7-2021

Effects of Genetics and Sex on Hippocampal Gene Expression and Adolescent Behaviors Following Neonatal Ethanol Exposure in BXD Recombinant Inbred Mice

Jessica A. Baker
University of Tennessee Health Science Center

Effects of Genetics and Sex on Hippocampal Gene Expression and Adolescent Behaviors Following Neonatal Ethanol Exposure in BXD Recombinant Inbred Mice

Abstract

Fetal alcohol spectrum disorders (FASD) are the leading preventable neurodevelopmental disorders in the western world. A hallmark symptom of FASD is cognitive and learning deficits that present in early childhood and continue throughout adulthood. Teratogenic effects of alcohol include increased cell death in the hippocampus, a brain region critically important in learning and memory. Genetics have been shown to have a role in the severity of alcohol's teratogenic effect on the developing brain. Previous work in our lab identified differential vulnerability to ethanol-induced call death in the hippocampus using fourteen BXD strains and the two parental strains. The goal of the present study was to examine the effect of genetics and sex on differential gene expression changes and behavioral responses in animals exposed to postnatal ethanol.

To test this, we examined multiple BXD strains that showed increased susceptibility to ethanol-induced cell death in the hippocampus, multiple BXD strains that were resistant to ethanol's effect on hippocampal cell death, and the parental B6 and D2 strains which showed moderate levels of cell death in the hippocampus after ethanol exposure. Neonatal mice were treated on postnatal day 7 (third trimester equivalent in humans). Animals received a subcutaneous injection of either 5.0g/kg ethanol in saline solution or isovolumetric saline given in two equal doses two hours apart. Animals were sacrificed 7 hours after initial ethanol exposure. Differential gene expression was examined using the Affymetrix Microarray platform across the strains. In another subset of animals exposed to the same alcohol paradigm, we investigated the long-term effects of developmental alcohol exposure on cognition and behavior in select BXD strains and parental strains. Adolescent animals exposed to postnatal ethanol were tested across the following behavioral tests: elevated plus maze, open field, Y-maze, and T-maze.

We identified gene expression changes after postnatal ethanol exposure in all BXD and parental strains with little overlap between males and females in the same strain. However, there were limited gene expression changes that showed a sex x treatment interaction. Sex-specific ethanol-induced gene expression changes were limited within each strain and these changes were not carried over across strains. Multiple genes showed a significant interaction between strain x treatment and/or strain x sex x treatment. Enrichment analysis of these genes revealed a number of significant over-represented biological categories involved in cell death and apoptosis. Genes that met our criteria and were also highly correlated with a number of apoptosis and learning and memory behaviors included Bcl2l11, Jun, Txnip, Chka, and Tgfb3. Interestingly, Tgfb3 has been previously linked to a significant QTL mediating strain-specific differences in hippocampal cell death after exposure to postnatal ethanol in BXD mice.

When comparing ethanol-induced gene expression changes in high cell death strains (HCD) and low cell death strains (LCD), we observed almost double the number of differentially ethanol-induced gene expression changes in the HCD strains compared to the LCD strains. Enrichment analysis revealed some overlap in significant over-represented categories between the HCD and LCD strains, though HCD showed more cell death and apoptosis categories. Significant ethanol-induced gene expression changes in the HCD and LCD strains were always regulated in the same direction suggesting 1) more perturbed effects of ethanol-induced gene expression changes in the HCD strains compared to LCD strains and 2) limited gene expression changes that confer resistance to ethanol-induced cell death in the hippocampus in the LCD strains.

In our behavioral study, our results demonstrate that the effects of developmental alcohol exposure on adolescent behavioral responses are highly dependent on strain, though the strains that showed the most behavioral alterations after exposure to postnatal alcohol were the B6 and D2 parental strains and the

BXD100 and BXD48a HCD strains. In these four strains, we observed many anxiety-like and activity-related behaviors that were significantly affected by postnatal ethanol exposure and in many of these measures there were sex-specific differences within the strain. The LCD strains, BXD60 and BXD71, showed minimal effect of treatment in all behavioral tests. Interestingly, the HCD strains, BXD100 and BXD48a, were the only strains that showed significant effect of postnatal ethanol exposure in hippocampal-dependent spatial learning and memory assessment. These results suggest that there are long-term effects of developmental alcohol exposure on adolescent behavior and that these effects are highly strain specific.

Overall, our study aimed to better understand genetic variation in ethanol-induced susceptibility to ethanol's teratogenic effects. Our results accomplish this by identifying differential gene expression changes and behavioral responses in animals exposed to postnatal ethanol using the BXD RI mice and parental strains. Additionally, our study identified sex differences in both ethanol-induced gene expression changes and adolescent behaviors in mice exposed to postnatal ethanol, though sex-specific effects were highly dependent on strain. To our knowledge, this is the first study using the BXD RI strains to examine the effects of genetics and sex on 1) ethanol-induced gene expression changes during development, and 2) adolescent behaviors in mice exposed to postnatal ethanol.

Document Type
Dissertation

Degree Name
Doctor of Philosophy (PhD)

Program
Biomedical Sciences

Research Advisor
Kristin M. Hamre, PhD

Keywords
Behavioral Neuroscience, Developmental Alcohol Exposure, Developmental Neuroscience, Fetal Alcohol Spectrum Disorders, Genetics, Mice

Subject Categories
Diseases | Medical Neurobiology | Medical Sciences | Medicine and Health Sciences | Nervous System Diseases | Neurosciences

UNIVERSITY OF TENNESSEE HEALTH SCIENCE CENTER

DOCTORAL DISSERTATION

Effects of Genetics and Sex on Hippocampal Gene Expression and Adolescent Behaviors Following Neonatal Ethanol Exposure in BXD Recombinant Inbred Mice

Author:
Jessica A. Baker

Advisor:
Kristin M. Hamre, PhD

A Dissertation Presented for The Graduate Studies Council of
The University of Tennessee Health Science Center
in Partial Fulfillment of the Requirements for the Doctor of Philosophy degree from
The University of Tennessee

in

Biomedical Sciences: Neuroscience
College of Graduate Health Sciences

July 2021

DEDICATION

To my husband, Morgun. I never could have done this without your constant love, support, and encouragement.

ACKNOWLEDGEMENTS

Thank you to my mentor Dr. Kristin Hamre. Thank you for believing in me, guiding me, and pushing me to be a better scientist. I will always be grateful for your encouragement and support during my graduate career.

I appreciate the time and help from my committee members: Dr. Viktor Chizhikov, Dr. Tauheed Ishrat, Dr. Lu Lu, and Dr. Megan Mulligan. Their advice and guidance helped shaped this project and my graduate studies. Thank you especially to Dr. Hamre and Dr. Lu who encouraged me to attend graduate school while I was an undergraduate in their lab. Their continuous support and guidance over the years has helped me become a better scientist. I would also like to thank Dr. Mulligan who acted as an additional mentor for me during my graduate career. Thank you, Dr. Mulligan, for your constant advice and guidance over the years.

This project was possible thanks to the support from the National Institute of Alcohol Abuse and Alcoholism which funded my F31 Ruth L. Kirschstein National Research Service Award Individual Predoctoral Fellowship during my second year of graduate school. This project was also allowed to grow thanks to funding support from multiple sources at the University of Tennessee Health Science Center including the CORNET Award and support from the Neuroscience Institute and the Genetics, Genomics, and Informatics Department.

ABSTRACT

Fetal alcohol spectrum disorders (FASD) are the leading preventable neurodevelopmental disorders in the western world. A hallmark symptom of FASD is cognitive and learning deficits that present in early childhood and continue throughout adulthood. Teratogenic effects of alcohol include increased cell death in the hippocampus, a brain region critically important in learning and memory. Genetics have been shown to have a role in the severity of alcohol's teratogenic effect on the developing brain. Previous work in our lab identified differential vulnerability to ethanol-induced call death in the hippocampus using fourteen BXD strains and the two parental strains. The goal of the present study was to examine the effect of genetics and sex on differential gene expression changes and behavioral responses in animals exposed to postnatal ethanol.

To test this, we examined multiple BXD strains that showed increased susceptibility to ethanol-induced cell death in the hippocampus, multiple BXD strains that were resistant to ethanol's effect on hippocampal cell death, and the parental B6 and D2 strains which showed moderate levels of cell death in the hippocampus after ethanol exposure. Neonatal mice were treated on postnatal day 7 (third trimester equivalent in humans). Animals received a subcutaneous injection of either 5.0g/kg ethanol in saline solution or isovolumetric saline given in two equal doses two hours apart. Animals were sacrificed 7 hours after initial ethanol exposure. Differential gene expression was examined using the Affymetrix Microarray platform across the strains. In another subset of animals exposed to the same alcohol paradigm, we investigated the long-term effects of developmental alcohol exposure on cognition and behavior in select BXD strains and parental strains. Adolescent animals exposed to postnatal ethanol were tested across the following behavioral tests: elevated plus maze, open field, Y-maze, and T-maze.

We identified gene expression changes after postnatal ethanol exposure in all BXD and parental strains with little overlap between males and females in the same strain. However, there were limited gene expression changes that showed a sex x treatment interaction. Sex-specific ethanol-induced gene expression changes were limited within each strain and these changes were not carried over across strains. Multiple genes showed a significant interaction between strain x treatment and/or strain x sex x treatment. Enrichment analysis of these genes revealed a number of significant over-represented biological categories involved in cell death and apoptosis. Genes that met our criteria and were also highly correlated with a number of apoptosis and learning and memory behaviors included *Bcl2l11, Jun, Txnip, Chka,* and *Tgfb3.* Interestingly, *Tgfb3* has been previously linked to a significant QTL mediating strain-specific differences in hippocampal cell death after exposure to postnatal ethanol in BXD mice.

When comparing ethanol-induced gene expression changes in high cell death strains (HCD) and low cell death strains (LCD), we observed almost double the number of differentially ethanol-induced gene expression changes in the HCD strains compared to the LCD strains. Enrichment analysis revealed some overlap in significant over-

represented categories between the HCD and LCD strains, though HCD showed more cell death and apoptosis categories. Significant ethanol-induced gene expression changes in the HCD and LCD strains were always regulated in the same direction suggesting 1) more perturbed effects of ethanol-induced gene expression changes in the HCD strains compared to LCD strains and 2) limited gene expression changes that confer resistance to ethanol-induced cell death in the hippocampus in the LCD strains.

In our behavioral study, our results demonstrate that the effects of developmental alcohol exposure on adolescent behavioral responses are highly dependent on strain, though the strains that showed the most behavioral alterations after exposure to postnatal alcohol were the B6 and D2 parental strains and the BXD100 and BXD48a HCD strains. In these four strains, we observed many anxiety-like and activity-related behaviors that were significantly affected by postnatal ethanol exposure and in many of these measures there were sex-specific differences within the strain. The LCD strains, BXD60 and BXD71, showed minimal effect of treatment in all behavioral tests. Interestingly, the HCD strains, BXD100 and BXD48a, were the only strains that showed significant effect of postnatal ethanol exposure in hippocampal-dependent spatial learning and memory assessment. These results suggest that there are long-term effects of developmental alcohol exposure on adolescent behavior and that these effects are highly strain specific.

Overall, our study aimed to better understand genetic variation in ethanol-induced susceptibility to ethanol's teratogenic effects. Our results accomplish this by identifying differential gene expression changes and behavioral responses in animals exposed to postnatal ethanol using the BXD RI mice and parental strains. Additionally, our study identified sex differences in both ethanol-induced gene expression changes and adolescent behaviors in mice exposed to postnatal ethanol, though sex-specific effects were highly dependent on strain. To our knowledge, this is the first study using the BXD RI strains to examine the effects of genetics and sex on 1) ethanol-induced gene expression changes during development, and 2) adolescent behaviors in mice exposed to postnatal ethanol.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

B6	C57BL/6J
D2	DBA/2J
EPM	Elevated plus maze
FASD	Fetal alcohol spectrum disorders
HCD	High cell death
LCD	Low cell death
NIAAA	National institute of alcohol abuse and alcoholism
OF	Open field
QTL	Quantitative trait locus
RI	Recombinant inbred
TM	T-maze
YM	Y-maze

CHAPTER 1. INTRODUCTION AND BACKGROUND

Background on Fetal Alcohol Spectrum Disorders

Alcohol was identified as a teratogen almost 50 years ago, yet exposure to alcohol during pregnancy is still a leading cause of abnormal developmental throughout the world (Jones & Smith, 1973; Jones, Smith, Ulleland, & Streissguth, 1973; May et al., 2014; Roozen et al., 2016). The umbrella term, fetal alcohol spectrum disorder (FASD), refers to the range of symptoms and effects due to exposure to alcohol during development (Bertrand et al., 2005). The severe end of the spectrum includes fetal alcohol syndrome (FAS) and partial fetal alcohol syndrome (PFAS) which are both associated with distinct facial anomalies, physical deficits, and neurobehavioral impairments (Hoyme et al., 2016). Other disorders in the continuum of FASD that do not present severe facial or physical abnormalities are alcohol-related neurobehavioral disorder (ARND), alcohol-related birth defects (ARBD), and neurobehavioral disorder associated with prenatal alcohol exposure (ND-PAE) (Hoyme et al., 2016).

FASD constitutes the leading preventable neurodevelopmental disorders in the United States. Neuropathology associated with prenatal alcohol exposure alters cognitive, emotional, motor, and behavioral functions that present in childhood and can persist throughout life (Kable et al., 2016). A recent meta-analysis estimates the global prevalence of FASD at 23 per 1000 live births, making the prevalence of FASD greater than that of autism spectrum disorders (Roozen et al., 2016). Overall rates are even higher in the United States, where it is estimated that 2-5% of live births are adversely affected by prenatal alcohol exposure (May et al., 2014; May et al., 2018; J. R. Wozniak, Riley, & Charness, 2019). In fact, some studies report approximately 13% of women consumed some alcohol during pregnancy; moreover, nearly half of all pregnancies are unplanned and with a majority of women of childbearing age drinking alcohol, the risk of fetal exposure to alcohol continues to be high (Floyd & Sidhu, 2004; Ryan, Williams, & Thomas, 2008). Excessive patterns of drinking, such as binge drinking, have been shown to have particularly detrimental effects on the developing brain (Bonthius & West, 1990). Binge drinking, defined as four or more drinks per occasion, is estimated to occur in 2-3% of pregnancies (Popova, Lange, Probst, Gmel, & Rehm, 2018). Thus, FASD remains a serious problem, despite prevention efforts. Considering the long-lasting medical, psychological, and social problems associated with prenatal alcohol exposure, developing ways to better identify children exposed to developmental alcohol is a high priority for public health.

Although the United States Surgeon General issued the first public health advisory that prenatal alcohol can cause birth defects in 1981, it was not until 2002 that the Centers for Disease Control and Prevention (CDC) developed diagnostic guidelines for FAS and related disorders (Bertrand et al., 2005; Williams, Smith, & Committee On Substance, 2015). Children on the severe end of the spectrum are usually diagnosed in infancy or early childhood by the presence of cardinal facial features and/or confirmed exposure to prenatal alcohol. Early diagnosis of FASD is associated with more positive

outcomes and has been found to reduce the risk of developing secondary disabilities (Peadon, Rhys-Jones, Bower, & Elliott, 2009; Streissguth et al., 2004). However, FASD in the absence of cardinal facial anomalies have proven difficult to identify and are normally diagnosed later in childhood or not at all. For example, a recent study in children who were referred to children's mental health center found that 86.7% of children either in the foster care system or legally adopted were misdiagnosed or undiagnosed with FASD (Chasnoff, Wells, & King, 2015). With estimates that 2.4% to 4.8% of school-aged children in the United States may have FASD, early screening tools to identify children most at risk of neurobehavioral impairments and those most likely to benefit from specific treatments is critical (May et al., 2014; May et al., 2009).

The Role of Genetics in FASD

Evaluation in Humans

One-way recent research has aimed to identify at-risk children and discover novel molecular pathways that can be used as therapeutics is by studying the role of genetics in FASD. Genetics has been shown to be an important factor in both the presence and severity of FASD. In humans, there is a higher concordance of deficits seen in human monozygotic twins compared to dizygotic twins (Chasnoff, 1985; Christoffel & Salafsky, 1975). For example, in an early study of twins exposed to in utero alcohol, all monozygotic twins examined showed concordance in FASD diagnosis while only 7 out of 11 dizygotic twin pairs showed similar concordance in FASD diagnosis (Streissguth & Dehaene, 1993). Another study of dizygotic twins found twin growth inconsistency was common after exposure to in utero alcohol and cited occasions where one twin showed multiple ethanol-related neurological phenotypes including neonatal withdrawal symptoms, delay in motor and cognitive function, and cortical and central brain atrophy while the other twin was unaffected and showed normal development (Riikonen, 1994). Consistent with the twin studies, the severity of alcohol-induced deficits in children varies even among mothers who consume approximately equivalent amounts of alcohol and at approximately the same time period during their pregnancies (Astley, 2010). Although there are many factors that can influence ethanol teratogenicity, these early twin studies suggest genetic variation may play a role in the severity of ethanol-induced changes during development.

Identification of genetic factors that contribute to the presence and severity of FASD could allow us to better identify at risk individuals and provide new routes for interventions and therapeutics for those affected. To date, there have been few genetic predictors identified in humans, although several studies have found an association between allelic variations in ethanol metabolizing enzymes and severity of FASD symptoms (as reviewed in (Warren & Li, 2005). Ethanol is metabolized by converting ethanol to acetaldehyde via the catalyzing enzyme alcohol dehydrogenase (ADH). Acetaldehyde is then converted to acetate via aldehyde dehydrogenase (ALDH). These by-products of the oxidative alcohol metabolism pathway can cause cellular damage,

especially acetaldehyde (Eberhart & Parnell, 2016). In humans, genetic FASD research has focused on allelic variations in the ADH class I family (ADH1) of enzymes which effect the rate of ethanol metabolism. Specifically, variants in ADH1B have been found to play a protective role against the teratogenic effects of alcohol (Das, Cronk, Martier, Simpson, & McCarver, 2004; Jacobson et al., 2006; McCarver, Thomasson, Martier, Sokol, & Li, 1997; Viljoen et al., 2001). A study from Western Cape Province, a South African region that has the highest rates of reported FAS, showed the ADH1B*2 allele was significantly more common in controls compared to FAS-affected children and their mothers (Viljoen et al., 2001). Another study found FASD characteristics were only present in alcohol-exposed infants of mothers lacking an ADH1B*3 allele while infants whose mothers drank equivalent amounts of alcohol and possessed at least one ADH1B*3 allele were more similar to non-exposed infants of either maternal genotype (McCarver et al., 1997). Presence of ADH1B*3 has also been shown to protect against neurobehavioral problems as adolescents exposed to prenatal alcohol whose mother lack an ADH1B*3 allele displayed significantly higher behavioral problems while adolescents whose mothers possessed at least one copy of ADH1B*3 showed no adverse behavioral effects of alcohol (Dodge, Jacobson, & Jacobson, 2014). These studies show allelic variation can influence susceptibility to FASD in humans. However, human studies on the role genetics in FASD have been limited, mostly focusing on ethanol metabolizing enzymes. Ethanol's teratogenic effects are much more complex and further research using animal models will lead to a better understanding of the influence of genetics in FASD.

