011, Smith et al., 2012). It is unclear why performance in this paradigm would be any different from performance in previous studies. It becomes even more perplexing when an effect of ETOH was found in our other tasks (see balance and water maze below). When considering possible explanations one path would be comparing activity levels in this study to previous studies conducted in this lab. For this, average activity in control offspring was compared between this study and the Smith et al., (2012) study; average activity was approximately the same as the current study (around 2000 cm. total distance traveled for both days). Interestingly, they did not find an effect of ETOH on total distance traveled for day 2 in respect to control performance, but ETOH did increase entries into the center of the apparatus for both days. The current study did not find an effect of ETOH in either distance traveled for either day or entries into the center. Additionally, neither Smith and colleagues (2012) or Lewis and colleagues (2011) found any differences between male and female offspring in activity in response to developmental ETOH exposure. Investigations into the BAC’s for this model,

though not conducted in the current study, demonstrated that this model produces BAC's up to 220 mg/dl 60 minutes after intubation (Lewis et al., 20011). As mentioned previously, BAC's in this range have reliably found deficits in the behaviors investigated for this study (see Painter et al., 2014 for review). Additionally, males exposed to DMXB-A and ethanol had reduced activity when compared to control offspring for the beginning of day 1 and day 2 testing. Also, center activity (distance traveled and time spent) was increased during day 2 above control levels when ETOH was combined with DMXB-A, but not in offspring exposed to ETOH alone. Though previous studies have not been conducted investigating DMXB-A's effects on activity level after third trimester ETOH exposure, or any model of developmental ETOH exposure, we expected the combination of DMXB-A and ETOH to have similar performance to that of control animals. Previous studies investigating DMXB-A's side-effect profile compared to nicotine found that DMXB-A had no effect on activity levels in healthy mice or rats while nicotine alone increased activity levels, which was reflected in the current study (Nanri et al., 2001). It is possible that the combination of ETOH and DMXB-A in the current model produces some type of sedation, however, other studies using α-7 nAChR activating compounds like choline, found that after exposing male rodents to ethanol during PND's 4-9, choline supplementation was able to reduce increased activity levels produced by ETOH exposure to control levels (Thomas et al., 2004). Similar findings were observed with prenatal ethanol exposure in male and female rodents, where ethanol exposed rodents had significantly increased activity compared to controls and prenatal supplementation with choline was able to reduce these deficits (Thomas et al., 2009). However, the studies investigating these compounds effects on developmental ETOH

exposure reliably found an effect of ETOH administration both in pre and postnatal models. Because of this, it becomes difficult to compare the results of DMXB-A in this test with other studies using compounds that target the α-7 nAChR. Future investigations in this lab using this exposure model with the open-field task should be completed to determine a possible cause for this difference because the use of this task is invaluable in determining the efficacy of compounds aimed at reducing the effects of developmental ETOH exposure.

DMXB-A was also investigated for its ability to reduce deficits in motor coordination after developmental ETOH exposure. This paradigm was used because deficits in motor function and coordination (as measured by distance traveled in the current study) are common in both clinical cases and animal models of FAS/FASD (Idrus et al., 2011; Lewis et al., 2007; Thomas et al., 2004). As previously mentioned, this task is believed to be largely mediated by the cerebellum, a structure that is sensitive to the effects of developmental ETOH exposure, particularly during the third trimester brain growth spurt (see Riley & McGee, 2005 for review). Additionally, the cerebellum is innervated by the cholinergic system and contains α7-nAChR (Hurst et al., 2013). In the current study, our hypothesis that developmental ETOH exposure would decrease distance achieved and a single injection of DMXB-A would reduce these deficits to control levels was supported, but only in female animals. However, our hypothesis was not supported in males where ETOH exposure alone had no effect and distance achieved was significantly decreased below that of controls after treatment with DMXB-A in ethanol exposed offspring. Previous research using the same model of ETOH exposure and methods for investigating motor function as the current study have typically found

coordination deficits in both male and female offspring exposed to ETOH (Lewis et al., 2007 & 2011). Interestingly, in one of these studies they observed sex differences in motor function, however, these differences were in untreated offspring where females performed better than males overall, with no effect on those treated with ETOH or ETOH + Drug (Lewis et al., 2007). They posited that these differences in sex occurred because of the weight discrepancies between males and females, where females generally weigh less than males and thus stand to do better on the dowel rod. Interestingly, we did not see this effect in the current study, even though males weighed significantly more than females, they performed at roughly equivalent levels (around 600 +/- cm total). Males exposed to ETOH and ETOH and DMXB-A also did not differ from controls regarding weight. Decreased performance on this task in males exposed to ETOH and treated with DMXB-A has not been supported by previous literature. Though DMXB-A has not yet been investigated for its ability to reduce motor deficits in developmental ETOH exposure; studies testing the ability of α7-nAChR activating compounds to reduce these deficits have not shown a reduction in performance when combined with ETOH exposure in either sex (Thomas et al., 2004 & 2016). In fact the results mirror what was demonstrated in the female's performance (Thomas et al., 2004 & 2016). It is promising that DMXB-A was able to reduce the deficit found in females exposed to ethanol on this balance task, however, it is obvious that further work needs to be completed to explore the potential effects of ETOH exposure on task performance in male and female offspring.

Finally, DMXB-A was investigated for its ability to reduce learning and memory deficits in a Hebb style water maze after developmental ETOH exposure. As mentioned

previously, this paradigm was chosen because it demonstrates both learning (during maze acquisition) and memory (during maze retention) in only two days (for review see Von Euler et al., 2006). This task is also useful because it tests a behavior (learning/memory) that is commonly deficient in both clinical cases and animals models of FAS/FASD's (Hunt et al., 2009; Thomas et al., 2009; Tiwari et al., 2012). The task is believed to use both spatial navigation and working memory which is thought to be largely mediated by the hippocampus and prefrontal cortex (Von Euler et al., 2006), structures that are sensitive to the teratogenic effects of developmental ETOH exposure, particularly during the third trimester (see Riley & McGee, 2005 for review). These structures are also innervated by the cholinergic system and contain α7-nAChR's (Hurst et al., 2012). In support of the hypothesis, ethanol exposure increased the total amount of trials required to reach criterion in males and a single injection of DMXB-A reduced this deficit to control levels during maze acquisition. This effect was not observed in females. Interestingly, during retention, there were no significant differences between any of the groups in males, but in females those offspring treated with DMXB-A alone required significantly fewer trials than their respective controls to complete the task. The sex differences observed in the current study in response to ETOH have not been found previously using this model or task. Studies in this lab using the same methodology and exposure model did not find sex differences in this task, and the offspring exposed to ETOH had retention deficits and not acquisition deficits like in the present study (Lewis, et al., 2011). Interestingly, Goodlett & Peterson (1995) suggested females performance on spatial navigation tasks may be less sensitive to ETOH exposure than males which we did observe. In regards to the acquisition deficit, it is possible that these results stem from

ETOH's effects on the hippocampus, the structure thought predominately responsible for spatial navigation tasks, where early maze learning seems to rely on the hippocampus proper but retention may be mediated more by the striatum (for review see Berman & Hannigan, 2000). However, research shows that both the striatum and hippocampus are sensitive to developmental ETOH exposure (Fryer et al., 2007). As for DMXB-A's ability to increase performance in retention in females compared to control; it is possible that activation of the α7-nAChR could increase cognition. In fact, previous studies have supported this claim; where activation of the receptor by choline supplementation improved executive functioning and learning and memory in control offspring (Thomas et al., 2000). However, this effect was seen in both males and females in the Thomas study and was only demonstrated in female offspring in the current study. Though not sex specific, other studies investigating DMXB-A in an animal model Alzheimer's model have demonstrated that it can increase cognitive deficits in other hippocampal including eyeblink conditioning (Kihara et al., 1997; Chen et al., 2010). Again, further work is necessary to elucidate the differences between acquisition and retention in response to developmental ETOH exposure, activation of the α7-nAChR, and the possible effect sex has on those behaviors.

These results are the first to report on DMXB-A's effects on behavior in a third trimester developmental ETOH exposure model. The results demonstrate the potential of DMXB-A to reduce some of the behavioral deficits associated with developmental ETOH exposure. However, there were several unexpected results and it is possible that the combination of DMXB-A and ETOH could be detrimental to certain behaviors. As previously mentioned, targeting EWD, a process that is severely damaging to the

developing nervous system (Riley et al., 2011) is one potential avenue for reducing deficits related to FAS/FASD and could be a one-time treatment. The mechanism for the damage caused by EWD, as reviewed above, is thought to be predominately produced by excitotoxicity, oxidative stress, and neuroinflammation (Alfonso-Loeches et al., 2011). DMXB-A, a compound that has shown affinity for binding at the alpha7 receptor has been shown to reduce these types of damage in cellular models of ETOH exposure (de Fiebre et al., 2003; Li et al., 2002; Shimohama et al., 1997; Tizabi et al., 2004). This is promising because as mentioned previously, there are currently no pharmacotherapies clinically approved to treat FAS/FASD. Clearly more work is necessary with DMXB-A to further elucidate the differences in our behavioral studies as well as to further understand its actions in the nervous system. However, the clinical utility of compounds that target the α7-nAChR seem clear, and because there are currently no clinically approved medications for the treatment of FAS/FASD further work must be done to explore this compound and other compounds that target this system.

Tables:

Table 2.1: DMXB-A Study Animal Weights

Body Weights (in g.)

Group		Treatment (PND 8) M ± SEM	Open Field (PND 21) M ± SEM	Balance (PND 33) ^ M ± SEM	Water Maze (PND 44) ^ M ± SEM
Control					
	Males	23.0 ± .6	67.0 ± 2.7	124.7 ± 5.4	223.8 ± 8.4
	Females	21.7 ± .8	61.1 ± 1.6	108.5 ± 2.7	172.4 ± 3.7
ETOH					
	Males	18.5 ± 1.2*	58.4 ± 3.0*	114.4 ± 4.1	199.6 ± 8.8*
	Females	18.1 ± 1.0*	53.6 ± 3.2*	98.2 ± 4.5*	157.8 ± 5.0
DMXB-A					
	Males	23.4 ± .3	71.3 ± 3.0	128.3 ± 4.9	223.7 ± 6.4
	Females	22.6 ± .5	62.8 ± 2.9	105.2 ± 3.5	171.7 ± 6.2
ETOH + DMXB-A					
	Males	18.8 ± .8*	60.9 ± 3.5	120.4 ± 5.5	217.0 ± 6.7
	Females	17.4 ± .8*	58.3 + 3.0	108.1 ± 3.6	168.4 ± 3.7

* Denotes significant difference from same sex control ($p<.05$)

^ Denotes sex difference in same treatment group ($p<.05$)

Figures:

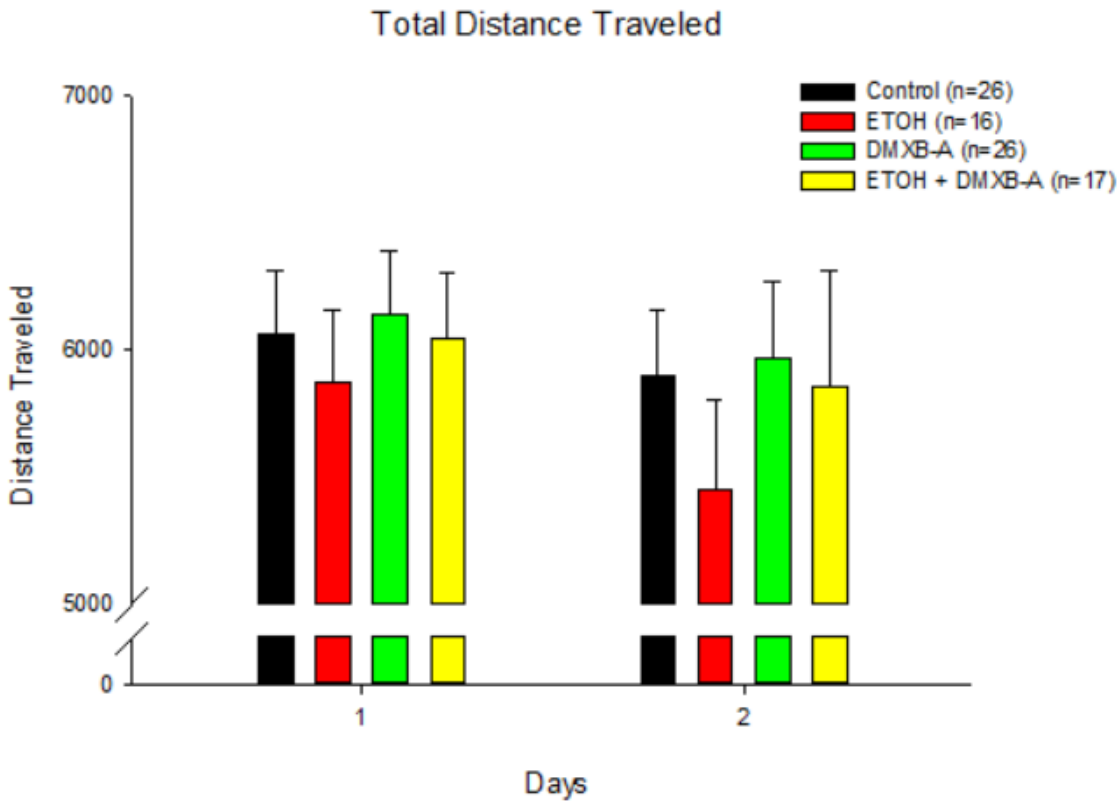

Figure. 2.1: Open-Field performance as measured by total distance traveled. None of the groups differed in total distance traveled for either day

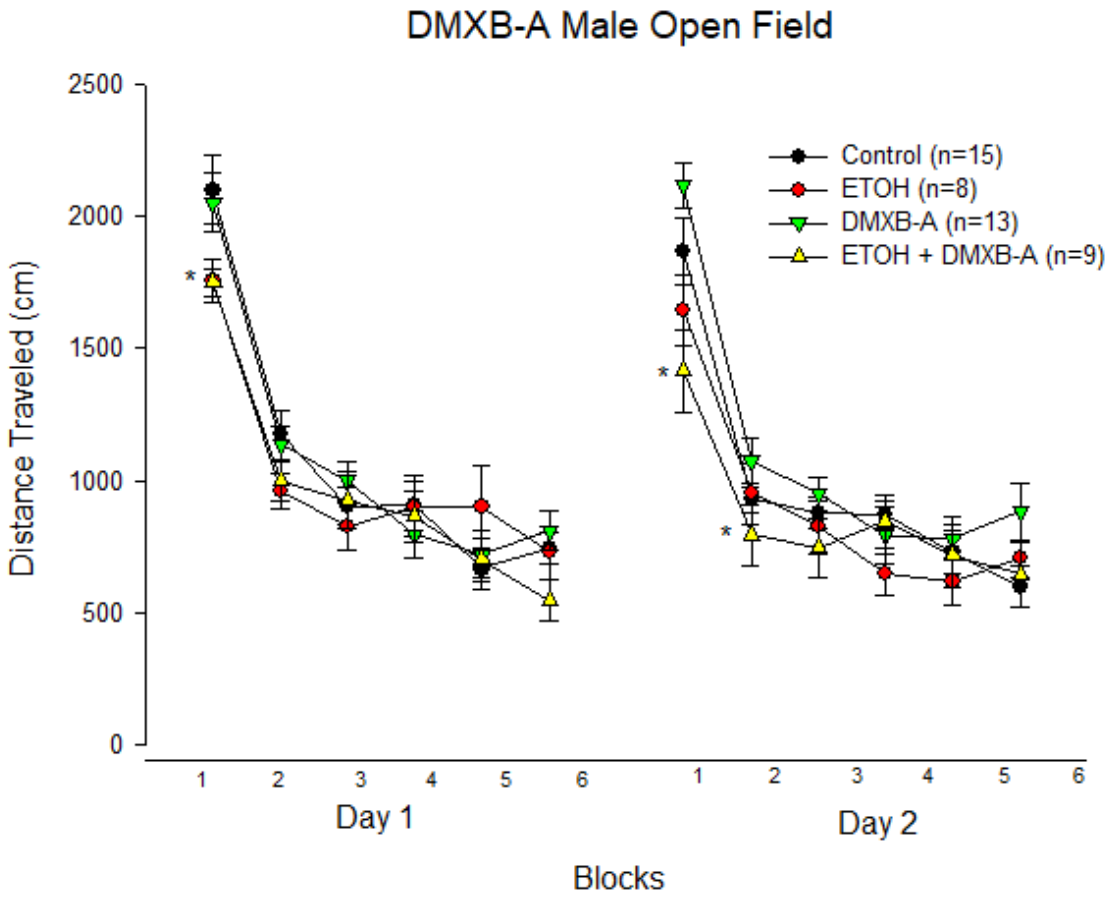

Figure. 2.2: Male Open-Field performance as measured by distance traveled. On day 1, ETOH & ETOH+DMXB-A offspring traveled significantly less distance than control offspring in the first 5 minutes of the trial. A similar pattern was seen on day two where ETOH+DMXB-A offspring traveled significantly less distance than control offspring. These differences dissipated as the trial continued. *p<.05, differs significantly from control offspring.

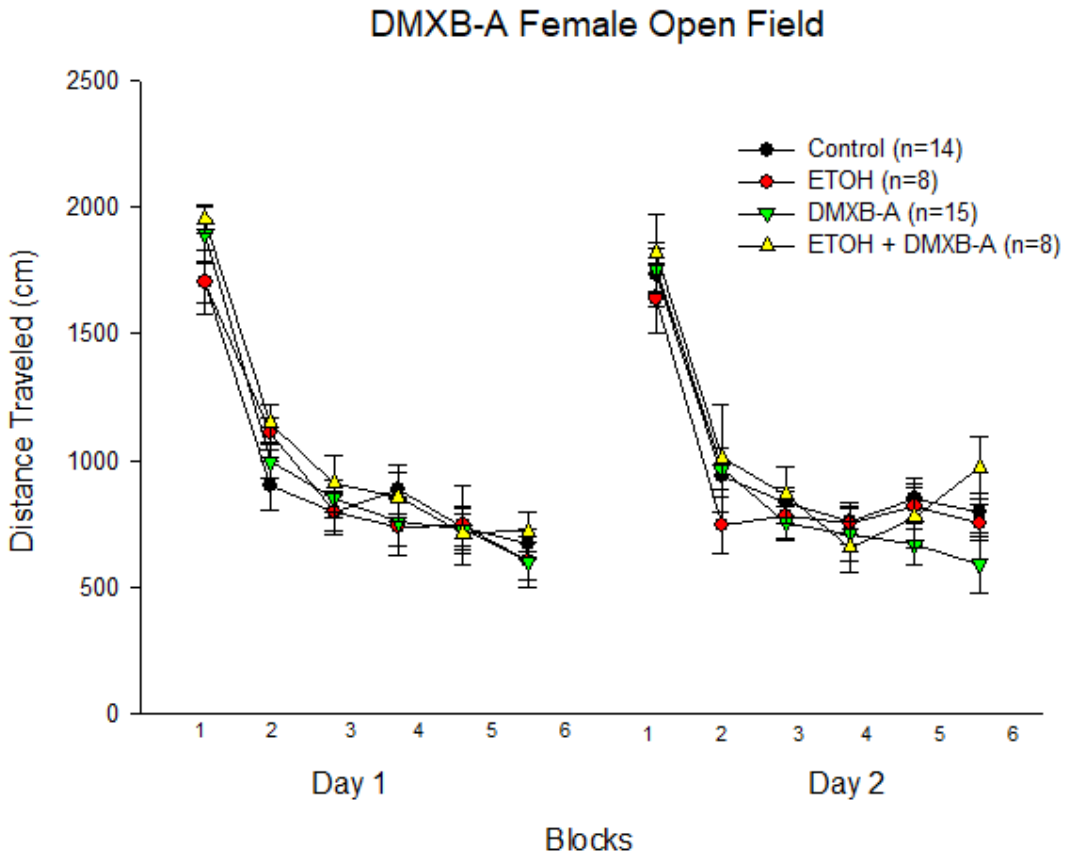

Figure. 2.3: Female Open-Field performance as measured by distance traveled. On day one and day two there no significant differences between any of the groups.

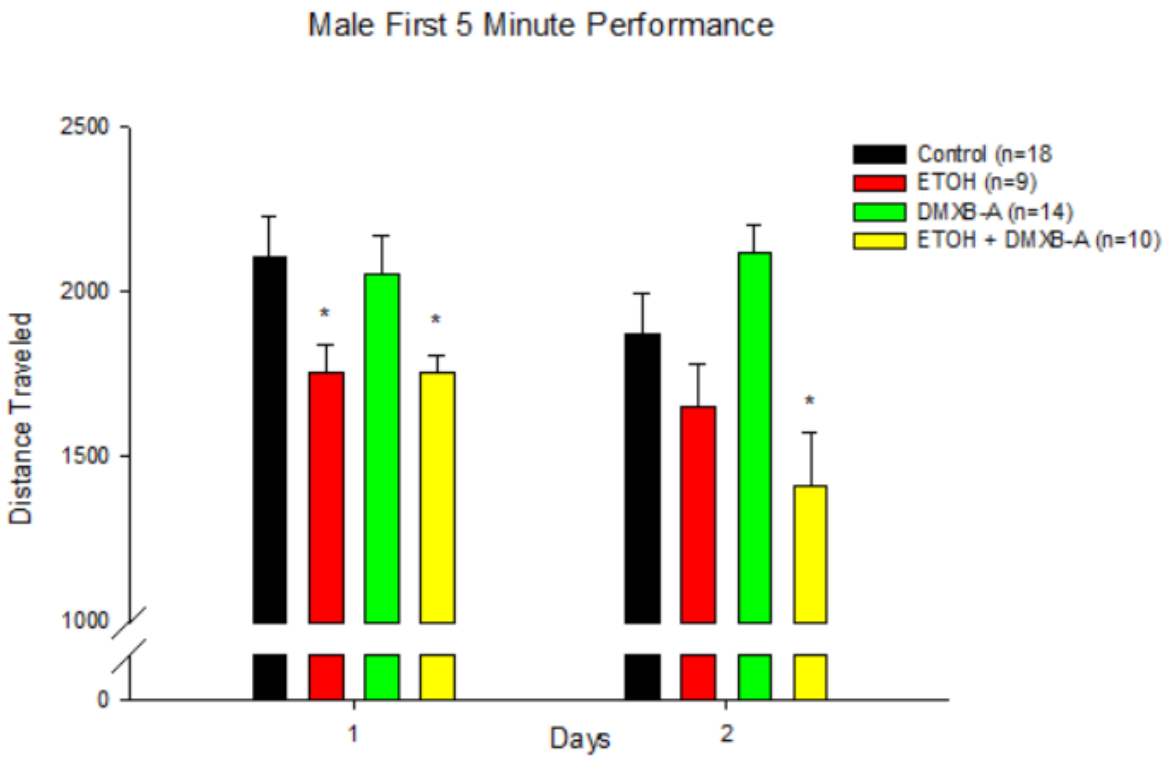

Figure. 2.4: Male Open-Field performance as measured by distance traveled in the first five minutes. On day 1, ETOH & ETOH+DMXB-A offspring traveled significantly less distance than control offspring in the first 5 minutes of the trial. A similar pattern was seen on day two where ETOH+DMXB-A offspring traveled significantly less distance than control offspring. These differences dissipated as the trial continued. *p<.05, differs significantly from control offspring.

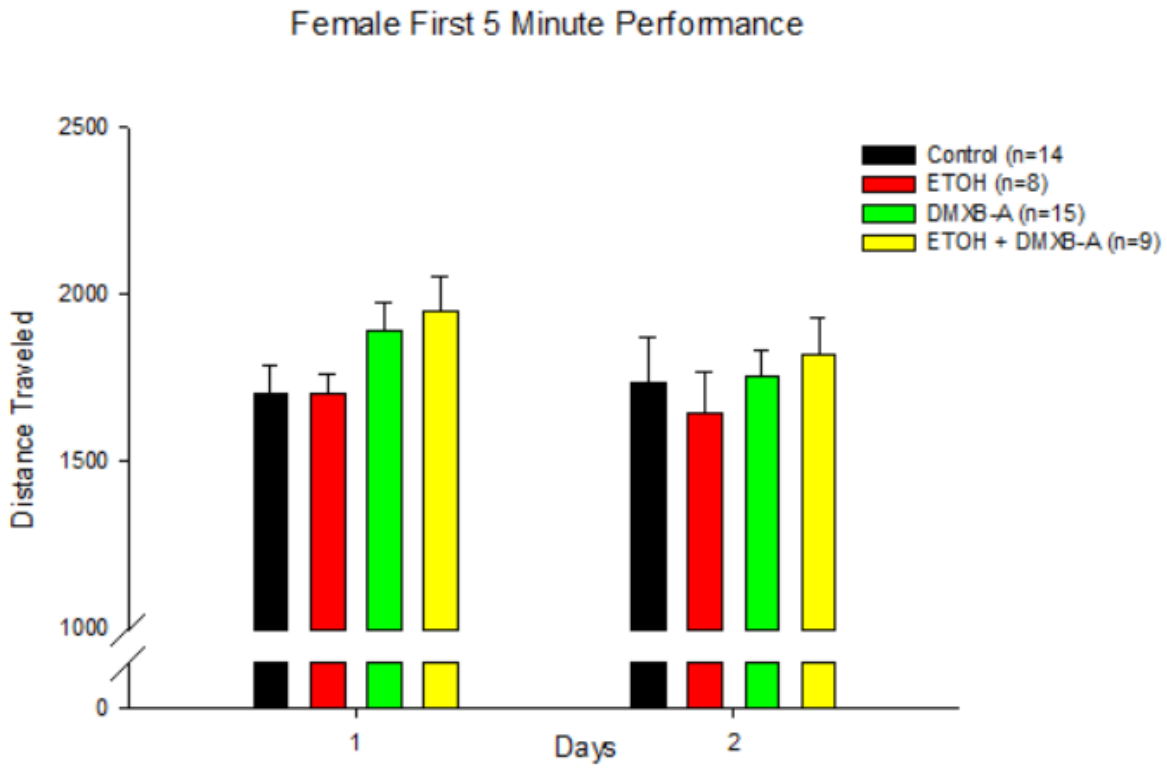

Figure. 2.5: Female Open-Field performance as measured by distance traveled in the first five minutes. There were no significant group differences between the groups on either day.