Animal Models of FASD

Identification of specific genetic factors have been difficult to ascertain in humans for multiple reasons. First, maternal drinking history is often either unreliable or unknown making it difficult to identify children with FASD without the cardinal facial deformities (Astley, 2006; Benz, Rasmussen, & Andrew, 2009). Drinking history is often unreliable because the amount of alcohol or timing of exposure can be difficult to recall, especially in mothers who participate in binge drinking, and drinking alcohol during pregnancy is a social taboo in many cultures and therefore often not accurately disclosed (Astley, 2006; Benz et al., 2009). Second, the amount, pattern, and timing of alcohol exposure in utero can influence the severity of developmental deficits, as discussed below (Alvik, Aalen, & Lindemann, 2013; Flak et al., 2014; Maier & West, 2001). Finally, there are limited genetic studies of FASD in humans and the range of genetic variation on FASD phenotypes is unknown. For these reasons, the identification of genetic factors that influence FASD phenotypes have been primarily studied in animal models. Animal models are advantageous as other factors that may influence the severity of ethanol-induced changes can be controlled such as, developmental timing of exposure, maternal health and nutrition, and dose and frequency of exposure during development.

Although many species have been used to study the effects of developmental alcohol exposure, rodent models are the most commonly used animal species in FASD research (Cudd, 2005). Both humans and rodents have similar physiological responses to

alcohol and rodent neurobehavioral outcomes to alcohol during development are comparable to human clinical studies (Driscoll, Streissguth, & Riley, 1990; Hannigan, 1996). Brain developmental occurs at different time periods in humans compared to rodent models and therefore needs to be considered. Human gestation is split into three trimesters all of which occur prenatally. While gestation in rodent models is significantly shorter than humans, their gestational stages are also split into three trimester equivalents and a large part of their brain development takes place during the neonatal period. In humans, gastrulation and neurulation takes place during the first trimester which is equivalent to embryonic days 0-10 in mice (Marquardt & Brigman, 2016; West, 1987). Animals exposed to alcohol at specific stages during this critical developmental period can exhibit facial dysmorphologies and brain malformations as seen in children with FASD (Astley, Magnuson, Omnell, & Clarren, 1999; Kotch & Sulik, 1992; Sulik, 2005). The second trimester in humans corresponds to embryonic days 11-21 in mouse models (Marquardt & Brigman, 2016). Exposure to alcohol at this developmental stage has been shown to alter cell proliferation and neuronal migration (Guerri, 1998). The third trimester in humans is equivalent to postnatal days 0-10 in most rodent models (Gil-Mohapel, Boehme, Kainer, & Christie, 2010). In humans the third trimester begins the brain growth spurts which continues after birth through the first year or two of life (Dobbing, 1974; Dobbing & Sands, 1979).

During the brain growth spurt, the brain is growing at its fastest rate, neurons are completing migration and differentiating, microneurons such as granule cells in the hippocampus and cerebellum are being generated, connections are established through synaptogenesis and dendritic arborization, and natural programmed cell death occurs (Alfonso-Loeches & Guerri, 2011; Gil-Mohapel et al., 2010; Marquardt & Brigman, 2016). Although this stage of development occurs exclusively postnatally in rodents, this third trimester equivalent model should not be discounted as human studies have reported some women continue drinking alcohol during this development period (Ethen et al., 2009). In fact, a recent study testing infant blood samples revealed that 8.4% were positive for a unique metabolite of ethanol, indicative of prenatal alcohol exposure within one month of delivery (Bakhireva et al., 2017). Moreover, the third trimester has shown to be particularly sensitive to ethanol-induced neuronal deficits in several late-developing brain regions (Bonthius & West, 1990; Coles et al., 1991; Goodlett, Marcussen, & West, 1990; Ikonomidou et al., 2000; Maier, Chen, Miller, & West, 1997). Though studies examining the effects of alcohol exposure in neonatal animals, should take into account the route of ethanol administration as postnatal exposure bypasses the mother and is given directly to the pups.

Along with controlling the developmental timing of exposure, the pattern and route of administration can also be controlled using animal models. In rodents, alcohol administration methods include ingestion, injection, or inhalation (as reviewed in (Patten, Fontaine, & Christie, 2014)). Each of these methods produce a wide range of intoxication levels as commonly measured by blood alcohol concentrations (BAC). There are some discrepancies when comparing BACs in humans to animal models such as rodents (Driscoll et al., 1990). For example, the legal intoxication limit in the United States is 80 mg/dl. In clinical research, repeated exposure to a BAC of 80 mg/dl is considered

moderate to high exposure (Marquardt & Brigman, 2016). However, in rodents this is considered a low exposure. A computational modeling study analyzed ethanol-induced neurodevelopmental toxicity across multiple species and found significantly higher BACs are required in rodent models to achieve comparable neurodevelopmental effects as humans (Gohlke, Griffith, & Faustman, 2007). In rodents, both high levels of alcohol exposure, as defined by BAC $\geq$ 150 mg/dl, and low to moderate levels of alcohol exposure, as defined by BAC $\leq$ 150, can have long-lasting detrimental effects on brain development (Patten et al., 2014; Valenzuela, Morton, Diaz, & Topper, 2012).

The route of administration, dose of ethanol, and pattern of exposure effect the level of intoxication. Ethanol exposure through dietary methods, voluntary drinking paradigms, or vapor inhalation normally produce low to moderate BACs while intragastric gavage or injection (subcutaneous or intraperitoneal) tend to produce higher BACs (Patten et al., 2014). The dose of ethanol and pattern of exposure is also important to consider in animal studies (Bonthius, Goodlett, & West, 1988; Bonthius & West, 1988). Binge-like ethanol exposure is a common method to achieve high BACs in a short amount of time. Even a single high dose of ethanol has been shown to produce ethanol-induced brain abnormalities and neurological dysfunction in adult animals exposed to developmental alcohol (Ieraci & Herrera, 2007; Parnell et al., 2013; Parnell et al., 2009). Animal models are also used to study chronic ethanol exposure paradigms during different developmental time points (Choi, Allan, & Cunningham, 2005; Wigal & Amsel, 1990). Of note, postnatally exposed animals (i.e., third trimester equivalent models) can achieve a higher BAC with a lower dose of ethanol (Livy, Miller, Maier, & West, 2003). This is partly due to decreased levels of alcohol dehydrogenase in neonates which only functions at one fourth the activity in neonates as compared to adults (Raiha, Koskinen, & Pikkarainen, 1967).

Evaluation in Animal Models

Numerous studies in animal models also support the strong role of genetics by showing differential vulnerability to ethanol's teratogenic effects across differing genetic backgrounds. A large range of phenotypes have shown differential sensitivity to developmental alcohol exposure including craniofacial dysmorphology (M. L. Green et al., 2007; Su, Debelak, Tessmer, Cartwright, & Smith, 2001), brain growth delays (Chen, Ozturk, Ni, Goodlett, & Zhou, 2011; Goodlett, Gilliam, Nichols, & West, 1989; Ogawa, Kuwagata, Ruiz, & Zhou, 2005), cell death (Chen et al., 2011; Debelak & Smith, 2000; Goldowitz et al., 2014), epigenetic regulation (Amiri, Davie, & Rastegar, 2020; Goldowitz et al., 2014), and gene expression (Downing, Flink, et al., 2012; M. L. Green et al., 2007; Lossie et al., 2014). Differential behavioral responses to alcohol exposure during development have also been found including activity (Riley, Barron, Melcer, & Gonzalez, 1993; Thomas, Melcer, Weinert, & Riley, 1998), motor coordination (Thomas, Burchette, Dominguez, & Riley, 2000; Thomas, Leany, & Riley, 2003), and learning and memory (Gilliam, Stilman, Dudek, & Riley, 1987).

Mouse models have been especially useful for studying the role of genetics in FASD (Driscoll et al., 1990). Both inbred and selectively bred strains have been used to show differential vulnerability to ethanol's teratogenic effects (Chen et al., 2011; Dunty, Chen, Zucker, Dehart, & Sulik, 2001; Gilliam & Kotch, 1990, 1996; Goodlett et al., 1989; Riley et al., 1993; Thomas et al., 1998). Inbred strains are created through brother-sister mating for over 20 generations (Peirce, Lu, Gu, Silver, & Williams, 2004; Taylor et al., 1999; X. Wang et al., 2016). They are homozygous at all gene loci and therefore considered to be genetically identical to one another (Peirce et al., 2004; Taylor et al., 1999; X. Wang et al., 2016). Genetic differences to ethanol's teratogenic effects can be determined by evaluating two or more inbred strains. Two inbred strains that have been extensively studied in developmental alcohol exposure research are the C57BL/6J (B6) and DBA/2J (D2) (Boehm, Lundahl, Caldwell, & Gilliam, 1997; Gora-Maslak et al., 1991). Multiple groups have shown differential vulnerability to developmental alcohol exposure including multiple skeletal and soft-tissue malformations, (Boehm et al., 1997; Chen et al., 2011; Downing, Balderrama-Durbin, et al., 2012; Ogawa et al., 2005), apoptotic response (Chen et al., 2011; Theberge et al., 2019), and gene expression changes (Downing, Flink, et al., 2012; Lossie et al., 2014; Zhou et al., 2011).

A common theme of these studies is that B6 strains are more susceptible to ethanol-induced developmental abnormalities including growth retardation, brain morphology, and anomalies such as malformations of the kidney, heart, and digits compared to D2 strains that have been found to be relatively resistant (Boehm et al., 1997; Downing, Balderrama-Durbin, Broncucia, Gilliam, & Johnson, 2009; Downing, Balderrama-Durbin, et al., 2012; Downing, Flink, et al., 2012). Studies have also found slight variation in the rate of development between B6 and D2 embryos as measured by number of somites, though there was large variability within strains (Ogawa et al., 2005; Thiel, Chahoud, Jurgens, & Neubert, 1993; Zhou et al., 2011). When developmental staging was controlled, ethanol's effects on neurulation in D2 embryos were similar to B6 embryos exposed to ethanol though, specific regional vulnerabilities between the two strains were found (Ogawa et al., 2005). For example, gestational ethanol exposure produced preferential vulnerability in the heart in B6 embryos and in the eye in D2 embryos (Ogawa et al., 2005). A follow up study by this lab found when developmental staging and maternal and intrauterine factors were controlled for, B6 embryos showed great vulnerability to alcohol-induced deficits in growth and apoptosis while D2 strains were more resistant to these effects (Chen et al., 2011). These studies show differential strain vulnerability to ethanol teratogenicity and demonstrate the use of B6 and D2 strains as FASD mouse models to further evaluate the roles of genetics in FASD.

The Developing Hippocampus and Alcohol Exposure

Throughout the FASD spectrum, each diagnostic category (FAS, PFAS, ARND, ARBD, ND-PAE) includes either a cognitive or behavioral impairment. Cognitive impairments are defined as deficits in executive function, learning, memory, or visual-spatial while behavioral impairments are defined as mood or behavioral regulation, attention, or impulse control. As the effects of alcohol on the developing brain appear to

be widespread, affecting many areas of the brain depending on the timing of exposure, this myriad of cognitive and behavioral deficits is not surprising (Lebel, Roussotte, & Sowell, 2011). Neuroimaging studies in children with FASD have shown reduced overall brain size as well as significantly smaller volume of multiple brain structures including the hippocampus, cerebral cortex, corpus callosum, and cerebellum (Archibald et al., 2001; Autti-Ramo et al., 2002; Sowell et al., 2002).

Hippocampal-Dependent Learning and Memory

The hippocampus is of particular interest as it plays a large role in many of the cognitive and behavioral abnormalities present in FASD, specifically impairments in learning, memory, and attention. In humans, hippocampal abnormalities and dysfunctions have been associated with impaired spatial working memory performance (Coles et al., 1991; D. A. Hamilton, Kodituwakku, Sutherland, & Savage, 2003; E. M. Moore et al., 2021; Willoughby, Sheard, Nash, & Rovet, 2008), decreased verbal learning skills (Willoughby et al., 2008), and deficits in episodic memory (du Plooy, Malcolm-Smith, Adnams, Stein, & Donald, 2016; Roediger et al., 2021; Streissguth et al., 1994). Impaired hippocampal-dependent behaviors are also seen in animal models including spatial learning and memory (Kelly, Goodlett, Hulsether, & West, 1988; Subbanna, Shivakumar, Psychoyos, Xie, & Basavarajappa, 2013; D. F. Wozniak et al., 2004; Zimmerberg, Sukel, & Stekler, 1991), and fear conditioning (Brady, Allan, & Caldwell, 2012; G. F. Hamilton et al., 2014; Hunt, Jacobson, & Torok, 2009; A. F. Wagner & Hunt, 2006). Recent neuroimaging work in an animal model of FASD shows similar findings as in human studies, showing significant decreases in whole brain volume as well as the hippocampus (Parnell et al., 2009). These studies show structural hippocampus abnormalities as well as impairments in hippocampal dependent learning and memory in both animal models and humans exposed to developmental alcohol.

Hippocampal Cell Loss

Research into the mechanisms behind the structural abnormalities present after exposure to alcohol during development, have extended past the hippocampus proper to also include other members of the hippocampal formation. Briefly, the hippocampal formation comprises the hippocampus proper, dentate gyrus (DG), subicular complex, and entorhinal cortex (Schultz & Engelhardt, 2014). The hippocampus proper is subdivided into four major subfields named *Cornu Ammonis* 1-4 (CA1-CA4). Although there are some differences in the orientation of the hippocampal formation between humans and rodents, inherent structure and connectivity is preserved in mammals (Leuner & Gould, 2010).

Animal models of FASD have allowed researchers to investigate ethanol-induced alternations in the structure and function of the hippocampal formation. The number of cells in hippocampal subfields and dentate gyrus have been extensively studied although ethanol's effect on these developing cell populations differed depending on dose and

timing of exposure. An initial study in adult rats exposed to ethanol during E10-E21 (second trimester-equivalent) found decreased pyramidal cells in the CA1 region but no differences in the other hippocampal subfields or granule cells of the DG (Barnes & Walker, 1981). Another study examined neuron numbers on P10 after chronic ethanol exposure P4-P10, finding a reduction of neurons in the CA4 region but not CA1 or CA3, and an increase of neurons in the DG (West, 1986). A different study using the same third-trimester equivalent model found a significant reduction in neuronal number in the CA1 region on P10 while CA3, CA4, and the DG showed no difference in neuron number (Bonthius & West, 1990). A subsequent study by this lab showed long-lasting effects of chronic postnatal ethanol exposure showing that neuron numbers were still reduced in the CA1 region at P90 (Bonthius & West, 1991). Binge-like exposure to third trimester-equivalent ethanol produced reduced cell numbers in the CA1, CA3, and DG on P10 (Livy et al., 2003). Cell number reductions in the CA1, CA3, and DG were also found when animals were exposed to both gestational (E1-E20) and postnatal (P4-10) ethanol. However, these reductions were not seen when exposed to gestational ethanol alone (Livy et al., 2003; Maier & West, 2001). Similarly, another study administered ethanol either exclusively during the neonatal period or in combination with exposure during gestation and found a reduced number pyramidal cells in the CA1 region in adult animals (Tran & Kelly, 2003). However, there were no differences in cell number observed in the CA3 region or DG (Tran & Kelly, 2003). Overall, these results suggest the hippocampus is particularly vulnerable to third trimester-equivalent ethanol exposure and that the CA1 region is highly susceptible cell loss while the CA3 region and DG seem to be more resilient.

Hippocampal Dendrites and Synapses

Developmental alcohol exposure has also been shown to affect dendritic architecture and synaptogenesis in the hippocampus. An early study of ethanol-induced functional and structural abnormalities showed learning impairments in animals prenatally exposed to ethanol as well as significant deficits in dendritic structure in the hippocampus of these animals (Abel, Jacobson, & Sherwin, 1983). Ethanol has been shown to inhibit the dendritic arborization in hippocampal pyramidal neurons exposed to ethanol, showing significantly shorter dendrites, decreased branching, and reduced number of dendrites per neuron (Davies & Smith, 1981; Lindsley, Comstock, & Rising, 2002; Yanni & Lindsley, 2000). Decreased dendritic spine density in both CA1 and CA3 pyramidal neurons has also been shown after exposure to developmental alcohol (Berman & Hannigan, 2000; Berman, Hannigan, Sperry, & Zajac, 1996; Ferrer, Galofre, Lopez-Tejero, & Llobera, 1988). Another study found over fifty percent less dendritic spines in ethanol treated pyramidal cell compared to controls as well as a predominance of stubby wide spines instead of the more mature mushroom or thin spines (Gonzalez-Burgos et al., 2006). Developmental ethanol exposure has been shown to affect the development and the maturation of synapses in the hippocampus. Prenatal ethanol exposure has been found to affect synapse turnover in the DG of the hippocampal formation (Hoff, 1988). Reduced synapse densities in the CA1 region of the hippocampus were also found in adult animals after chronic ethanol exposure during development (Kuge et al., 1993).

Altogether, these studies indicate developmental ethanol exposure influences dendritic morphology and arborization as well as synaptogenesis in the hippocampal formation.

Adult Hippocampal Neurogenesis

In addition to alterations in cell number and dendrite and spine morphology, developmental alcohol exposure has been reported to reduce adult hippocampal neurogenesis. The subgranular zone of the DG in the hippocampal formation is one of only two regions in the entire brain that can produce new neurons in adulthood (as reviewed in (Gil-Mohapel et al., 2010)). Newly generated neurons differentiate, migrate to the granular zone of the DG, and integrate into the preexisting circuitry (Kempermann, Jessberger, Steiner, & Kronenberg, 2004). Many factors have been shown to influence adult neurogenesis, including alcohol exposure in adult animals; however, the long-lasting effects of development alcohol exposure on the ability to produce new neurons during adulthood is just beginning to be explored (Nixon & Crews, 2002). An early investigation found decreased cell proliferation in adult mice exposed to first and second trimester-equivalent alcohol compared to non-handled controls while no significant difference was found in maltose-dextrin, pair-fed controls (Redila et al., 2006). Similarly, another prenatal model found no changes in adult hippocampal neurogenesis using a voluntary drinking paradigm; though ethanol-exposed animals showed significant decreases in neurogenic response to environmental enrichment (Choi et al., 2005). In contrast to prenatal models, exposure to third trimester-equivalent alcohol has more deleterious effects on adult hippocampal neurogenesis. Reductions in total number of granule cells and decreased survival of newly generated neurons in the DG were found in adolescent and adult animals exposed to chronic binge-like alcohol postnatally (D. A. Hamilton et al., 2014; G. F. Hamilton et al., 2014; G. F. Hamilton et al., 2011; Klintsova, Hamilton, & Boschen, 2012; Klintsova et al., 2007; Miller, 1995). Another study found even an acute exposure to ethanol on postnatal day 7, decreased the number of hippocampal progenitor cells and reduced cell survival in adult animals (Ieraci & Herrera, 2007). These studies show that developmental alcohol exposure has long-lasting effects on adult hippocampal neurogenesis and that similar to cell loss in the CA1 region, exposure during the third trimester-equivalent is a particularly sensitive period.

Ethanol-Induced Apoptosis and the Developing Brain

Ethanol-induced brain malformations and structural abnormalities were first identified through postmortem samples of individuals exposed to heavy prenatal alcohol (Clarren, 1981; Clarren, Alvord, Sumi, Streissguth, & Smith, 1978). Early evidence of brain dysmorphology was examined in the most severe cases of FAS which was fatal to the fetus or infant (Clarren et al., 1978; Peiffer, Majewski, Fischbach, Bierich, & Volk, 1979). Modern neuroimaging studies has provided non-invasive examination of brain structure and function in humans and has allowed investigation across the full spectrum of FASD (for review see (E. M. Moore, Migliorini, Infante, & Riley, 2014)). Analysis of specific structural and functional malformities seen in children with FASD can help

researchers identify regions behind life-long neurobehavioral abnormalities (Mattson, Bernes, & Doyle, 2019; E. M. Moore et al., 2014). As previously stated, studies have found significant reductions in total brain volume (Chen et al., 2011; Rajaprakash, Chakravarty, Lerch, & Rovet, 2014; Zhou et al., 2011) as well as reductions in specific brain regions such as the corpus callosum (Y. Yang et al., 2012), cerebellum (Fryer et al., 2012), caudate nucleus (Archibald et al., 2001), and hippocampus (Willoughby et al., 2008). Recent advances in structural MRI analyses have gone a step further to examine specific subfields in affected brain structures such as the recent study that identified significantly smaller subfields of the hippocampus in children with prenatal alcohol exposure (Roediger et al., 2021). Overall, these neuropathological studies have identified reduced brain volume and abnormalities in specific bran structures that may underlie cognitive and behavioral phenotypes seen in children with FASD.

A proposed mechanism behind these neuropathological findings and neurobehavioral impairments is ethanol-induced programmed cell death or apoptosis (Creeley & Olney, 2013; Guerri, Bazinet, & Riley, 2009). This naturally occurring phenomenon is a highly regulated mode of cell deletion and alterations to cell death or cell survival pathways can have deleterious consequences in the developing brain (Dikranian et al., 2001; Farber, Creeley, & Olney, 2010; Farber & Olney, 2003; Ikonomidou, 2009; Johnston et al., 2009). Ethanol exposure during brain development has been shown to cause neuronal apoptosis in numerous brain regions though neuronal populations show varying susceptibility to ethanol-induced cell death depending on the developmental time of exposure (Dunty et al., 2001; Ikonomidou et al., 2000; Olney et al., 2002). For example, animal models have found exposure to ethanol during early gestation results in increased apoptosis in brain and craniofacial areas that are associated with FAS (Astley et al., 1999; Dunty et al., 2001; Sulik, 2005; Sulik, Cook, & Webster, 1988). The development period equivalent to the third trimester has shown to be particularly vulnerable to ethanol-induced neuroapoptosis in animal models (Ikonomidou et al., 2000; Olney et al., 2002). Ethanol exposure during the postnatal period in rodents has shown to elicit apoptosis in a number brain regions including the cerebral cortex, thalamus, retina, cerebellum; and hippocampus (Dikranian et al., 2001; Heaton et al., 2003; Ikonomidou et al., 2000; Mooney & Miller, 2001; Olney et al., 2002; Tenkova, Young, Dikranian, Labruyere, & Olney, 2003; Young et al., 2003).

Hippocampal Apoptotic Response to Developmental Ethanol Exposure

As the hippocampus is a structure highly involved in many of the cognitive and neurobehavioral deficits present in FASD and that can exhibit large levels of cell loss, this region has been a focus of for studying ethanol-induced neuroapoptosis (Olney, 2004). Many of these studies have focused on the CA1 region of the hippocampus as it has shown to be more vulnerable to the ethanol induced cell loss (Tran & Kelly, 2003). In fact, apoptotic cell death was significantly greater in the CA1 region of the hippocampus compared to the CA3 region or DG (Smith, Guevremont, Williams, & Napper, 2015). Third trimester equivalent models have demonstrated that even a single day exposure to ethanol can produce varying levels of hippocampal apoptosis depending on the postnatal

day of exposure (Ikonomidou et al., 2000). For example, exposure on P4 shows over 250% increase of hippocampal apoptotic response to ethanol while later postnatal exposure exhibits more severe apoptotic cell death of up to 11,000% increase in response to ethanol (Boschen & Klintsova, 2017; Ikonomidou et al., 2000; Smith et al., 2015). In addition to increased hippocampal apoptotic neurodegeneration, adolescent animals exposed to ethanol on a single postnatal day exhibited spatial learning and memory impairments (D. F. Wozniak et al., 2004). Neurogenesis in the hippocampal formation and extended members of the hippocampal circuit did not show any signs of these deleted neurons being replaced through neurogenesis (D. F. Wozniak et al., 2004). These results suggest extensive hippocampal apoptosis in response to developmental ethanol exposure and subsequent behavioral deficits.

Ethanol-Induced Apoptotic Mechanisms During Development

Apoptosis is defined by a series of very specific morphological and biochemical changes (Dikranian et al., 2001; Kerr, Wyllie, & Currie, 1972). Ethanol exposure during development produces these unique changes rapidly, in a period of 6-16 hours, ending in programmed cell death (Ikonomidou et al., 2000; Olney et al., 2002; Young et al., 2003). One hypothesis is that ethanol induces apoptosis through its antagonistic effect on NMDA receptors and its hyperactivation of GABA receptors, both which reduce neuronal activity (Ikonomidou et al., 2000; Olney et al., 2002). Once initiated, apoptosis occurs through a series of gene-regulated pathways and mechanisms (Kerr et al., 1972). Developmental ethanol exposure has been shown to activate the intrinsic apoptotic pathway rather than the extrinsic pathway (Young et al., 2003). Briefly, the intrinsic apoptotic pathway involves translocation of members of the Bcl-2 family from the cytosol to the mitochondrial membranes increasing membrane permeability and triggering cytochrome c release. Activating factor-1 (APAF-1) and procaspase-9 bind to released cytochrome c, activating caspase-9 which activates other caspases such as caspase-3, caspase-6, and caspase-7 (D. R. Green & Amarante-Mendes, 1998; D. R. Green & Reed, 1998; Young et al., 2003; Young et al., 2005). The Bcl-2 family consists of several counterbalancing factors such as the anti-apoptotic factor, Bcl-2, and the pro-apoptotic factor, Bax (Jurgensmeier et al., 1998; Kluck, Bossy-Wetzel, Green, & Newmeyer, 1997). The expression ratio of these two specific molecules has shown to be affected by developmental ethanol exposure (Mooney & Miller, 2001; Smith et al., 2015; Ullah et al., 2011). Both Bcl-2 and Bax have been shown to be altered by exposure to ethanol during development with Bax-deficient mice showing resistance to ethanol-induced neuroapoptosis while Bcl-2 over-expressing cells show protection against ethanol-induced cell death (Britton & Miller, 2018; Camargo Moreno, Mooney, & Middleton, 2017; Mooney & Miller, 2001; Siler-Marsiglio et al., 2005; Young et al., 2003). Induction of molecules in the intrinsic pathway and apoptotic cell death in response to alcohol exposure also have downstream consequences such as changes in neuroinflammation and neurotrophic factors that can influence the developing brain (Ahlers, Karacay, Fuller, Bonthius, & Dailey, 2015; Boschen & Klintsova, 2017). Better understanding of the effect of ethanol-induced neuroapoptosis and subsequent

downstream pathways will help identify possible avenues for intervention to alleviate neurobehavioral impairments seen in FASD.

Sex Differences in FASD

While neurodevelopmental abnormalities and behavioral deficits after exposure to developmental alcohol have been extensively studied in both humans and animals, the effect of sex on these ethanol responses have been considerably less investigated. In general females have been understudied in both animal and pre-clinical research and it was not until 2014 that the National Institute of Health issued polices encouraging the use of both sexes as a biological variable in animal research (Beery, 2018; Beery & Zucker, 2011; Clayton, 2016; Clayton & Collins, 2014; Clayton & Sullivan, 2016; Klein et al., 2015). The field of neuroscience has been particularly male predominate with a recent study finding one study in females for every five studies in male (Beery, 2018). The human and animal neuroscience research papers in this same sample found that only 5.5% included both males and females and analyzed results using sex as a factor (Beery, 2018). These examples indicate that females are understudied, specifically in the neuroscience field, and that more studies including both male and females are needed to address the effect of sex.

Evaluation of Sex Differences in Humans with FASD

Overall a diagnosis of FASD is not prevalent in one sex or the other, though recent research indicates there are salient sex differences in neurological and behavioral response to developmental alcohol exposure. A recent study addressed whether one sex is more affected by prenatal alcohol exposure by comparing several physical and neurobehavioral traits in boys and girls exposed to various amounts of alcohol during development (May et al., 2017). Similar to earlier studies, sex ratio analysis revealed that boys were significantly less prevalent in FASD groups exposed to severe binge drinking during development (May et al., 2005; May et al., 2009; May et al., 2017). They conclude that compared to girls, boys are more susceptible to mortality due to prenatal alcohol exposure either by reduced survivability during prenatal development (i.e., unsuccessful pregnancies resulting in miscarriage) or increased vulnerability during early neonatal and childhood periods (May et al., 2017). While boys and girls were comparable for many physical and behavioral responses, girls exposed to prenatal alcohol exposure exhibited significantly more dysmorphology and cardinal facial features and performed significantly worse on non-verbal IQ tests compared to males (May et al., 2017). Another recent study in humans examined sex differences in a place learning task and associated neural correlates (Woods et al., 2018). This study found prenatal alcohol exposure was associated with impairments in behavioral performance in boys but not in girls (Woods et al., 2018). Boys and girls also showed marked differences in activated brain regions and navigational strategies during place learning (Woods et al., 2018). They suggest that prenatal alcohol exposure might have a greater effect on behavioral performance in boys because the regions boys rely on for spatial navigation are more vulnerable to alcohol

exposure compared to the regions activated in girls (Woods et al., 2018). Other studies have assessed comorbidities associated with FASD and found boys were more likely to be diagnosed with ADHD than girls while girls were more vulnerable to mental health problems (Herman, Acosta, & Chang, 2008; Sayal, Heron, Golding, & Emond, 2007). These recent finding suggest sex plays a role in the structural, functional, and neurobehavioral abnormalities seen in FASD and future research should employ both sexes to better understand sex-specific differences seen in prenatal alcohol exposure.

Evaluation of Sex Differences in Animal Models of FASD

Emerging evidence in animal models also emphasizes sex-specific differences in ethanol-induced neurobehavioral deficits and developmental abnormalities. For example, locomotor hyperactivity was observed in female animals exposed to prenatal alcohol but not in male animals (Hellemans, Sliwowska, Verma, & Weinberg, 2010). Conflicting results were found in a third trimester equivalent model in which a single day exposure to postnatal ethanol elicited hyperactivity in male but not female adult animals (D. F. Wozniak et al., 2004). Increases in anxiety, as tested by percentage of time spent near the edge in the open field test, have been found in male mice exposed to prenatal ethanol while no significant change in anxiety was found in female mice (Fidalgo et al., 2017). Adolescent and adult females exposed to prenatal alcohol also showed an increase of depressive-like behaviors as measured by greater immobility during a forced swim test while males showed no difference from their controls (Hellemans, Sliwowska, et al., 2010; Hellemans, Verma, et al., 2010). Both male and female animals exposed to prenatal alcohol displayed impaired memory in a social recognition test though sex differences were present depending on the delay and duration of the task (Kelly et al., 1988).

Sex differences are also seen in hippocampal-dependent memory tests though results have not been consistent. For example, exposure to high levels of ethanol during postnatal development showed significant spatial navigation impairments in adult female rats while alcohol exposure did not affect performance in adult male rats (Kelly et al., 1988). Both female and male animals exposed to prenatal alcohol showed deficits in spatial navigation using a Morris Water Maze but more salient effects in spatial processing were seen in males (Blanchard, Riley, & Hannigan, 1987). Chronic ethanol exposure during postnatal days 4-9 results in acquisition impairments and trial performance deficits in both male and female adolescent animals (Goodlett & Peterson, 1995). However, exposure to alcohol for a shorter time postnatal period (P7-9) produced place learning deficits in male but not female mice, suggesting sex-dependent temporal vulnerability (Goodlett & Peterson, 1995). Adult male and female mice exposed to prenatal alcohol displayed significant deficits in reference memory as measured by number of errors in a spatial T-maze however, only male animals showed ethanol-induced deficits in working memory (Zimmerberg et al., 1991). An acute exposure to postnatal ethanol also elicited male-specific deficits in working memory performance as measured by a spatial discrimination test in the radial arm maze (D. F. Wozniak et al., 2004). Similarly, a study using the same alcohol exposure paradigm found only male animals showed impairments in hippocampal-dependent memory measured by both a

water maze and fear conditioning tests (Ieraci & Herrera, 2007). These studies show complex sex differences in hippocampal-dependent learning and memory after exposure to developmental alcohol with males often exhibiting greater impairments and vulnerability.

The sexually dimorphic effects of developmental alcohol exposure are also beginning to be explored at the mechanistic level. For example, sex-specific gene expression changes and DNA hypomethylation after developmental alcohol exposure have been identified (Amiri et al., 2020; Lunde-Young et al., 2019; Schaffner et al., 2020). Sex-specific neuroimmune responses in the neonatal hippocampus have been found after developmental alcohol exposure. Specifically, male rats had significantly higher number of microglia and up-regulation of TNFα after acute ethanol exposure, whereas female rats showed significant increases in other neuroinflammatory molecules such as CCL4 (Ruggiero, Boschen, Roth, & Klintsova, 2018). Animals prenatally exposed to ethanol show increased stress responsiveness and hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis and sex differences are seen in these responses (as reviewed in (Weinberg, Sliwowska, Lan, & Hellemans, 2008). For example, females exposed to prenatal ethanol show increased corticosterone levels in response to acute restraint stress while no significant effect is found in prenatally exposed males (Weinberg, 1988). In contrast, males exposed to prenatal ethanol demonstrated HPA hyperactivity in response to prolonged restraint stress while females both exposed to ethanol and non-exposed controls had similar HPA activity in response to prolonged restraint stress (Weinberg, 1992). Sex differences have also been found in hippocampal cell survival with reduced cell survival in males after exposure to developmental ethanol while this effect was not seen in females (Sliwowska et al., 2010; Uban et al., 2010). Other studies show male specific differences in processes related to learning and memory such as reduced long-term potentiation (LTP) in the DG and downregulation of NMDA receptor subunits in adult male but not female mice exposed to developmental alcohol (Ieraci & Herrera, 2007; Sickmann et al., 2014). While another study found prenatal exposure to ethanol reduces LTP in adolescent males while enhancing LTP in adolescent females (Titterness & Christie, 2012). These animal studies and the aforementioned human studies reveal salient sex differences in response to developmental alcohol exposure and these functional, structural, and neurobehavioral changes are prevalent in the hippocampus.

Specific Aims

Recombinant inbred (RI) strains are highly used to study the effect of genetics for numerous complex phenotypes including alcohol responses. The largest and most well characterized family of RI strains is the BXDs generated by crossing B6 and D2 mice and inbreeding for over 20 generations (**Figure 1-1**; (Taylor et al., 1999; X. Wang et al., 2016)). There are now over 150 BXD RI strains that each represents a unique combination of homozygous parental alleles (Peirce et al., 2004). The BXD RI strains can be used to study natural genetic variation observed over a population in contrast to genetically engineered knockout animals (Morse et al., 1979; Peirce et al., 2004). Over a

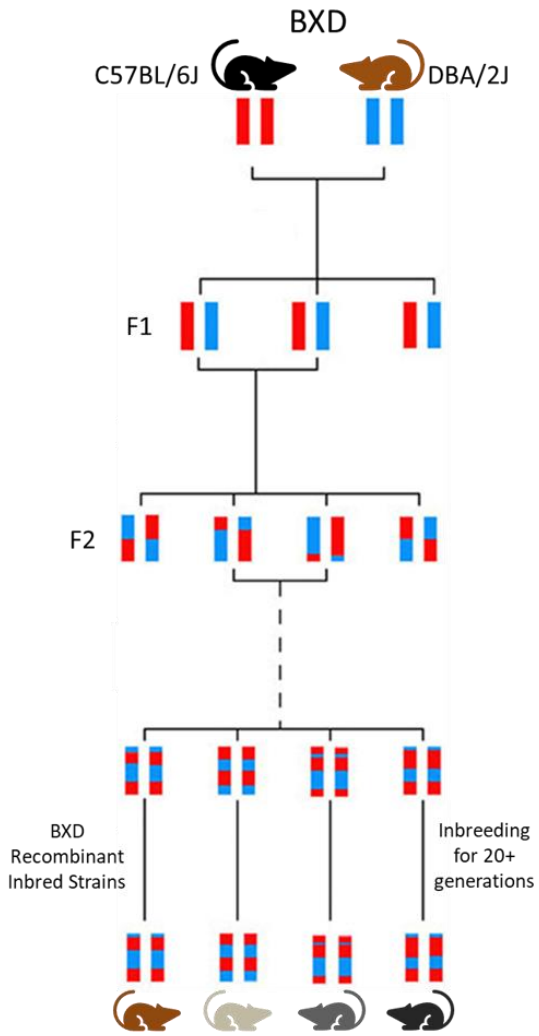

Figure 1-1. Generation of the BXD recombinant inbred mice.

The BXD recombinant inbred mice were created by crossing the C57BL/6J (B6) and DBA/2J (D2) inbred strains of mice. This initial cross results in F1 offspring that are then crossed to produce the F2 generation. Inbreeding of the F2 mice for over 20 generations results in homozygosity at almost every loci. The resulting stable and reproducible strains are known as the BXD RI strains. There are now over 150 BXD RI strains.

Sources: Wang, X., Pandey, A. K., Mulligan, M. K., Williams, E. G., Mozhui, K., Li, Z., . . . Williams, R. W. (2016). Joint mouse-human phenome-wide association to test gene function and disease risk. Nat Commun, 7, 10464. doi:10.1038/ncomms1. Taylor, B. A., Wnek, C., Kotlus, B. S., Roemer, N., MacTaggart, T., & Phillips, S. J. (1999). Genotyping new BXD recombinant inbred mouse strains and comparison of BXD and consensus maps. Mamm Genome, 10(4), 335-348. doi:10.1007/s003359900980464.

hundred BXD strains have been reliably genotyped and over 6 million variants have been characterized, to date (Chesler et al., 2003; X. Wang et al., 2016). The BXD RI family is a powerful tool to investigate variation in gene expression and phenotypic responses to different stimuli. Both B6 and D2 parental strains have been extensively studied and show marked differences in responses to alcohol exposure, making the BXD strains an ideal model to examine genetic differences in neonatal ethanol exposure (Chen et al., 2011; Downing, Balderrama-Durbin, et al., 2012; Downing, Flink, et al., 2012).

Recent work by Dr. Hamre's lab identified differential vulnerability to ethanol-induced cell death in the hippocampus using fourteen BXD strains and the two parental strains (Goldowitz et al., 2014). By identifying mean levels of caspase-3 positive cells, they identified four BXD strains that show high susceptibility to ethanol-induced cell death and three BXD strains that show low vulnerability after exposure to neonatal ethanol. Examining these BXD strains that show differential hippocampal cell death after postnatal ethanol exposure could lead to better understanding of the genetic and mechanistic factors involved in differential susceptibility to hippocampal cell death after developmental ethanol exposure. Additional examination of the effect of sex in these strains will help identify any sex-specific changes within or between strains that show differential vulnerability to ethanol-induced cell death in the postnatal hippocampus.

Aim 1: Evaluate the Effect of Genetics and Sex on Hippocampal Gene Expression Following Neonatal Ethanol Exposure in BXD RI Strains

Hippocampal gene expression was evaluated in parental B6 and D2 strains and BXD RI strains that display differential vulnerability to cell death in the hippocampus after exposure to neonatal ethanol. In the present study, we examined three BXD strains that showed increased susceptibility to ethanol-induced cell death in the hippocampus, three BXD strains that were resistant to ethanol's effect on hippocampal cell death, and the parental strains which showed moderate levels of cell death in the hippocampus after ethanol exposure. Male and females were examined separately to address the effect of sex on ethanol-induced gene expression changes in the neonatal hippocampus.