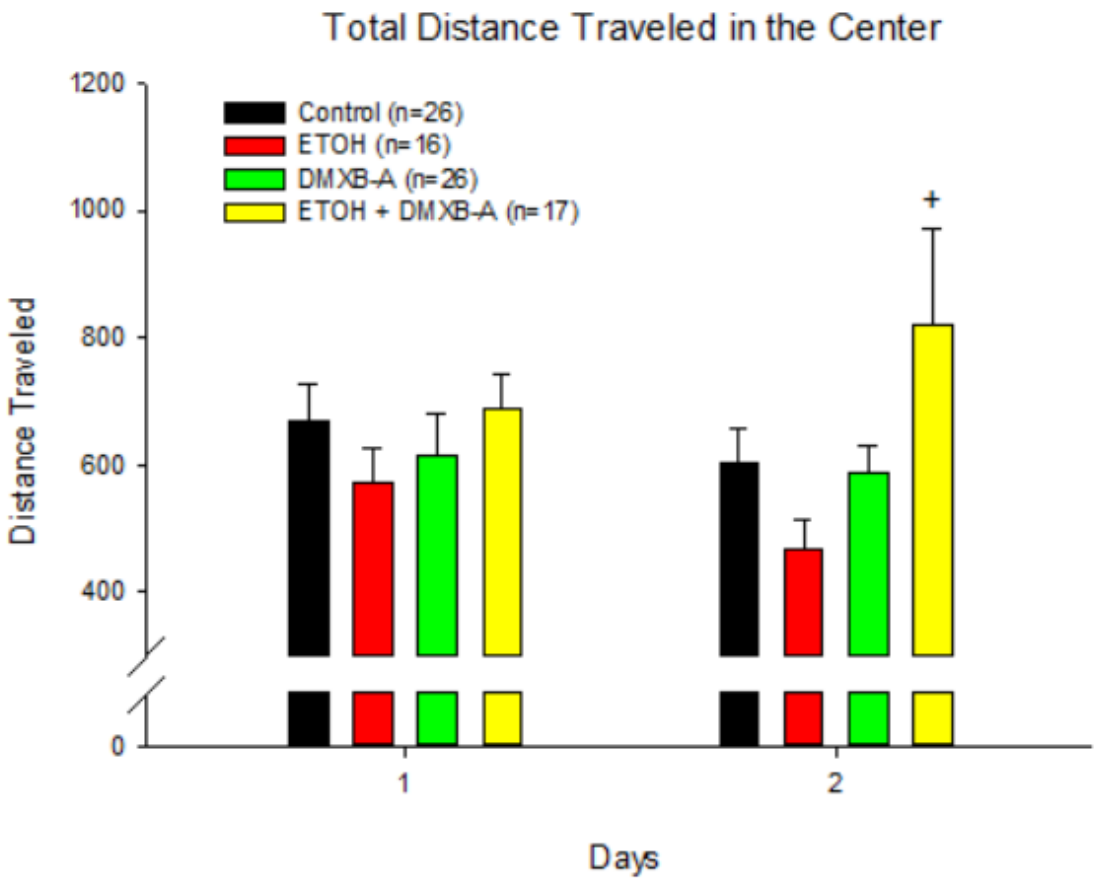

Figure. 2.6: Open-Field performance as measured by distance traveled in the center of the apparatus. On day 1, there were no significant difference between groups. However, on day two those offspring exposed to ethanol and treated with DMXB-A traveled significantly more distance than those offspring exposed to ETOH alone. Neither group differed from controls. += $p<.05$, differs significantly from ETOH offspring.

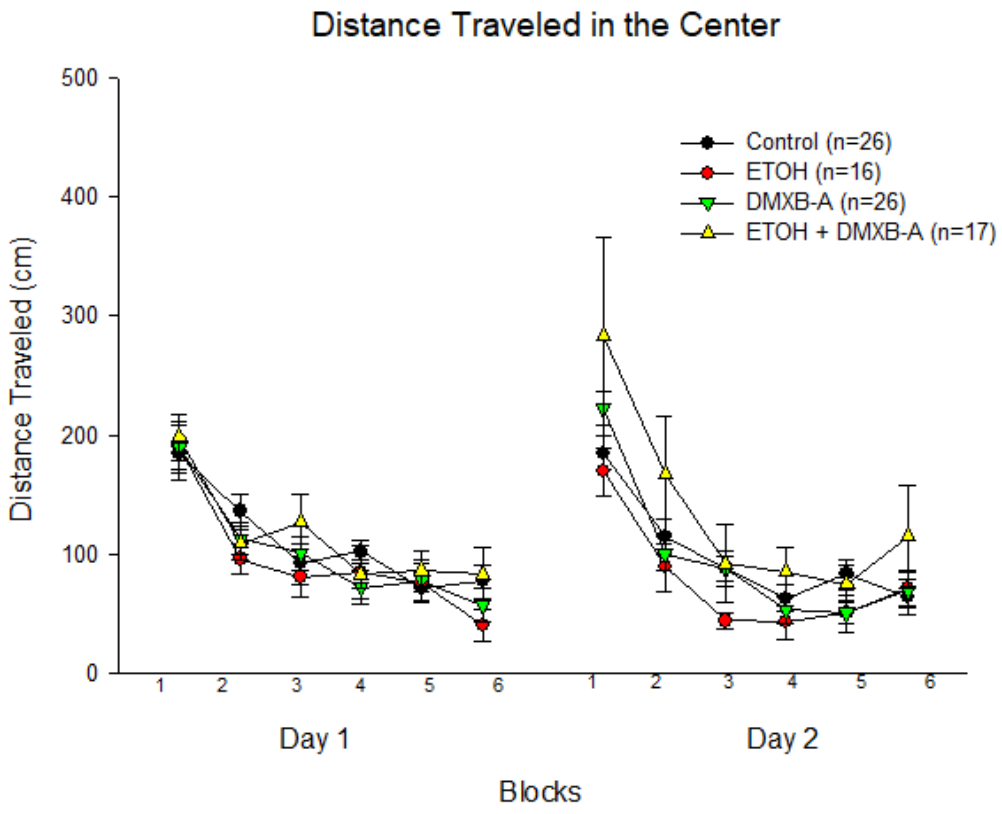

Figure. 2.7: Open-Field performance as measured by distance traveled in the center of the apparatus. There were no significant group differences between the groups on either day.

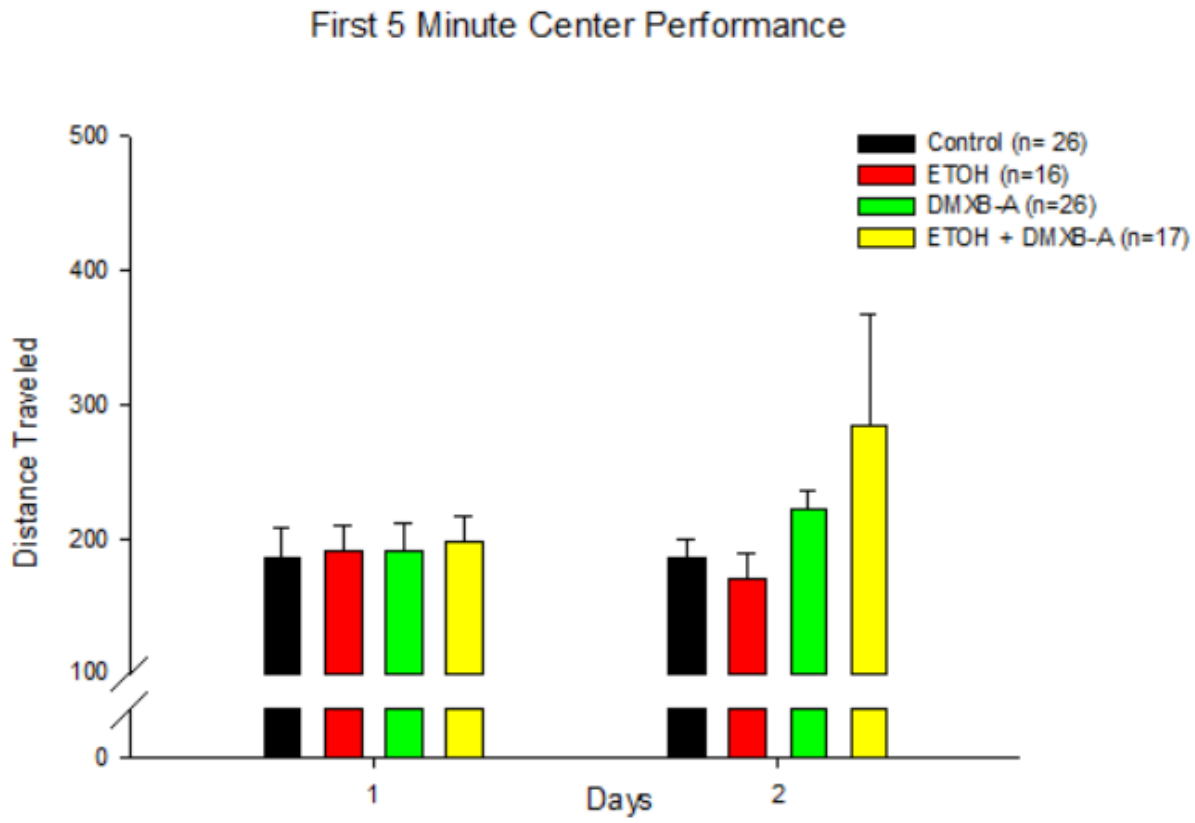

Figure. 2.8: Open-Field performance as measured by distance traveled in the center of the apparatus for the first 5 minutes of testing. There were no significant group differences between the groups on either day.

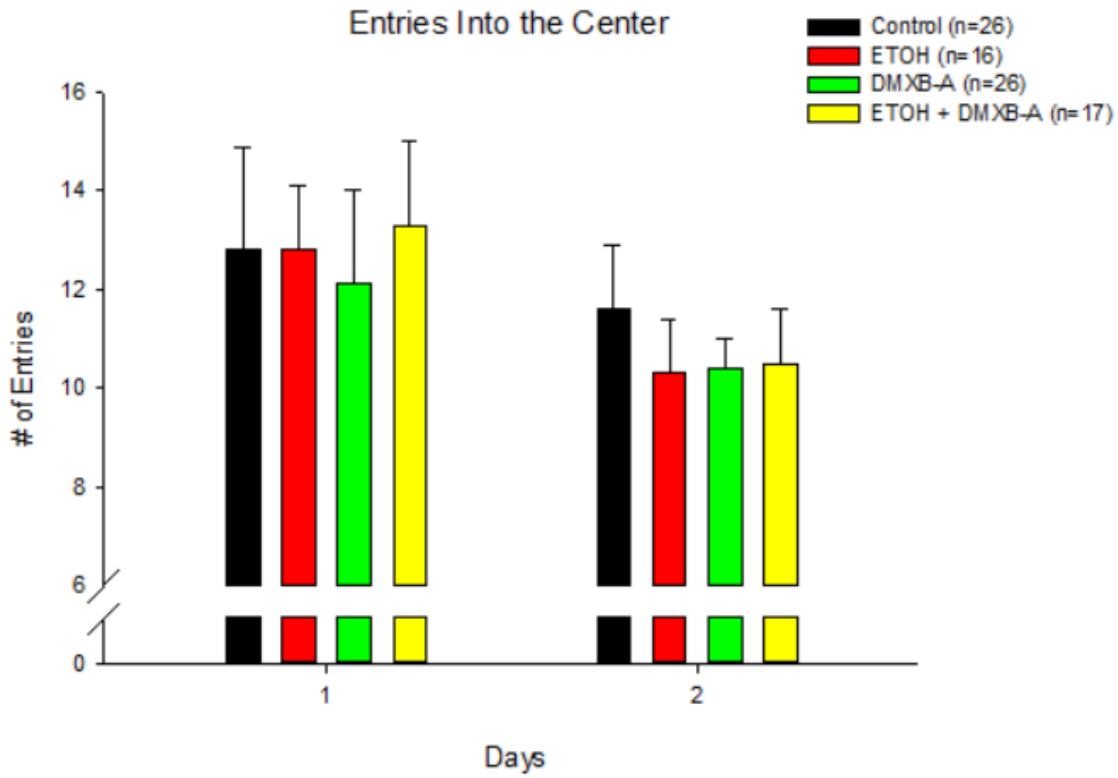

Figure. 2.9: Entries into the center of the open-field chamber. There were no significant group differences for either day.

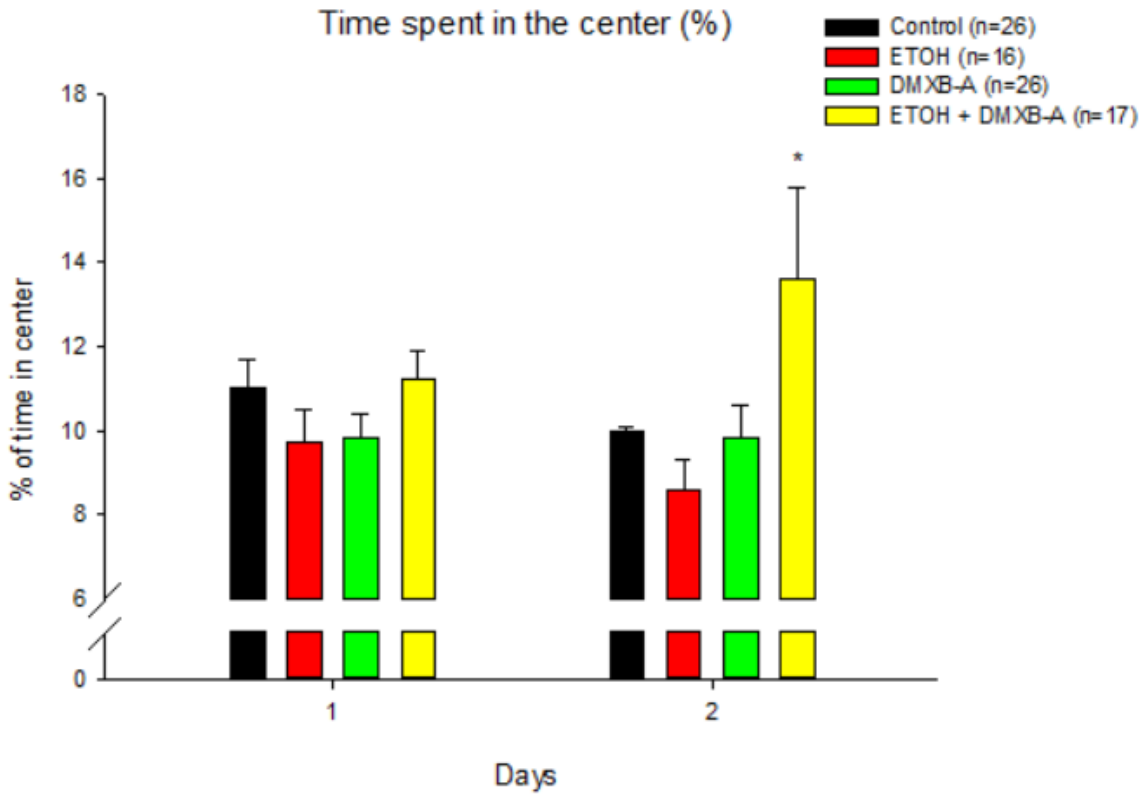

Figure. 2.10: Time spent in the center (%) of the open-field chamber. There were no significant group differences for day 1. However, offspring exposed to ETOH and treated with DMXB-A had an increased amount of time spent in the center compared to control offspring. *p<.05 from control

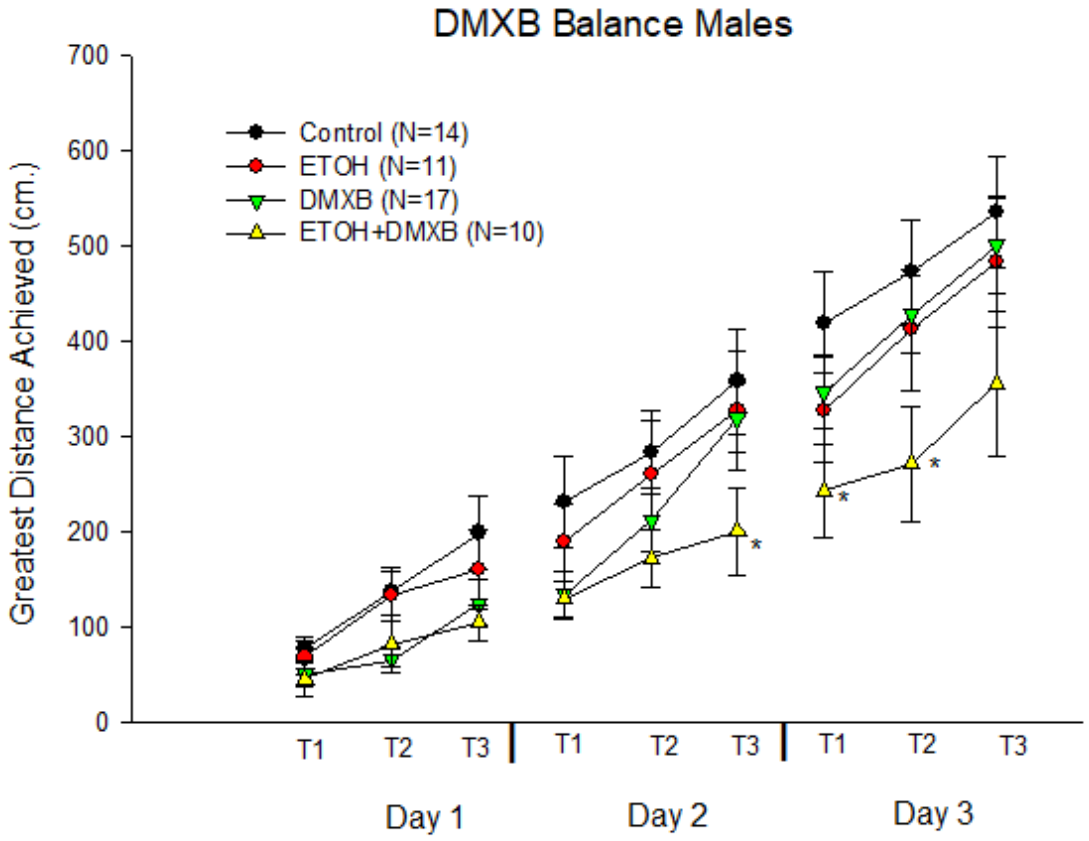

Figure. 2.11: Male Balance performance as measured by distance traveled. Ethanol exposed males treated with DMXB traveled significantly less distance than control males for three trials, however this difference was not present during any other trial. *p<.05, differs significantly from control offspring.

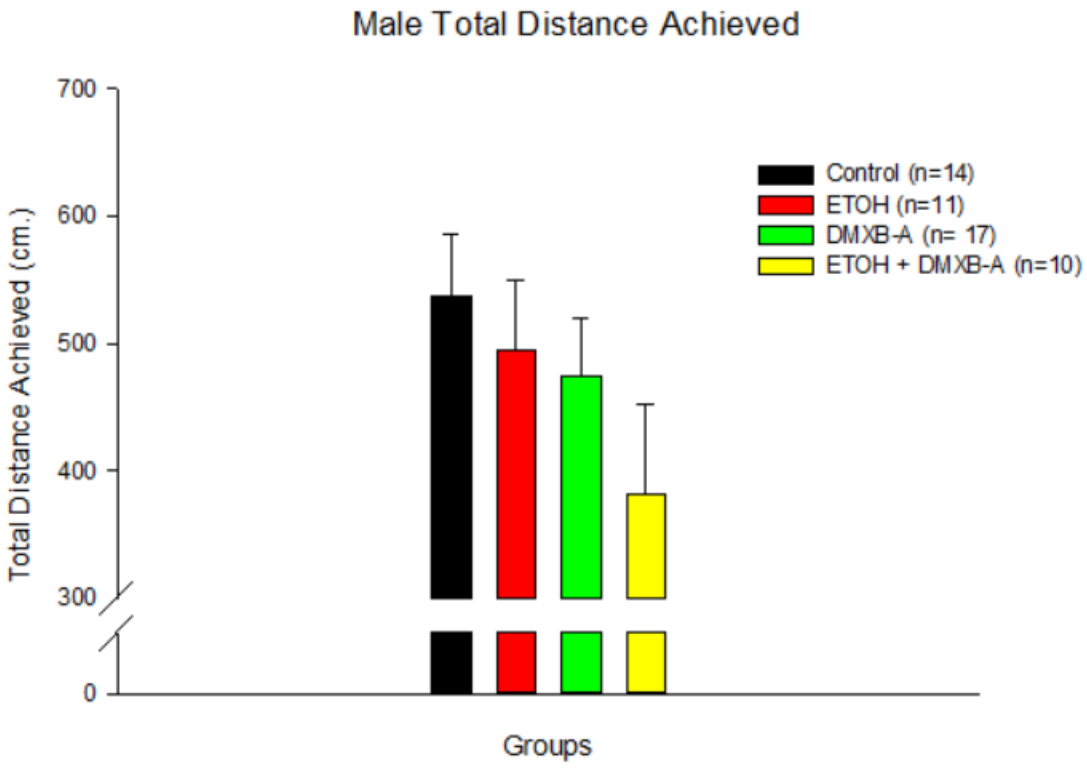

Figure. 2.12: Male Balance performance as measured by distance traveled. There were no significant differences in total distance traveled between any of the groups.

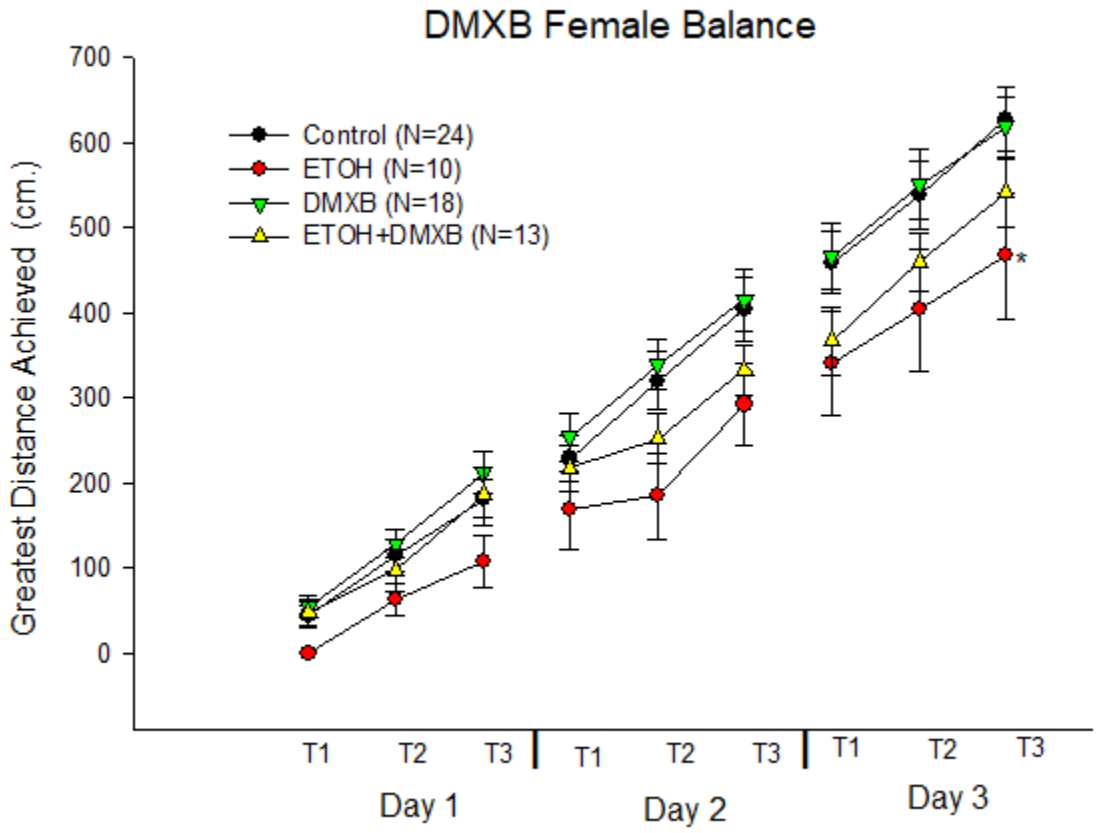

Figure. 2.13: Female Balance performance as measured by distance traveled. The pattern of reduction in performance in ETOH exposed offspring was consistent throughout testing, however this difference was significant during the last trial of testing. *p<.05, differs significantly from control offspring.

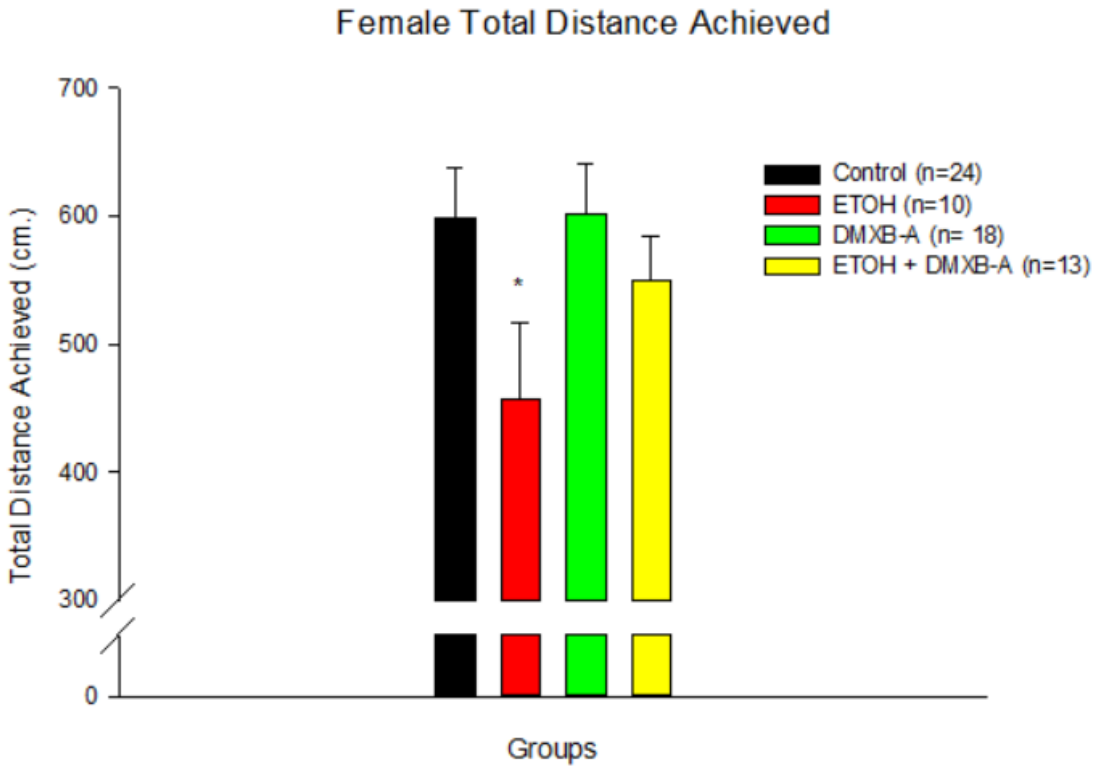

Figure. 2.14: Female Balance performance as measured by distance traveled. Ethanol exposed females traveled significantly less distance than control females. A single injection of DMXB-A reduces this deficit to control levels. *p<.05, differs significantly from control

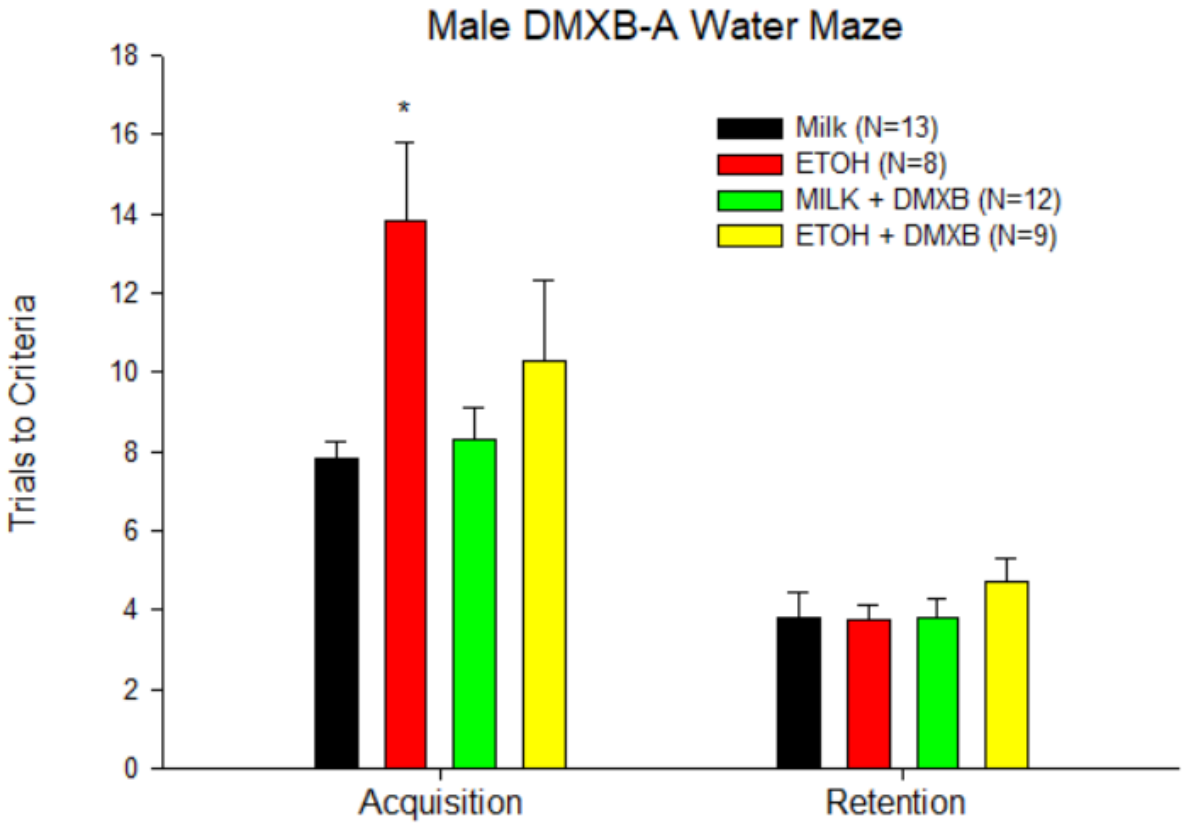

Figure. 2.15: Male water maze performance as measured by trials to completion. ETOH males required significantly more trials than control males during acquisition to reach criterion and the addition of DMXB-A to ethanol exposed offspring reduced this deficit to control levels. *p=.001, differs significantly from control offspring.

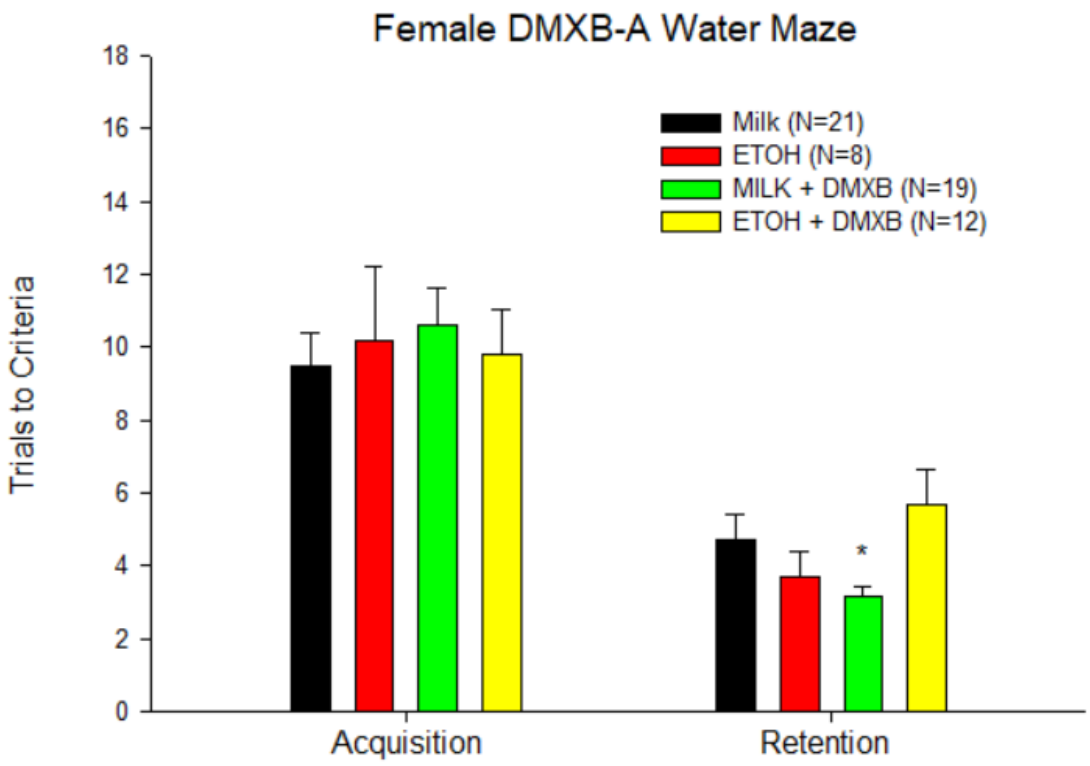

Figure. 2.16: Female water maze performance as measured by trials to completion. Females treated with DMXB required significantly less trials than control females during retention to reach criterion. *p<.05, differs significantly from control offspring.

Chapter 3: *Solidago Nemoralis*

Introduction:

Alcohol is one of the most commonly used drugs in the western world, with an estimated 87% of individuals (18 years of age or older) reporting that they had consumed alcohol at some point in their lifetime, 70% in the last year, and 56 % in the last month (NIAAA, 2015). In addition, approximately 10% of pregnant women consume alcohol at some point during pregnancy with a third of these women binge drinking (Center for Disease Control (CDC), 2015). Prenatal ethanol (ETOH) exposure has serious consequences for the developing offspring which at times manifests as Fetal Alcohol Syndrome/ Spectrum Disorders (FAS/FASD) (Hoyme, et al. 2005). Estimates of the prevalence of these disorders has been conservatively estimated to be 1-5% in first grade children, with a cost of approximately $4 billion annually in the United States (CDC, 2015; May et al., 2018).

Exposure to ETOH during development can sometimes lead to a multitude of both behavioral and physiological problems throughout the offspring's lifetime (for review see Riley et al., 2011). Such behavioral impairments include issues with executive functioning, motor coordination, spatial learning, attention, and hyperactivity (Jones, 2011; Mattson & Riley, 1998). Because of this, considerable amounts of research have been focused on treating these disorders, however no medications aimed at treating FAS/FASD specifically have been clinically approved. Of the many possible targets for treatment currently being investigated, treating ETOH withdrawal (EWD) in the neonate has proven to be a promising path for possibly negating the effects of developmental

ETOH exposure. Previous work has shown that EWD is a potential mediator in much of the damage in the central nervous system (CNS) that inevitably leads to many of these deficits (Barron et al., 2008; Goodlett & Horn 2001; Thomas et al., 1997). This damage caused by EWD is thought to be facilitated, in part, by excitotoxicity, neuroinflammation, and oxidative stress (Lutz et al., 2015; Barron et al., 2017; Crews & Nixon, 2009). Targeting these forms of toxicity associated with EWD is promising because it could potentially be a one-time treatment that would protect the fetus or newborn during a time when ETOH is absent from the system. This treatment could either be administered during parturition, or when a mother is seeking help for an alcohol use disorder.

The cholinergic system, a system that is critical in mediating behaviors related to wakefulness and attention, as well as learning and memory, has shown considerable promise in reducing some of the effects of developmental ETOH exposure on the fetus and newborn in various animal models (Barron et al., 2016, Thomas et al., 2003, 2004 & 2009). Activation of receptor subsystems within the cholinergic system, particularly those found in the CNS, have been useful in their ability to reduce various excitotoxic challenges. Activation of the nicotinic system can increase cell survival after hypoxic events, ETOH withdrawal and beta-amyloid-mediated cell death in cortical cultures (Hejmadi et al., 2003; Shimohama et al., 2001; Prendergast et al., 2000), and also reduce NMDA induced excitotoxicity in the hippocampus after chronic nicotine administration (Ferchmin et al., 2003; Prendergast et al., 2001). Within the nicotinic system, the alpha 7 nicotinic acetylcholine receptor (α7-nAChR), a calcium permeable ion channel, is particularly interesting because of its high concentration in regions of the CNS associated with FAS/FASD's, including the hippocampus, frontal cortex, and cerebellum (Hurst et

al., 2013). The receptor is also commonly found on neuroimmune cells that mediate neuroinflammatory responses (Xiu et al., 2005). Activation of the α7-nAChR has also been shown to reduce cellular toxicity during ethanol withdrawal (de Fiebre et al., 2003 & 2005; Yangxin et al., 2002). Supplementation with choline, an essential nutrient, which has a high affinity for the α7-nAChR, has shown considerable promise in reducing deficits related to fetal ETOH exposure in rodents (Thomas, 2009). Choline supplementation has been shown to reduce hyperactivity, spatial working memory deficits, eye-blink and trace fear conditioning, and executive functioning deficits following developmental ETOH exposure (Monk, 2012; Waddell & Mooney, 2017). Thomas and colleagues (2009) reported that choline supplementation during early development increased birth and brain weight after ETOH exposure. Interestingly, a recent study using choline supplementation in pregnant women who had consumed alcohol reduced the amount of adverse effects caused by prenatal ETOH exposure (low birth weight, and cognitive deficits) compared to women given a placebo (Jacobson et al., 2018). Though treating deficits related to developmental ETOH exposure using compounds like choline are promising, it is relatively non-specific and to better understand the role of the α7-nAChR in this model it is beneficial to use compounds that specifically target this receptor. If an agonist of the α7-nAChR's can reduce deficits produced by developmental ETOH exposure it is worth pursuing pharmacotherapies that agonize this receptor for treatment of human populations with prenatal ETOH exposure.

Solidago nemoralis (*SN*) is a flavonoid enriched extract derived from the Gray Goldenrod. *SN* was extracted at the University of Kentucky by Naprogenix Inc while exploring various plant extracts for their potential α7-nAChR binding properties

(Littleton et al., 2005). *SN* demonstrated an increased ability to displace MLA (a selective α7-nAChR antagonist) in rodent hippocampal cells which demonstrated its specificity for the receptor (Lutz, 2014). One important characteristic of *SN* is its high concentration of flavonoids which are highly associated with anti-inflammatory responses in the periphery, CNS, and for their neuroprotective effects in models of neurodegeneration (Gonzalez, et al., 2011; Spencer et al., 2012; Dajas et al., 2013). Flavonoids have also been studied extensively in models of ETOH exposure as well. For example, quercetin reversed hepatotoxicity and neurotoxicity induced by 90 days of ETOH administration (Ambadath et al., 2010). One of the flavonoids found in *SN*, rhamnetin, demonstrated an ability to reduce both neuroinflammation and excitotoxicity in response to EWD in an organotypic hippocampal slice culture (Lutz, et al., 2015 b). Goulart and colleagues (2007) also found that *SN* was able to reduce neuroinflammation in mouse model of pleurisy. Though previous work with this novel compound is limited, the amount of literature demonstrating that selective activation of the α7-nAChR has the ability to reduce both neuroinflammation and excitotoxicity provided a strong rationale for using *SN* in the current study. We hypothesized that *SN* would be able to reduce or eliminate the behavioral deficits found after "third trimester" ETOH exposure in rodents.

Methods:

Subjects & ETOH Exposure Paradigm:

Male and female Sprague Dawley rats were used for all experiments. Offspring were bred in the University of Kentucky Psychology Departments breeding facility. Once born, half of the offspring were exposed to ETOH (6 g/kg/day) administered by oral

gavage from postnatal days (PND) 1-7. This model uses a split litter design with 1:1 male/female ratio designated to each of the following treatment groups: alcohol (6 g/kg/day), an intubated control, and a non-treated control. All intubated offspring received .0278 ml/g of body weight. No more than one male and one female were included in any cell of the experimental design to avoid potential litter effects (Abbey & Howard, 1973). Intubations were administered in a milk-based diet designed to mimic the pups mothers milk (West and Harne, 1984). Two daily feeds were administered at 10AM and 2PM and the ETOH exposed offspring received a 3rd intubation at 4PM of milk only to account for decreased bodyweight in the ETOH exposed offspring possibly due to intoxication or decreased feeding while non-ETOH pups received a sham intubation. The intubation schedule was used to achieve a binge-like pattern of exposure which is particularly harmful for the developing fetus (West et al., 1989). Blood ethanol concentrations collected previously from this lab have found that BEC's with this type of exposure regime typically peak at 230 mg/dl which returns to 0 within 10 hours after intubation (Lewis et al., 2007). These levels of alcohol have been shown to reliably produce behavioral deficits in a number of tasks (See Patten et al., 2014) and are clinically relevant to the study of developmental ETOH exposure in offspring because binge drinkers often display this level of BEC (Cherpitel, 2007).

Offspring weight was recorded daily. On PND 8, approximately 21 hours after receiving their last intubation, offspring were injected subcutaneously with either a vehicle control of 50mg/kg *SN*. This design allowed for six treatment groups: milk, milk + drug, ETOH, ETOH + drug, non-treated control (NTC), and NTC +drug. The NTC group contained two pups to avoid investigate potential intubation effects. At PND 21 the

rats were weaned from their mother and moved from the nursery room to the colony room of Kastle Hall.

All offspring were kept on a 12 hour light/dark cycle, and were allowed continuous access to food and water. The care of the offspring was carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996), as well as the University of Kentucky's Institutional Animal Care and Use Committee.

Drugs:

For the following experiment *SN* was generously provided to the Barron lab by Dr. John Littleton of the University of Kentucky. *SN* was administered subcutaneously on PND 8, 21 hours after the final intubation period. This time was chosen because previous research has found that this period of ETOH withdrawal in neonates appears to be particularly sensitive to administration of compounds that may reduce the excitotoxicity associated with ETOH withdrawal (see Thomas et al., 2006 for review). *SN* was administered acutely at a dose of 50 mg/kg, a dose previously found efficacious in reducing inflammation in a mouse model of pleurisy (Goulart et al., 2007). The drug was suspended in a saline solution and offspring not receiving the drug received an injection of saline alone.

Behavioral Paradigms:

Open Field:

The Open-Field paradigm was used to investigate rodent hyperactivity and impulse control as measured by total distance traveled and distance traveled in the center

of the apparatus. As previously mentioned, hyperactivity and deficits in impulse control are common in individuals with FAS/FASD (see Riley et al., 2005). These deficits have been observed using animal models of developmental ETOH exposure as well (Mattson et al., 2011). Thomas and colleagues (2004) demonstrated that perinatal choline supplementation reduced hyperactivity in neonatally exposed rodents. Because of this, we hypothesize that *SN* will reduce hyperactivity in our model of developmental ETOH exposure.

During open field testing (PND 20-21), offspring were housed with their mothers and litter-mates until testing began. On PND 20, pups were taken into the testing room alone for 10 min to habituate. The experimental room was illuminated with red lights, and a muffin fan which provided white noise. After habituation, each rat was placed into a round 55 cm open-field chamber for 30 minutes. The rats' activity was recorded using SMART real time video tracking (San Diego Instruments). For analysis, the open field was divided into center and outer zones, comprising 25% and 75% of the total field area respectively. Total distance traveled, and distance traveled in the center of the open field over a 30-minute period was measured. Total activity and activity in the center is considered a potential marker for impulsivity and/or anxiety (for review see Royce, 1977). Once testing was completed, bodyweights were recorded, and the subjects were returned to their home cage. Both chambers were then cleaned with a 30% ETOH solution to eliminate any possible odors prior to the next test session.

Dowel Balance Task:

This task was chosen because previous studies in this lab using this same task,

and other labs using similar tasks have reliably demonstrated deficits in motor function and coordination in rodent models of developmental ETOH exposure (Lewis et al., 2007; Thomas et al., 2004). Thomas and colleagues (2009), showed that offspring exposed to ETOH and choline on gestational days (5-20) showed similar levels of performance as controls on a motor function and coordination task, while ETOH alone offspring performed significantly worse. Because of this we hypothesized that DMXB-A would be able to reduce deficits in this task produced by our model of developmental ETOH exposure.

On PND 31-33, offspring were tested on a dowel balance paradigm. The apparatus consisted of one dowel rod (114cm long, 1.85cm diameter) that was elevated (75cm above the ground). Pads were placed under the dowel to avoid injury in case of falling. At the end of the dowel there was a black escape box (21x10x17cm). Before testing, each rat was handled for three minutes, weighed, and individually housed in the colony room. Testing began the following day and continued for three days (PND 31-33). The experimental room for the balance task was illuminated with a red light, a muffin fan helped reduce extraneous environmental noise during testing. Before testing, each rat was brought into the experimental room and habituated to the room for 10 min. On day 1, the subject was placed in the escape box for 1 minute to habituate and then returned to their home cage for 30 sec. After habituation, the subject was placed on the dowel 10 cm away from the escape box and allowed to enter the escape box. Once the subject successfully completed a trial, the distance to the goal box was increased by 13cm. A correct trial was defined as reaching the escape box without slipping or falling. An incorrect trial was defined as a subject slipping (two or more paws falling off the dowel) or falling off the

dowel completely. Once the animal reached the escape box it was left in the escape box for an additional 10 sec. The subject was then returned to their home cage for 30 sec until the next trial began. During the first trial of test days 2 and 3 the animal was placed on the dowel 13 cm further than their last successful distance traveled on the previous testing day. Total distance traveled on the dowel rod was the dependent measure for this task. Once a rat completed the three trials for that day, the dowel was cleaned with 30% ETOH before the next subjects were tested.

Hebb Style Water-Maze

This task was chosen because previous studies in this lab using this same task, and other labs using similar tasks have reliably demonstrated deficits in spatial navigation and working memory in rodent models of developmental ETOH exposure (Lewis et al., 2011; Thomas et al., 2009; Hunt et al., 2009). Lewis and colleagues demonstrated that offspring exposed to ETOH in the same manner as the current study required significantly more trials to complete the task on the second day of testing. Also, Thomas and colleagues (2004) demonstrated in a similar maze, the Morris Water Maze (a paradigm that requires offspring to use spatial navigation and working memory), that prenatal ETOH exposure reduced performance compared to controls and concomitant choline treatment reduced these deficits. Because of this we hypothesized that DMXB-A would be able to reduce deficits in this task produced by our model of developmental ETOH exposure.

The maze was a 130 x 90 x 40 cm black Plexiglass chamber divided into multiple divergent paths, with each path 18 cm wide (Fig. 1) The end of the maze was characterized with a submerged platform approximately 1cm below the water. The water

was mixed with non-toxic black powder tempura paint to obscure the platform. The temperature of the water in the maze was kept at 76 degrees +/- 2 degrees F and temperature of the room was 75 degrees +/- 3 degrees F. A plastic sheet surrounded the maze to reduce potential external cues from surroundings and the experimenter. The performance of the offspring was recorded by the experimenter and SMART real time video tracking software (San Diego Instruments). Testing was conducted between PND 40-45 for two days; day 1 was termed acquisition day and day 2 was termed retention day. One day prior to testing, each rat was handled for 3 min and weighed. On day 1, subjects were habituated to the test room for 5 minutes prior to the start of testing. For each trial, the rat was placed at the start of the maze. The rat's swim path was recorded until it reached the platform or after a 1 minute ceiling had passed (resulting in a failed attempt). If the rat failed to reach the platform on a given trial, it was guided to the platform by hand. The rat was allowed to stay on the platform for 10 seconds at which time it was removed from the maze and placed in a holding cage under a heat lamp until the next trial commenced.

This task required thee correct left/right discriminations. A correct trial required reaching the platform with no errors. The learning criterion was defined as two consecutive trials with zero errors with a ceiling of 20 trials. At the conclusion of acquisition training, the rat was left under the heat lamp for 5min to warm up/dry off and then returned to its home cage. The maze was cleaned before the next subject begins testing. For day two, the retention period, the same procedure was used as day one.

Attentional Set-Shifting Task (ASST):

This task was chosen because individuals with prenatal ETOH exposure commonly have deficits in executive function including reduced ability to shift attentional sets and ability to inhibit responses and make more errors (McGee et al., 2008; Riley et al. 2011; Jones et al., 2011). These deficits in executive function have also been found in animal models of developmental ETOH exposure (Kingdon et al., 2016). Previous studies using a similar task have shown that administration of an α7-nAChR agonist can both reduce deficits in an animal model of schizophrenia and improve cognitive flexibility in control offspring (Wood, et al., 2016). Recently, choline supplementation after prenatal ETOH exposure improved deficits in a set-shifting task very similar to the task used in the current study (Waddell et al., 2017).

Testing began on PND 50-55. In this paradigm offspring had to differentiate between odor and media to receive food reward. The apparatus was approximately 35 x 60 cm with one side containing a dividing wall were on each side (17.5cm) a cup containing the scent and media was placed. A start gate was placed between the animal and the two sides to stop exploration until a trial begins.

One week before testing began, offspring were placed on a restricted diet in order to reach a body weight of approximately 80-85% of their free-feeding weight. Once the offspring reached the desired weight day 1 training could begin. On day 1 of training offspring were first shaped to the task of digging in unscented cups for a food reward (1/2 of a cheerio). The unscented media was wood chips, similar to that found in the offspring's home cage. After 10 successful trials of digging and retrieving the food reward the animal was then tested on two forms of simple discrimination. For the first

simple discrimination (SD1) the animal was tasked with differentiating between two scents (garlic or dill) and one media (wood chips). This discrimination was done in order to determine if the animal could properly distinguish between scents without the distraction of different medias. An additional simple discrimination (SD2) was completed after the first where the animal was tasked with differentiating between two different media's (beads or rubber bands) with the same scent (celery) in order to receive food. This discrimination was completed to determine whether the animal could properly distinguish between media without the distraction of scent. For each simple discrimination the selection of only one scent (garlic or dill for SD1) or one media (beads or rubber bands for SD2) resulted in food reward. For the first four trials the animal was allowed to explore both cups in order to find the food reward. For all remaining trials the first cup the animal explored, whether it had a food reward or not, will end the trial. Criterion for completion during these and all following discrimination tasks was six correct trials not including the initial four exploratory trials. When the animal completed both SD1 and SD2 they could move on to day 2 testing.

During day 2 testing the animal was tasked with attending to one of two scents or media in the presence of both. This phase of testing explored their ability to shift between these different scent or media sets. Testing was comprised of six total components including complex discrimination (CD), CD reversal (CDR), interdimensional shift (ID), ID reversal (IDR), extradimensional shift (ED), and ED reversal (EDR)(See Table. 3.1). During the CD phase offspring attended to a scent (paprika or nutmeg) while ignoring the media the scents were in. To ensure the offspring were not attending to the media multiple sets of cups were made that had each scent in both types of media. During

testing these media/scent combinations were randomly assigned so the animal could not predict which media the rewarding scent would be in. The side of the rewarding scent was also randomized. For CD Reversal the scent that the animal was attending to was switched to the opposite scent. The media remained the same in this phase. This reversal challenged subjects to inhibit the learned response to attend to a previously rewarded cue and instead shift to the other now rewarded cue. As described above, for all day two phases offspring were allowed 4 initial trials to explore both cups and following these trials the offspring first choice would end the trial. The animal needed to complete six correct trials in a row after the initial four exploratory trials to meet criterion to go onto the next phase. During the ID and ID reversal phases, testing was the same to that of CD and CD reversal except that both scents and media were different. During ED and ED reversal, like in the other phases, two new scents and media were used, however the animal was only rewarded for attending to media instead of scent which challenged offspring to inhibit previous attentional strategies and adapt to new cue sets to receive reward.

Statistical Analysis

The data were analyzed using SPSS analysis of variance (ANOVA) with either a univariate or repeated measure where appropriate. Unless stated otherwise, the milk and NTC groups were compared first for similarity; if they showed no significant variation they were then collapsed into a single control group. This was done because the NTC group was used to investigate the effect of intubations, if no significant variation was found between the two groups it was assumed that intubation itself had no meaningful effect and these offspring could be considered control. Combining the control and NTC

groups also allows direct investigation of potential interactions between our compound and ETOH in a 2 x 2 analysis. Variables included sex (2), x ETOH (2) x drug (2). Subsequent ANOVAs were based on the interactions from this overall analysis. If there was no main effect or interaction with sex, the data was collapsed across this variable for ease of presentation. Significant main effects were broken down when needed using Least Significant Difference (LSD) post hoc analyses. All tests were investigated for outliers and litter effects before statistical analysis, for this study no offspring were removed from analysis and there were no significant differences between litters regarding performance. Significance for all analyses was set at $p<0.05$.

Results

Open-Field Performance:

Group performance was investigated using total distance traveled for each day and distance traveled for each 5-minute time block for each day. The first 5 minutes of each day was also investigated as a measure of reactivity to initial placement into the chamber. Entries into the center and percentage of time spent in the center of the chamber was also investigated because this is a common measure for impulse control (lack of inhibition).

Overall there were no significant differences between the groups in the open-field. Our hypothesis that ETOH offspring would be more active than control offspring and that treatment with *Solidago nemoralis (SN)* could reduce that increased activity was not supported. Also, as predicted, *SN* alone had no effect on performance.

Total Distance Traveled:

A 2 x 2 x 2 x 2 (Sex x ETOH x *SN* x day) repeated measure ANOVA revealed no

significant interactions or main effects between any of the groups. Because of the a-priori hypothesis and previous work in this lab, we predicted that ETOH offspring would be more hyperactive than control offspring and a LSD post-hoc analysis was used to investigate potential group differences. No differences were found between the groups for either day. (Fig. 3.1)

Total Distance Traveled by Day and 5 Minute Time Block:

The sex x ETOH x *SN* x day x block (2 x 2 x 2 x 2[6]) analysis using a repeated measures ANOVA also showed no significant interactions or main effects. However, because of previous findings in this lab an a-prior hypothesis that groups would differ based on treatment group, an LSD post-hoc analysis was run and confirmed the previous analysis that there was no significant difference between any of the groups (Fig. 3.2 & 3.3)

Total Distance Traveled in the Center:

A 2 x 2 x 2 x 2 (Sex x ETOH x *SN*x day) repeated measure ANOVA revealed a similar pattern specifically that there were no significant interactions or main effects between any of the groups or group differences as assessed by an LSD post-hoc analysis, ETOH exposed offspring had increased variability in time spent in the center for day 1 when compared to controls, however this difference was not significant. This was not observed in offspring treated with both *SN* and exposed to ETOH (Fig. 3.4).