Aim 2: Evaluate Effect of Genetics and Sex on Adolescent Behavior Following Neonatal Ethanol Exposure in BXD RI Strains

In order to better understand the long-term effects of postnatal ethanol exposure, a series of neurobehavioral tests were performed in adolescent mice exposed to neonatal ethanol. Strain differences were evaluated by examining BXD RI strains that showed differential vulnerability to ethanol-induced cell death in the hippocampus and their parental strains. Male and females were examined separately to address the effect of sex on adolescent behavior following neonatal ethanol exposure in these strains. Anxiety and activity were measured during early adolescence using an elevated plus maze and open field. Hippocampal-dependent learning and memory were examined using a Y-maze and

T-maze. Results help us better understand the effect of genetics and sex on adolescent behavior following neonatal ethanol exposure.

CHAPTER 2. EFFECTS OF GENETICS AND SEX ON GENE EXPRESSION IN THE HIPPOCAMPUS FOLLOWING NEONATAL ETHANOL EXPOSURE IN BXD RECOMBINANT INBRED STRAINS

Introduction

While alcohol exposure has been shown to affect the developing brain leading to abnormalities and dysfunction associated with cognitive and behavioral deficits, the molecular mechanisms behind these alterations is less well understood. Neuro-development is a highly regulated and organized molecular process controlled by gene expression in response to developmental cues. Yet this well controlled system is extremely vulnerable to alcohol during development which has been shown to alter expression of genes involved in cell proliferation, differentiation, signaling, neurotransmission, and apoptosis (Chater-Diehl, Laufer, Castellani, Alberry, & Singh, 2016; Hard, Abdolell, Robinson, & Koren, 2005; Kleiber, Mantha, Stringer, & Singh, 2013; Lunde-Young et al., 2019; Lussier et al., 2015; Mandal, Park, Jung, & Chai, 2015; Marjonen et al., 2015). Numerous genes have been implicated in abnormal neurodevelopment after alcohol exposure though the widespread effects of ethanol across the central nervous system and the wide-ranging cognitive and behavioral abnormalities in FASD are likely do to complex interactions between multiple genes and alterations in their regulatory and biological pathways (Lunde-Young et al., 2019; Mandal et al., 2015; Zhou et al., 2011). Examination of gene expression profiles in specific tissues after exposure to developmental alcohol is important to understand molecular mechanisms underlying FASD pathogenesis and identify possible therapeutic interventions.

Developmental alcohol exposure has been shown to have both short-term (Lunde-Young et al., 2019; Mandal et al., 2015) and long-term consequences on the transcriptome (Chater-Diehl et al., 2016; Kleiber et al., 2013; Marjonen et al., 2015). These ethanol-induced gene expression changes have also been found to be dependent on tissue (Downing, Flink, et al., 2012; Lussier et al., 2015) and developmental time of exposure (Kleiber et al., 2013). A study examined long-term effects of whole brain gene expression after acute alcohol exposure in mice at three separate neurodevelopmental timepoints equivalent to the first, second, or third trimester in humans (Kleiber et al., 2013). Gene expression profiling in adults found significant ethanol-induced alterations in all three timepoints though, there was little overlap in gene expression suggesting distinct molecular pathways dependent on time of exposure (Kleiber et al., 2013). Recently, a study also found sex-dependent changes in ethanol-induced gene expression and molecular pathways in the hippocampus of mice exposed to gestational alcohol (Lunde-Young et al., 2019).

Additionally, genetic variation has also been shown to effect gene expression changes after exposure to developmental alcohol (Downing, Flink, et al., 2012; M. L. Green et al., 2007; Lossie et al., 2014). One of the earliest studies of ethanol-induced gene expression changes in mice examined two related strains of C57BL/6 mice that showed differential susceptibility to alcohol (M. L. Green et al., 2007). They found the

two strains differed in alcohol-induced malformations, gene expression changes, and response to a pharmacological therapeutic in which treatment in one strain protected against alcohol-induced malformations while treatment in the other strain exacerbated alcohol's deleterious effect on the developing embryo (M. L. Green et al., 2007). Differences in ethanol-induced gene expression have also been found in two well studied strains of mice, the B6 and D2 strains which have demonstrated differential susceptibility the teratogenic effects of ethanol (Downing, Flink, et al., 2012; Lossie et al., 2014). The B6 strain has been found to be more vulnerable to ethanol-induced developmental abnormalities while D2 mice have been found to be more resistant (Chen et al., 2011; Downing et al., 2009; Downing, Balderrama-Durbin, et al., 2012). Transcriptomic changes have also been compared in differential alcohol-induced phenotypes (Lossie et al., 2014). In this study, embryos in both B6 and D2 strains showed two distinct morphological phenotypes, either an opened or closed neural tube, after developmental alcohol exposure (Lossie et al., 2014). They identified differential gene expression between these two phenotypes and across both strains demonstrating genetic and alcohol interactions (Lossie et al., 2014). Although these studies support the role of genetic background in susceptibility to neurodevelopmental abnormalities after developmental ethanol exposure, there have been limited studies evaluating gene expression changes across these strains (Downing, Flink, et al., 2012; Lossie et al., 2014).

A great tool for studying genetic variation and differences in phenotypic response is the BXD recombinant inbred (RI) strains of mice which are generated by crossing B6 and D2 strains and inbreeding for over 20 generations (Taylor et al., 1999; X. Wang et al., 2016). The BXD strains have shown differential vulnerability to several developmental phenotypes and malformations after exposure to developmental alcohol (Downing, Balderrama-Durbin, et al., 2012; Goldowitz et al., 2014). A study by our lab found differential vulnerability to ethanol-induced apoptosis in the hippocampus using fourteen BXD strains and the two parental, B6 and D2 strains (Goldowitz et al., 2014). By identifying mean levels of caspase-3 positive cells, we identified four BXD strains that showed high susceptibility to ethanol-induced cell death and three BXD strains that showed low vulnerability after exposure to neonatal ethanol (Goldowitz et al., 2014). Examination of these BXD strains could lead to a better understanding of the genetic and mechanistic factors involved in differential susceptibility to hippocampal cell death after developmental alcohol exposure. Additional examination of both males and females will help identify sex-specific differences within or between strains that show differential vulnerability to ethanol-induced cell death in the postnatal hippocampus.

In the present study, hippocampal gene expression was examined in parental B6 and D2 strains and BXD RI strains that display differential vulnerability to cell death in the hippocampus after exposure to neonatal ethanol. Gene expression was evaluated using microarrays in three BXD strains that showed increased susceptibility to ethanol-induced cell death in the hippocampus, three BXD strains that were resistant to ethanol's effect on hippocampal cell death, and the parental strains which showed moderate levels of cell death in the hippocampus after ethanol exposure. Males and females were examined separately to address the effect of sex on ethanol-induced gene expression changes in the neonatal hippocampus. Enrichment analysis was used to identify

biological and molecular pathways associated with differentially expressed genes. We hypothesize that the previously identified high cell death strains will show greater differential gene expression after exposure to developmental alcohol compared to the low cell death strains. We also theorize that there will be some sex-specific differences in hippocampal gene expression in these strains as sex-dependent gene expression changes after gestational alcohol exposure has recently been reported as well as some sex-by-genotype interactions (Lunde-Young et al., 2019).

Materials and Methods

Animals

Original breeders were obtained from either Dr. Robert Williams at the University of Tennessee Health Science Center (UTHSC) or Jackson Laboratory (City, State). All treatments and experiments were approved by the Institutional Animal Care and Use Committee at UTHSC. The present study aims to identify genetic networks that influence susceptibility to ethanol-induced cell death in the hippocampus of male and female neonatal mice. To test this, mouse strains were examined including, C57BL/6J (B6), DBA/2J (D2), and BXD recombinant inbred (RI) strains that showed differential susceptibility to ethanol-induced cell death in the developing hippocampus (Goldowitz et al., 2014). BXD2, BXD48a, and BXD100 showed higher susceptibility to ethanol-induced cell death in the hippocampus while BXD60, BXD71, and BXD73 showed low vulnerability to ethanol-induced cell death in hippocampus (**Figure 2-1**).

Once all strains were acquired, breeding was conducted at UTHSC. Breeders were the products of on-site mating and thus breeders were not affected by excess stressors such as travel and relocation. Mice were maintained on a 12:12 light:dark cycle and given food and water ad libitum. Environmental enrichments (igloo house and paper bedding) were placed in each mouse cage throughout all experiments. Breeding cages were maintained with multiple male and female mice over 60 days of age. Breeders were checked multiple times per week to assess female mice. When female mice appeared pregnant, they were placed alone in a clean cage and monitored daily for pups. Pregnant dams were separated to 1) acclimate the dam to new cage and reduce stress, 2) control for differences in pup rearing with other adult male and female mice in the original breeding cage, and 3) to allow for close monitoring of pups without disturbing other breeders. On average dams were placed in cage alone a week prior to birth. The date of birth was recorded and defined as postnatal day 0 (P0). The first litter from each mother was skipped and not used for experiments to control for differences in maternal care for first time mothers. Only litters of 4 or more were kept while litters greater than 8 were culled. Pups were undisturbed until treatment on postnatal day 7 (P7).

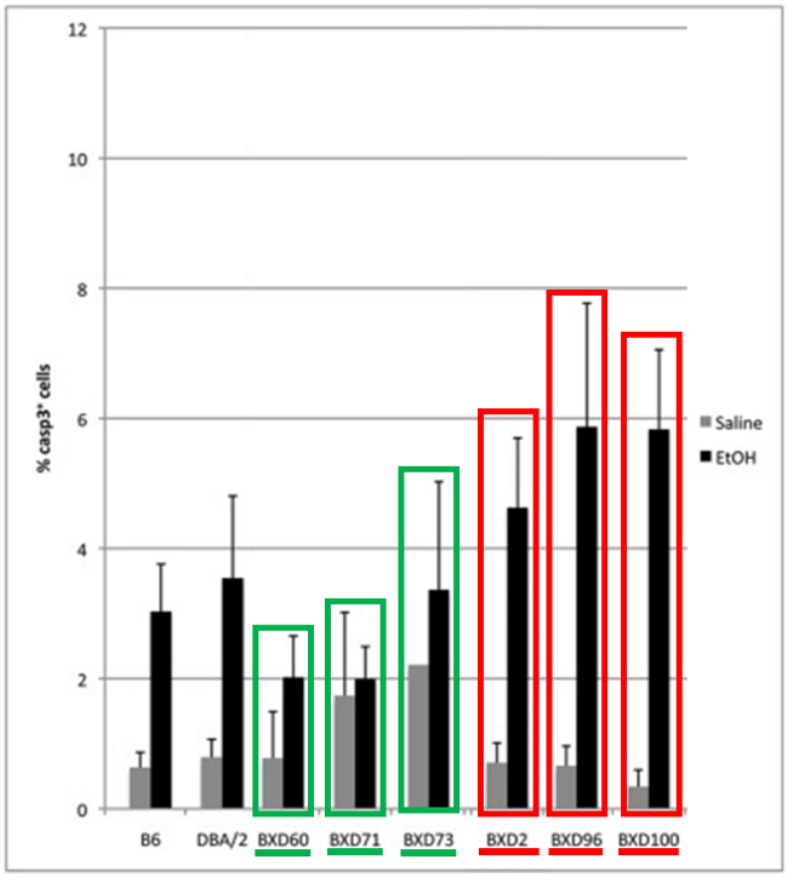

Figure 2-1. Cell death in the CA1 region of the hippocampus and identification of the strains used in the current study.

Cell death in the CA1 region of the hippocampus determined by caspase-3 immunostaining. Cell death was examined in postnatal day 7 mice seven hours after initial ethanol exposure during the peak of ethanol-induced cell death. Controls were given an isovolumetric dose of saline. Strains shown are the two parental, B6 and D2 strains, the three low cell death strains: BXD60, BXD71, BXD73 (outlined in green), and the three high cell death strains: BXD2 BXD48a (aka BXD96), BXD100 (outlined in red).

Ethanol Exposure

Neonatal mice were treated on postnatal day (P) 7 which is a developmental time point during the third trimester in humans. For mice, P7 is the middle of the brain growth spurt, a time during which neurons are completing migration, differentiation, establishing connections through synaptogenesis and dendrite arborization and natural programmed cell death is occurring (Alfonso-Loeches & Guerri, 2011; Gil-Mohapel et al., 2010; Marquardt & Brigman, 2016). Pups were brought to a separate testing room in their cage with their mother between 9:00AM and 10:00AM. Litters were then placed in clean cage on a heating pad while they were weighed, dosed, then promptly placed back in their home cage with their mother. Pups were split into either an ethanol or control group (**Figure 2-2**). As in previous studies, ethanol treated animals received 20% ethanol in sterile saline though subcutaneous injection. The total dose of ethanol was 5.0 g/kg split in two 2.5 g/kg doses, given two hours apart while controls received an isovolumetric volume injection of sterile saline (Goldowitz et al., 2014). This ethanol exposure represents an acute neonatal binge which has been shown to produce BACs of approximately 350 mg/dl in P7 neonatal mice (Goldowitz et al., 2014; Schaffner et al., 2020). Early prenatal and postnatal rodent studies of blood alcohol concentrations found no differences in BAC levels across multiple strains including B6 and D2 mouse strains (Boehm et al., 1997; Goodlett et al., 1989). As parental B6 and D2 strains do not differ in BACs and because collection of enough blood for BAC is lethal to neonatal pups, additional pups were not produced for this measure. Littermates were used when possible and a maximum of one male and one female per group were used per litter. A minimum of four litters per strain were used in this study.

Tissue Harvest and RNA Extraction

Animals were sacrificed 7 hours after the first injection which is the peak of ethanol-induced cell death in the hippocampus (Olney et al., 2002). Pups were briefly exposed to isoflurane and quickly decapitated. The hippocampus was dissected, and flash frozen in liquid nitrogen and stored in an -80°C freezer until processing. Purification of total RNA was accomplished with the RNeasy Mini Kit (Qiagen, Maryland, United States) using the Qiagen QIAcube (Qiagen, Maryland, United States) following the manufactures protocol for purification of total RNA from easy-to-lyse animal tissues and cells. RNA concentration and purity were measured using NanoDrop 1000 Spectrophotometer (NanoDrop Technologies Delaware, United States). Samples were prepared for Affymetrix arrays and was completed by experienced technicians in the Molecular Resource Center, an Institutional Core at the University of Tennessee Health Science Center. RNA integrity was evaluated using Eukaryote Total RNA Nano Chip and measured using the Agilent 2100 Bioanalyzer (Agilent, California, United States). The RNA Integrity Number (RIN) was used to as quality control. The average RIN was 9.87 + 0.17, indicating excellent sample quality.

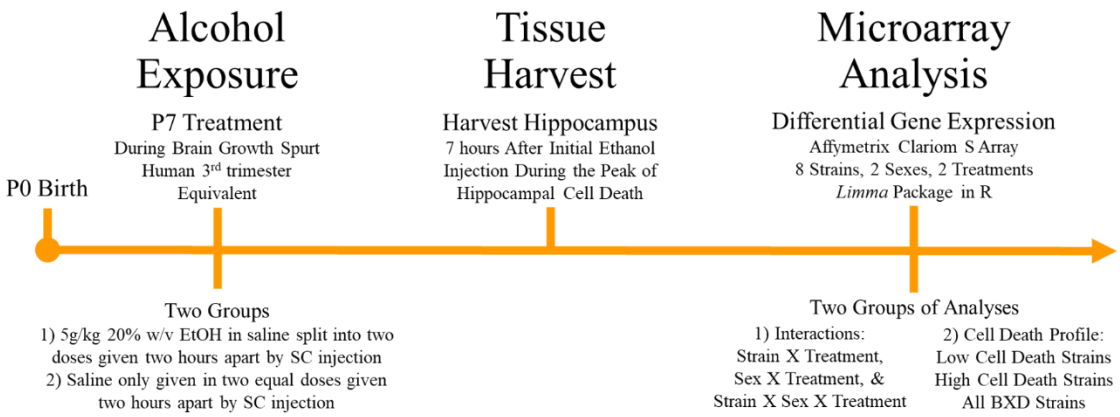

Figure 2-2. Overview of experimental design for the gene expression study.

An overview of the alcohol exposure paradigm (left), tissue harvest (middle), and microarray analysis (right).

Gene Expression Microarray and Data Processing

The Affymetrix Genechip Mouse Clariom S was used to examine gene expression (Affymetrix, California, United States). Two hundred nanograms of DNased total RNA was amplified, labeled, and fragmented using Ambion Whole Transcript (WT) Expression Kit according to manufacturer's protocol (Thermo Fisher Scientific, Santa Clara, California United States). Briefly, samples are hybridized overnight according to manufacturer's protocols; samples are then washed and stained on Affymetrix GeneChip Fluidics Station 450 (Affymetrix, California, United States). Samples were then scanned using the GeneChip Scanner 3000 (Applied Biosystems, California, United States). Data was normalized and analyzed for quality control in Affymetrix Expression Console Software using RMA-sketch normalization (Affymetrix, California, United States). After normalization and quality control, a total number of 22,203 probe sets were used for subsequent data analysis. A total of 128 samples were used—4 samples per treatment (control, ethanol), sex (male, female), and strain (B6, D2, BXD2, BXD48a, BXD60, BXD71, BXD73, BXD100) (**Figure 2-2**). Samples were matched for treatment and sex within a litter when possible. Principle component analysis (PCA) was used to visualize the data and identify sources of variation as well as identify potential sample outliers. PCA was conducted in R software environment (version 4.1) with the PCA package and ggplot2 package (version 3.3.3) using the function *comput.pca* (Blighe and Lun, 2021). PCA analysis did show some variation that was not due to strain, sex, or treatment and was not limited to few samples (**Appendix A, Figure A-1**). Due to the inclusion of eight strains and two sexes, our sample size was relatively small and therefore no samples were excluded from the analysis.

Differential Expression Analysis

Differential expression was analyzed using the limma package (version 3.13) in the R (version 4.1) software environment (Ferguson et al., 2019; Ritchie et al., 2015). Expression differences between saline-treated and ethanol-treated samples were conducted for each strain and sex (e.g., BXD2Male, BXD2Female, etc.). Benjamini-Hochberg post-hoc test was used to correct for multiple testing or False Discovery Rate (FDR) (Benjamini, Drai, Elmer, Kafkafi, & Golani, 2001; Chipman & Tibshirani, 2006). Significant differential expression within strain and sex was defined as an adjusted p-value ≤ 0.05 and Fold Change (FC) ≥ 1.5. Due to the extensiveness of this project across eight strains, two sexes, and two treatments, as well as limited tissue availability, microarray validation was not included in this study (Osterndorff-Kahanek et al., 2015; Vornholt et al., 2020). The microarray data is publicly available and can be accessed on Gene Network (www.genenetwork.org).

Analysis 1. The effect of ethanol treatment across all strains and sexes was analyzed as well as the following interactions: strain x treatment, sex x treatment, and strain x sex x treatment (**Figure 2-2**). Significant interactions were defined as adj$p < 0.05$ and the number of genes with FC ≥ 1.5 were determined. For some interactions,

differentially expressed genes lists were extended to include those significant at nominal p-values ($p < 0.05$) for expanded descriptive analyses (Terenina et al., 2019).

Analysis 2. To analyze the relationship between differential hippocampal cell death and differential hippocampal gene expression after exposure to neonatal ethanol, BXD strains were grouped based on previously identified cell death profile, i.e., high cell death or low cell death (Goldowitz et al., 2014). Due to significant sex differences, males and females were analyzed separately though any overlap between the sexes was reported. Significantly expressed genes across all high cell death strains (HCDS) (BXD2, BXD48a, BXD100) and all low cell death strains (LCDS) (BXD60, BXD71, BXD73) were identified. Of specific interest were genes that were 1) only significantly differentially expressed in the LCDS but not in the HCDS, 2) only significantly differentially expressed in the HCDS but not the LCDS, and 3) significantly differentially expressed genes across all BXD strains regardless of previously identified cell death profile (**Figure 2-2**).

Gene Enrichment Analysis

Enrichment analysis was performed using tools available at WebGestalt (www.webgestalt.org) (Y. Liao, Wang, Jaehnig, Shi, & Zhang, 2019; L. Wang et al., 2013; Zhang & Horvath, 2005). Gene Ontology (GO) analysis was performed to determine over-representation by functional categories. Gene Symbols were used as inputs for all lists and suggested parameters were used—at least 5 genes per category and significance of adjusted $p < 0.05$ (FDR) based on Benjamini-Hochberg adjustment for multiple testing (Benjamini et al., 2001).

Results

Treatment Effect Across Strains and Sexes

All strains showed significant hippocampal gene expression changes after exposure to neonatal ethanol. As hypothesized, the significant genes were very specific within each strain. However, a little more surprising was the limited overlap in significant ethanol-induced gene expression changes between males and females of the same strain. Even parental strains showed differential effects of sex with greater ethanol-induced gene expression changes in B6 females compared to B6 males while ethanol-induced gene expression changes were greater in D2 males compared to D2 females (**Figure 2-3**).

The following provides an overview of the number of significantly differentially expressed genes in males, females, and overlap between the sexes in parental and BXD strains (**Figure 2-4**). B6 males (B6M) show 166 genes significantly expressed ($adjp < 0.05$) while B6 females (B6F) show 231 genes ($adjp < 0.05$), with 92 genes significant in both B6M and B6F (**Figure 2-3A**). D2 males (D2M) show 1,026 genes significantly expressed ($adjp < 0.05$) while D2 females (D2F) show 332 genes ($adjp < 0.05$), with 237

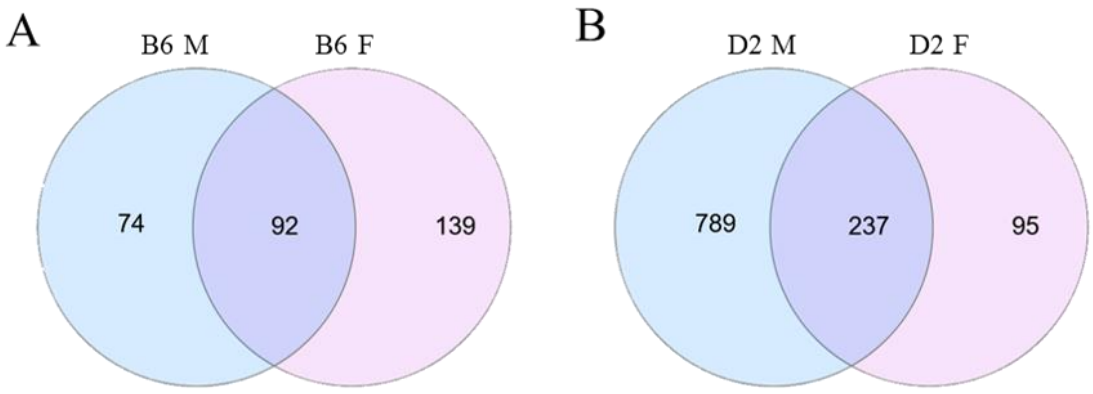

Figure 2-3. Sex-specific ethanol-induced gene expression changes in parental B6 and D2 strains.

Differential effects of sex in the parental (A) B6 and (B) D2 strains. For both strain, blue circles (left) represent number of significant (*adjp* < 0.05) ethanol-induced gene expression changes in males. Pink circles (right) represent number of significant (*adjp* < 0.05) ethanol-induced gene expression changes in females. The purple overlap between the two circles (middle) represents number of significant (*adjp* < 0.05) ethanol-induced gene expression changes that were present in both males and females.

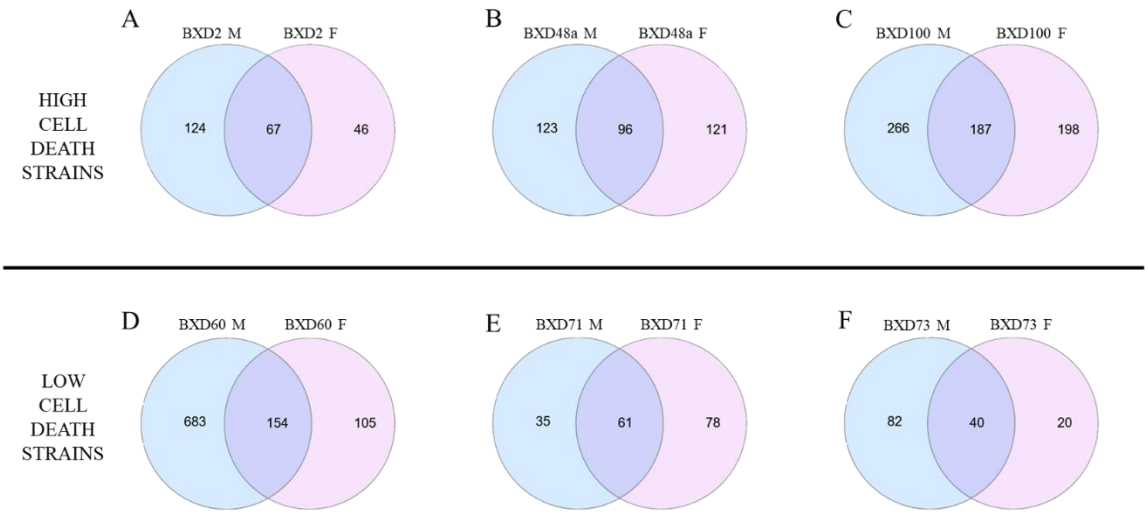

Figure 2-4. Sex-specific ethanol-induced gene expression changes in BXD strains.

Differential effects of sex in the high cell death strains (top): (A) BXD2, (B) BXD48a, (C) BXD100, and low cell death strains (bottom): (D) BXD60, (E) BXD71, (F) BXD73. For each strain, blue circles (left) represent number of significant (*adjp* < 0.05) ethanol-induced gene expression changes in males. Pink circles (right) represent number of significant (*adjp* < 0.05) ethanol-induced gene expression changes in females. The purple overlap between the two circles (middle) represents number of significant (*adjp* < 0.05) ethanol-induced gene expression changes that were present in both males and females.

genes significant in both D2M and D2F (**Figure 2-3B**). BXD2 males (BXD2M) show 191 genes significantly expressed (*adjp* < 0.05) while BXD2 females (BXD2F) show 113 genes (*adjp* < 0.05), with 67 genes significant in both BXD2M and BXD2F (**Figure 2-4A**). BXD48a males (BXD48aM) show 219 genes significantly expressed (*adjp* < 0.05) while BXD48a females (BXD48aF) show 217 genes (*adjp* < 0.05), with 96 genes significant in both BXD48aM and BXD48aF (**Figure 2-4B**). BXD100 males (BXD100M) show 453 genes significantly expressed (*adjp* < 0.05) while BXD100 females (*BXD100F*) show 385 genes (*adjp* < 0.05), with 187 genes significant in both BXD100M and BXD100F (**Figure 2-4C**). BXD60 males (BXD60M) show 837 genes significantly expressed (*adjp* < 0.05) while BXD60 females (BXD60F) show 259 genes (*adjp* < 0.05), with 154 genes significant in both BXD60M and BXD60F (**Figure 2-4D**). BXD71 males (BXD71M) show 96 genes significantly expressed (*adjp* < 0.05) while BXD71 females (BXD71F) show 139 genes (*adjp* < 0.05), with 61 genes significant in both BXD71M and BXD71F (**Figure 2-4E**). Finally, BXD73 males (BXD73M) show 122 genes significantly expressed (*adjp* < 0.05) while BXD73 females (BXD73F) show 60 genes (*adjp* < 0.05), with 40 genes significant in both BXD73M and BXD73F (**Figure 2-4F**).

Strain x Treatment Interactions

Strain x Treatment Interactions were examined using all strains and both sexes. There were 6,863 genes that were significantly differentially expressed (adj*p* < 0.05) across the BXD and parental strains after exposure to neonatal ethanol. Gene Ontology was performed to identify over-represented categories within the list of genes by parameters listed above. With the parameters described above, genes that were significant for strain x treatment interactions were found to be significantly (FDR < 0.05) over-expressed in 2,119 gene ontology pathways (**Chap. 2 Supplemental Table 1**). Certain pathways involved in apoptosis, development, learning, and sex differences were closely examined. Numerous cell death and apoptotic pathways were found including regulation of neuron apoptotic process, positive regulation of neuron death, apoptotic mitochondrial changes, intrinsic apoptotic signaling pathway in response to DNA damage, regulation of intrinsic apoptotic signaling pathway, and intrinsic apoptotic signaling pathway by p53 class mediator. There were also multiple development pathways found such as central nervous system maturation, hindbrain development, forebrain development, regulation of dendrite development, regulation of dendrite morphogenesis, neuron projection guidance and hippocampal development. Behavioral categories were also found including learning or memory, long-term memory, and behavioral fear response. Several neuroimmune pathways were identified such as cellular response to transforming growth factor beta stimulus, regulation of tumor necrosis factor production, tumor necrosis factor superfamily cytokine production, positive regulation of dendritic cell cytokine production, and cellular response to IL-6. Various epigenetic pathways found such as histone lysine methylation, positive regulation of histone deacetylation, and regulation of histone H4 acetylation. Alcohol metabolism pathways were also found including cellular response to alcohol, alcohol metabolic process, and primary alcohol metabolic process.

Finally, sex differentiation pathway was found to be significantly over-represented in genes that were significant for Strain x Treatment interactions.

To further narrow down the thousands of genes that were significant for Strain x Treatment Interactions, we examine the fold-change (FC) differences between control and ethanol treated animals. There were 210 genes that were significant at $adjp < 0.05$ and showed a FC > 1.5 (**Table 2-1**).

Sex x Treatment Interactions

Sex x Treatment Interactions were examined using all strains and both sexes. There were 1,297 genes that were significantly differentially expressed ($p < 0.05$) across males and females after exposure to neonatal ethanol. The nominal p-value was used in the Sex x Treatment interaction as the adjusted *p*-value yielded 0 significant interactions. There were no significant categories that were identified through Gene Ontology analysis in the 1,297 ($p < 0.05$) significant genes. Of the 1,297 genes, only 9 genes showed FC > 1.5: *4930519F16Rik*, *A630073D07Rik*, *Axdnd1*, *Col1a2*, *Foxp2*, *Gm11115*, *Hscb*, *Htr1d*, and *Klhl14* (**Table 2-2**).

Strain x Sex x Treatment Interactions

Strain x sex x treatment Interactions were examined using all strains and both sexes. There were 4,866 genes that were significantly differentially expressed ($p < 0.05$) across all strains and both sexes after exposure to neonatal ethanol. Gene Ontology analysis was again used to identify over-represented pathways from the 4,866 significantly differentially expressed genes. With the parameters described above, genes that were significant for strain x sex x treatment interaction were found to be significantly (FDR < 0.05) over-expressed in 1,144 gene ontology pathways (**Chap. 2 Supplemental Table 2**). Over-represented categories involving proliferation and apoptosis were closely examined. Many cell proliferation and cell migration categories were identified including regulation of neural precursor cell proliferation, cerebral cortex radial glia guided migration, telencephalon cell migration, neural precursor cell proliferation, regulation of stem cell proliferation, and glial cell migration. There were 28 cell death and apoptotic pathways were also identified such as positive regulation of apoptotic process, apoptotic signaling pathway, neuron apoptotic process, positive regulation of cell death, intrinsic apoptotic signaling pathway, and necrotic cell death.

Over-represented categories involving gene expression regulation as well as aspects of brain development and organization were also closely examined. Categories involving gene expression regulation included posttranscriptional regulation of gene expression, mitochondrial gene expression, and circadian regulation of gene expression. Numerous categories involved in brain development and organization including neuron projection organization, neuron projection morphogenesis, axon development, regulation

Table 2-1. Differentially expressed genes that showed significant strain x treatment interaction and fold change > 1.5.

Accession	Gene Symbol	Gene Name	*Adj p*-Value
NR_015488	*A930003A15Rik*	RIKEN cDNA A930003A15 gene	4.29E-26
NR_033609	*A930017M01Rik*	RIKEN cDNA A930017M01 gene	4.49E-23
NM_030210	*Aacs*	acetoacetyl-CoA synthetase	3.23E-27
NM_001166556	*Abca6*	ATP binding cassette subfamily A member 6	2.43E-15
NM_013851	*Abca8b*	ATP-binding cassette, sub-family A (ABC1), member 8b	6.79E-26
NM_178162	*Agfg2*	ArfGAP with FG repeats 2	7.23E-27
NM_007428	*Agt*	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)	7.82E-15
NM_001172146	*Aimp2*	aminoacyl tRNA synthetase complex-interacting multifunctional protein 2	2.43E-23
NM_019764	*Amotl2*	angiomotin-like 2	1.94E-17
NM_020581	*Angptl4*	angiopoietin-like 4	6.11E-27
NM_001024851	*Ankrd34a*	ankyrin repeat domain 34A	1.84E-19
NM_028390	*Anln*	anillin, actin binding protein	5.2E-13
NM_001109914	*Apold1*	apolipoprotein L domain containing 1	1.16E-18
NM_009705	*Arg2*	arginase type II	2.83E-16
NM_001172205	*Arid5a*	AT rich interactive domain 5A (MRF1-like)	6.65E-29
NM_023598	*Arid5b*	AT rich interactive domain 5B (MRF1-like)	1.8E-33
NM_001042591	*Arrdc3*	arrestin domain containing 3	1.48E-47
NM_001042592	*Arrdc4*	arrestin domain containing 4	1.68E-29
NM_016847	*Avpr1a*	arginine vasopressin receptor 1A	1.21E-29
NM_001159407	*B3gnt5*	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5	1.13E-25
NM_001284410	*Bcl2l11*	BCL2-like 11 (apoptosis facilitator)	7.31E-40
NM_007570	*Btg2*	BTG anti-proliferation factor 2	1.05E-33
NM_009770	*Btg3*	BTG anti-proliferation factor 3	2.19E-27
NM_016859	*Bysl*	bystin-like	2.35E-18
NM_144817	*Camk1g*	calcium/calmodulin-dependent protein kinase I gamma	3.04E-28
NM_178396	*Car12*	carbonic anhydrase 12	5.9E-06
NM_144820	*Ccdc28a*	coiled-coil domain containing 28A	1.43E-22
NM_011337	*Ccl3*	chemokine (C-C motif) ligand 3	1.47E-14
NM_007631	*Ccnd1*	cyclin D1	1.59E-24
NM_001170395	*Cd163*	CD163 antigen	6.04E-10
NM_007646	*Cd38*	CD38 antigen	8.27E-18
NM_001111060	*Cd59a*	CD59a antigen	1.05E-13
NM_001081345	*Chd2*	chromodomain helicase DNA binding protein 2	1.32E-26

Table 2-1. Continued.

Accession	Gene Symbol	Gene Name	*Adj p*-Value
NM_001271496	*Chka*	choline kinase alpha	2.3E-13
NM_001033302	*Ciart*	circadian associated repressor of transcription	2.05E-15
NM_001243762	*Clcn5*	chloride channel, voltage-sensitive 5	4.55E-28
NM_016674	*Cldn1*	claudin 1	0.000118
NM_172469	*Clic6*	chloride intracellular channel 6	0.044261
NM_153384	*Clrn1*	clarin 1	3.27E-19
NM_009898	*Coro1a*	coronin, actin binding protein 1A	1.63E-18
NM_001252525	*Cpeb1*	cytoplasmic polyadenylation element binding protein 1 [*Mus musculus*	2.06E-24
NM_011957	*Creb3l1*	cAMP responsive element binding protein 3-like 1 [*Mus musculus*	6.47E-15
NM_172728	*Creb5*	cAMP responsive element binding protein 5	1E-26
NM_001110850	*Crem*	cAMP responsive element modulator	2.33E-33
NM_007762	*Crhr1*	corticotropin releasing hormone receptor 1	7.82E-32
NM_001145799	*Ctla2a*	cytotoxic T lymphocyte-associated protein 2 alpha	9.6E-17
NM_153775	*Ctu2*	cytosolic thiouridylase subunit 2	7.39E-17
NM_009994	*Cyp1b1*	cytochrome P450, family 1, subfamily b, polypeptide 1	2.67E-23
NM_001177713	*Cyp26b1*	cytochrome P450, family 26, subfamily b, polypeptide 1	6.96E-14
NM_028979	*Cyp2j9*	cytochrome P450, family 2, subfamily j, polypeptide 9	3.5E-28
NM_010516	*Cyr61*	cellular communication network factor 1	2.2E-18
NM_007837	*Ddit3*	DNA-damage inducible transcript 3	1.24E-15
NM_053272	*Dhcr24*	24-dehydrocholesterol reductase	3.8E-48
NR_002854	*Dlx1as*	distal-less homeobox 1	2.48E-17
NM_013642	*Dusp1*	dual specificity phosphatase 1	2.94E-47
NM_019819	*Dusp14*	dual specificity phosphatase 14	6.09E-16
NM_001048054	*Dusp16*	dual specificity phosphatase 16	6.68E-35
NM_176933	*Dusp4*	dual specificity phosphatase 4	9.31E-19
NM_026268	*Dusp6*	dual specificity phosphatase 6	7.13E-26
NM_153459	*Dusp7*	dual specificity phosphatase 7	1.11E-16
NM_008748	*Dusp8*	dual specificity phosphatase 8	5.63E-41
NM_010104	*Edn1*	endothelin 1	9.3E-23
NM_001289925	*Egr3*	early growth response 3	6.61E-34
NM_018781	*Egr3*	early growth response 3	3.46E-15
NM_175522	*Elfn1*	leucine rich repeat and fibronectin type III, extracellular 1	1.53E-16
NM_008815	*Etv4*	ets variant 4	6.1E-27
NM_023794	*Etv5*	ets variant 5	3.01E-30
NM_183187	*Fam107a*	family with sequence similarity 107, member A	6.75E-42

Table 2-1. Continued.