Total Distance Traveled by 5 Minute Time Block in the Center:

Again, sex x ETOH x *SN* x day x block (2 x 2 x 2 x 2[6]) repeated measures ANOVA revealed similar pattern as total distance, specifically that there were no significant interactions or main effects and not significant differences between any of the

groups as assessed by LSD post-hoc analysis. However, ETOH offspring performance toward the end of day 1 testing appeared more active compared to controls, but this difference was non-significant (Fig. 3.6). This increase in activity was not observed in offspring treated with *SN* after ethanol exposure (Fig. 3.5)

Entries into the Center:

A 2 x 2 x 2 x 2 (Sex x ETOH x DMXB-A x Day) repeated measures ANOVA revealed that there were no significant interactions or main effects for entries into the center of the apparatus. An LSD post-hoc investigating individual group differences also revealed no significant difference between any of the group for either day. (Fig . 3.7)

Percentage of Time Spent in the Center:

A 2 x 2 x 2 x 2 (Sex x ETOH x DMXB-A x Day) repeated measures ANOVA revealed that there were no significant interaction or main effects. An LSD post-hoc analysis confirmed that none of the groups differed on either day (Fig. 3.8)

Balance Performance:

Treatment with *SN* in ETOH exposed offspring resulted in no significant differences between this group and control animals. Offspring exposed to ETOH alone had significantly reduced performance below that of control animals for a single trial in day three, however ETOH offspring's performance for the reminder of the trails was similar to that of control animals. A 2 x 2 x 2 x 3(3) repeated measures ANOVA (sex x ETOH x *SN* x day[trial]) was conducted and yielded a main effect of ETOH $F(1,101)=4.245$, $p<.05$. Group differences were investigated using a LSD post-hoc analysis, offspring exposed to ETOH performed more poorly than control offspring during the first trial of day 3 ($p<.05$), when treated with *SN*, performance in ETOH

offspring was similar to that of control animals (see Fig. 3.9). The greatest distance achieved was also investigated (Fig. 3.10) and no significant differences were found.

Water Maze Performance:

In support of the hypothesis, *SN* treatment in ETOH exposed males significantly reduced the number of trials required to reach criterion compared to males exposed to ETOH alone during acquisition. This trend continues into retention where both *SN* alone and *SN* treatment in ETOH exposed males require significantly less trials to reach criterion than males exposed to ETOH alone. In females, there is a trend for the ETOH exposed offspring to require more trials to reach criterion during acquisition that is not present in any of the other groups. No differences were found during retention in females.

A 2 x 2 x 2 x 2 (sex x ETOH x *SN* x day) repeated measures ANOVA revealed a main effect of sex $F(1,90)=5.212$, $p<.05$, and a main effect of *SN* $F(1, 90)=5.988$, $p<.05$. Because of this each sex was investigated separately. In males, a 2 x 2 x 2 (ETOH x DMXB x day) repeated measures ANOVA revealed a main effect of *SN* $F(1,46)=7.746$, $p<.01$. Group differences were explored using LSD post-hoc analysis. Males exposed to ethanol and treated with *SN* took fewer ($p<.05$) trials to reach criterion than ethanol alone offspring during both acquisition and retention. Also, males treated with *SN* alone required significantly less trials to reach criterion than ETOH offspring during retention but did not differ from control offspring (see Fig. 3.11). In females, the 2 x 2 x 2 ANOVA revealed no interactions or main effects. Though not significant, there was a trend for the ETOH exposed females to require more trials to reach criterion than any other group during acquisition (see Fig. 3.12).

Attentional Set-Shifting Task Performance:

Because of the multifaceted and complex nature of the ASST task, each phase of testing was unique and thus warranted investigation using a univariate ANOVA between ETOH, *SN*, and sex (2 x 2 x 2) (see Wood et al., 2016 & Hawkey, 2017). Additionally, trials to criterion and number of errors made during trials will be discussed separately because they are different measures investigating how long it takes to learn an attentional set (rodent demonstrates greater cognitive ability if they require less trials to acquire a new attentional set) and how well the animal can inhibit previous learning (the more errors made implies more difficulty inhibiting previously learned attentional sets) (Waddell et la., 2017; Gursky & Klinstova 2017). Each main effect and interaction will be provided in sequential order by phase (starting with SD1 and ending with EDR when applicable). Also, with subsequent analyses, because an a-prior hypothesis that groups would differ in performance based on treatment group, an LSD post-hoc analysis was used to investigate group differences.

Trials to Criterion:

In support of our hypothesis, ETOH exposed offspring required more trials to reach criterion than control offspring in several phases. This was particularly true in female offspring. Treatment with *SN* in ETOH exposed offspring reduced the number of trials to criterion to control levels in every phase that ETOH exposed offspring displayed deficits. However, *SN* alone increased the number of trials required to reach criterion during simple discrimination, a phase that was unaffected by ETOH exposure alone, in male and female offspring and complex discrimination reversal in males. Treatment with *SN* in ETOH exposed males also increased the number of trials required to reach criterion during both simple discrimination trials, while ETOH alone had no effect.

Because there was a difference between male and female offspring for several of the phases including a main effect of sex for the first simple discrimination $F(1,79)=3.686$, $p<.05$, and an interaction between sex and *SN* during complex discrimination reversal $F(1,79)= 5.816$, $p<.05$, the results for each sex will be presented separately for ease of presentation.

In males, for the first simple discrimination (SD1) there was a main effect of *SN*, $F(1,39)=7.221$, $p<.05$ and no interactions. An LSD post-hoc analysis revealed that males treated with *SN* and *SN* and ETOH both required significantly more trials ($p<.05$) to reach criterion than control offspring during SD1. For the second simple discrimination (SD2) there was a main effect of ETOH, $F(1,39)=6.358$, $p<.05$ and no interactions. For this phase, an LSD post-hoc analysis revealed that males exposed to ETOH and treated with *SN* required significantly more ($p<.05$) trials to reach criterion than control offspring. Additionally, those treated with ETOH alone appeared to require more trials during SD2 than controls, though this difference was not significant, there were no other group differences. For the complex discrimination (CD) phase there were no main effects or interaction, however, an LSD post-hoc test revealed that ETOH exposed males required significantly more ($p<.05$) trials to reach criterion than controls during this phase and treatment with *SN* in ETOH animals reduced this deficit to control levels. For complex discrimination reversal (CDR) there were also no main effects or interactions, however an LSD post-hoc analysis revealed males treated with *SN* alone required significantly more ($p<.05$) trials to reach criterion than control offspring, however, this effect was not seen in ethanol exposed offspring treated with *SN*. For all other phases, including

Intradimensional shift (ID), ID reversal (IDR), extradimensional shift (ED), and ED reversal (EDR) there were no main effects or interactions for males. (see Fig. 3.13)

For females, there were no significant main effects or interactions for the first simple discrimination (SD1). LSD post-hoc analyses revealed that females treated with *SN* required significantly more ($p<.05$) trials to reach criterion than controls, however, this group did not differ from either ETOH or ETOH + *SN* offspring during this phase. For the second simple discrimination (SD2) there were no main effects or interaction. For complex discrimination (CD) there was a main effect of ETOH, $F(1,41)=4.321$, $p<.05$, *SN*, $F(1,41)=7.142$, $p<.05$, and an interaction between ETOH and *SN*, $F(1,41)=5.822$, $p<.05$. An LSD post-hoc analysis revealed that females exposed to ETOH required significantly more trials ($p<.005$) than controls to achieve criterion during this phase and that treatment with *SN* reduced this deficit to control levels. For both complex discrimination reversal (CDR) and Intradimensional shift (ID) phases there were no main effects or interactions. However, ETOH exposed offspring, though not significant, performed similarly to previous phases and required additional trials to reach criterion for both of these phases. During the intradimensional reversal (IDR) and extradimensional shift (ED) phases there were no main effects, however, an LSD post-hoc analysis revealed that females exposed to ETOH during both of these phases required more trials ($p<.05$) than controls to reach criterion and that treatment with *SN* reduced these deficits to control levels. Finally, during extradimensional reversal (EDR) there were no main effects or interactions. (see Fig. 3.14)

Errors:

Our hypothesis was supported in that offspring exposed to ETOH made more errors than control offspring during several phases. Treatment with *SN* in ETOH exposed offspring reduced this deficit in some of the phases affected but did not reduce this in others.

Again, because there was a difference between male and female offspring for several of the phases including an interaction between sex and *SN* for complex discrimination (CD) $F(1,79)=4.058$, $p<.05$, for complex discrimination reversal (CDR) $F(1,79)= 4.723$, $p<.05$, and for extradimensional shift (ED) $F(1,79)=4.976$, $p<.05$, the results for each sex will be presented separately for ease of presentation.

In males, for the first and second simple discrimination phases (SD1 & SD2), there were no main effects or interactions in errors made during these phases. For complex discrimination (CD), there was a main effect of ETOH, $F(1,39)=6.264$, $p<.05$, and an interaction between ETOH and *SN*, $F(1,39)=9.108$, $p=.005$. An LSD post-hoc analysis revealed that all three treatment groups (ETOH, *SN*, and ETOH + *SN*) made more errors ($p<.05$) than control offspring during this phase. However, those treated with *SN* alone and in addition to ETOH, though not significant, were closer to control performance than those treated with ETOH alone. For complex discrimination reversal (CDR) there were no main effects or interactions, however LSD post-hoc revealed a significant increase ($p<.05$) in errors in males treated with ETOH compared to controls. For Intradimensional shift (ID), ID reversal (IDR), extradimensional shift (ED), and ED reversal (EDR) there were no main effects or interactions. (see Fig. 3.15).

In females, for the first simple discrimination (SD1), there was a main effect of

ETOH, F(1,41)=4.092, p=.05, and an LSD post-hoc analysis revealed that females exposed to ETOH, and those exposed to ETOH and treated with *SN* made more mistakes ($p<.05$) than controls. However, differences between groups in amount of errors made are in the order of 1 which may be statistically significant though biologically insignificant for complex animal behavior. For the second simple discrimination (SD2), there were no main effects or interactions, however LSD post-hoc analysis revealed that females exposed to ETOH and treated with *SN* made more mistakes ($p<.05$) than controls. For complex discrimination (CD) there was a main effect of *SN*, F(1,41)=4.204, $p<.05$, and an LSD post-hoc analysis revealed there were no differences between the controls and any of the other groups, however, ETOH exposed females made significantly more errors than *SN* treated females. For complex discrimination reversal (CDR) there were no main effects or interactions. For Intradimensional shift (ID) there was a main effect of ETOH, F(1,41)=5.025, $p<.05$, and an LSD post-hoc analysis revealed that females exposed to ETOH made more errors than control offspring during this phase. For Intradimensional shift reversal (IDR), extradimensional shift (ED), and extradimensional shift reversal (EDR) there were no main effects or interactions. (see Fig. 3.16)

Body Weights:

For all tasks weight was recorded after the completion of each behavioral test. For open-field weights were recorded on PND 21 and analyzed with neonatal treatment and sex as grouping variables (see Table 3.2). As predicted, a 2 x 4 ANOVA revealed a main effect of both sex F(1,100)=28.873, $p<.01$, where males generally weight more than females of the same group, and treatment group F(1,100)=71.592, $p<.01$ but no interaction. Post-hoc analysis revealed that both male and female offspring treated with

ETOH and ETOH & DMXB-A weighed less than control offspring ($p<.05$). For balance the ANOVA revealed a main effect of sex $F(1,101)=11.930$, $p<.005$, where males consistently weighed more than females, and a main effect of group $F(1,101)=7.414$, $p<.005$, where both ETOH and ETOH + *SN* offspring weighed significantly less than control offspring. This was true in both male and female offspring. For water maze the ANOVA revealed a main effect of sex $F(1,90)=191.647$, $p<.001$ and a main effect of group $F(1,90)=4.591$, $p=.005$, and no interaction. Males, as predicted, were reliably heavier than their female counterparts in all groups. Additionally, ETOH males weighed significantly less than their respective control offspring, also ETOH females treated with *SN* weighed significantly less than their respective control offspring. For the ASST task the ANOVA revealed a main effect of sex $F(1,78)=35.434$, $p<.001$ and no interaction. Males, as predicted, were reliably heavier than their female counterparts in all groups. There was no longer an effect of ETOH exposure on weight during ASST.

Discussion:

This study was aimed at determining if *SN* could reduce the behavioral deficits produced by developmental ETOH exposure during the 3rd trimester brain growth spurt in rodents. In short, *SN* appeared to reduce some of the deficits found in motor coordination, learning/memory, and executive functioning. However, these results appeared to be dependent on sex, and in some cases SN alone, or in combination with ETOH was detrimental to behavior. These results are explored further below.

As discussed previously, previous research in this lab and others have demonstrated that developmental ETOH exposure, particularly during the third trimester, can impair behaviors related to attention/hyperactivity (Smith et al., 2012; Thomas et al.,

2007; Lewis et al., 2012), learning/memory (Hunt et al., 2009; Thomas et al., 2009; Tiwari et al., 2012), coordination/balance (Idrus et al., 2011; Lewis et al., 2007; Thomas et al., 2004), and executive function (reviewed in Gurskey & Klinstova 2017). Along with these deficits in behavior, this period is also sensitive to anatomical deficits in regions associated with these behaviors including the cerebellum, hippocampus, and prefrontal cortex are undergoing great growth and development during the third trimester and are more sensitive to ETOH's teratogenic effects (for review see Dobbing & Sands, 1979; Riley & McGee, 2005 & Patten et al., 2014). These areas are innervated by the cholinergic system and contain α7-nAChR's (Hurst et al., 2013). Previous studies have demonstrated the potential of α7-nAChR activation to reduce behavioral deficits associated with various neurological disorders including developmental ETOH exposure (Yu et al., 2012; Koukouli & Maskos, 2015; Suzuki et al., 2006, Thomas et al., 2016). Because of this we believed *SN*, a plant derived flavonoid extract that targets the α7-nAChR would be able to reduce the behavioral deficits associated with developmental ETOH exposure.

The first test offspring were ran in was an open-field task. This task was used in the current study because like humans, animals who are exposed to ETOH during development typically demonstrate inattention/hyperactivity (for review see Mattson et al., 2011 & Patten et al., 2014). In the current study however, our hypothesis was not supported, and offspring exposed to ETOH did not display the typical pattern of hyperactivity where path length and time spent in the center of the apparatus was significantly increased over control offspring. Because there was no effect of ETOH in this task the addition of *SN* by itself and in ETOH exposed offspring could not be

properly investigated for group differences. Previous studies in this lab using identical methods and exposure model (Lewis et al., 2012; Smith et al., 2012) and other labs who have used similar models (Thomas et al., 2007 & 2016) have reliably found that ETOH animals are more hyperactive/inattentive than control animals. It is unclear why performance in this paradigm would be any different from performance in previous studies from the same lab, but it did not appear to be an outlier or litter effect. When considering possible explanations for lack of an ETOH effect average activity in control offspring was compared between this study and previous studies in this lab, for Smith et al., (2012), average activity was approximately the same as the current study (around 2000 cm. total distance traveled for both days). Interestingly, they did not find an effect of ETOH on total distance traveled for day 2 in respect to control performance, but ETOH did increase entries into the center of the apparatus for both days. As mentioned previously, investigations into the BAC's for this model have been performed and it has shown that this model produces BAC's up to 220 mg/dl 60 minutes after intubation (Lewis et al., 20011). BAC's in this range have reliably produced deficits in the behaviors investigated for this study (see Painter et al., 2014 for review). Future investigations using this model with the open-field task should be completed to determine a possible cause for this difference because the use of this task is invaluable in determining the efficacy of compounds aimed at reducing some of the behavioral effects of developmental ETOH exposure.

SN was also investigated for its ability to reduce deficits in motor coordination after developmental ETOH exposure. As previously discussed, this paradigm was used because deficits in coordination (as measured by distance traveled in the current study)

are common in both clinical cases and animal models of FAS/FASD (Idrus et al., 2011; Lewis et al., 2007; Thomas et al., 2004). This task is believed to be mediated by the cerebellum, a structure that is particularly sensitive to the effects of developmental ETOH exposure (see Riley & McGee, 2005 for review). This structure is also innervated by the cholinergic system and contains α7-nAChR (Hurst et al., 2013). Previous studies have shown that activation of the α7-nAChR through choline supplementation have reduced deficits in motor function in animals exposed to ethanol during development (Thomas et al., 2004 & 2016). In the current study, performance in offspring treated with *SN* and exposed to ETOH was at or near control levels during the entirety of the test. However, ETOH exposure alone only reduced performance significantly below control levels during a single trial of day three. However, though not significant, ETOH offspring did appear to have reduced performance for the remainder of the test. A reduction in deficits produced by ETOH exposure as a result of activation of the α7-nAChR agrees with previous studies such as Thomas and colleagues (2009) who found that supplementation with choline (a α7-nAChR agonist) was able to reduce deficits in motor ability and coordination in rodents exposed to ETOH during development.

Deficits in learning and memory were also investigated during the current study using a Hebb style water maze developed by Von Euler and colleagues (2006). This test is unique in that it demonstrates learning (acquisition of the task) and memory (retention of the task) in just two days. The use of a paradigm investigating learning and memory is useful because clinical populations with FAS/FASD often demonstrate deficits in these abilities (Hunt et al., 2009; Thomas et al., 2009; Tiwari et al., 2012). In the current study, males treated with *SN* after ethanol exposure required significantly less trials to reach

criterion than offspring exposed to ethanol alone during both acquisition and retention. Additionally, treatment with *SN* alone in males significantly reduced the trials required to reach criterion below that of offspring treated with ethanol during retention. Though not significant, a similar pattern was observed in females during acquisition where ETOH exposed offspring took more trials to reach criterion than control or *SN* treated ETOH exposed offspring. These results support the hypothesis that a single dose of *SN* during EWD can reduce learning and memory deficits using this model of ETOH exposure. Thomas and colleagues (2009 & 2016) have supported the role of activating the α7-nAChR through choline supplementation in reducing learning and memory deficits in other water maze tasks. However, this is the first study to use this task to investigate *SN*'s ability to reduce deficits produced by 3rd trimester ETOH exposure followed by EWD. Interestingly, there also appeared to be a difference in performance between male and female offspring regarding acquisition and retention. Female offspring had no significant differences between the groups regarding trials to criterion. Previous studies from this lab using an identical model of ETOH exposure and the same task found that animals exposed to ETOH only displayed a deficit during acquisition and not retention for both male and females (see Lewis et al., 2007 & 2011). However, in the current study males appeared to show a deficit in both acquisition and retention after ethanol exposure compared to offspring treated with *SN* and exposed to ethanol, and females showed no differences in performance at all. Goodlett & Peterson (1995) suggested that males may be more affected by developmental ETOH exposure than females in learning tasks like the Morris water maze, which would support the current findings.

SN's ability to reduce deficits in executive function after developmental ETOH exposure was also investigated. To study this, we used the attentional set shifting task, also referred to as the ASST. ASST is considered to be an animal correlate to the human Wisconsin card sorting task (see Barnese et al., 2002), where the participant is expected to shift between changing rules for reward. In our task as described above, the animal is required to shift its attention from odor to odor or media to media, while ignoring the other odor and media cues present to receive reward. Executive function has been shown to be deficient in many individuals with FAS/FASD and has also been shown in animal models of this disorder as well where deficits appear to be in ability to shift attentional sets and ability to inhibit previous learning (for review see Kingdon, Cardoso, & McGrath, 2016 & Gurskey & Klinstove 2017). Performance of this task appears to be largely controlled by communication between the prefrontal cortex and striatum (Birrell & Brown et al., 2000; Barnese et al., 2002), both structures that contain α7-nAChR (Hurst et al., 2013) and are sensitive to developmental ETOH exposure (Barron et al., 2017; Goodlett et al., 1997; Riley et al., 1993), especially during the third trimester (Ikonomidou et al., 2000). These deficits are thought to arise from aberrant connectivity from the PFC to other structures critical for the performance of set-shifting like the hippocampus (Gorsky & Klinstova 2017). Previous studies using a similar task have shown that administration of an α7-nAChR agonist can both reduce deficits in an animal model of schizophrenia and improve cognitive flexibility in control animals (Wood, et al., 2016). Recently, choline supplementation after prenatal ETOH exposure improved deficits in a set-shifting task very similar to the task used in the current study (Waddell et al., 2017). However, this task had never been used in our third trimester model of ETOH

exposure. In the current study we investigated the number of trials required to complete the task (6 trials correct in a row to move to the next phase) and number of errors made in each phase. Offspring exposed to ETOH required more trials to achieve criteria and made more errors during complex discrimintation (CD) in both male and female offspring, and Intradimensional shift reversal (IDR) and extradimensional shift (ED) in females. A single administration of *SN* in ETOH exposed offspring reduced the number of trials required to reach criterion to control levels for each of these phases in both male and female offspring. Deficits in complex discrimination are common because this is the first phase where an animal is required to attend to a specific stimuli (scent) and ignore other stimuli (media and unrewarded scent) (Waddell et al., 2017 & Gursky & Klinstova 2017). Reversal ability in animals exposed to developmental ETOH also appears to be a common deficit (Kingdon et al., 2016). To be able to reverse an animal must be able to inhibit response to an old stimuli that was previously rewarded to a newly rewarded stimuli (Bissonette et al., 2013), this trouble with response inhibition leading to issues in reversal learning are common in individuals and rodents exposed to ETOH during development (Kingdon et al., 2016). Deficiencies in reversal learning are believed to be mediated by damage to the medial PFC (mPFC), where Mihalick and colleagues (2001) found that rodents with a lower number of mPFC neurons (in response to prenatal ETOH exposure) were more deficient at reversal learning than control animals. Deficits in extradimensional (ED) shifts are also common after developmental ETOH exposure (Gurskey & Klinstova 2017). ED shifts test the strength of attentional sets formed for a previously rewarded set, which is odor in the present study, an increase in the number of trials to complete ED demonstrates a deficit in ability to shift attentional set to attend to a

new set (Waddell et al., 2016). Another interesting finding was that males exposed to ETOH and treated with *SN* required significantly more trials to reach criterion during the SD1 and SD2 phases, but females exposed to ETOH alone made more errors during these phases a result not found in the remaining phases. These initial simple discriminations only require the animal to attend to a single stimulus at a time. Previous studies using an ASST model similar to our own have found that animals exposed to ETOH are deficient in simple discriminations when compared to control (Waddell et al., 2016). However, the current study only found this deficit when ETOH and *SN* were combined. It's possible that this combination is detrimental for these simple discriminations, however, this deficit did not continue in any of the other phases. Also, studies using compounds that target the cholinergic system in combination with developmental ETOH exposure have not found deficits of this nature (Waddell et al., 2016) Also, in both males and females exposed to *SN* alone, there were times that these offspring required significantly more trials to complete the task. Interestingly, SD1 (their first discrimination) was an issue for *SN* offspring for both sexes, this effect was not observed in females for the remaining phases but was observed in males during the CDR phase. Again, this is not supported by the literature where compounds that target the α7-nAChR have commonly found improvement in performance over controls (Wood et al., 2016 & Waddell et al., 2016). This difference between control offspring and those treated with *SN* should be explored further to determine if this is a continuing effect or simply due to random variation.

We also investigated errors made in the ASST task, males exposed to ETOH made more errors during complex discrimination (CD) and CD reversal (CDR), and females exposed to ETOH made more errors during simple discrimination 1 and 2 and

also Intradimensional shift. Again, these deficits in performance were expected due to previous findings by Waddell and colleagues (2017). Errors made commonly correlate to a difficulty in shifting to a new set, or difficulty inhibiting previous response sets (Kingdon et al., 2016). An unexpected finding was that females and males who were exposed to ETOH and treated with *SN* both made more errors during several of the phases. However, these differences were not supported by the results of previous literature using other α7-nAChR activating drugs (Waddell et la., 2017), and many of these differences were less than 1 error which may not be biologically relevant. Again, because this is the first time using this task to investigate any compound in our third trimester ETOH exposure model it is important to further investigate these potential differences between groups to determine if this is an effect of the drug or just a random event.

The present study demonstrates the ability of the flavonoid extract, *Solidago nemoralis* (*SN*), derived from the grey goldenrod, in reducing some of the behavioral deficits produced by "3rd trimester" ETOH exposure in rodents. Though it is clear further work needs to be completed with this compound to understand its variable effects on sex and the possibility of it producing deficits in certain behaviors, these initial results lend evidence to the cholinergic systems involvement in reducing deficits produced by developmental ETOH exposure. As discussed above, there are currently no pharmacotherapies clinically approved to treat FAS/FASD. As previously mentioned, targeting EWD, a process that is severely damaging to the developing nervous system (Riley et al., 2011) is one potential avenue for reducing deficits related to FAS/FASD and could be a one-time treatment. The mechanism for the damage caused by EWD, as

reviewed above, is thought to be predominately produced by excitotoxicity, oxidative stress, and neuroinflammation (Alfonso-Loeches et al., 2011). *SN*, an extract rich in flavonoids and a compound that has shown affinity for binding at the alpha7 receptor has been shown to reduce these types of damage in cellular models of ETOH exposure (Lutz et al., 2015). Because *SN* was able to reduce some of the behavioral deficits associated with developmental ETOH exposure in our model, it appears that agonizing the alpha7 receptor is a promising avenue for reducing deficits in FAS/FASD. Clearly more work is necessary with this compound to further elucidate the differences between treatment groups in our behavioral studies as well as to further understand its actions in the nervous system. But this is a promising step and the clinical utility of this compound seems apparent and should be investigated further in hopes that it or another compound like it will one day be used to treat the harmful effects of developmental ETOH exposure in human populations.

Tables:

Table. 3.1, Phases of ASST.

DAY	PHASE	ODOR		TEXTURE	
1	Shaping	N/A	N/A	Wood Chips	N/A
1	SD1	Garlic (+)	Dill (-)	N/A	N/A
	SD2	N/A	N/A	R. Bands (+)	Beads (-)
2	CD	Paprika (+)	Nutmeg (-)	Sponge	Paper
	CD Reversal	Paprika (-)	Nutmeg (+)	Sponge	Paper
2	ID	Cumin (+)	Oregano (-)	Wax Paper	Raffia
	ID Reversal	Cumin (-)	Oregano (+)	Wax Paper	Raffia
2	ED	Sage	Basil	Straw (+)	Aluminum (-)
	ED Reversal	Sage	Basil	Straw (-)	Aluminum (+)

Table. 3.2, *Solidago Nemoralis* Study Animal Weights

Body Weights (in g.)