Accession	Gene Symbol	Gene Name	*Adj p*-Value
NM_153574	*Fam13a*	family with sequence similarity 13, member A	5.34E-17
NM_178908	*Fam26e*	calcium homeostasis modulator family member 5	3.31E-36
NM_175104	*Fam53c*	family with sequence similarity 53, member C	1.05E-27
NM_011812	*Fbln5*	fibulin 5	0.033886
NM_026346	*Fbxo32*	F-box protein 32	1.33E-14
NM_010191	*Fdft1*	farnesyl diphosphate farnesyl transferase 1	2.21E-42
NM_001253751	*Fdps*	farnesyl diphosphate synthetase	2.05E-23
NM_010197	*Fgf1*	fibroblast growth factor 1	1.61E-13
NM_001163215	*Fgfr3*	fibroblast growth factor receptor 3	2.78E-24
NM_001164259	*Fgfrl1*	fibroblast growth factor receptor-like 1	5.65E-24
NM_001159706	*Folh1*	folate hydrolase 1	4.16E-26
NM_011817	*Gadd45g*	growth arrest and DNA-damage-inducible 45 gamma	3.55E-16
NM_028022	*Gatsl3*	cytosolic arginine sensor for mTORC1 subunit 1	2.97E-19
NM_001010937	*Gjb6*	gap junction protein, beta 6	1.87E-11
NM_029102	*Glt8d2*	glycosyltransferase 8 domain containing 2	1.77E-22
NM_001110809	*Gpatch4*	G patch domain containing 4	1.7E-20
NM_027518	*Gpr137c*	G protein-coupled receptor 137C	1.33E-46
NM_010338	*Gpr37*	G protein-coupled receptor 37	2.49E-27
NM_145066	*Gpr85*	G protein-coupled receptor 85	6.2E-26
NM_153419	*Grwd1*	glutamate-rich WD repeat containing 1	1.08E-21
NM_008216	*Has2*	hyaluronan synthase 2	6.21E-16
NM_198962	*Hcrtr2*	hypocretin (orexin) receptor 2	7.02E-22
NM_144835	*Heatr1*	HEAT repeat containing 1	2.73E-26
NM_175256	*Heg1*	heart development protein with EGF like domains 1	6.46E-21
NM_001162950	*Hif3a*	hypoxia inducible factor 3, alpha subunit	2.61E-17
NM_008252	*Hmgb2*	high mobility group box 2	1.16E-18
NM_008255	*Hmgcr*	3-hydroxy-3-methylglutaryl-Coenzyme A reductase	1.14E-33
NM_008256	*Hmgcs2*	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	1.58E-27
NM_007545	*Hrk*	harakiri, BCL2 interacting protein (contains only BH3 domain)	9.28E-20
NM_010476	*Hsd17b7*	hydroxysteroid (17-beta) dehydrogenase 7	2.95E-38
NM_019564	*Htra1*	HtrA serine peptidase 1	2.19E-30
NM_015790	*Icosl*	icos ligand	1E-17
NM_010495	*Id1*	inhibitor of DNA binding 1, HLH protein	4.58E-17
NM_031166	*Id4*	inhibitor of DNA binding 4	1.3E-14
NM_145360	*Idi1*	isopentenyl-diphosphate delta isomerase	1.85E-48
NM_001101605	*Ifit1bl1*	interferon induced protein with tetratricpeptide repeats 1B like 1	6.77E-16

Table 2-1. Continued.

Accession	Gene Symbol	Gene Name	*Adj p*-Value
NM_013562	*Ifrd1*	interferon-related developmental regulator 1	5.04E-24
NM_172439	*Inpp5j*	inositol polyphosphate 5-phosphatase J	7.71E-25
NM_153526	*Insig1*	insulin induced gene 1	1.44E-42
NM_016851	*Irf6*	interferon regulatory factor 6	5.41E-19
NM_010591	*Jun*	Jun proto-oncogene, AP-1 transcription factor subunit	3.2E-60
NM_001286944	*Jund*	JunD proto-oncogene, AP-1 transcription factor subunit	1.05E-34
NM_001081134	*Kcng1*	potassium voltage-gated channel, subfamily G, member 1	7.56E-30
NM_001110227	*Kcnj13*	potassium inwardly-rectifying channel, subfamily J, member 13	0.026908
NR_045177	*Kctd16*	potassium channel tetramerisation domain containing 16	1.43E-27
NM_026135	*Kctd16*	potassium channel tetramerisation domain containing 16	9.88E-21
NM_078477	*Klf16*	Kruppel-like factor 16	6.82E-34
NM_001252658	*Ldlr*	low density lipoprotein receptor	2.07E-37
NM_001083125	*Lhx6*	LIM homeobox protein 6	3.47E-15
NM_028894	*Lonrf3*	LON peptidase N-terminal domain and ring finger 3	1.79E-14
NM_181470	*Ltv1*	LON peptidase N-terminal domain and ring finger 3	3.8E-18
NM_001271416	*Ly6a*	lymphocyte antigen 6 complex, locus A	1.4E-13
NM_001252055	*Ly6c1*	lymphocyte antigen 6 complex, locus C1	3.71E-24
NM_001171187	*Mal*	myelin and lymphocyte protein, T cell differentiation protein	3.92E-17
NM_011737	*Map3k19*	mitogen-activated protein kinase kinase kinase 19	6.66E-09
NM_008563	*Mcm3*	minichromosome maintenance complex component 3	1.28E-16
NM_001012335	*Mdk*	midkine	4.22E-14
NM_008587	*Mertk*	MER proto-oncogene tyrosine kinase	1.83E-26
NM_001163833	*Msl3l2*	MSL3 like 2	2.4E-15
NM_025436	*Msmo1*	methylsterol monoxygenase 1	5.2E-43
NM_013602	*Mt1*	metallothionein 1	4.56E-34
NM_008630	*Mt2*	metallothionein 2	1.98E-43
NM_138656	*Mvd*	mevalonate diphosphate decarboxylase	6.34E-38
NM_001008542	*Mxi1*	MAX interactor 1, dimerization protein	1.63E-46
NM_178728	*Napepld*	N-acyl phosphatidylethanolamine phospholipase D	2.98E-14
NM_022565	*Ndst4*	N-deacetylase/N-sulfotransferase (heparin glucosaminyl) 4	2.63E-17
NM_028995	*Nipal3*	NIPA-like domain containing 3	6.65E-23
NM_024193	*Nop56*	NOP56 ribonucleoprotein	5.39E-23
NM_010342	*Npbwr1*	neuropeptides B/W receptor 1	1.85E-20

Table 2-1. Continued.

Accession	Gene Symbol	Gene Name	*Adj p*-Value
NM_178644	*Oaf*	out at first homolog	5.42E-16
NM_011859	*Osr1*	odd-skipped related transcription factor 1	1.91E-05
NM_001286481	*Otx2*	orthodenticle homeobox 2	0.030486
NM_001008497	*P2ry14*	purinergic receptor P2Y, G-protein coupled, 14	1.22E-11
NM_153594	*Pcmtd2*	protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2	3.5E-25
NM_016861	*Pdlim1*	PDZ and LIM domain 1 (elfin)	6.76E-17
NM_001159367	*Per1*	period circadian clock 1	4.91E-24
NM_178149	*Pik3ip1*	phosphoinositide-3-kinase interacting protein 1	5.02E-37
NM_001024955	*Pik3r1*	phosphoinositide-3-kinase regulatory subunit 1	2.81E-52
NM_145478	*Pim3*	proviral integration site 3	1.05E-13
NM_008872	*Plat*	plasminogen activator, tissue	1.5E-19
NM_152813	*Plcd3*	phospholipase C, delta 3	4.03E-28
NM_001033253	*Plekhg1*	pleckstrin homology domain containing, family G (with RhoGef domain) member 1	1.02E-38
NM_013807	*Plk3*	polo like kinase 3	1.7E-31
NM_001164630	*Pwwp3b*	PWWP domain containing 3B	7.21E-14
NM_029494	*Rab30*	RAB30, member RAS oncogene family	4.38E-44
NM_001099624	*Rapgef2*	Rap guanine nucleotide exchange factor 2	4.6E-46
NM_001252494	*Rapgef6*	Rap guanine nucleotide exchange factor (GEF) 6	8.64E-46
NM_009062	*Rgs4*	regulator of G-protein signaling 4	1.98E-23
NM_153514	*Rhobtb2*	Rho related BTB domain containing 2	3.77E-24
NM_172612	*Rnd1*	Rho family GTPase 1	1.45E-21
NM_001166553	*Rnf145*	ring finger protein 145	1.01E-33
NM_133982	*Rpp25*	ribonuclease P/MRP 25 subunit	4.57E-22
NM_146244	*Rps6kl1*	ribosomal protein S6 kinase-like 1	1.01E-33
NM_011521	*Sdc4*	syndecan 4	6.79E-33
NM_030261	*Sesn3*	sestrin 3	4.25E-32
NM_016687	*Sfrp4*	secreted frizzled related protein 4	1E-15
NM_001168525	*Sgms1*	sphingomyelin synthase 1	8.19E-14
NM_028943	*Sgms2*	sphingomyelin synthase 2	3.96E-05
NM_027921	*Slc16a14*	solute carrier family 16 (monocarboxylic acid transporters), member 14	3.51E-27
NM_001029842	*Slc16a6*	solute carrier family 16 (monocarboxylic acid transporters), member 6	1.28E-23
NM_025807	*Slc16a9*	solute carrier family 16 (monocarboxylic acid transporters), member 9	2.35E-07
NM_011400	*Slc2a1*	solute carrier family 2 (facilitated glucose transporter), member 1	4.41E-28
NM_178934	*Slc2a12*	solute carrier family 2 (facilitated glucose transporter), member 12	3.84E-08

Table 2-1. Continued.

Accession	Gene Symbol	Gene Name	*Adj p*-Value
NM_001199283	*Slc43a2*	solute carrier family 43, member 2	9.51E-26
NM_001081263	*Slc44a5*	solute carrier family 44, member 5	4.36E-38
NM_139142	*Slc6a20a*	solute carrier family 6 (neurotransmitter transporter), member 20A	9.75E-06
NM_001038643	*Slco3a1*	solute carrier organic anion transporter family, member 3a1	3.95E-27
NM_134133	*Smim3*	small integral membrane protein 3	4.01E-16
NM_182927	*Spred3*	sprouty-related EVH1 domain containing 3	1.66E-31
NM_011898	*Spry4*	sprouty RTK signaling antagonist 4	3.04E-31
NM_009270	*Sqle*	squalene epoxidase	2.68E-34
NM_011358	*Srsf2*	serine and arginine rich splicing factor 2	3.51E-30
NM_001195485	*Srsf7*	serine and arginine-rich splicing factor 7	2.69E-27
NM_133774	*Stard4*	StAR-related lipid transfer (START) domain containing 4	8.5E-33
NM_027399	*Steap1*	six transmembrane epithelial antigen of the prostate 1	0.001305
NM_019675	*Stmn4*	stathmin-like 4	2.23E-22
NM_013515	*Stom*	stomatin	2.98E-13
NM_133670	*Sult1a1*	sulfotransferase family 1A, phenol-preferring, member 1	1.42E-13
NM_017465	*Sult2b1*	sulfotransferase family, cytosolic, 2B, member 1	2.44E-11
NM_001040085	*Sytl2*	synaptotagmin-like 2	1.16E-28
NM_001082976	*Tc2n*	tandem C2 domains, nuclear	0.003006
NM_009368	*Tgfb3*	transforming growth factor, beta 3	3.69E-14
NM_174989	*Ticam1*	toll-like receptor adaptor molecule 1	2.03E-22
NM_133211	*Tlr7*	toll-like receptor 7	1.72E-17
NM_001160385	*Tmem196*	transmembrane protein 196	1.01E-18
NM_177344	*Tmem203*	transmembrane protein 203	5.7E-18
NM_133706	*Tmem97*	transmembrane protein 97	1.57E-23
NM_001170855	*Trim36*	tripartite motif-containing 36	2.58E-14
NM_001170912	*Trim66*	tripartite motif containing 66	1E-15
NM_012035	*Trpc7*	transient receptor potential cation channel subfamily C member 7	1.27E-13
NM_001009935	*Txnip*	thioredoxin interacting protein	1.51E-43
NM_001169576	*Ube2h*	ubiquitin conjugating enzyme E2 H	5.01E-33
NM_175158	*Utp20*	UTP20 small subunit processome component	3.61E-18
NM_001039385	*Vgf*	VGF nerve growth factor inducible	1.37E-26
NM_172372	*Wdr45*	WD repeat domain 45	5.48E-20
NM_020603	*Wdr46*	WD repeat domain 46	8.66E-18
NM_001005342	*Ypel4*	yippee like 4	2.16E-34
NM_027166	*Ypel5*	yippee like 5	3.8E-34

Table 2-1. Continued.

Accession	Gene Symbol	Gene Name	*Adj p*-Value
NM_001033324	*Zbtb16*	zinc finger and BTB domain containing 16	3.7E-16
NM_001110309	*Zfp426*	zinc finger protein 426	3.11E-26
NM_001005425	*Zfp663*	zinc finger protein 663	3.6E-12
NM_001252584	*Zmynd8*	zinc finger MYND-type containing 8	3.03E-23

Table 2-2. Differentially expressed genes that showed significant sex x treatment interaction and fold change > 1.5.

Accession	Gene Symbol	Gene Name	*p*-Value
NM_029170	*4930519F16Rik*	RIKEN cDNA 4930519F16 gene	0.004
NM_001142969	*A630073D07Rik*	RIKEN cDNA A630073D07 gene	0.0025
BC150932	*Axdnd1*	axonemal dynein light chain domain containing 1	0.001
NM_007743	*Col1a2*	collagen, type I, alpha 2	0.0177
NM_001286607	*Foxp2*	forkhead box P2	0.0499
NM_153571	*Hscb*	HscB iron-sulfur cluster co-chaperone	0.0124
NM_001285482	*Htr1d*	5-hydroxytryptamine (serotonin) receptor 1D	0.0049
NM_001081403	*Klhl14*	kelch-like 14	0.0008
ENSMUST00000113255	*LOC6620586*	active breakpoint cluster region-related protein	0.0324

of developmental growth, brain development, hippocampus development, dentate gyrus development, positive regulation of neurogenesis, negative regulation of neurogenesis, forebrain cell migration, and development of primary sexual characteristics.

Developmental alcohol has been shown to affect hippocampal dendrites and synapses, and therefore we closely examined over-represented categories involving dendrite and synapse function. Almost 50 dendritic and synaptic categories were over-represented in our genes also identified including dendrite development, dendritic spine maintenance, dendritic spine organization, positive regulation of dendritic cell cytokine production, regulation of dendritic spine development, regulation of synapse assembly, regulation of synapse organization, chemical synaptic transmission- postsynaptic, synaptic vesicle transport, synaptic vesicle exocytosis, regulation of long-term synaptic potentiation, regulation of postsynaptic membrane potential, postsynaptic specialization organization, and regulation of synaptic transmission-GABAergic.

Neuroimmune response and epigenetic changes to developmental alcohol exposure have been areas of focus in recent fetal alcohol research. We found multiple neuroimmune and epigenetic over-represented categories from our list of genes significant for a strain x sex x treatment interaction. Neuroimmune categories that were identified such as immune system development, regulation of cytokine production, regulation of interferon-beta production, regulation of tumor necrosis factor secretion, and response to leukemia inhibitory factor. A few categories involving epigenetics were found including positive regulation of histone methylation, histone H4 acetylation and positive regulation of histone modification.

Growth factor categories as well as cognitive and behavioral categories were also found to be significantly over-represented in our genes significant for an interaction between strain x sex x treatment. Many growth factor categories were identified including regulation of cellular response to growth factor stimulus, platelet-derived growth factor receptor signaling pathway, response to transforming growth factor beta, cellular response to nerve growth factor stimulus, and cellular response to vascular endothelial growth factor stimulus. Cognitive and behavioral over-represented categories included behavioral fear response, locomotion involved in locomotory behavior, learning, and memory. Finally, a handful of over-represented categories were identified such as response to alcohol, alcohol metabolic process, and alcohol biosynthetic process.

Comparison Between High Cell Death Strains and Low Cell Death Strains

Previously, BXD strains showed differential response to cell death in the CA1 region of the hippocampus after an acute binge-like alcohol exposure on P7 (Goldowitz et al., 2014). BXD2, BXD48a, and BXD100 were found to be highly susceptible to ethanol-induced cell death in the hippocampus while BXD60, BXD71, and BXD73 were found to be resistant to ethanol-induced hippocampal cell death (see **Figure 2-1**). For this second analysis, we only focused on BXD strains that showed either resistance or vulnerability to ethanol-induced cell death in the CA1 region of the hippocampus

(Goldowitz et al., 2014). We did not include the parental strains who showed moderate levels of ethanol-induced cell death in this analysis and instead focus on the differences and similarities between BXD strains that showed either low or high cell death in the hippocampus after postnatal ethanol exposure.

As discussed above, marked sex differences were found with little overlap within strains. For this reason, male and females were also separated in this second analysis on BXD strains that show differential cell death in the hippocampus. Thus the following results include discussion of significant ethanol-induced gene expression changes in high cell death males (HCD-M: BXD2M, BXD48Am, BXD100M), low cell death males (LCD-M: BXD60M, BXD71M, BXD73M), high cell death females (HCD-F: BXD2F, BXD48aF, BXD100F), and low cell death females (LCD-F: BXD60F, BXD71F, BXD73F). There were 75 genes significantly (*adjp* < 0.05) expressed genes across all three HCD-M strains after exposure to developmental alcohol while there were only 41 significantly (*adjp* < 0.05) expressed genes across all three LCD-M strains. Overlapping significantly expressed genes were lower in both the HCD-F and LCD-F compared to males with 58 significantly (*adjp* < 0.05) expressed genes across all three HCD-F and 38 significantly (*adjp* < 0.05) expressed genes across all three LCD-F strains after postnatal ethanol exposure.

Due to the few number of overlapping genes using FDR adjusted p-value less than 0.05, the nominal p-value threshold less than 0.05 was used for enrichment analysis (Terenina et al., 2019). The HCD-M had 528 significantly ($p < 0.05$) expressed genes across all three strains after exposure to developmental alcohol while 325 genes were significantly ($p < 0.05$) expressed across all three LCD-M strains (**Figure 2-5A, B**). The HCD-F had 484 significantly ($p < 0.05$) expressed genes across all three HCD-F after postnatal ethanol exposure while 239 genes were significantly ($p < 0.05$) expressed across all three LCD-F strains (**Figure 2-5C, D**).

Enrichment analysis identified 236 significantly (FDR < 0.05) over-represented gene ontology categories in HCD-M and identified 116 in LCD-M (**Table 2-3**). There were 90 over-represented categories that HCD-M and LCD-M had in common including cellular regulation to stress, regulation of cell proliferation, regulation of cell differentiation, regulation of cell migration, and regulation of locomotion. Enrichment analysis identified 146 over-represented categories that were unique to HCD-M including over 15 categories involving regulation of cell death such as positive regulation of neuron apoptotic process, autophagic cell death, positive regulation of programmed cell death, and cell death in response to oxidative stress. Other categories that were unique to HCD-M included locomotion involved in locomotory behavior, response to a toxic substance, positive regulation of cell migration by vascular endothelial growth factor signaling pathway, Ras protein signal transduction, and regulation of transcription from RNA polymerase II promoter in response to stress. In contrast, only 26 over-represented categories were unique to LCD-M with none related to cell death. Categories that were unique to LCD-M included regulation of alternative mRNA splicing via spliceosome,

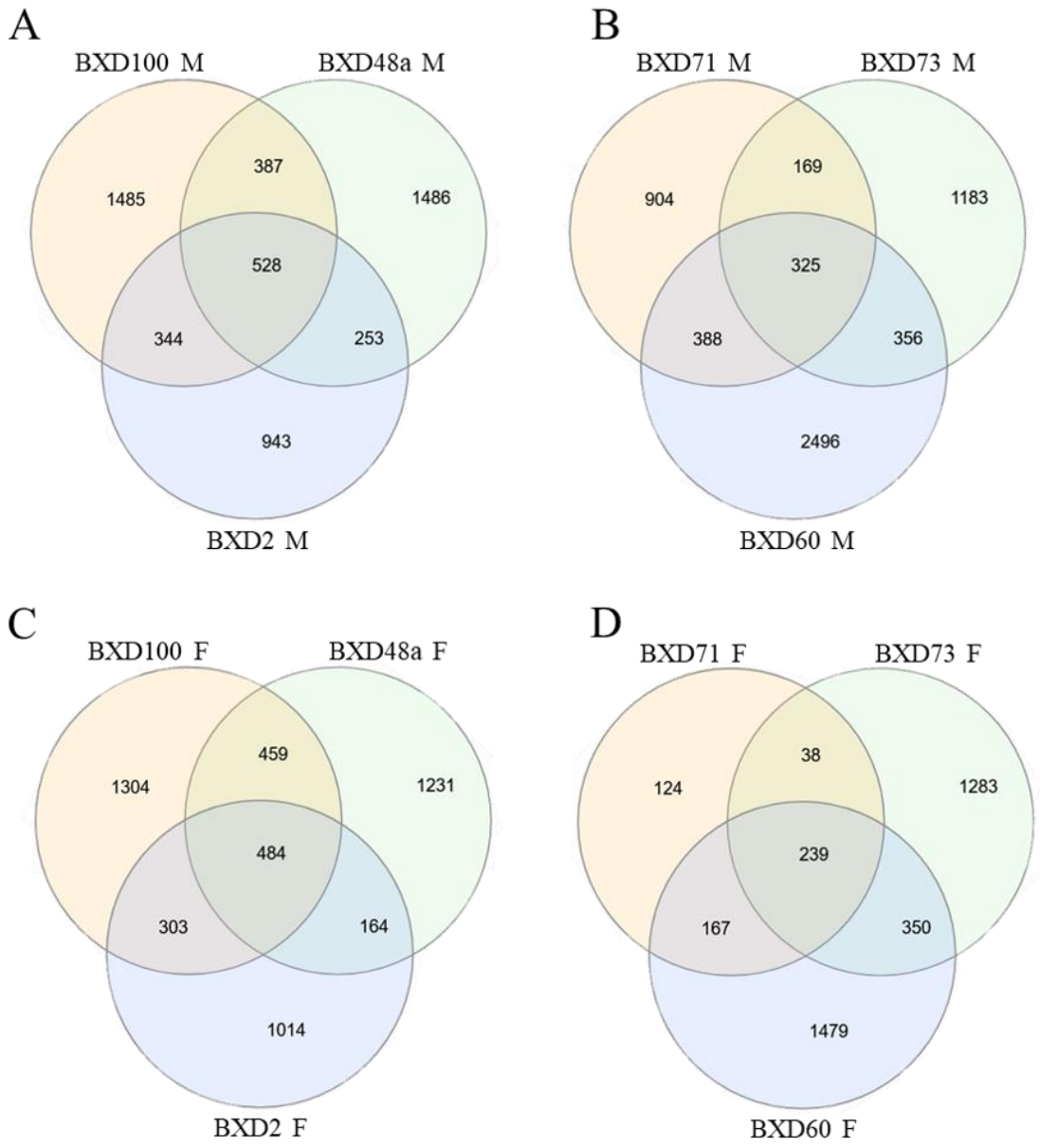

Figure 2-5. Venn diagrams of significant differentially expressed genes after ethanol exposure in high cell death strains and low cell death strains.

Overlap of differentially expressed genes after postnatal ethanol exposure in males (top) and females (bottom) of high cell death strains (left) and low cell death strains (right). (A) Differential ethanol-induced gene expression changes in males of high cell death strains: BXD100, BXD48a, and BXD2. (B) Differential ethanol-induced gene expression changes in males of low cell death strains: BXD71, BXD73, and BXD60. (C) Differential ethanol-induced gene expression changes in females of high cell death strains: BXD100, BXD48a, and BXD2. (D) Differential ethanol-induced gene expression changes in females of high cell death strains: BXD71, BXD73, and BXD60.

Table 2-3. Comparison of significant gene ontology (GO) categories in high cell death males (HCD-M) and low cell death males (LCD-M).

GO ID	Description
Both High Cell Death Males & Low Cell Death Males	
GO:0006629	lipid metabolic process
GO:0008610	lipid biosynthetic process
GO:0016126	sterol biosynthetic process
GO:0009719	response to endogenous stimulus
GO:0016125	sterol metabolic process
GO:0042127	regulation of cell proliferation
GO:0008203	cholesterol metabolic process
GO:1902652	secondary alcohol metabolic process
GO:0006695	cholesterol biosynthetic process
GO:1902653	secondary alcohol biosynthetic process
GO:1901615	organic hydroxy compound metabolic process
GO:0008202	steroid metabolic process
GO:0006066	alcohol metabolic process
GO:0031327	negative regulation of cellular biosynthetic process
GO:0071495	cellular response to endogenous stimulus
GO:1901700	response to oxygen-containing compound
GO:0044281	small molecule metabolic process
GO:0009890	negative regulation of biosynthetic process
GO:0009725	response to hormone
GO:0031328	positive regulation of cellular biosynthetic process
GO:0009891	positive regulation of biosynthetic process
GO:0051338	regulation of transferase activity
GO:0008283	cell proliferation
GO:0010628	positive regulation of gene expression
GO:0010629	negative regulation of gene expression
GO:0009991	response to extracellular stimulus
GO:0010558	negative regulation of macromolecule biosynthetic process
GO:0010557	positive regulation of macromolecule biosynthetic process
GO:0006694	steroid biosynthetic process
GO:2000113	negative regulation of cellular macromolecule biosynthetic process
GO:1901617	organic hydroxy compound biosynthetic process
GO:0031667	response to nutrient levels
GO:0014070	response to organic cyclic compound
GO:0000188	inactivation of MAPK activity
GO:0045944	positive regulation of transcription by RNA polymerase II
GO:0019220	regulation of phosphate metabolic process
GO:0051174	regulation of phosphorus metabolic process
GO:0043549	regulation of kinase activity

Table 2-3. Continued.

GO ID	Description
GO:0033554	cellular response to stress
GO:0010563	negative regulation of phosphorus metabolic process
GO:0045936	negative regulation of phosphate metabolic process
GO:0044283	small molecule biosynthetic process
GO:0008285	negative regulation of cell proliferation
GO:0032870	cellular response to hormone stimulus
GO:0051254	positive regulation of RNA metabolic process
GO:0045595	regulation of cell differentiation
GO:0045893	positive regulation of transcription, DNA-templated
GO:1903508	positive regulation of nucleic acid-templated transcription
GO:1902680	positive regulation of RNA biosynthetic process
GO:0043407	negative regulation of MAP kinase activity
GO:0042594	response to starvation
GO:1903507	negative regulation of nucleic acid-templated transcription
GO:1902679	negative regulation of RNA biosynthetic process
GO:0010648	negative regulation of cell communication
GO:0040011	locomotion
GO:0023057	negative regulation of signaling
GO:0009267	cellular response to starvation
GO:0042326	negative regulation of phosphorylation
GO:0051253	negative regulation of RNA metabolic process
GO:1902532	negative regulation of intracellular signal transduction
GO:0045934	negative regulation of nucleobase-containing compound metabolic process
GO:0009968	negative regulation of signal transduction
GO:0006357	regulation of transcription by RNA polymerase II
GO:0031669	cellular response to nutrient levels
GO:0045892	negative regulation of transcription, DNA-templated
GO:0071901	negative regulation of protein serine/threonine kinase activity
GO:0071900	regulation of protein serine/threonine kinase activity
GO:0051240	positive regulation of multicellular organismal process
GO:0051270	regulation of cellular component movement
GO:0042325	regulation of phosphorylation
GO:0045935	positive regulation of nucleobase-containing compound metabolic process
GO:0006366	transcription by RNA polymerase II
GO:0031668	cellular response to extracellular stimulus
GO:0051348	negative regulation of transferase activity
GO:0045338	farnesyl diphosphate metabolic process
GO:0040012	regulation of locomotion
GO:0001933	negative regulation of protein phosphorylation
GO:0046165	alcohol biosynthetic process

Table 2-3. Continued.

GO ID	Description
GO:0033673	negative regulation of kinase activity
GO:0030334	regulation of cell migration
GO:1902531	regulation of intracellular signal transduction
GO:0008284	positive regulation of cell proliferation
GO:0000122	negative regulation of transcription by RNA polymerase II
GO:0043405	regulation of MAP kinase activity
GO:0043409	negative regulation of MAPK cascade
GO:0008299	isoprenoid biosynthetic process
GO:0034613	cellular protein localization
GO:0070727	cellular macromolecule localization
GO:1903311	regulation of mRNA metabolic process
GO:0071496	cellular response to external stimulus
High Cell Death Males Only	
GO:0044255	cellular lipid metabolic process
GO:0042592	homeostatic process
GO:0006979	response to oxidative stress
GO:0010941	regulation of cell death
GO:0006644	phospholipid metabolic process
GO:1901701	cellular response to oxygen-containing compound
GO:0008654	phospholipid biosynthetic process
GO:0006665	sphingolipid metabolic process
GO:0019216	regulation of lipid metabolic process
GO:1901216	positive regulation of neuron death
GO:0006643	membrane lipid metabolic process
GO:0006687	glycosphingolipid metabolic process
GO:1901566	organonitrogen compound biosynthetic process
GO:0019637	organophosphate metabolic process
GO:0045444	fat cell differentiation
GO:0046677	response to antibiotic
GO:0000302	response to reactive oxygen species
GO:0012501	programmed cell death
GO:0031987	locomotion involved in locomotory behavior
GO:0031399	regulation of protein modification process
GO:0009636	response to toxic substance
GO:0046337	phosphatidylethanolamine metabolic process
GO:0071236	cellular response to antibiotic
GO:0006468	protein phosphorylation
GO:0042981	regulation of apoptotic process
GO:1901214	regulation of neuron death

Table 2-3. Continued.

GO ID	Description
GO:0044087	regulation of cellular component biogenesis
GO:0009628	response to abiotic stimulus
GO:0006915	apoptotic process
GO:0033002	muscle cell proliferation
GO:0048660	regulation of smooth muscle cell proliferation
GO:0048585	negative regulation of response to stimulus
GO:0043067	regulation of programmed cell death
GO:0006650	glycerophospholipid metabolic process
GO:0007610	behavior
GO:0034599	cellular response to oxidative stress
GO:0048878	chemical homeostasis
GO:0048659	smooth muscle cell proliferation
GO:0090407	organophosphate biosynthetic process
GO:0016477	cell migration
GO:0010942	positive regulation of cell death
GO:0042493	response to drug
GO:0010243	response to organonitrogen compound
GO:0006446	regulation of translational initiation
GO:0006646	phosphatidylethanolamine biosynthetic process
GO:0042542	response to hydrogen peroxide
GO:0051094	positive regulation of developmental process
GO:0009888	tissue development
GO:0032269	negative regulation of cellular protein metabolic process
GO:0048870	cell motility
GO:0051674	localization of cell
GO:0046486	glycerolipid metabolic process
GO:0070997	neuron death
GO:0006664	glycolipid metabolic process
GO:0051384	response to glucocorticoid
GO:0034614	cellular response to reactive oxygen species
GO:0033043	regulation of organelle organization
GO:1903509	liposaccharide metabolic process
GO:0070301	cellular response to hydrogen peroxide
GO:0033993	response to lipid
GO:0038033	positive regulation of endothelial cell chemotaxis by VEGF-activated vascular endothelial growth factor receptor signaling pathway
GO:0048545	response to steroid hormone
GO:0051248	negative regulation of protein metabolic process
GO:0010608	posttranscriptional regulation of gene expression
GO:0045017	glycerolipid biosynthetic process

Table 2-3. Continued.

GO ID	Description
GO:0001101	response to acid chemical
GO:1901135	carbohydrate derivative metabolic process
GO:0043603	cellular amide metabolic process
GO:0006983	ER overload response
GO:0001666	response to hypoxia
GO:0043618	regulation of transcription from RNA polymerase II promoter in response to stress
GO:2000145	regulation of cell motility
GO:0034248	regulation of cellular amide metabolic process
GO:0023014	signal transduction by protein phosphorylation
GO:0031960	response to corticosteroid
GO:2000278	regulation of DNA biosynthetic process
GO:0036293	response to decreased oxygen levels
GO:0043525	positive regulation of neuron apoptotic process
GO:1901654	response to ketone
GO:0006413	translational initiation
GO:0035295	tube development
GO:0046474	glycerophospholipid biosynthetic process
GO:1901698	response to nitrogen compound
GO:0036003	positive regulation of transcription from RNA polymerase II promoter in response to stress
GO:0035690	cellular response to drug
GO:0043085	positive regulation of catalytic activity
GO:0051247	positive regulation of protein metabolic process
GO:0043620	regulation of DNA-templated transcription in response to stress
GO:1901031	regulation of response to reactive oxygen species
GO:0038089	positive regulation of cell migration by vascular endothelial growth factor signaling pathway
GO:0001932	regulation of protein phosphorylation
GO:0000165	MAPK cascade
GO:0072359	circulatory system development
GO:0032270	positive regulation of cellular protein metabolic process
GO:0007049	cell cycle
GO:0031400	negative regulation of protein modification process
GO:0071407	cellular response to organic cyclic compound
GO:0048871	multicellular organismal homeostasis
GO:1901657	glycosyl compound metabolic process
GO:0051726	regulation of cell cycle
GO:1990845	adaptive thermogenesis
GO:0046890	regulation of lipid biosynthetic process
GO:0060548	negative regulation of cell death
GO:0070482	response to oxygen levels

Table 2-3. Continued.

GO ID	Description
GO:0060613	fat pad development
GO:0061614	pri-miRNA transcription by RNA polymerase II
GO:0016137	glycoside metabolic process
GO:1902895	positive regulation of pri-miRNA transcription by RNA polymerase II
GO:0007010	cytoskeleton organization
GO:2000379	positive regulation of reactive oxygen species metabolic process
GO:0050918	positive chemotaxis
GO:0048661	positive regulation of smooth muscle cell proliferation
GO:1902115	regulation of organelle assembly
GO:1901652	response to peptide
GO:0045859	regulation of protein kinase activity
GO:0034504	protein localization to nucleus
GO:0006928	movement of cell or subcellular component
GO:0010506	regulation of autophagy
GO:0033865	nucleoside bisphosphate metabolic process
GO:0033875	ribonucleoside bisphosphate metabolic process
GO:0034032	purine nucleoside bisphosphate metabolic process
GO:0035239	tube morphogenesis
GO:0007033	vacuole organization
GO:0006914	autophagy
GO:0061919	process utilizing autophagic mechanism
GO:0072331	signal transduction by p53 class mediator
GO:0006984	ER-nucleus signaling pathway
GO:0048732	gland development
GO:0048102	autophagic cell death
GO:1903726	negative regulation of phospholipid metabolic process
GO:0002091	negative regulation of receptor internalization
GO:0019752	carboxylic acid metabolic process
GO:0006417	regulation of translation
GO:0044089	positive regulation of cellular component biogenesis
GO:0043065	positive regulation of apoptotic process
GO:0007265	Ras protein signal transduction
GO:0060326	cell chemotaxis
GO:0010866	regulation of triglyceride biosynthetic process
GO:0006082	organic acid metabolic process
GO:0043068	positive regulation of programmed cell death
GO:0055082	cellular chemical homeostasis
GO:0006637	acyl-CoA metabolic process
GO:0035383	thioester metabolic process
GO:0036473	cell death in response to oxidative stress

Table 2-3. Continued.

GO ID	Description
GO:2000573	positive regulation of DNA biosynthetic process
GO:0006084	acetyl-CoA metabolic process
Low Cell Death Males Only	
GO:0051591	response to cAMP
GO:0006084	acetyl-CoA metabolic process
GO:0035335	peptidyl-tyrosine dephosphorylation
GO:0060419	heart growth
GO:0035265	organ growth
GO:0006396	RNA processing
GO:0050684	regulation of mRNA processing
GO:0000381	regulation of alternative mRNA splicing, via spliceosome
GO:0016311	dephosphorylation
GO:0045943	positive regulation of transcription by RNA polymerase I
GO:0051241	negative regulation of multicellular organismal process
GO:0046889	positive regulation of lipid biosynthetic process
GO:0043687	post-translational protein modification
GO:0062012	regulation of small molecule metabolic process
GO:0014074	response to purine-containing compound
GO:0055017	cardiac muscle tissue growth
GO:0097435	supramolecular fiber organization
GO:1903299	regulation of hexokinase activity
GO:0036314	response to sterol
GO:0031645	negative regulation of neurological system process
GO:0010977	negative regulation of neuron projection development
GO:0048024	regulation of mRNA splicing, via spliceosome
GO:0000380	alternative mRNA splicing, via spliceosome
GO:0043484	regulation of RNA splicing
GO:0051549	positive regulation of keratinocyte migration
GO:0018410	C-terminal protein amino acid modification
GO:0046683	response to organophosphorus

GO – Gene Ontology Identification

C-terminal protein amino acid modification, negative regulation of neurological system process, and negative regulation of neuron projection development.

Enrichment analysis identified 214 (FDR < 0.05) over-represented gene ontology categories in HCD-F and identified 100 in LCD-F (**Table 2-4**). There were 72 over-represented categories that HCD-F and LCD-F had in common including regulation of apoptotic process, regulation of growth, negative regulation of cell communication, negative regulation of cell proliferation, alcohol metabolic process, and associative learning. Enrichment analysis identified 142 over-represented categories that were unique to HCD-F including 9 categories involving cytokines such as response to cytokine, cellular response to leukemia inhibitory factor, and dendritic cell cytokine production. Other categories that were unique to HCD-F included regulation of cell development, regulation of programmed cell death, response to oxidative stress, cell migration, visual behavior, visual learning, and hippocampus development. While there were only 28 over-represented categories unique to LCD-F, three were related to positive regulation of programmed cell death. Other categories that were unique to LCD-F included regulation of transcription from RNA polymerase II promoter in response to stress, learning, cognition, and regulation of synaptic plasticity.

Next, we examined specific differences and similarities in significant ($p < 0.05$) gene expression changes between the four groups, HCD-M, HCD-F, LCD-M, and LCD-F (**Figure 2-6**). There were 484 significant ($p < 0.05$) gene expression changes after developmental alcohol exposure that were found in all three HCD strains but not in all LCD strains (**Table 2-5**). Of these, 88 genes were significant for both male and female HCD strains, while 218 were specific for HCD males and 178 were specific for HCD females. In contrast, there were only 109 significant ($p < 0.05$) gene expression changes after exposure to postnatal ethanol that were found in all three LCD strains (**Table 2-6**). Of these, 17 genes were significant for both male and female LCD strains, while 68 were specific for LCD-M and 24 specific for LCD-F. We also analyzed sex-specific gene expression changes that were similar in both HCD and LCD strains of one sex but not the other. We found 30 genes that were significantly expressed in both HCD and LCD males but not females while 8 genes were significantly expressed in both HCD and LCD females but not males (**Table 2-7**). Finally, we analyzed gene expression changes that were significant ($p < 0.05$) across all four groups. There were 115 genes that were significantly ($p < 0.05$) in HCD-M, LCD-M, HCD-F, LCD-F (**Table 2-8**).

Table 2-4. Comparison of significant gene ontology (GO) categories in high cell death females (HCD-F) and low cell death females (LCD-F).