Group		Treatment (PND 8) M ± SEM	Open Field (PND 21) M ± SEM	Balance (PND 33)^ M ± SEM	Water Maze (PND 44)^ M ± SEM	ASST (PND 55)^ M ± SEM
Control						
	Males	20.0 ± .7	57.8 ± .6^	117.6 ± 4.6	207.2 ± 3.7	264.9 ± 4.8
	Females	19.2 ± .5	54.4 ± .6	103.7 ± 2.7	162.4 ± 2.6	212.4 ± 8.0
ETOH						
	Males	15.8 ± 1.0*	50.6 ± 1.7*^	100.7 ± 8.1*	187.4 ± 4.3*	246.3 ± 8.0
	Females	15.8 ± 1.0*	43.0 ± 1.0*	85.0 ± 5.2*	156.8 ± 3.8	218 ± 9.8
SN						
	Males	19.1 ± .6	59.8 ± 1.1^	122.0 ± 6.4	198.9 ± 4.0	250.8 ± 9.1
	Females	18.9 ± .7	56.1 ± 1.0	107.6 ± 4.2	164.2 ± 2.7	206 ± 9.8
ETOH + SN						
	Males	15.1 ± .9*	46.0 ± 1.1*	106.4 ± 5.2*	199.8 ± 8.3	260.2 ± 8.5
	Females	14.2 ± 1.0*	44.4 ± 1.2*	94.4 ± 5.6*	146.37 ± 2.3*	192.5 ± 9.4

* Denotes significant difference from control of same sex ($p<.05$)

^ Denotes sex difference in same treatment group ($p<.05$)

Figures:

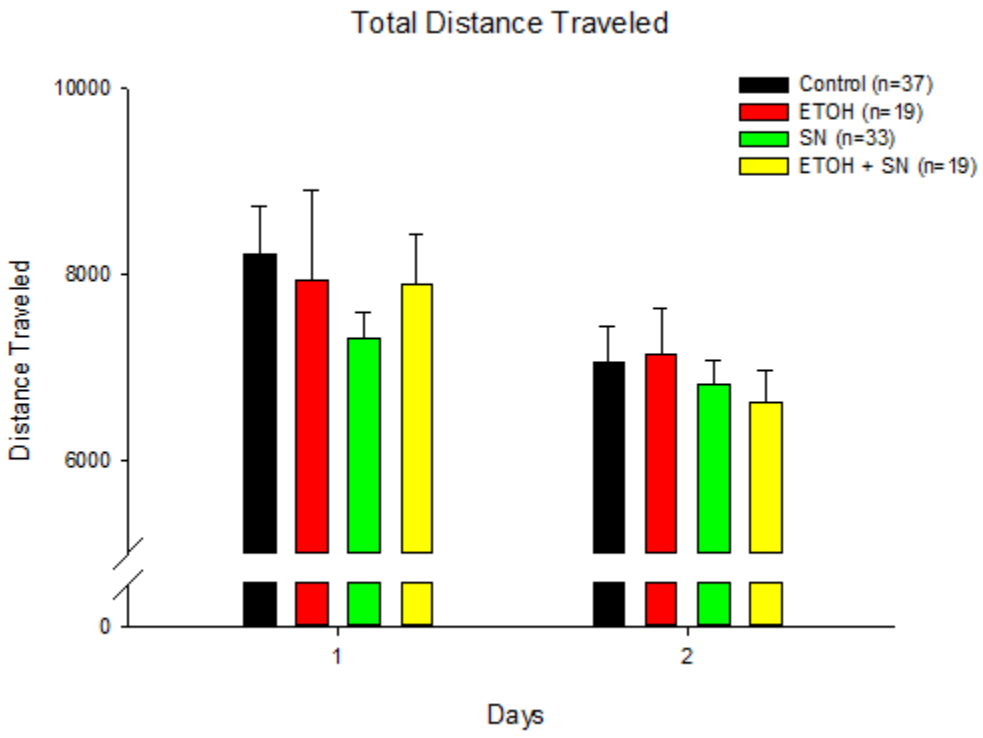

Figure 3.1: Open-Field performance as measured by total distance traveled. There were no groups differences in distance traveled for either day of the

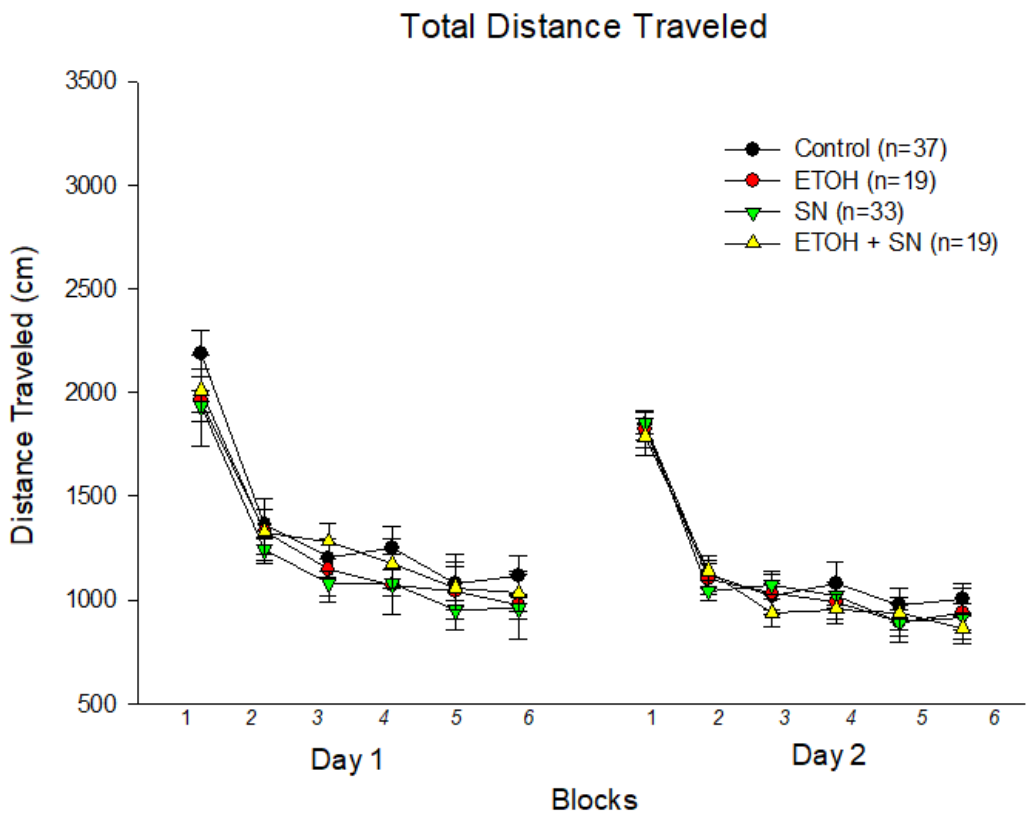

Figure 3.2: Open-Field performance as measured by total distance traveled and broken down into 5-minute blocks. Again, there were no groups differences in distance traveled for either day of the open-field task.

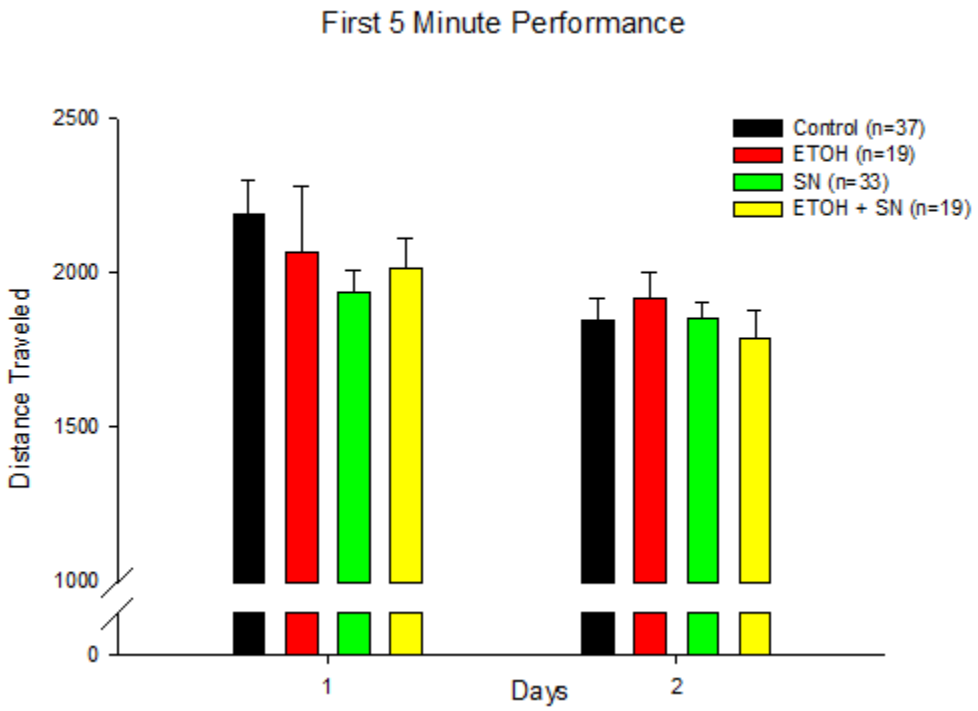

Figure. 3.3: Open-Field performance as measured by total distance traveled in the first five minutes of testing for each day. There were no groups differences in distance traveled for either day of the open-field task.

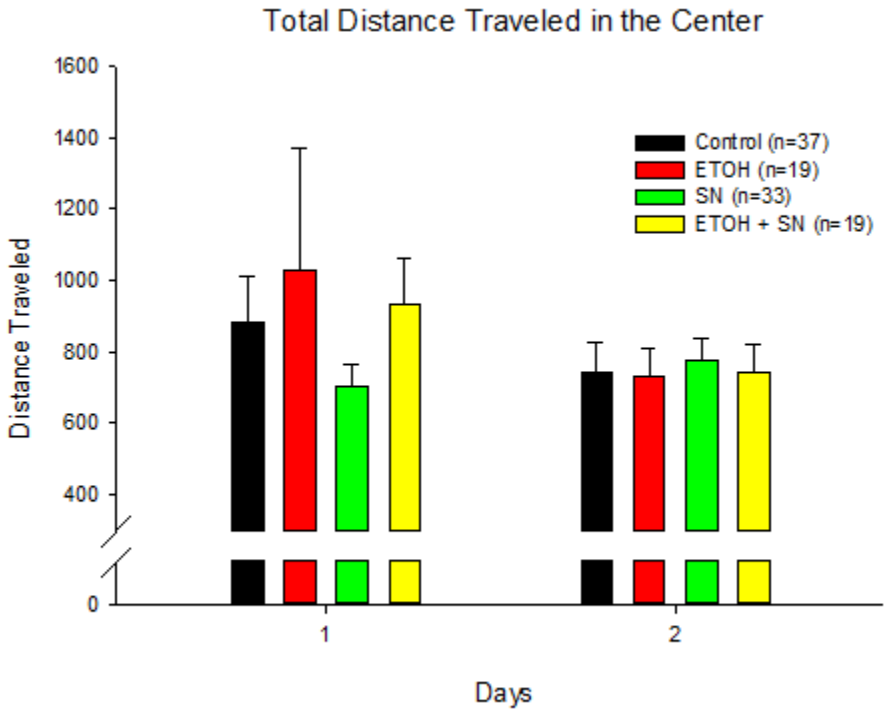

Figure. 3.4: Open-Field performance as measured by total distance traveled in the center of the open-field for each day. Groups did not differ from one another for either day.

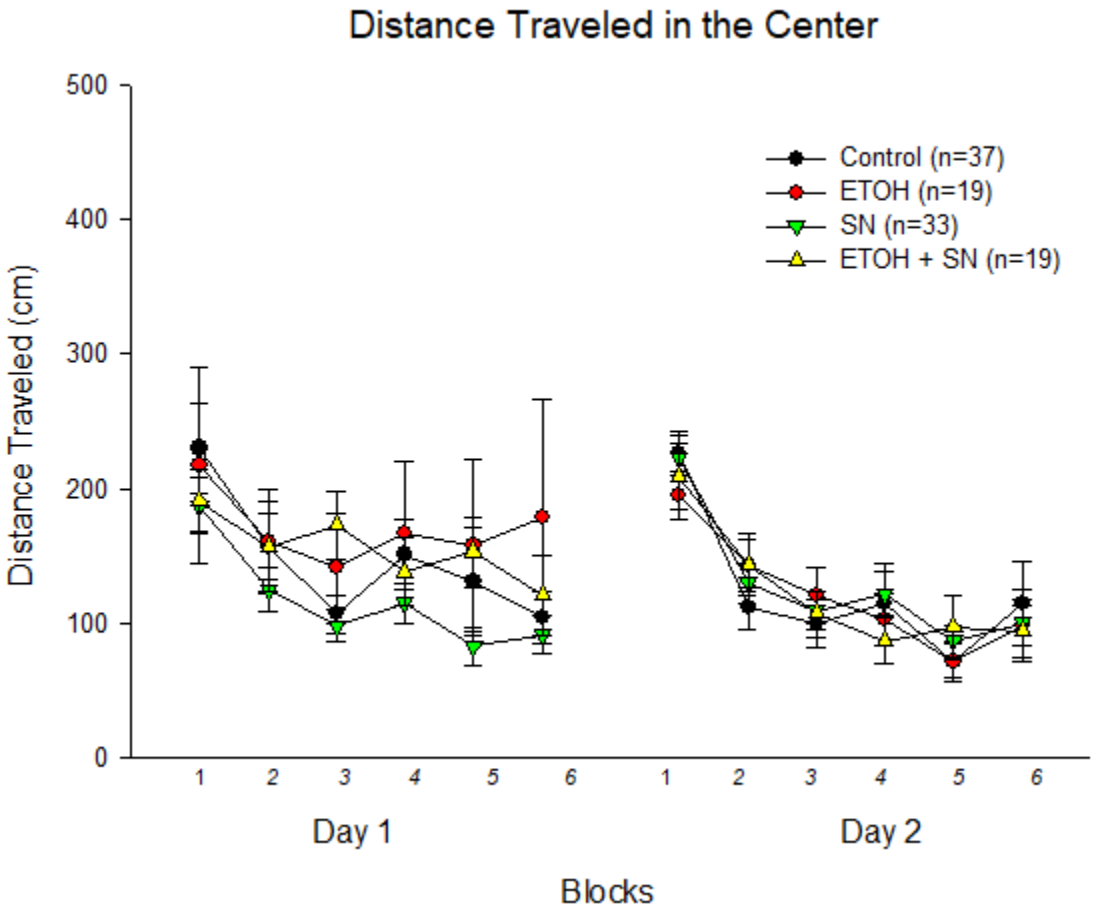

Figure. 3.5: Open-Field performance as measured by total distance traveled in the center of the open-field in 5-minute time blocks for each day. There were no groups differences in distance traveled in the center for either day of the open-field task.

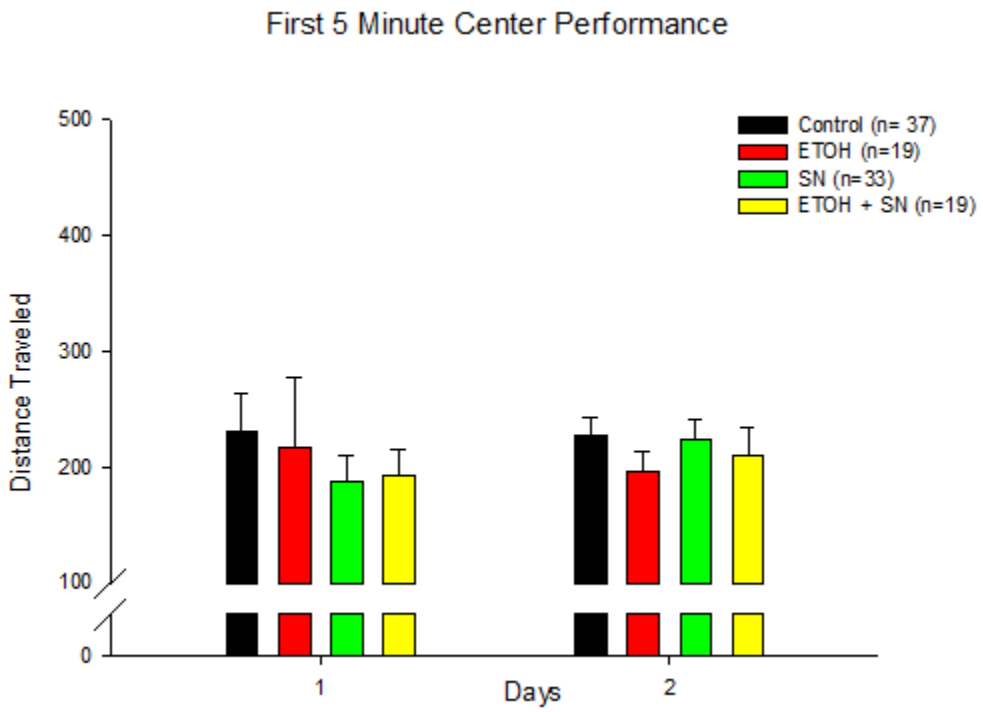

Figure. 3.6: Open-Field performance as measured by total distance traveled in the center of the open-field in the first five minutes for each day. No significant differences were found between groups for either day of testing.

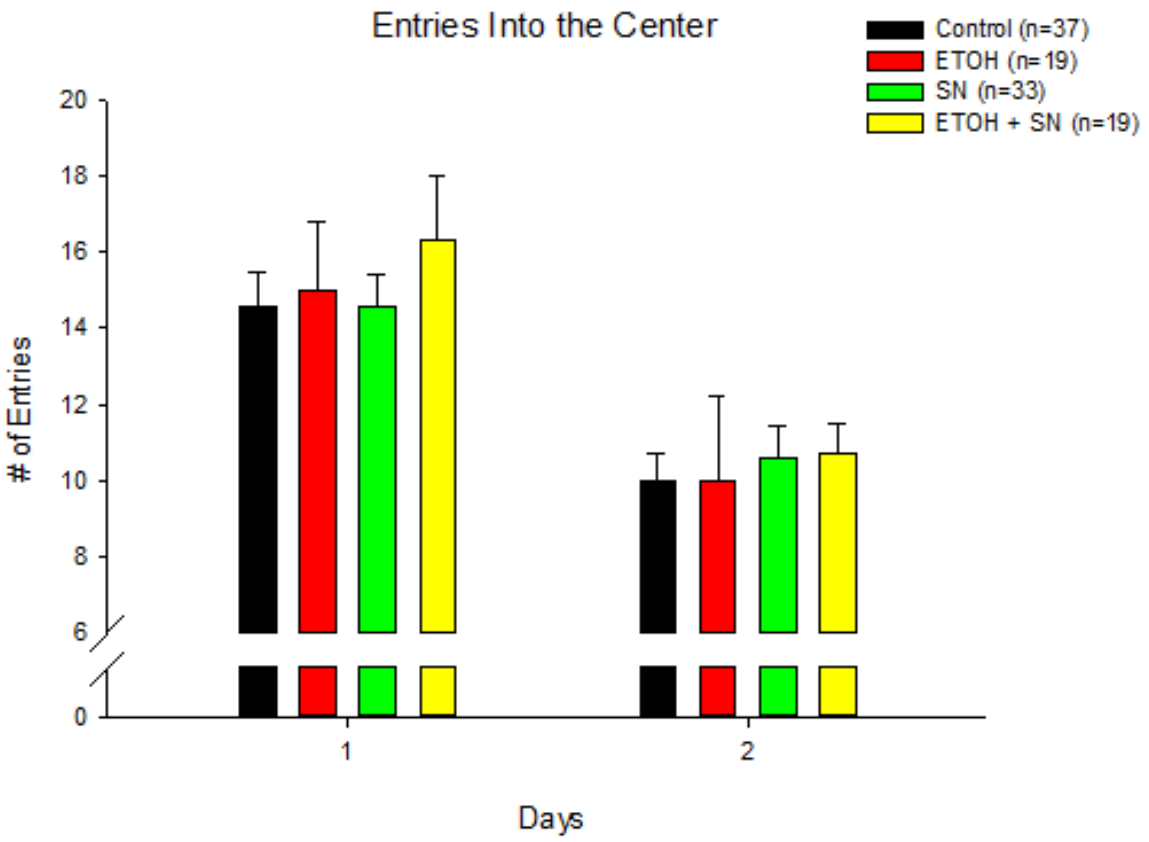

Figure. 3.7: Open-Field performance as measured by entries into the center of the open-field. No significant differences were found between groups for either day of testing.

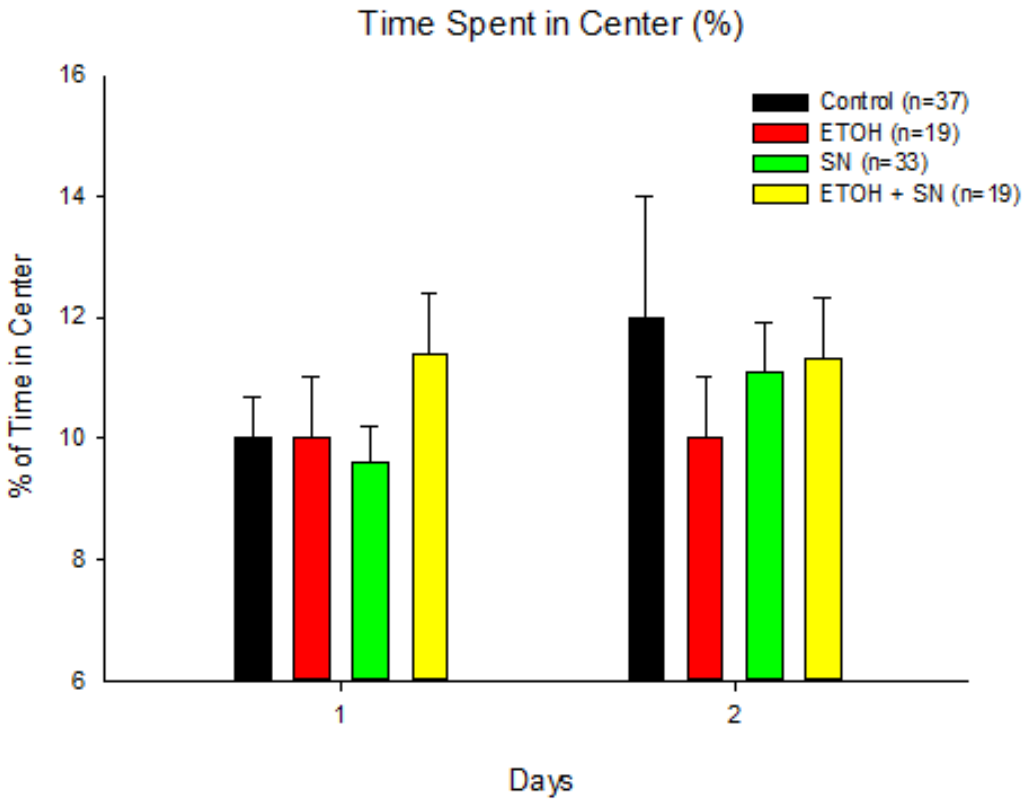

Figure. 3.8: Open-Field performance as measured by percentage of time spent in the center of the open-field for each day. No significant differences were found between groups for either day of testing.

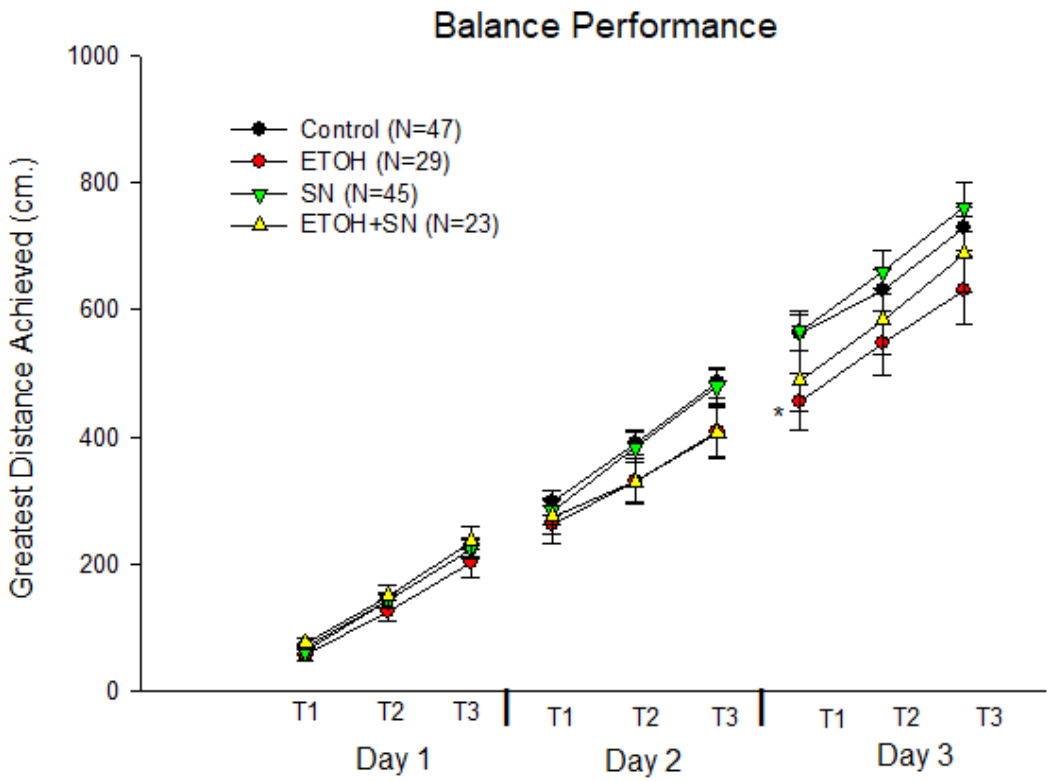

Figure. 3.9: Balance performance as measured by distance achieved. On day 3 trial 1 performance ETOH offspring did not perform as well as Control offspring ($p<.05$). This difference was not seen in ETOH offspring treated with *SN*. *$p<.05$, differs significantly from

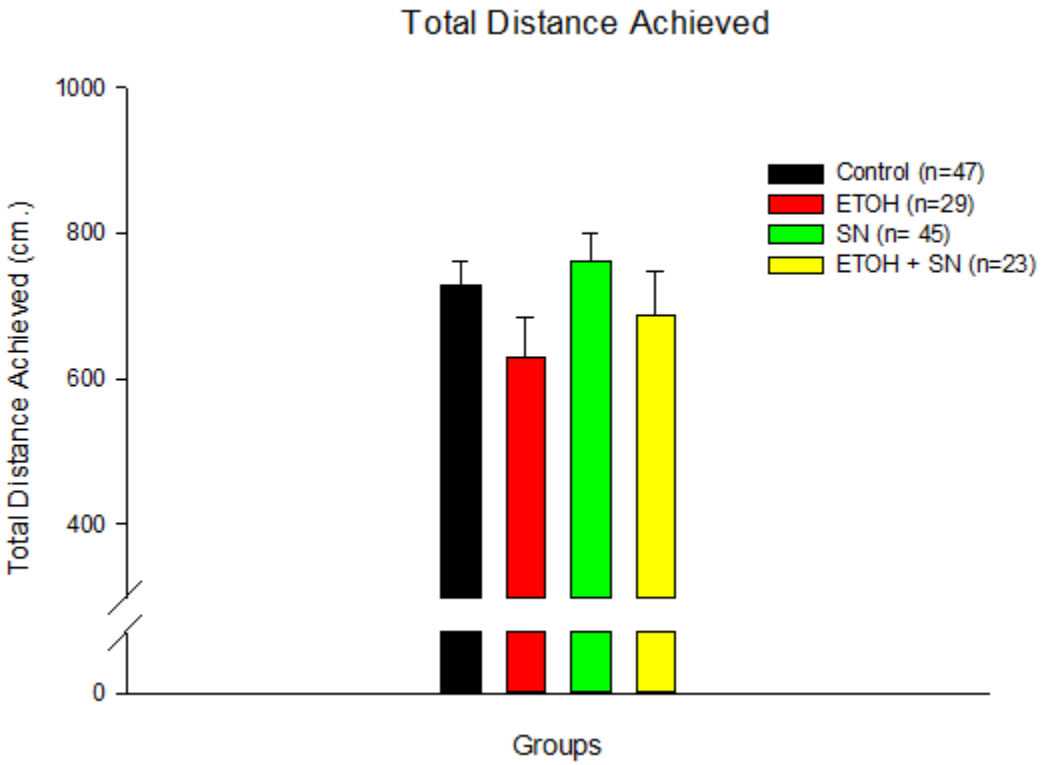

Figure. 3.10: Balance performance as measured by greatest distance traveled. Though not significant, ETOH offspring appeared to have decreased performance compared to controls. A difference not seen in ETOH offspring treated with *SN*.

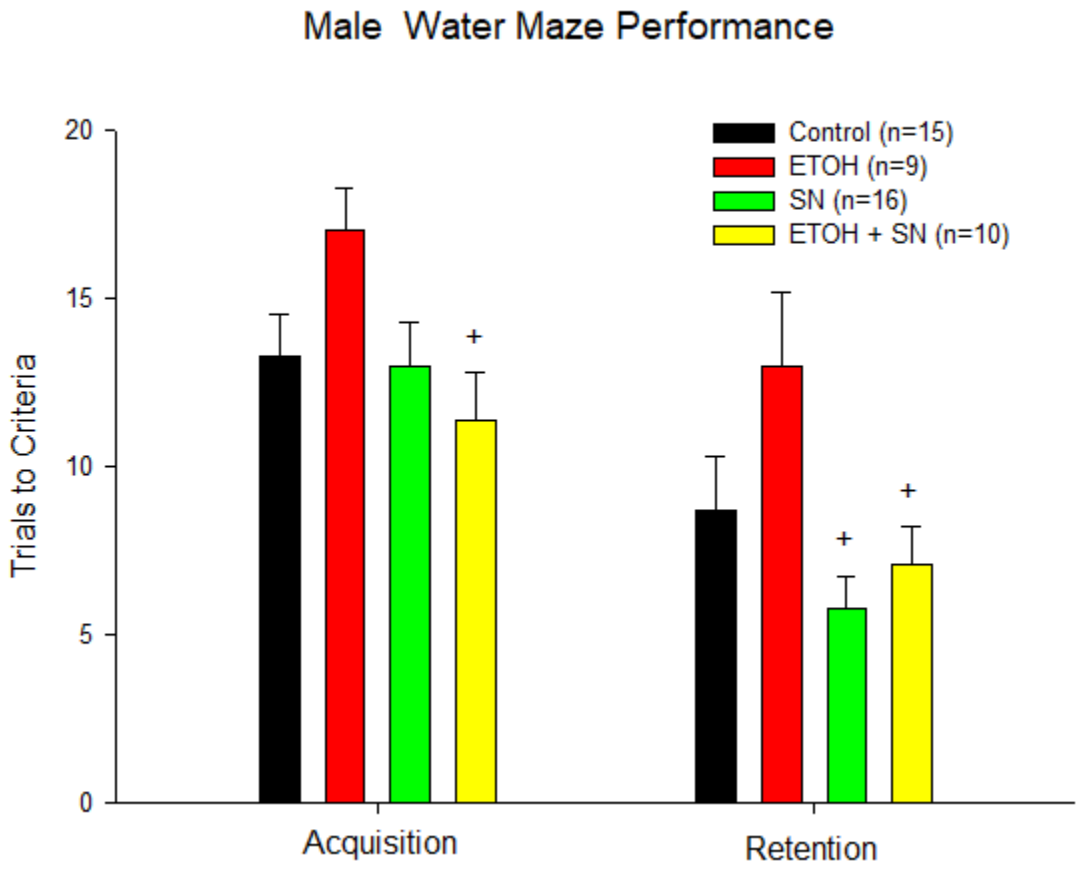

Figure. 3.11: Water Maze performance as measured by trials required to reach criterion. During acquisition and retention ETOH offspring treated with *SN* required significantly fewer trials to reach criterion than did ETOH offspring ($p<.05$). During retention, offspring treated with *SN* alone required significantly less trials to reach criterion than did ETOH offspring. + $p<.05$, differs significantly from ETOH offspring.

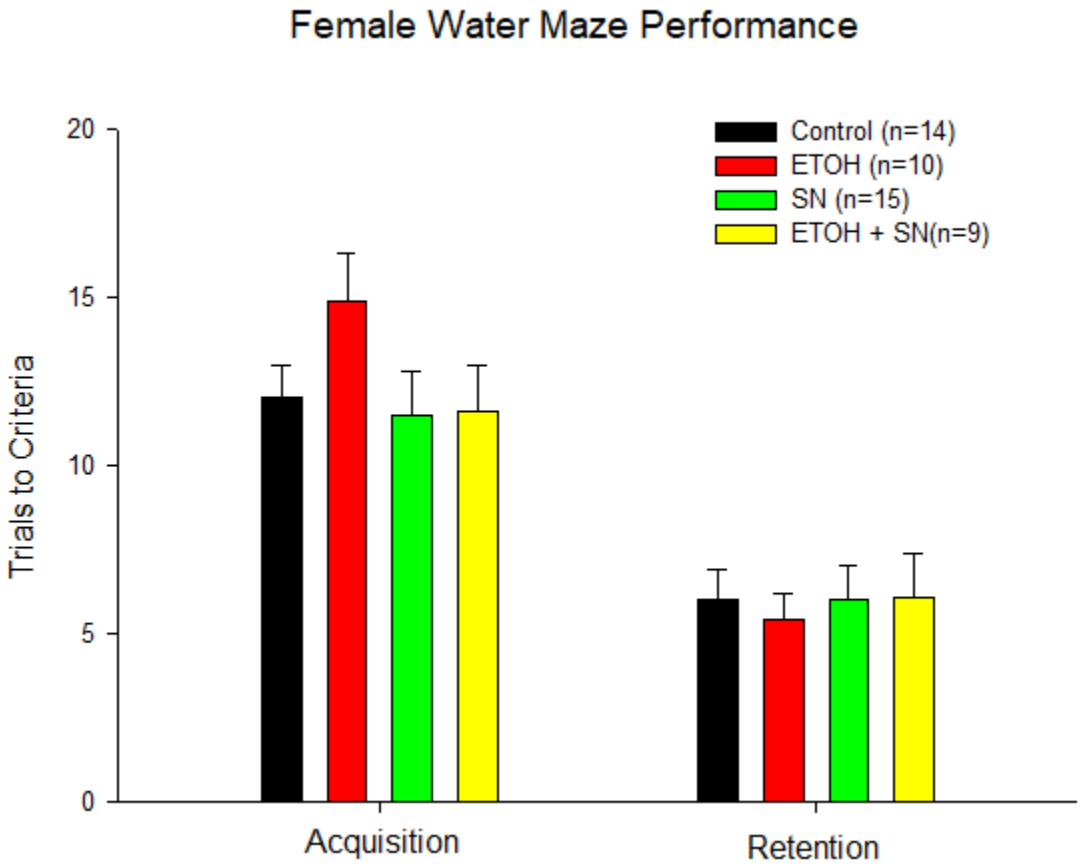

Figure. 3.12: Water Maze performance as measured by trials required to reach criterion. There is a trend for the ETOH exposed females to require more trials to reach criterion than all other groups during acquisition. There were no group differences in performance at the 24 hr retention test.

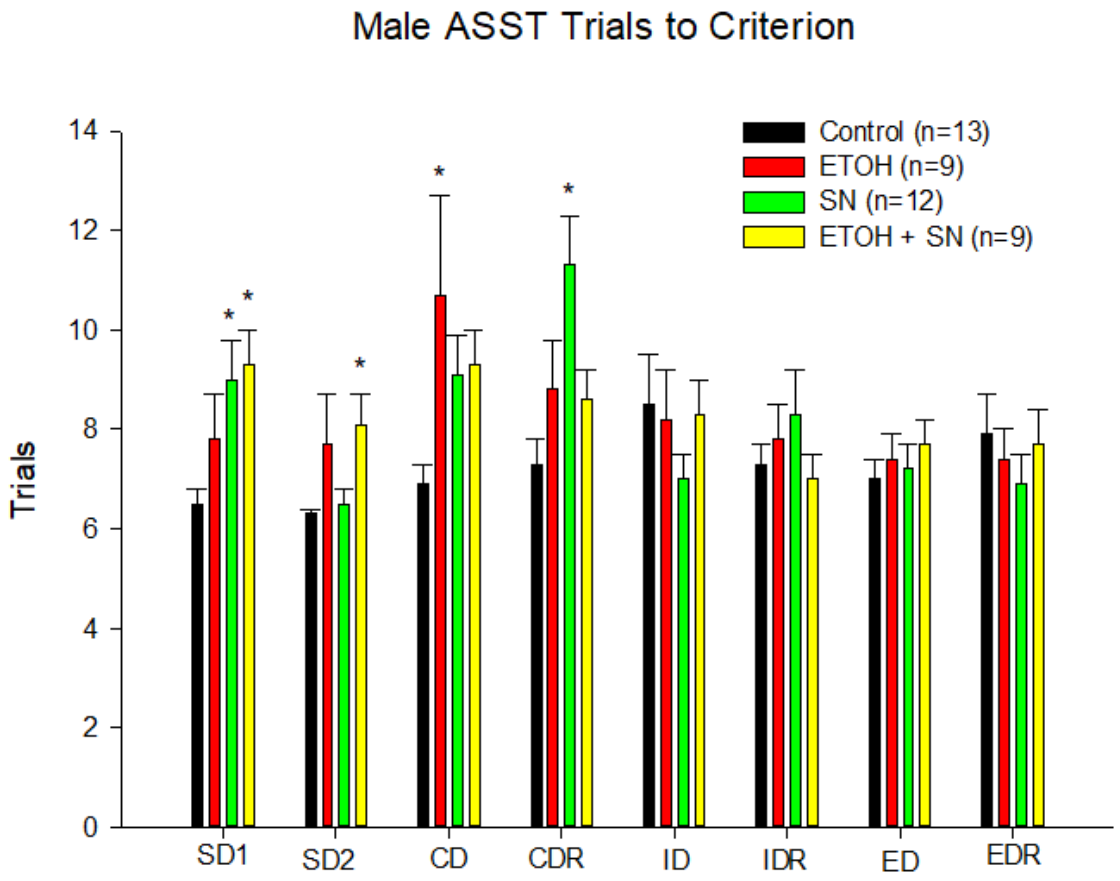

Figure. 3.13: ASST performance as measured by trials required to reach criterion. ETOH offspring required significantly more trials to reach criterion during the CD phase. *SN* offspring required significantly more trials to reach criterion during the SD1 and CDR phases. offspring exposed to both ETOH and *SN* required significantly more trials to reach criterion during the SD1 and SD2 phases. *p<.05, differs significantly from respective control.

Legend: SD1 & SD2 = simple discrimination, CD & CDR = Complex discrimination & reversal, ID & IDR = Intradimensional shift & reversal, ED & EDR = extradimensional shift & reversal.

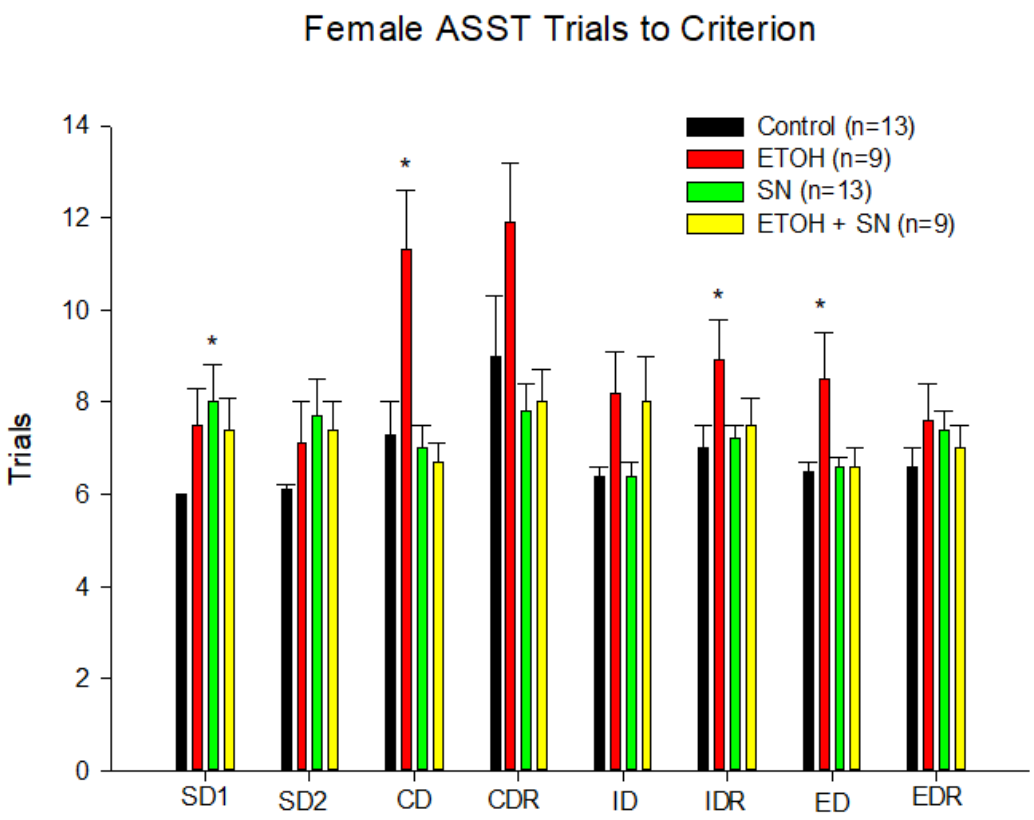

Figure 3.14: ASST performance as measured by trials required to reach criterion. ETOH offspring required significantly more trials to reach criterion during the CD, IDR, and ED phases. *SN* offspring required significantly more trials to reach criterion during the SD1 phase. *p<.05, differs significantly from respective control.

Legend: SD1 & SD2 = simple discrimination, CD & CDR = Complex discrimination & reversal, ID & IDR = Intradimensional shift & reversal, ED & EDR = extradimensional shift & reversal.

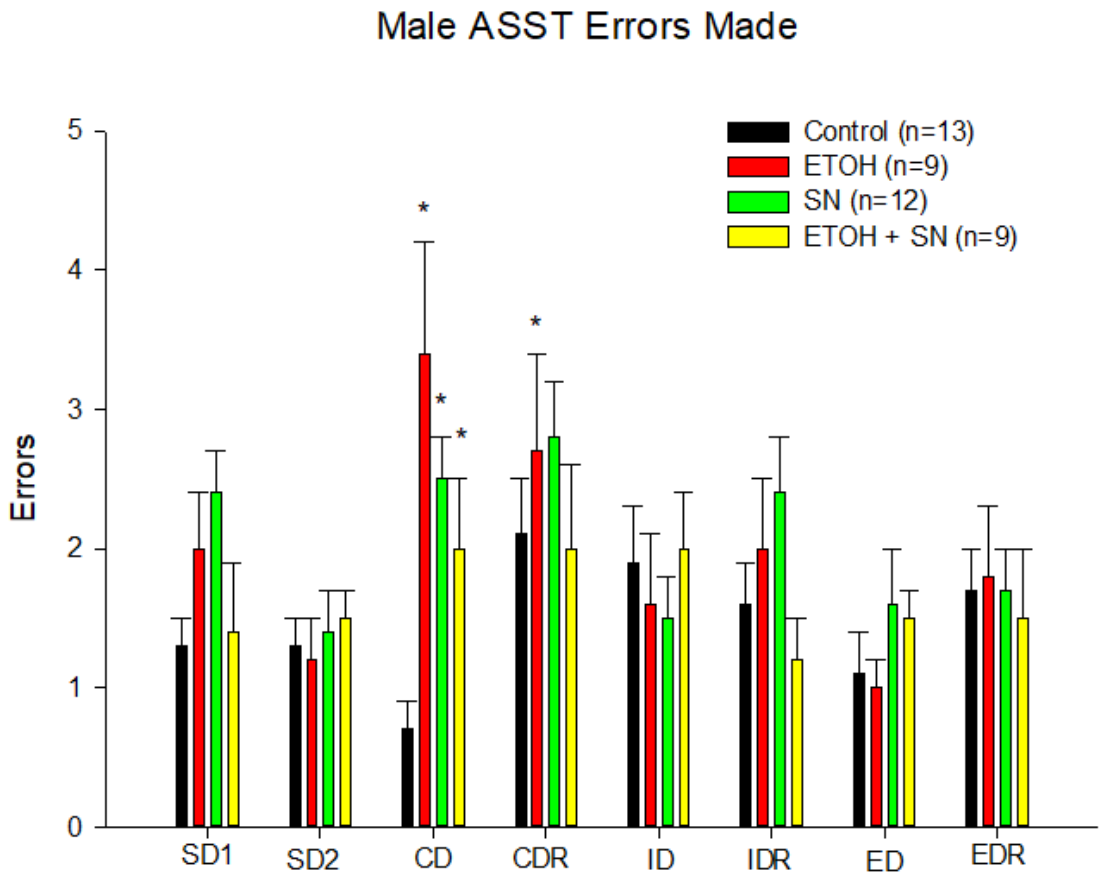

Figure 3.15: ASST performance as measured by errors made. ETOH, *SN*, and ETOH + *SN* offspring made significantly more errors during CD. However only ETOH offspring made significantly more errors during the CDR phase. *p<.05, differs significantly from respective control.

Legend: SD1 & SD2 = simple discrimination, CD & CDR = Complex discrimination & reversal, ID & IDR = Intradimensional shift & reversal, ED & EDR = extradimensional shift & reversal.

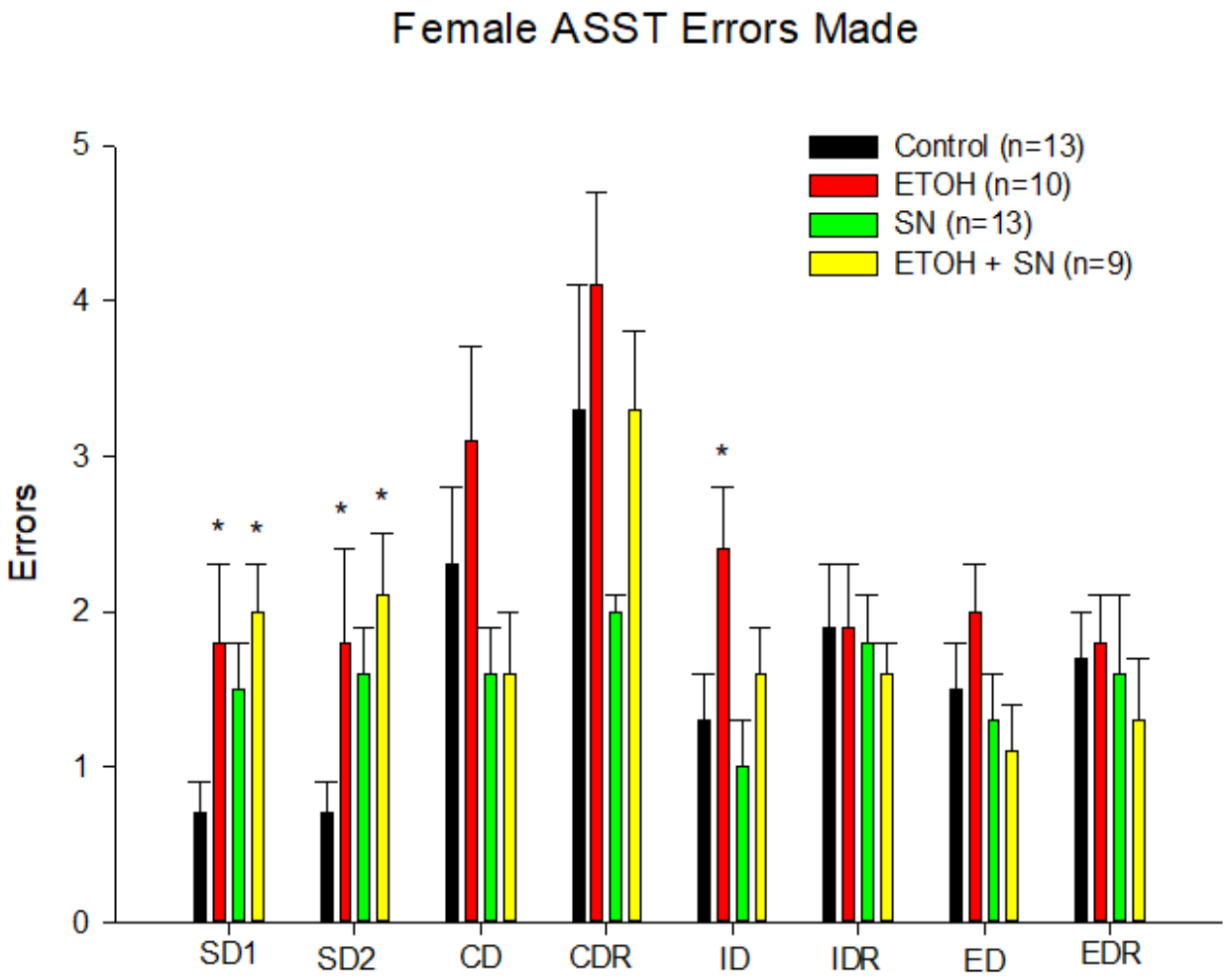

Figure 3.16: ASST performance as measured by errors made. ETOH and ETOH + *SN* offspring made significantly more errors during SD1 and SD2. However only ETOH offspring made significantly more errors during the ID phase. *p<.05, differs significantly from respective control.

Legend: SD1 & SD2 = simple discrimination, CD & CDR = Complex discrimination & reversal, ID & IDR = Intradimensional shift & reversal, ED & EDR = extradimensional shift & reversal.

Chapter 4:

Overall Discussion:

This dissertation focused on the ability of two separate compounds (DMXB-A and SN) that selectively target the α7 nAChR to reduce behavioral deficits associated with "third trimester" ETOH exposure in male and female rodents. Both studies demonstrated that these compounds can provide some form of protection from developmental ETOH exposure in several of the behavioral paradigms investigated. DMXB-A reduced deficits produced by ETOH exposure in motor function in females and learning and memory deficits in males. *SN* reduced deficits produced by ETOH exposure in motor function and executive function in both sexes and learning and memory deficits in males. These results provide further support for the role of activation of the α7 nAChR in reducing the damage associated with developmental ETOH exposure and withdrawal. However, with both compounds, it appeared that their effects were sex specific and that treatment with the compounds alone, or in combination with ETOH, could produce deficits in specific tasks.

The model of developmental ETOH exposure used in the current studies targeted the "brain growth spurt" which co-occurs with the third trimester in humans. An equivalent time of rapid brain growth occurs in rodents just before birth and into the first postnatal weeks (Dobbing & Sands). Targeting this period of rapid development is useful because it is a time where the CNS is particularly sensitive to the teratogenic effects of ETOH exposure (Barron et al., 2016; Goodlett et al., 1997; Riley et al., 1993). ETOH exposure during this period have reliably produced behavioral deficits in motor

coordination (Idrus et al., 2011; Lewis et al., 2007; Thomas et al., 2004), attention/activity (Smith et al., 2012; Thomas et al., 2007; Lewis et al., 2012), learning and memory (Hunt et al., 2009; Thomas et al., 2009; Tiwari et al., 2012), and executive function (Thomas et al., 2000). Along with these deficits in behavior, ETOH exposure during this period has also produced anatomical deficits in regions associated with these behaviors including the cerebellum, hippocampus, and prefrontal cortex (for review see Dobbing & Sands, 1979; Riley & McGee, 2005 & Patten et al., 2014). Currently, no clinically approved medication exists to treat these deficits produced by developmental ETOH exposure and so development of a compound is critical. ETOH withdrawal is believed to mediate a considerable amount of the damage associated with developmental ETOH toxicity by producing neurotoxicity, oxidative stress, and neuroinflammation (Livy et al., 2003, Painter, et al., 2014; Alfosno-Loeches & Guerri, 2011). Development of a compound aimed at reducing the damage associated with EWD during a period of rapid brain growth is appealing because it is potentially a one-time treatment that would protect the fetus or newborn after ETOH has left the system. Previous studies have demonstrated the potential of α7-nAChR agonization to reduce neurotoxicity, neuroinflammation, and oxidative stress (de Jonge & Ulloa, 2007; Prendergast et al., 2000 & 2001; Li et al., 1999; Suzuki et al., 2006; Wang et al., 2003; Xiu et al., 2005; Shytle et al., 2004). Activation of this receptor has also reduced behavioral deficits associated with various neurological disorders and developmental ETOH exposure (Yu et al., 2012; Koukouli & Maskos, 2015; Suzuki et al., 2006, Thomas et al., 2016). The primary hypothesis of the current study was that an injection of DMXB-A or *SN*, 21 hours after the animal's final exposure to ETOH (during a period of withdrawal), would

be able to reduce deficits caused by ETOH exposure. Both compounds used in the current studies have demonstrated potential in reducing the damage associated with developmental ETOH exposure. Data from *in vitro* studies suggest that DMXB-A dose-dependently reduced cell death following ETOH exposure in E17 (embryonic) cells, PC12 (pheochromocytoma) cells, and in adult male rodents (DMXB-A preexposure 24 h prior to ETOH) (de Fiebre et al., 2003; Li et al., 2002; Shimohama et al., 1997; Tizabi et al., 2004). Additionally, rhamnetin, a flavonoid found in *SN*, has demonstrated an ability to reduce both neuroinflammation and excitotoxicity in response to EWD in an organotypic hippocampal slice culture (Lutz, et al., 2015).

First, both DMXB-A and SN demonstrated some ability to reduce deficits in motor coordination after ETOH exposure, though it appeared to be sex dependent. These results were not surprising because many α7-nAChR containing cholinergic neurons innervate key structures related to motor coordination and balance like the cerebellum (Hurst et al., 2013; Koukouli & Maskos 2015), a structure that is also particularly sensitive to the effects of developmental ETOH exposure (Thomas et al., 2004). Thomas and colleagues (2009) also found that choline was able to reduce deficits in motor function produced by prenatal ETOH exposure. They postulated that one of the possible mechanisms that could have produced this effect is α7-nAChR modulation of glutamatergic neurons in the cerebellum preventing excitotoxicity during ETOH exposure and withdrawal. Previous studies have supported a role in α7-nAChR reducing cell damage produced by NMDA-induced toxicity in PC12 cell lines, primary hippocampal cultures and in organotypic hippocampal cultures (Prendergast et al., 2000 & 2001; de Fiebre et al., 2003 & 2005; Yangxin et al., 2002). This activity is thought to

possibly be mediated by activation of the α7 nAChR which is believed to increase calbindin-D28K (a calcium buffer) activity which in turn reduces intercellular calcium and increases cell survival (Mullholland et al., 2003). Another interesting possibility is through cross-desensitization with the NMDAR where activation of the α7 nAChR by donepezil hydrochloride appeared to mediate phosphorylation and subsequent internalization of specific NMDAR receptor subunits preventing excitotoxicity (Kihara et al., 2010). Next, both compounds were able to reduce some deficits in learning and memory. Tasks involving learning and memory are mediated by many structures in the CNS, namely the hippocampus and prefrontal cortex, both of which are sensitive to developmental ETOH exposure (Lebel et al., 2011), and contains α7-nAChR's (Hurst et al., 2013). The reduction of learning deficits in this study are supported by previous research demonstrating postnatal choline supplementation was able to rescue deficits in a spatial navigation task (the Morris Water Maze) after ethanol exposure during the third trimester equivalent in rodents (Thomas et al., 2016). DMXB-A has also previously demonstrated an ability to rescue spatial navigation deficits in the Morris task in rodents who had bilateral lesions of the Nucleus Basalis (a structure rich in cholinergic neurons) (Meyer et al., 1997). Additionally, DMXB-A was also shown to be neuroprotective against global ischemia and glutamate-induced excitotoxicity in rodents while also improving cognitive function on a passive avoidance task (Nanri et al., 1998). In this study, Nanri and colleagues theorized that this protection against cellular damage and cognitive deficits was provided by the α7 nAChR's reduction of excitotoxicity and neuroinflammation. Though they did not give supporting details about this mechanism, however, activation of the α7 nAChR has been implicated in reducing these mechanisms

of damage (excitotoxicity discussed above). In neuroinflammation, activation of α7 nAChR's found on microglia, have been shown to reduce microglial activation and reduce LPS-induced TNF-alpha release (Suzuki et al., 2006; Shytle et al., 2004). Activation of the α7 nAChR has also been shown to reduced LPS-induced TNF-alpha release in an *in-vitro* model of EWD in the hippocampus of neonatal rodents (Lutz et al., 2015 a & b). It is believed that activation of the receptor creates a neuroprotective state in the microglia and in turn reduces transcription and phosphorylation of critical components required for activation of an inflammatory response like nuclear factor kappa-b, and mitogen-activated protein kinase (see Lutz, 2015 & Suzuki et al., 2006 for review). Finally, *SN* demonstrated an ability to reduce some of the deficits found in executive function produced by ETOH administration. Performance of this task appears to be largely dependent on the proper functioning of the prefrontal cortex (Birrell & Brown et al., 2000; Barnese et al., 2002), a structure that contains many α7-nAChR (Hurst et al., 2013) and is particularly sensitive to developmental ETOH exposure (Barron et al., 2017; Goodlett et al., 1997; Riley et al., 1993), especially during the third trimester (Ikonomidou et al., 2000). These deficits are thought to arise from aberrant connectivity from the PFC to other structures critical for the performance of set-shifting like the hippocampus (Gorsky & Klinstova 2017). Previous studies using a similar task have shown that administration of an α7-nAChR agonist can both reduce deficits in an animal model of schizophrenia and improve cognitive flexibility in control animals (Wood, et al., 2016). Recently, choline supplementation after prenatal ETOH exposure improved deficits in a set-shifting task very similar to the task used in the current study (Waddell et al., 2017). This study stipulated that one of the possible mechanisms of

protection provided by choline could possibly be a reduction of NMDA-induced excitotoxicity by activation of the α7-nAChR. Again, as discussed above, activation of the α7-nAChR leading to decreases in excitotoxicity has been supported by previous literature. Though the exact mechanisms of protection provided by the α7-nAChR is not well understood, it is clear that this receptor is involved in the modulation of and subsequent neuroprotection against processes related to excitotoxicity, oxidative stress, and neuroinflammation (for further review see Lutz et al., 2015 & Benchrief et al., 2014).

Though the two compounds demonstrated potential in reducing deficits associated with developmental ETOH exposure, there were also several unexpected findings. One unexpected finding was during the assessment for hyperactivity and impulse control in the Open-Field chamber. Unexpectedly, there was a lack of ETOH response in the open-field paradigm for both studies. As mentioned previously, this task was used because like humans, rodents who are exposed to ETOH during development commonly demonstrate hyperactivity (for review see Mattson et al., 2011 & Patten et al., 2014). Previous studies in this lab using the same model of ETOH exposure and the same task for assessing activity levels have reliably found increased activity in offspring exposed to ETOH both in overall activity, activity in the center, and entries into the center (Lewis et al., 2011, Smith et al., 2012). This hyperactivity in humans and animals, expressed as increased path length and entries into the center in the current study, is believed to be caused in part by an inability to inhibit responses that may not be appropriate to the situation (Riley et al., 2011; Thomas et al., 2009). To explore potential explanations, we considered our dose of ETOH. We administer a total of 6 g/kg/day of ETOH (3g/kg twice daily) by oral gavage from postnatal days (PND) 1-7. BAC's for this dose of ETOH peak at 220 mg/dl

(+/- 6mg/dl) approximately 60 minutes after intubation (Lewis et al., 2011). This level of ETOH exposure has been efficacious in producing behavioral deficits in the open-field task, dowel balance rod, and water maze (Lewis et al., 2007 & 2011, Smith et al., 2012). We also compared control animal activity in the current study to previous studies in this lab. Controls had approximately the same level of activity in both studies (20000 cm +/- 100)(Smith et al., 2012). It is also possible that these animals in the current study were especially hardy to the effects of developmental ETOH exposure in our model, however, this is doubtful due to the homogenous nature of animals used in this study (Sprague Dawley), the fact that no litter or outlier effects were detected, and the fact that we have reliably found activity deficits in these animals in the past (see above). Future investigations in this lab using this exposure model with the open-field task should be completed to determine a possible cause for this difference because the use of this task is invaluable in determining the efficacy of compounds aimed at reducing the effects of developmental ETOH exposure

An additional unexpected finding was that both DMXB-A and *SN* appeared to have variable effects on performance in several of the behavioral studies. DMXB-A, when combined with ETOH, in the open-field task appeared to reduce overall activity but increase activity in the center of the apparatus. Though DMXB-A's effects on this task have not been previously investigated in this or any model of ethanol exposure, these results were unexpected. As discussed previously, choline, a compound believed to activate the α7-nAChR, in combination with ethanol exposure both pre and postnatally have reliably produced control levels of activity, while ETOH alone resulted in increased activity (Thomas et al., 2004 & 2007). This behavior is believed to be mediated in part

by the basal forebrain and hippocampus because lesions of these areas have produced increases in activity level (for discussion see Thomas et al., 2004). These areas are also innervated by nAChR's, including the α7-nAChR's. Thomas and colleagues stipulated that the reduction of activity level produced by choline in reaction to developmental ETOH exposure could possibly be due to activation of the α7-nAChR and its subsequent decrease in NMDA-mediated toxicity (Thomas et al., 2004, and discussed above). It may be possible that DMXB-A does not provide this type of protection for this specific task in response to developmental ETOH exposure, a possibility that has not been explored in the literature. But because there is support for the α7-nAChR's protection of this and other tasks against developmental ETOH exposure combined with the fact that we did not see the usual ETOH mediated increase in activity in this task leads to the conclusion that this result may not be meaningful. However, further work is necessary to address this potential issue. DMXB-A in combination with ETOH also appeared to reduce balance performance in male animals, a finding that was also unexpected. Again, previous literature supports the role of the α7-nAChR in reducing motor function deficits in this task (Thomas et al., 2004 & 2009). Also, the combination of DMXB-A and ETOH was able to rescue deficits in this task in females in the current study. Interestingly, there were no sex differences in any of the groups in Thomas and colleagues (2004) investigation of balance deficits produced by third trimester ETOH and subsequent rescue of this deficit by choline. Our own lab has found sex differences in performance between male and female animals on this task where females generally performed better than male subjects, which they believed was produced by differences in body weights (Lewis et al., 2007). However, in the current study, though body weights did differ between the sexes for each

treatment group, there was no meaningful difference in performance between males and females in control groups or those treated with DMXB-A alone. Again, it may be possible that DMXB-A does not provide this type of protection for this specific task in response to developmental ETOH exposure in males, a possibility that has not been explored in the literature. Though there is support for the α7-nAChR's protection of this and other tasks against developmental ETOH exposure, it is clear that this possibility of sex specific effects of treatment with DMXB-A in response to developmental ETOH exposure should be further investigated. Finally, *SN* treatment alone and in combination with ETOH produced increases in the amount of trials required to reach criterion and errors made in several of the phases in the ASST paradigm. Previous studies using a similar task have shown that administration of an α7-nAChR agonist (a novel compound SSRI180711 [compound-A]) can both reduce deficits in an animal model of schizophrenia and improve cognitive flexibility in control animals (Wood, et al., 2016). Recently, choline supplementation after prenatal ETOH exposure improved deficits in a set-shifting task very similar to the task used in the current study (Waddell et al., 2017). However, neither of these studies demonstrated any deficits produced by treatment with the α7-nAChR itself, in fact both studies supported the role of these compounds to increase performance in certain phases when administered alone. Also, Waddell did not find that administration of choline after developmental ETOH exposure increased deficits in any of the phases of ASST, instead it only decreased it. However, this is the first time this compound has been investigated for its effects to reduce developmental ETOH exposure in this task. So, these deficits caused by SN by itself and in combination with ETOH may be meaningful which indicates that this possible interaction should be

investigated further because previous literature does not support the role of α7-nAChR activating compounds reducing performance in this task either by itself or in combination with ETOH.

Another somewhat unexpected finding in several of the studies was that there appeared to be a difference in the way males and females responded to both ETOH administration and treatment with either compound. As previously mentioned, during the DMXB-A study, males appeared to have reduced activity in response to ETOH treatment as well as the combination of ETOH and DMXB-A. This result was unexpected because ETOH exposure reliably produces increases in activity but not in a sex specific way (Lewis et al., 2011; Smith et al., 2012). Also previous studies using a compound that is believed to target the α7-nAChR (choline) found that the combination of this drug with ETOH resulted in activity being at control levels in both males and females (Thomas et al., 2004 & 2009). As discussed above, this behavior is believed to be mediated in part by the basal forebrain and hippocampus because lesions of these areas have produced increases in activity level (for discussion see Thomas et al., 2004). These areas are also innervated by nAChR's, including the α7-nAChR's (Hurst et al., 2013). The mechanism of protection provided by activation of the α7-nAChR in this task was theorized to be from its reduction in glutamate-induced neurotoxicity (Thomas et al., 2004). Previous studies have supported the theory that α7-nAChR activation can reduce glutamate toxicity and neuroinflammation in response to ETOH exposure in both male and female organotypic hippocampal explants, but this mechanism did not appear to be sex specific (Lutz et al., 2015). However, this was the first time DMXB-A was investigated for its ability to reduce activity in response to developmental ETOH exposure. There could

potentially be an unexplored sex-specific effect produced by this compound in combination with ETOH in this model that clearly needs further investigation. Additional differences were found in males exposed to ETOH and treated with DMXB-A where they had reduced performance on the dowel task, a result not found in females (however this difference was discussed at length in the previous section). Another interesting sex effect was for both the DMXB-A study and *SN* study females in the water maze task exposed to ETOH performed at control levels, while males exposed to ETOH required more trials to reach criterion. For the SN study, males and females differed in water maze performance where males, like in the DMXB-A study, treated with ETOH required more trials to reach criterion. Studies in this lab using the same methodology and exposure model have not previously found sex differences in this task (Lewis et al., 2007 & 2011; Smith et al., 2012). Other labs investigating developmental ETOH's effects on similar tasks have also not found sex specific differences in response to ETOH exposure (Thomas et al., 2004, 2007 & 2009; Riley et al., 2005). However, Goodlett & Peterson (1995) suggested females performance on spatial navigation tasks (in their case, the morris water maze) may be less sensitive to ETOH exposure than males which does support the finding in both studies of this dissertation. Additional studies exploring prenatal ETOH exposure in wistar rats (Hamilton et al., 2014; Savage et al., 2010) and long-evans rats (Hannigan et al., 1987) have also found that males are typically more affected than females in navigation tasks. Interestingly, in none of the above studies were the causes for this difference explained. As mentioned previously, this task is believed to be mediated largely by the hippocampus and prefrontal cortex (Lebel et al., 2011) and these structures are particularly sensitive to developmental ETOH administration (Schneider et al., 2011

& Gursky & Klinstova 2017). Waddell and colleagues (2017) found that males were more sensitive to deficits in performance on an executive function task that involves the use of the prefrontal cortex after prenatal ETOH exposure than females. They postulated this was because prenatal ETOH appears to disrupt engagement of the frontal cortices in males more than females which could lead to deficits in executive functioning tasks as well as learning and memory tasks. With this, it is clear further work needs to be conducted to explore the possible difference between male and females in reaction to developmental ETOH exposure. Another interesting finding that is contradictory to the evidence discussed above is that in the ASST task, females appeared to be more sensitive to the effects of ETOH than males were. Again, in the study by Waddell & Mooney (2016), they found that males were more sensitive to ETOH's effects in the ASST than females. Perhaps this difference could be because of the timing of developmental ETOH exposure (prenatal for the Waddell study and neonatal for the current), however Gursky & Klinstova (2017) found that males and females were similarly deficient in executive tasks after third trimester ETOH exposure. It is clear that there are differences between males and females in response to ETOH for several of these tasks, and future work in this lab or others should investigate these differences further to elucidate the potential explanation and mechanism for these differences.

It appears that both DMXB-A and *SN* were able to reduce some of the deficits associated with developmental ETOH exposure. However, as discussed throughout this discussion there are many things that we do not yet have a clear answer for where future investigations in this lab and others who want to understand the role of the α7 nAChR in developmental ETOH exposure should address. For one our compounds at the current

dose had variable effects on performance alone and when combined with ETOH. These effects appeared to be dependent on sex in several of the tasks. Future studies should investigate these compounds effects on microscopic and macroscopic brain structures in male and female animals exposed to ETOH during different developmental timepoints to see if they produce varied effects in structure or regions associated with behavioral deficits demonstrated in the current study like the prefrontal cortex, hippocampus, and cerebellum. This could help determine if their effects are varied by region and what role sex potentially plays in these differences. As mentioned before, these compounds have been investigated for their effects on ETOH exposure and withdrawal *in-vitro* (de Fiebre et al., 2003; Li et al., 2002; Shimohama et al., 1997; Tizabi et al., 2004; Lutz et al., 2015) and found that these compounds can increase cell survival produced by various forms of damage including neurotoxicity, neuroinflammation, and oxidative stress. However, these studies are typically conducted on specific cell lines, or in explants of specific regions of the brain like the hippocampus. It would benefit this and other studies using these compounds to understand how these specific compounds (or other selective α7 nAChR agonists) effect neuronal development in an intact nervous system exposed to ETOH during development. With these types of findings, we could then try to correlate our findings in these behavioral studies with changes in the intact brain of male and female animals exposed to ETOH during the same developmental period. Future studies may also benefit from further investigations into the exact mechanisms in which the α7 nAChR receptor produces neuroprotection. As mentioned previously, activation of the α7 nAChR's found on microglia, when activated, have been shown to reduce microglial activation and reduce LPS-induced TNF-alpha release (Suzuki et al., 2006; Shytle et al.,

2004). Activation of the α7 nAChR has also been shown to reduced LPS-induced TNF-alpha release in an *in-vitro* model of EWD in the hippocampus of neonatal rodents (Lutz et al., 2015 a & b). This process is believed to occur because the α7 nAChR receptor creates a neuroprotective state in the microglia and in turn reduces transcription and phosphorylation of critical components required for activation of an inflammatory response like nuclear factor kappa-b, and mitogen-activated protein kinase (see Lutz, 2015 & Suzuki et al., 2006 for review). Modulation of this receptor has also been associated with reductions in NMDA-induced toxicity (Dajas-Bailador 2000; Lutz et al., 2015; Prendergast et al., 2000 & 2001; de Fiebre et al., 2003 & 2005; Yangxin et al., 2002). This is thought to occur beacause activation of the α7 nAChR increases calbindin-D28K (a calcium buffer) activity which in turn reduces intercellular calcium and increases cell survival (Mullholland et al., 2003). This could also be from activation of the α7 nAChR resulting in phosphorylation and subsequent internalization of specific NMDAR receptor subunits preventing excitotoxicity (Kihara et al., 2010). However, these mechanisms are just theories and a clear answer is not available. What is clear is that the protection provided by this receptor is varied and involves many processes. Discovering the particular mechanisms that modulate this receptors protection could help explain some of the current findings, but more importantly help direct future investigation and possibly medication development aimed at reducing the effects of developmental ETOH exposure and other neurological disorders.

Taken together it appears that both DMXB-A and *SN* can reduce some behavioral deficits associated with "3rd trimester" ETOH exposure in rodents. This project lends support to the theory that compounds that act as agonists at the nicotinic receptor system,

specifically the α7 nAChR, is a promising route to reducing or eliminating ETOH's adverse effects on developing offspring. It also provides justification to further explore these or other compounds that target the α7 nAChR in other models of ETOH exposure and CNS disease/disorder as well as possibly lead to clinical development of a pharmacotherapy to treat FAS/FASD.

Reference List:

Abreu-Villaca, Y., Filgueiras, C., Manhaes, A. (2011). Developmental Aspects of the Cholinergic System. Behavioral Brain Research. 221:367

Alfonso-Loeches S., Pascual-Lucas M., Blanco A.M., Sanchez-Vera I., Guerri C. Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. J Neurosci 2010; 30: 8285-95.

Ambadath V., Venu R.G., Madambath I. Comparative study of the efficacy of ascorbic acid, quercetin, and thiamine for reversing ethanol-induced toxicity. J Med Food 2010; 13: 1485-9.

Archibald, S. L., Fennema-Notestine, C., Gamst, A., Riley, E. P., Mattson, S. N., & Jernigan, T.L. (2001). Brain dysmorphology in individuals with severe prenatal alcohol exposure. Developmental Medicine and Child Neurology, 43(3), 148–154

Atluri P, Fleck MW, Shen Q, Mah SJ, Stadfelt D, Barnes W, et al. Functional nicotinic acetylcholine receptor expression in stem and progenitor cells of the early embryonic mouse cerebral cortex. Dev Biol 2001;240:143–56.

Bannerman, D. M., Rawlins, J. N. P., McHugh, S. B., Deacon, R. M. J., Yee, B. K., Bast, T., ... & Feldon, J. (2004). Regional dissociations within the hippocampus—memory and anxiety. Neuroscience & Biobehavioral Reviews, 28(3), 273-283.

Barnese, M., Fox, M., Baxter, M. (2002). Aged rats are impaired on an attentional set-shifting taks sensitive to medial frontal cortex damage in young rats. Research

Barron, S., Mulholland, P. J., Littleton, J. M., & Prendergast, M. A. (2008). Age and gender differences in response to neonatal ethanol withdrawal and polyamine challenge in organotypic hippocampal cultures. [Comparative Study Research Support, N.I.H., Extramural]. *Alcohol Clin Exp Res, 32*(6), 929-936. doi: 10.1111/j.1530-0277.2008.00649.x

Barron S, Hawkey A, Fields L, Littleton JM. Chapter Thirteen- Animal Models for Medication Development and Application to Treat Fetal Alcohol Effects. 2016, International Review of Neurobiology. 126; 423-440.

Benchrerif, M. (2014). Neuronal nicotinic receptors as novel targets for inflammation and neuroprotection: mechanistic considerations and clinical relevance. Acta Pharma (6):702-714.

Berman, R. F. & Hannigan, J. H. (2000). Effects of Prenatal Alcohol Exposure on the Hippocampus: Spatial Behavior, Electrophysiology, and Neuroanatomy. *Hippocampus*, 10:94-110.

Birrell, M.; Brown, V.J. Medial frontal cortex mediates perceptual attentional set shifting in the rat. J. Neurosci. 2000, 20, 4320–4324. [PubMed]

Bishop, S., Gahagan, S., Lord, C. (2007). Re-examining the Core Features of Autism: A Comparison of Autism Spectrum Disorder and Fetal Alcohol Spectrum Disorder. *Journal of Child Psychology and Psychiatry*; 48:11, 1111-1121.

Bondy S.C. Ethanol toxicity and oxidative stress. Toxicol Lett 1992; 63: 231-41.

Briggs, C., ….. & Arneric, S. (1997). Functional Characterization of the Novel Neuronal Nicotinic Acetylcholine Receptor Ligand GTS-21 In Vitro and *In-Vivo*. Pharmacology Biochemistry and Behavior, 57(1):231-241.

Burden, M. J., Jacobson, S. W., Sokol, R. J., & Jacobson, J. L. (2005). Effects of prenatal alcohol exposure on attention and working memory at 7.5 years of age. Alcoholism, Clinical and Experimental Research, 29(3), 443–452.

Cantacorps L, Alfonso-Loeches S, Moscoso-Castro M, Cuitavi J, Garcia-Rubio I, Lopex-Arnau R, Escubedo E, Guerri C, Valverde O. Maternal Alcohol Binge Drinking Induces Persistent Neuroinflammation Associated with Myelin Damage and Behavioral Dysfunctions in Offspring Mice. 2017, Neuropharmacology, 368-384.

Callahan, P., Terry, A., Tehim, A. Effects of the nicotinic α7 receptor partial agonist GTS-21 on NMDA-glutamatergic receptor related deficits in sensorimotor gating and recognition memory in rats, Psychopharmacology, 231:3695-3706.

Cantacorps, L. Alfonso-Loeches, S…., Valverde, O. (2017). Maternal Alcohol Binge Drinking Induces Persistent Neuroinflammation Associated with Myelin Damage and Behavioral Dysfunctions in Offpspring Mice. Neuropharmacology:123. 368-384.

Centers for Disease Control Prevention (2015). Alcohol Use and Binge Drinking Among Women of Childbearing Age-United States, 2008-2015. *Morbidity and Mortality Weekly Report*, 61:534-538

Chen, L., Wang, H., Zhang, Z., Li, Z.,….& Chen, L. (2010). DMXB (GTS-21 Amelorates the Cognitive Deficits in Beta Amyloid Injected Mice through Preventing the Dysfunction of Alpha 7 Nicotinic Receptor. *Journal of Neuroscience Research*. 88, 1784-1794.

Cherpitel CJ. Alcohol and injuries: a review of international emergency room studies since 1995. Drug Alcohol Rev. 2007;26:201–14.

Climent E, Pascual M, Renau-Piqueras J, Guerri C. Ethanol exposure enhances cell death in the developing cerebral cortex: role of brain-derived neurotrophic factor and its signaling pathways. J Neurosci Res 2002;68:213-225.

Cohen-Keren M, Koren G (2003). Antioxidants and fetal protection against ethanol teratogenicity. I. Review of the experimental data and implication to humans. Neurotoxicol Teratol 25: 1–9

Coles, C. D., Goldstein, F. C., Lynch, M. E., Chen, X., Kable, J. A., Johnson, K. C., et al. (2011). Memory and brain volume in adults prenatally exposed to alcohol. Brain & Cognition, 75(1), 67–77.

Costa ET, Savage DD, Valenzuela CF. A review of the effects of prenatal or early postnatal ethanol exposure on brain ligand-gated ion channels. Alcohol Clin Exp Res 2000;24:706–715.

Dajas F., Andres A.C., Florencia A., Carolina E., Felicia R.M. Neuroprotective actions of flavones and flavonols: mechanisms and relationship to flavonoid structural features. Cent Nerv Syst Agents Med Chem 2013; 13: 30-5.

Dajas-Bailador, F.A., P.A. Lima & S. Wonnacott. 2000. The alpha7 nicotinic acetylcholine receptor subtype mediates nicotine protection against NMDA excitotoxicity in primary hippocampal cultures through a Ca(2+) dependent mechanism.*Neuropharmacology* **39**: 2799–2807

Dani, J. A. (2001). Overview of Nicotinic Receptors and Their Roles in the Central Nervous System. *Society of Biological Psychiatry*, 49, 166-174.

Deacon, R. (2013). Measuring Motor Coordination in Mice. Journal of Visualized Experiments. (75)2609.

de Fiebre, N. C., & de Fiebre, C. M. (2003). α7 Nicotinic Acetylcholine Receptor-Mediated Protection Against Ethanol-Induced Neurotoxicity. *Alcohol*, 31, 149-153.

de Fiebre, N. C., & de Fiebre C. M. (2005). α7 Nicotinic Acetylcholine Receptor Knockout Selectively Enhances Ethanol-, but not B-Amyloid-Induced Neurotoxicity. *Neuroscience Letters*, 373, 42-47.

de Jonge, W. J., Ulloa, L. (2007). The Alpha7 Nicotinic Acetylcholine receptor as a pharmacological target for inflammation. *British Journal of Pharmacology*. 151, 915-929.

Deneberg, V. (1969). Open-Field Behavior in the Rat: What Does it Mean? Annals of the New York Academy of Sciences. 159: 852-859.

Dineley, K., Pandya, A., Yakel, J., (2015). Nicotinic ACh Receptors as Theraputic Targets in CNS Disorders. Trends Pharm Sci. 36(2):96-108

Dobbing, J., & Sands, J. (1979). Comparative aspects of the brain growth spurt. *Early Hum Dev, 3*(1), 79-83.

Ehrhart, F., Roozen, S.,…..Curfs, L. (2018). Review and Gap Analysis: Molecular Pathways Leading to Detal Alcohol Spectrum Disorders. Molecular Psychiatry. 1-8.

Ferchmin, P.A., D. Perez, V.A. Eterovic & J. de Vellis. (2003). Nicotinic receptors differentially regulate N-methyl-D-aspartate damage in acute hippocampal slices. *J. Pharmacol. Exp. Ther.* **305**: 1071–1078.

Freedman, R. (2014). α7-Nicotinic Acetylcholine Receptor Agonists for Cognitive Enhancement in Schizophrenia. *The Annual Review of Medicine*. 65:245-261.

Fryer, S. L., Schweinsburg, B. C., Bjorkquist, O. A., Frank, L. R., Mattson, S. N., Spadoni, A. D., & Riley, E. P. (2009). Characterization of white matter microstructure in fetal alcohol spectrum disorders. [Research Support, N.I.H., Extramural]. *Alcohol Clin Exp Res, 33*(3), 514-521. doi: 10.1111/j.1530-0277.2008.00864.x

Fryer SL, T.S., Mattson SN, Paulus MP, Spadoni AD, Riley EP. Prenatal alcohol exposure
affects frontal-striatal BOLD response during inhibitory control. Alcohol Clin Exp Res. 2007;31:1415–24.

Gonzalez R., Ballester I., Lopez-Posadas R., Suarez M.D., Zarzuelo A., Martinez-Augustin O., Sanchez de Medina F. Effects of flavonoids and other polyphenols on inflammation. Crit Rev Food Sci Nutr 2011; 51: 331-62. 155.

Goodlett, C. R., Peterson, S. D. (1995). Sex Differences in Vulnerability to Developmental Spatial Learning Deficits Induced by Limited Binge Alcohol Exposure in Neonatal Rats. *Neurobiology of Learning and Memory*, 64;265-275.

Goodlett, C. R., Johnson, T., B. (1997). Neonatal Binge Ethanol Exposure Using Intubation: Timing and Dose Effects on Place Learning. *Neurotoxicology & Teratology*, 19(6);435-446.

Goodlett, C. R., Horn, K. H. (2001). Mechanisms of Alcohol-Induced Damage to the Developing Nervous System. *Alcohol Research and Health*, 25(3):175-184.

Goodlett, C. R., Horn, K. H., Zhou, F. C. (2005). Alcohol Teratogenesis: Mechanisms of Damage and Strategies for Intervention. Experimental Biological Medicine. (230,6): 394-406.

Goulart, S., Izabel, M….Frode, T., (2007). Anit-inflammatory Evaluation of Solidago chilensis Meyen in a Murine Model of Plurisy. Ethno-pharmacology 113.

Covernton PJO, Connolly JG. Differential modulation of rat neuronal nicotinic receptor subtypes
by acute application of ethanol. Brit J Pharmacol. 1997; 122(8):1661–1668. [PubMed: 9422812]

Green, C. R., Mihic, A. M., Nikkel, S. M., Stade, B. C., Rasmussen, C., Munoz, D. P., et al. (2009). Executive function deficits in children with fetal alcohol spectrum disorders (FASD) measured using the Cambridge Neuropsychological Tests Automated Battery (CANTAB). Journal of Child Psychology and Psychiatry, 50(6), 688–697

Guizzetti M, Zhang X, Goeke C, Gavin DP. Glia and Neurodevelopment: Focus on Fetal Alcohol Spectrum Disorders. 2014, Frontiers in Pediatrics, 2, 123: 1-12

Gursky, Z. & Klinstova, A. (2017). Frontal Lobe Dysfunction After Developmental Alcohol Exposure: Implications From Animal Models. Addictive Substances and Neurological Diseases 139-147

Hejmadi, M. V., Dajas-Bailador, F., Barns, S. M., Jones, B., & Wonnacott, S. (2003). Neuroprotection by Nicotine against Hypoxia-Induced Apoptosis in Cortical Cultures Involves Activation of Multiple Nicotinic Acetylcholine Receptor Subtypes. *Molecular and Cellular Neuroscience*, 24, 779-786. Doi:10.1016/S1044-7431(03)00244-6.

Hendricson AW, Maldve RE, Salinas AG, Theile JW, Zhang TA, Diaz LM, Morrisett RA.
Aberrant synaptic activation of N-methyl-D-aspartate receptors underlies ethanol withdrawal
hyperexcitability. J Pharmacol Exper Ther. 2007; 321(1):60–72. [PubMed: 17229881]

Hoyme, H. E., May, P. A., Kalberg, W. O., Kodituwakku, P., Gossage, P., Trujiloo,…& Robinson, L. (2004). A Practical Clinical Approach to Diagnosis of Fetal Alcohol Spectrum Disorders: Clarification of the 1996 Institute of Medicine Criteria. *Pediatrics*; 115-139.

Hunt, P. S., Jacobson, S. E., & Torok, E. J. (2009). Deficits in trace fear conditioning in a rat model of fetal alcohol exposure: dose-response and timing effects. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Alcohol, 43*(6), 465-474. doi: 10.1016/j.alcohol.2009.08.004

Hurst, R., Rollema, H., Bertrand, D. (2013). Nicotinic Acetylcholine Receptors: From Basic Science to Theraputics. Pharmacology & Theraputics; (137):22-54.

Idrus, N. M., McGough, N. N., Riley, E. P., & Thomas, J. D. (2011). Administration of memantine during ethanol withdrawal in neonatal rats: effects on long-term ethanol-induced motor incoordination and cerebellar Purkinje cell loss. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Alcohol Clin Exp Res, 35*(2), 355-364. doi: 10.1111/j.1530-0277.2010.01351.x

Ikonomidou, C., Bittigau, P.,Koch, C., Genz, K., Hoerster, F., Felderhoff- Mueser, U., et al. (2001). Neurotransmitters and apoptosis in the developing brain. Biochemical Pharmacology, 62(4), 401–405.

Jacobson, S., Carter, R., Molteno, C.,…..Jacobson, J., Efficacy of Maternal Choline Supplementation During Pregnancy in Mitigating Adverse Effects of Prenatal Alcohol Exposure on Growth and Cognitive Function: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial. (2018). Alcoholism: Clinical and Experimental Research, 0:0,

Jacobson, S.,…Jacobson, J. (2018). Feasibility and Acceptability of Maternal Choline Supplementation in Heavy Drinking Pregnant Woman: A Randomized, Double-blind, Placebo Controlled Clinical Trial. Alcohol Clin Exp Res. 42(7).

Jones, K. L. (2011). The effects of alcohol on fetal development. [Review]. *Birth Defects Res C Embryo Today, 93*(1), 3-11. doi: 10.1002/bdrc.20200

Jones, K. L., & Smith, D. W. (1973). Recognition of the fetal alcohol syndrome in early infancy. *Lancet, 302*(7836), 999-1001.

Kalluri HSG, Mehta AK, Ticku MK. Up-regulation of NMDA receptor subunits in rat brain
following chronic ethanol treatment. Mol Brain Res. 1998; 58(1-2):221–224. [PubMed: 9685652]

Kem, W., Soti, F., Wildeboer, K.,...& Arias, H. R. (2006). The Nemertine Toxin Anabaseine and Its Derivative DMXBA (GTS-21): Chemical and Pharmacological Properties. *Marine Drugs*. 4:255-273.

Kem, W. R. (2000). The Brain α7 nicotinic receptor may be an important therapeutic target for the treatment of Alzheimers Disease: Studies with DMXBA (GTS-21). *Behavioral Brain Research,* 113:169-181.

Kihara, T., Shimohama, S., Sawada, H., Kimura, J., Kume, T., Kochiyama, H., Maeda, T., Akaike, A. (1997) Nicotinic Receptor Stimulation Protects Neurons Against Beta-Amyloid Toxicity, *Ann. Neurol*. 42, 159–163.

Koukouli, F., Maskos, U. (2015). The multiple roles of the alpha7 receptor in modulating glutamatergic systems in the normal and diseased nervous system. Biochem Pharm. 97.

Lauder JM, Schambra UB. Morphogenetic roles of acetylcholine. Environ Health Perspect 1999;107(Suppl. 1):65–9.

Lebel, C. (2011). Imaging the Impact of Prenatal Alcohol Exposure on the Structure of the Developing Human Brain. Neuropsych Rev. 21:102-118

Lemoine, P., Harousseau, H., Borteyru, J. P., & Menuet, J. C. (2003). Children of alcoholic parents - Observed anomalies: Discussion of 127 cases (Reprinted from Quest Med, vol 8, pg 476-482, 1968). *Therapeutic Drug Monitoring, 25*(2), 132-136. doi: Doi 10.1097/00007691-200304000-00002

Lewis, B., Wellmann, K. A., & Barron, S. (2007). Agmatine reduces balance deficits in a rat model of third trimester binge-like ethanol exposure. [Research Support, N.I.H., Extramural]. *Pharmacol Biochem Behav, 88*(1), 114-121. doi: 10.1016/j.pbb.2007.07.012

Lewis, B., Wellmann, K.A., Kehrberg, A.H., Carter, M.L., Baldwin, T., Cohen, M., Barron, S. (2011). Behavioral deficits and cellular damage following developmental ethanol exposure in rats are attenuated by CP-101,606, an NMDAR antagonist with uniqueNR2B specificity. Pharmacology, *Biochemistry and Behavior*, 100, 545-553.

Li, Y., King, M. A., Meyer, E. M. (2000). Alpha7 Nicotinic Receptor-Mediated Protection Against Ethanol-Induced Oxidative Stress and Cytotoxicity in PC12 Cells. *Brain Research*, 861, 165-167.

Li, Y., Meyer, E. M., Walker, D. W., Millard, W. J., He, Y., King, M. A. (2002). Alpha 7 Nicotinic Receptor Activation Inhibits Ethanol-Induced Mitochondiral Dysfunction, Cytochrome C Release and Neurotoxicity in Primary Rat Hippocampal Neuronal Cultures. *Journal of Neurochemistry*, 81:853-858.

Littleton J., Rogers T., Falcone D. Novel approaches to plant drug discovery based on high throughput pharmacological screening and genetic manipulation. Life Sciences 2005; 78: 467-475.

Livy, D. J., Miller, E. K., Maier, S. E., & West, J. R. (2003). Fetal alcohol exposure and temporal vulnerability: effects of binge-like alcohol exposure on the developing rat hippocampus. [Comparative Study Research Support, U.S. Gov't, P.H.S.]. *Neurotoxicol Teratol, 25*(4), 447-458.

Lutz JA, Carter M, Fields L, Barron S, Littleton JM. Altered Relation Between Lipopolysaccharide-induced Inflammatory Response and Excitotoxicity in Rat Organotypic Hippocampal Slice Cultures During Ethanol Withdrawal. 2015, Alcohol: Clinical and Experimental, 39(5): 827-835.

Lutz., J., Carter, M., Fields., L., Barron, S., Littleton, J.M. (2015). The Dietary Flavonoid Rhamnetin Inhibits Both Inflammation and Excitotoxicity During Ethanol Withdrawal in

Rat Organotypic Hippocampal Slice Cultures. Alcoholism: Clinical and Experimental Research: 39-12.

Mattson, S. N., Riley, E. P., Jernigan, T. L., Garcia, A., Kaneko, W. M., Ehlers, C. L., & Jones, K. L. (1994). A decrease in the size of the basal ganglia following prenatal alcohol exposure: a preliminary report. [Comparative Study Research Support, U.S. Gov't, Non-P.H.S. Research Support, U.S. Gov't, P.H.S.]. *Neurotoxicol Teratol, 16*(3), 283-289.

Mattson, S. N., & Riley, E. P. (1998). A review of the neurobehavioral deficits in children with fetal alcohol syndrome or prenatal exposure to alcohol. [Research Support, U.S. Gov't, P.H.S. Review]. *Alcohol Clin Exp Res, 22*(2), 279-294.

Mattson, S. N., Riley, E. P., Gramling, L., Delis, D. C., & Jones, K. L. (1998). Neuropsychological comparison of alcohol-exposed children with or without physical features of fetal alcohol syndrome. [Clinical Trial Comparative Study Research Support, U.S. Gov't, P.H.S.]. *Neuropsychology, 12*(1), 146-153.

Mattson, S. N., Roesch, S. C., Fagerlund, A., Autti-Ramo, I., Jones, K. L., May, P. A., . . . Riley, E. P. (2010). Toward a neurobehavioral profile of fetal alcohol spectrum disorders. [Evaluation Studies Multicenter Study Research Support, N.I.H., Extramural]. *Alcohol Clin Exp Res, 34*(9), 1640-1650. doi: 10.1111/j.1530-0277.2010.01250.x

May, P., Chambers, C., Kalberg, W., Zellner, J., et al. (2018). Prevalence of Fetal Alcohol Spectrum Disorder in 4 US Communities. JAMA Network. 319(5):474-482.

McGee, C. L., Schonfeld, A. M., Roebuck-Spencer, T. M., Riley, E. P., & Mattson, S. N. (2008). Children with heavy prenatal alcohol exposure demonstrate deficits on multiple measures of concept formation. Alcoholism: Clinical and Experimental Research, 32(8), 1388-1397.

Meyer, E. M., Tay, E.T., Papke, R.L., Meyers, C., Huang, G., and De Fiebre, C.M. (1997). Effects of 3-[2,4-dimethoxybenzylidene]anabaseine (DMXB) on rat nicotinic receptors and memory-related behaviors. Brain Res 768:49–56.

Meyer, E.M., Kuryatov, A., Gerzanich, V., Lindstrom, J., Papke, R.L. (1998).Analysis of 3-(4-hydroxy, 2-Methoxybenzylidene)anabaseine selectivity and activity at human and rat alpha-7 nicotinic receptors.J Pharmacol Exp Ther 287: 918–925.

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. Annual review of neuroscience, 24(1), 167-202.

Monk, B. R, Leslie, F. R., Thomas, J. D. (2012). The Effects of Perinatal Choline Supplementation on Hippocampal Cholinergic Development in Rats Exposed to Alcohol During the Brain Growth Spurt. *Hippocampus*, 22: 1750-1757.

Moore, E. M., Migliorini, R., Infante, M. A., & Riley, E. P. (2014). Fetal alcohol spectrum disorders: recent neuroimaging findings. Current developmental disorders reports, 1(3), 161-172.

Nanri, M., Yamamoto, J., Miyake, H., Watanabe, H. (1998). Protective effect of GTS-21, a novel nicotinic receptor agonist, on delayed neuronal death induced by ischemia in gerbils. Journal of Pharmacology; 76(1):23-29.

National Institute on Alcohol Abuse and Alcoholism. (2014) Moderate and Binge Drinking. Retreived from: http://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/moderate-binge-drinking

Nguyen TT, Risbud RD, Mattson SN, Chambers CD, Thomas JD. Randomized, Double-Blind, Placebo-Controlled Clinical Trial of Choline Supplementation in School-Aged Children with Fetal Alcohol Spectrum Disorders. 2016, American Journal of Clinical Nutrition. 104,6; 1683-1692.

Olincy, A., Stevens, K. E. (2007). Treating Schizophrenia with an Alpha7 Nicotinic Agonist, From Mice to Men. *Biochemical Pharamcology*, 74, 1192-1201.

Olney JW, Tenkova T, Dikranian K, Qin YQ, Labruyere J, Ikonomidou C. Ethanol-induced apoptotic neurodegeneration in the developing C57BL/6 mouse brain. Brain Res Dev Brain Res 2002;133:115–126.

Olson, H. C., Oti, R., Gelo, J., & Beck, S. (2009). “Family Matters:” Fetal Alcohol Spectrum Disorders and The Family. *Dev Disabil Res Rev*, 15(3), 235-249. DOI: 10.1002/ddrr.65

Paintner, A., Williams, A. D., & Burd, L. (2012). Fetal alcohol spectrum disorders--implications for child neurology, part 1: prenatal exposure and dosimetry. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Review]. *J Child Neurol, 27*(2), 258-263. doi: 10.1177/0883073811428376

Pauly, J. R., Charriez, C. M., Guseva, M., V., & Scheff, S. W. (2004). Nicotinic Receptor Modulation for Neuroprotection and Enhancement of Functional Recovery Following Brain Injury or Disease. *N.Y. Acad. Sci*. 1035, 316-334. Doi:10.1196/annals.1332.019.

Peng J.H., Fryer J.D., Hurst R.S., Schroeder K.M., George A.A., Morrissy S., Groppi V.E., Leonard S.S., Lukas R.J. High-affinity epibatidine binding of functional, human alpha7-nicotinic acetylcholine receptors stably and heterologously expressed de novo in human SH-EP1 cells. J Pharmacol Exp Ther 2005; 313: 24-35.

Picciotto M.R., Zoli M. Neuroprotection via nAChRs: the role of nAChRs in neurodegenerative disorders such as Alzheimer's and Parkinson's disease. Front Biosci 2008; 13: 492-504.

Popova, S., Lange, S., Probst, C., Rehm, J. (2017) Prevalence of Alcohol Consumption During Pregnancy and Fetal Alcohol Spectrum Disorders Among the General Population and Aboriginal Populations in Canada and the United States. *European Journal of Medical Genetics*, 60(1):32-48.

Pourtois, G., Schettino, A., & Vuilleumier, P. (2013). Brain mechanisms for emotional influences on perception and attention: what is magic and what is not. Biological psychology, 92(3), 492-512.

Prendergast, M. Harris, B., Mullholland, P., Blanchard, J., Gibson, D., Holley, R., & Littleton, J. (2004). Hippocampal CA1 Region Neurodegeneration Produced by Ethanol Withdrawal Requires Activation of Intrinsic Polysynaptic Hippocampal Pathways and Function of N-Methyl-D-Aspartate Receptors. Neuroscience, 124:869-877.

Prendergast, M. A., Harris, B. R., Mayer, S., Holley, R. C., Hauser, K.F., & Littleton, J. M. (2001a). Chronic nicotine exposure reduces *N*methyl-d-aspartate receptor–mediated damage in the hippocampus without altering calcium accumulation or extrusion: evidence of calbindin-D28K overexpression. *Neuroscience 102*, 75–85.

Prendergast, M. A., Harris, B. R., Mayer, S., Holley, R. C., Pauly, J.R., & Littleton, J. M. (2001b). Nicotine exposure reduces *N*-methyl-D-aspartate toxicity in the hippocampus: relation to distribution of the α-7 nicotinic acetylcholine receptor subunit. *Med Sci Monit 7*, 1153–1160.

Prendergast, M., Harris, B., Blanchard, J., Mayer, S., Gibson, A., Littleton, J. (2000). In Vitro Effects of Ethanol Withdrawal and Spermidine on Viability of Hippocampus From Male and Female Rat. Alcoholism: Clinical and Experimental Research, 24(12).1855-1861.

Prendergast, M., Harris, B., Mayer, S., Littleton, J. (2000). Chronic, But not Acute, Nicotine Exposure Attenuates Ethanol Withdrawal-Induced Hippocampal Damage in Vitro. Alcoholism: Clinical and Experimental Research, 24(10):1583-1592.

Proctor WR, Diao L, Freund RK, Browning MD, Wu PH. Synaptic GABAergic and glutamatergic mechanisms underlying alcohol sensitivity in mouse hippocampal neurons. J
Physiol. 2006; 15(575pt1):145–159. [PubMed: 16762999]

Quirk, G. J., Likhtik, E., Pelletier, J. G., & Paré, D. (2003). Stimulation of medial prefrontal cortex decreases the responsiveness of central amygdala output neurons. The Journal of Neuroscience, 23(25), 8800-8807.

Riley, E. P., Barron, S., Melcer, T., & Gonzalez, D. (1993). Alterations in activity following alcohol administration during the third trimester equivalent in P and NP rats. [Research Support, U.S. Gov't, P.H.S.]. *Alcohol Clin Exp Res, 17*(6), 1240-1246.

Riley, E. P., Infante, M. A., & Warren, K. R. (2011). Fetal alcohol spectrum disorders: an overview. [Research Support, N.I.H., Extramural Review]. *Neuropsychol Rev, 21*(2), 73-80. doi: 10.1007/s11065-011-9166-x

Riley, E. P., & McGee, C. L. (2005). Fetal alcohol spectrum disorders: an overview with emphasis on changes in brain and behavior. [Research Support, N.I.H., Extramural Research Support, U.S. Gov't, P.H.S. Review]. *Exp Biol Med (Maywood), 230*(6), 357-365.

Rossetti, Z. L., Carboni, S., Fadda, F. Glutamate induced increase of extracellular glutamate through N-methyl-D-aspartate receptors I ethanol withdrawal. Neuroscience 1993; 93:1135-40

Schneider, M. Moore, C., Adkins, M. (2011) The effects of Prenatal Alcohol Exposure on Behavior: Rodent and Primate studies. Neuropsych Reviews 21:186-203.

Shapiro L, Love J, Colman DR. Adhesion molecules in the nervous system: structural insights into function and diversity. Annu Rev Neurosci 2007;30:451–474.

Shimohama, S., Greenwald, D. L., Shafron, D. H.,….Meyer, E. H. (1998). Nicotinic a 7 receptors protect against glutamate neurotoxicity and neuronal ischemic damage. *Brain Research*, 779, 359-363

Shimohama, S. & T. Kihara. 2001. Nicotinic receptor-mediated protection against beta-amyloid neurotoxicity. *Biol. Psychiatry* **49**:233–239

Shytle R.D., Mori T., Townsend K., Vendrame M., Sun N., Zeng J., Ehrhart J., Silver A.A., Sanberg P.R., Tan J. Cholinergic modulation of microglial activation by α7 nicotinic receptors. Journal of Neurochemistry 2004; 89: 337-343. 89. Borovikova L.V., Ivanova S., Zhang M

Smith, A. M., Wellmann, K. A., Lundblad, T. M., Carter, M. L., Barron, S., & Dwoskin, L. P. (2012). Lobeline attenuates neonatal ethanol-mediated changes in hyperactivity and dopamine transporter function in the prefrontal cortex in rats. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. *Neuroscience, 206*, 245-254. doi: 10.1016/j.neuroscience.2011.11.018

Smothers C.T., Mrotek J.J., Lovinger D.M. Chronic ethanol exposure leads to a selective enhancement of N-methyl-D-aspartate receptor function in cultured hippocampal neurons. J Pharmacol Exp Ther 1997; 283: 1214-22.

Spencer J.P., Vafeiadou K., Williams R.J., Vauzour D. Neuroinflammation: modulation by flavonoids and mechanisms of action. Mol Aspects Med 2012; 33: 83-97. 156.

Stepanyan T.D., Farook J.M., Kowalski A., Kaplan E., Barron S., Littleton J.M. Alcohol withdrawal-induced hippocampal neurotoxicity in vitro and seizures in vivo are both reduced by memantine. Alcohol Clin Exp Res 2008; 32: 2128-35.

Stratton, K. R. (1996). Fetal Alcohol Syndrome: Diagnosis, Epidemiology, Prevention and Treatment. *National Academy Press*.

Suzuki, T., Hide, I., ….Nakata, Y. (2006). Microglial alpha7 Nicotinic Acetylcholine Receptors Drive a Phospholipase C/IP3 Pathway and Modulate the Cell Activation Toward a Neuroprotective Role. Neuroscience Research 83.

Thomas, J.D., Weinert, S.P., Sharif, S., Riley, E.P. (1997). MK-801 Administration During Ethanol Withdrawal in Neonatal Rat Pups Attenuates Ethanol-Induced Behavioral Deficits. *Alcoholism: Clinical and Experimental Research*, 21(7):1218-1225.

Thomas, J.D. & Riley, E.P. (1997). Fetal Alcohol Syndrome: Does Alcohol Withdrawal Play a Role? *Alcohol Health & Research World,* 22:47-53.

Thomas, J. D., Abou, E. J., & Dominguez, H. D. (2009). Prenatal choline supplementation mitigates the adverse effects of prenatal alcohol exposure on development in rats. [Research Support, N.I.H., Extramural]. *Neurotoxicol Teratol, 31*(5), 303-311. doi: 10.1016/j.ntt.2009.07.002

Thomas, J. D., Garrison, M., O'Neill, T. M. (2004). Perinatal Choline Supplementation Attenuates Behavioral Alterations Associated with Neonatal Alcohol Exposure in Rats. *Neurotoxicology and Teratology*, 26: 35-45.

Thomas, J. D., Biane, J. S., O'Bryan, K. A., O'Neill, T. M., & Dominguez, H. D. (2007). Choline supplementation following third-trimester-equivalent alcohol exposure attenuates

behavioral alterations in rats. [Comparative Study Research Support, N.I.H., Extramural]. *Behav Neurosci, 121*(1), 120-130. doi: 10.1037/0735-7044.121.1.120

Ticku MK. The effects of acute and chronic ethanol administration and its withdrawal on gammaaminobutyricacid receptor binding in rat brain. British J Pharmacol. 1980; 70(3):403–410.

Tiwari, V., Arora, V., & Chopra, K. (2012). Attenuation of NF-kappabeta mediated apoptotic signaling by tocotrienol ameliorates cognitive deficits in rats postnatally exposed to ethanol. *Neurochem Int, 61*(3), 310-320. doi: 10.1016/j.neuint.2012.05.010

Tizabi, Y., Al-Namaeh, M., Manaye, K., Taylor, R. (2003). Protective Effects of Nicotine on Ethanol-Induced Toxicity in Cultured Cerebellar Granuale Cells. *Neurotoxicity Research*. 5(5); 315-321.

Vertes, R. P. (2006). Interactions among the medial prefrontal cortex, hippocampus and midline thalamus in emotional and cognitive processing in the rat. Neuroscience, 142(1), 1-20.

Waddell, J., Mooney, S., (2017).Choline and Working Memory Training Improve Cognitive Deficits Caused by Prenatal Exposure to Ethanol. Nutrients:2-17.

Wang H., Yu M., Ochani M., Amella C.A., Tanovic M., Susarla S., Li J.H., Yang H., Ulloa L., Al-Abed Y., Czura C.J., Tracey K.J. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 2003; 421: 384- 8. 87.

West, J. R., Chen, W. A., Pantazis, N. J. (1994). Fetal Alcohol Syndrome: The Vulnerability of the Developing Brain and Possible Mechanisms of Damage. *Metabolic Brain Disease*; 9(4)291-322.

Wood, C., Kohli, S.,…Shoaid, M. (2016). Subtype-selective nicotinic acetylcholine receptor agonist can improve cognitive flexibility in an attentional set shifting task. Neuropharm 105.

Woodruff, D., Li, Y., Kem, W. A nicotinic agonist (GTS-21), eyeblink classical conditioning and nicotinic receptor binding in rabbit brain. 1994, Brain Research: 309-317.

Wilkins LH, Prendergast MA, Blanchard J, Holley RC, Chambers ER, & Littleton JM. Potential value of changes in cell markers in organotypic hippocampal cultures associated with chronic EtOH exposure and withdrawal: comparison with NMDA-induced changes. 2006, [Comparative Study Research Support, N.I.H., Extramural]. *Alcohol Clin Exp Res, 30*(10), 1768-1780. doi: 10.1111/j.1530-0277.2006.00210.x

Wu D., Cederbaum A.I. Alcohol, oxidative stress, and free radical damage. Alcohol Res Health 2003; 27: 277-84.

Xiu J., Nordberg A., Zhang J.T., Guan Z.Z. Expression of nicotinic receptors on primary cultures of rat astrocytes and up-regulation of the alpha7, alpha4 and 113 beta2 subunits in response to nanomolar concentrations of the beta-amyloid peptide(1-42). Neurochem Int 2005; 47: 281-90. 88.

Yang X, Zhao C, Chen X, Jiang L, Su X. Monocytes Primed with GTS-21/Alpha7 nAChR (Nicotinic Acetylcholine Receptor) Agonist Develop Anti-Inflammatory Memory, 2017. International Journal of Medicine, 437-445.

Yin, H. H., & Knowlton, B. J. (2006). The role of the basal ganglia in habit formation. Nature Reviews Neuroscience, 7(6), 464-476.

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Lutz, J.A., Carter, M.L., Fields, L., Barron, S., & Littleton, J.M. Altered Relation Between Lipopolysaccharide-Induced Inflammatory Response and Excitotoxicity in Rat Organotypic Hippocampal Slice Cultures during Ethanol Withdrawal. Alcoholism Clinical and Experimental Research. 2015; 39(5): 827-835

Lutz, J. A., Carter, M. L., Fields, L., Barron, S., & Littleton, J.M. The Dietary Flavonoid Rhamnetin Inhibits both Inflammation and Excitotoxicity during Ethanol Withdrawal in Rat Organotypic Hippocampal Slice Cultures. Alcoholism Clinical and Experimental Research. 2015; 39(12): 2345-2353.