GO ID	Description
Both High Cell Death Females & Low Cell Death Females	
GO:0016126	sterol biosynthetic process
GO:0008610	lipid biosynthetic process
GO:0016125	sterol metabolic process
GO:0008203	cholesterol metabolic process
GO:0006695	cholesterol biosynthetic process
GO:1902653	secondary alcohol biosynthetic process
GO:1902652	secondary alcohol metabolic process
GO:0009719	response to endogenous stimulus
GO:0006629	lipid metabolic process
GO:1901615	organic hydroxy compound metabolic process
GO:0042127	regulation of cell proliferation
GO:1901700	response to oxygen-containing compound
GO:0008202	steroid metabolic process
GO:1901617	organic hydroxy compound biosynthetic process
GO:004428	small molecule metabolic process
GO:0000188	inactivation of MAPK activity
GO:0006066	alcohol metabolic process
GO:0006694	steroid biosynthetic process
GO:0008283	cell proliferation
GO:0008654	phospholipid biosynthetic process
GO:1901701	cellular response to oxygen-containing compound
GO:0044255	cellular lipid metabolic process
GO:0071495	cellular response to endogenous stimulus
GO:0090407	organophosphate biosynthetic process
GO:0045338	farnesyl diphosphate metabolic process
GO:0031328	positive regulation of cellular biosynthetic process
GO:0009891	positive regulation of biosynthetic process
GO:0009725	response to hormone
GO:0008299	isoprenoid biosynthetic process
GO:0010557	positive regulation of macromolecule biosynthetic process
GO:0010628	positive regulation of gene expression
GO:0019637	organophosphate metabolic process
GO:0043407	negative regulation of MAP kinase activity
GO:0036314	response to sterol
GO:0031327	negative regulation of cellular biosynthetic process
GO:0006644	phospholipid metabolic process
GO:0046165	alcohol biosynthetic process
GO:0014070	response to organic cyclic compound

Table 2-4. Continued.

GO ID	Description
GO:0009890	negative regulation of biosynthetic process
GO:0010648	negative regulation of cell communication
GO:0023057	negative regulation of signaling
GO:0051254	positive regulation of RNA metabolic process
GO:0040008	regulation of growth
GO:0051240	positive regulation of multicellular organismal process
GO:0045935	positive regulation of nucleobase-containing compound metabolic process
GO:0044283	small molecule biosynthetic process
GO:0008285	negative regulation of cell proliferation
GO:0010563	negative regulation of phosphorus metabolic process
GO:0045936	negative regulation of phosphate metabolic process
GO:1903508	positive regulation of nucleic acid-templated transcription
GO:1902680	positive regulation of RNA biosynthetic process
GO:0008306	associative learning
GO:0051247	positive regulation of protein metabolic process
GO:0043409	negative regulation of MAPK cascade
GO:2000113	negative regulation of cellular macromolecule biosynthetic process
GO:0032870	cellular response to hormone stimulus
GO:0032269	negative regulation of cellular protein metabolic process
GO:0045944	positive regulation of transcription by RNA polymerase II
GO:0045893	positive regulation of transcription, DNA-templated
GO:0051348	negative regulation of transferase activity
GO:0051338	regulation of transferase activity
GO:0042326	negative regulation of phosphorylation
GO:0060613	fat pad development
GO:0051248	negative regulation of protein metabolic process
GO:0009991	response to extracellular stimulus
GO:0031667	response to nutrient levels
GO:0042981	regulation of apoptotic process
GO:0071901	negative regulation of protein serine/threonine kinase activity
GO:1903726	negative regulation of phospholipid metabolic process
GO:0032270	positive regulation of cellular protein metabolic process
GO:0019216	regulation of lipid metabolic process
GO:0033673	negative regulation of kinase activity
High Cell Death Females Only	
GO:1901566	organonitrogen compound biosynthetic process
GO:0022613	ribonucleoprotein complex biogenesis
GO:0043603	cellular amide metabolic process
GO:0042254	ribosome biogenesis

Table 2-4. Continued.

GO ID	Description
GO:0006364	rRNA processing
GO:0006665	sphingolipid metabolic process
GO:0016072	rRNA metabolic process
GO:0006396	RNA processing
GO:1990823	response to leukemia inhibitory factor
GO:1990830	cellular response to leukemia inhibitory factor
GO:1901698	response to nitrogen compound
GO:0034097	response to cytokine
GO:0006403	RNA localization
GO:0010243	response to organonitrogen compound
GO:0036315	cellular response to sterol
GO:0006595	polyamine metabolic process
GO:0006913	nucleocytoplasmic transport
GO:0051169	nuclear transport
GO:0009628	response to abiotic stimulus
GO:0016477	cell migration
GO:0006643	membrane lipid metabolic process
GO:0000165	MAPK cascade
GO:1901652	response to peptide
GO:0015931	nucleobase-containing compound transport
GO:0006720	isoprenoid metabolic process
GO:0006084	acetyl-CoA metabolic process
GO:0040011	locomotion
GO:0006598	polyamine catabolic process
GO:0033865	nucleoside bisphosphate metabolic process
GO:0033875	ribonucleoside bisphosphate metabolic process
GO:0034032	purine nucleoside bisphosphate metabolic process
GO:0061061	muscle structure development
GO:0034470	ncRNA processing
GO:0023014	signal transduction by protein phosphorylation
GO:0070723	response to cholesterol
GO:0050657	nucleic acid transport
GO:0050658	RNA transport
GO:0072359	circulatory system development
GO:0051028	mRNA transport
GO:0051236	establishment of RNA localization
GO:0006607	NLS-bearing protein import into nucleus
GO:0002371	dendritic cell cytokine production
GO:0006637	acyl-CoA metabolic process
GO:0035383	thioester metabolic process

Table 2-4. Continued.

GO ID	Description
GO:0043405	regulation of MAP kinase activity
GO:0048585	negative regulation of response to stimulus
GO:0060420	regulation of heart growth
GO:0033993	response to lipid
GO:0043933	protein-containing complex subunit organization
GO:1902532	negative regulation of intracellular signal transduction
GO:0019752	carboxylic acid metabolic process
GO:0040012	regulation of locomotion
GO:0002372	myeloid dendritic cell cytokine production
GO:0002732	positive regulation of dendritic cell cytokine production
GO:0002733	regulation of myeloid dendritic cell cytokine production
GO:0002735	positive regulation of myeloid dendritic cell cytokine production
GO:0030325	adrenal gland development
GO:1901135	carbohydrate derivative metabolic process
GO:0009117	nucleotide metabolic process
GO:0006606	protein import into nucleus
GO:0043434	response to peptide hormone
GO:0006732	coenzyme metabolic process
GO:0006687	glycosphingolipid metabolic process
GO:0009968	negative regulation of signal transduction
GO:0008284	positive regulation of cell proliferation
GO:0071397	cellular response to cholesterol
GO:0055094	response to lipoprotein particle
GO:0006753	nucleoside phosphate metabolic process
GO:0008542	visual learning
GO:0017038	protein import
GO:0034660	ncRNA metabolic process
GO:0048870	cell motility
GO:0051674	localization of cell
GO:0051170	import into nucleus
GO:0001944	vasculature development
GO:0010558	negative regulation of macromolecule biosynthetic process
GO:0060419	heart growth
GO:0034504	protein localization to nucleus
GO:0007632	visual behavior
GO:0019919	peptidyl-arginine methylation, to asymmetrical-dimethyl arginine
GO:0046907	intracellular transport
GO:0006576	cellular biogenic amine metabolic process
GO:0043604	amide biosynthetic process
GO:0006417	regulation of translation

Table 2-4. Continued.

GO ID	Description
GO:0042493	response to drug
GO:0065003	protein-containing complex assembly
GO:0006082	organic acid metabolic process
GO:0009259	ribonucleotide metabolic process
GO:0048514	blood vessel morphogenesis
GO:0001525	angiogenesis
GO:0051186	cofactor metabolic process
GO:0072358	cardiovascular system development
GO:0046620	regulation of organ growth
GO:0071402	cellular response to lipoprotein particle stimulus
GO:0042542	response to hydrogen peroxide
GO:0048878	chemical homeostasis
GO:0043436	oxoacid metabolic process
GO:0006220	pyrimidine nucleotide metabolic process
GO:0060284	regulation of cell development
GO:0009147	pyrimidine nucleoside triphosphate metabolic process
GO:0000302	response to reactive oxygen species
GO:1902531	regulation of intracellular signal transduction
GO:0051649	establishment of localization in cell
GO:0001558	regulation of cell growth
GO:0034248	regulation of cellular amide metabolic process
GO:0006979	response to oxidative stress
GO:0001666	response to hypoxia
GO:0046677	response to antibiotic
GO:0071404	cellular response to low-density lipoprotein particle stimulus
GO:0055021	regulation of cardiac muscle tissue growth
GO:0019693	ribose phosphate metabolic process
GO:0048732	gland development
GO:0055086	nucleobase-containing small molecule metabolic process
GO:0001568	blood vessel development
GO:0015833	peptide transport
GO:0008216	spermidine metabolic process
GO:0035247	peptidyl-arginine omega-N-methylation
GO:0001667	ameboidal-type cell migration
GO:0015918	sterol transport
GO:0050684	regulation of mRNA processing
GO:0032366	intracellular sterol transport
GO:0032367	intracellular cholesterol transport
GO:0042592	homeostatic process
GO:0010941	regulation of cell death

Table 2-4. Continued.

GO ID	Description
GO:0045934	negative regulation of nucleobase-containing compound metabolic process
GO:0036293	response to decreased oxygen levels
GO:0009636	response to toxic substance
GO:0046686	response to cadmium ion
GO:0055017	cardiac muscle tissue growth
GO:0035265	organ growth
GO:0043067	regulation of programmed cell death
GO:0071345	cellular response to cytokine stimulus
GO:0006412	translation
GO:0097164	ammonium ion metabolic process
GO:0051591	response to cAMP
GO:0042886	amide transport
GO:0006646	phosphatidylethanolamine biosynthetic process
GO:0035246	peptidyl-arginine N-methylation
GO:0017148	negative regulation of translation
GO:0021766	hippocampus development
GO:0033002	muscle cell proliferation
GO:0015031	protein transport
Low Cell Death Females Only	
GO:0043618	regulation of transcription from RNA polymerase II promoter in response to stress
GO:0006984	ER-nucleus signaling pathway
GO:0043620	regulation of DNA-templated transcription in response to stress
GO:0046889	positive regulation of lipid biosynthetic process
GO:0046890	regulation of lipid biosynthetic process
GO:0031668	cellular response to extracellular stimulus
GO:0031669	cellular response to nutrient levels
GO:0042594	response to starvation
GO:0009267	cellular response to starvation
GO:1990440	positive regulation of transcription from RNA polymerase II promoter in response to endoplasmic reticulum stress
GO:0060612	adipose tissue development
GO:0043065	positive regulation of apoptotic process
GO:0043068	positive regulation of programmed cell death
GO:0036003	positive regulation of transcription from RNA polymerase II promoter in response to stress
GO:0048167	regulation of synaptic plasticity
GO:0051241	negative regulation of multicellular organismal process
GO:0006366	transcription by RNA polymerase II
GO:0007612	learning
GO:0006357	regulation of transcription by RNA polymerase II

Table 2-4. Continued.

GO ID	Description
GO:0044087	regulation of cellular component biogenesis
GO:0050890	cognition
GO:0071496	cellular response to external stimulus
GO:0010942	positive regulation of cell death
GO:0071900	regulation of protein serine/threonine kinase activity
GO:0006468	protein phosphorylation
GO:0042149	cellular response to glucose starvation
GO:0010867	positive regulation of triglyceride biosynthetic process
GO:0051961	negative regulation of nervous system development

GO ID – Gene Ontology Identification

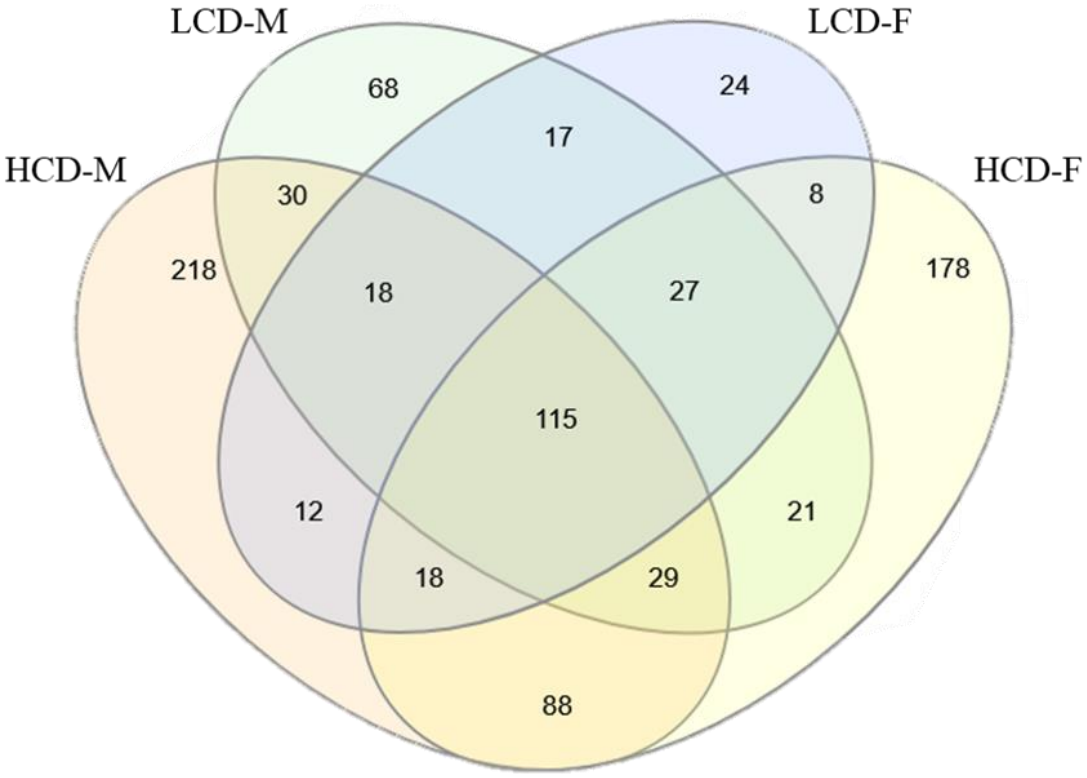

Figure 2-6. Venn diagram of differential gene expression changes in high cell death males (HCD-M), low cell death males (LCD-M), low cell death females (LCD-F), and high cell death females (HCD-F).

Similarities and differences in significant ethanol-induced gene expression changes in all four groups: HCD-M, LCD-M, LCD-F, and HCD-F (from left to right).

Table 2-5. Significant ethanol-induced gene expression changes that were unique to high cell death strains.

Accession	Gene Symbol	Gene Name
Both High Cell Death Males and High Cell Death Females		
NM_172133	*Adap2*	ArfGAP with dual PH domains 2
NM_001080798	*Aff1*	AF4/FMR2 family, member 1
NM_007428	*Agt*	angiotensinogen (serpin peptidase inhibitor, clade A, member 8)
NM_001172146	*Aimp2*	aminoacyl tRNA synthetase complex-interacting multifunctional protein 2
NM_017476	*Akap8l*	A kinase (PRKA) anchor protein 8-like
NM_001276301	*Ampd3*	adenosine monophosphate deaminase 3
NM_020581	*Angptl4*	angiopoietin-like 4
NM_001024851	*Ankrd34a*	ankyrin repeat domain 34A
NM_001109914	*Apold1*	apolipoprotein L domain containing 1
NM_029933	*Bcl9*	B cell CLL/lymphoma 9
NM_001122683	*Bdh1*	3-hydroxybutyrate dehydrogenase, type 1
NM_016859	*Bysl*	bystin-like
NM_026192	*Calcoco1*	calcium binding and coiled coil domain 1
NM_001168304	*Cdk19*	cyclin-dependent kinase 19
NM_001271496	*Chka*	choline kinase alpha
NM_134141	*Ciapin1*	cytokine induced apoptosis inhibitor 1
NM_025854	*Cir1*	corepressor interacting with RBPJ, 1
NM_024217	*Cmtm3*	CKLF-like MARVEL transmembrane domain containing 3
NM_009898	*Coro1a*	coronin, actin binding protein 1A
NM_178379	*Cox10*	cytochrome c oxidase assembly protein 10
NM_023565	*Cse1l*	chromosome segregation 1-like (S. cerevisiae)
NM_001145799	*Ctla2a*	cytotoxic T lymphocyte-associated protein 2 alpha
NM_009994	*Cyp1b1*	cytochrome P450, family 1, subfamily b, polypeptide 1
NM_001285947	*Cyp39a1*	cytochrome P450, family 39, subfamily a, polypeptide 1
NM_010516	*Cyr61*	cysteine rich protein 61
NM_001163026	*Dnajc13*	DnaJ heat shock protein family (Hsp40) member C13
NM_001110331	*Eci2*	enoyl-Coenzyme A delta isomerase 2
NM_010104	*Edn1*	endothelin 1
NM_001177883	*Elavl2*	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 2 (Hu antigen B)
NM_175313	*Eogt*	EGF domain-specific O-linked N-acetylglucosamine (GlcNAc) transferase
NM_007970	*Ezh1*	enhancer of zeste 1 polycomb repressive complex 2 subunit
NM_007988	*Fasn*	fatty acid synthase
NM_011817	*Gadd45g*	growth arrest and DNA-damage-inducible 45 gamma
NM_001033300	*Gmps*	guanine monophosphate synthetase
NM_198962	*Hcrtr2*	hypocretin (orexin) receptor 2
NM_175256	*Heg1*	heart development protein with EGF-like domains 1

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_010422	*Hexb*	hexosaminidase B
NM_008252	*Hmgb2*	high mobility group box 2
NM_001002012	*Hspa2*	heat shock protein 2
NM_010495	*Id1*	inhibitor of DNA binding 1
NM_031166	*Id4*	inhibitor of DNA binding 4
NM_010515	*Igf2r*	insulin-like growth factor 2 receptor
NM_016851	*Irf6*	interferon regulatory factor 6
NM_173441	*Iws1*	IWS1 homolog (S. cerevisiae)
NM_001033298	*Kiz*	kizuna centrosomal protein
NM_001077398	*Ldb2*	LIM domain binding 2
NM_008494	*Lfng*	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase
NM_001113386	*Lifr*	leukemia inhibitory factor receptor
NM_181074	*Lingo1*	leucine rich repeat and Ig domain containing 1
NM_145152	*Lrrc3*	leucine rich repeat containing 3
NM_172632	*Mapk4*	mitogen-activated protein kinase 4
NM_001012335	*Mdk*	midkine
NM_001081392	*Mdn1*	midasin AAA ATPase 1
NM_008587	*Mertk*	c-mer proto-oncogene tyrosine kinase
NM_019946	*Mgst1*	microsomal glutathione S-transferase 1
NM_001040395	*Nadk2*	NAD kinase 2, mitochondrial
NM_021303	*Noc2l*	NOC2 like nucleolar associated transcriptional repressor
NM_001164363	*Nt5c2*	5'-nucleotidase, cytosolic II
NM_001161430	*Nxt2*	nuclear transport factor 2-like export factor 2
NM_001285839	*Osgepl1*	O-sialoglycoprotein endopeptidase-like 1
NM_026420	*Paip2*	polyadenylate-binding protein-interacting protein 2
NM_001177980	*Pde4b*	phosphodiesterase 4B, cAMP specific
NM_016861	*Pdlim1*	PDZ and LIM domain 1 (elfin)
NM_181585	*Pik3r3*	phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3 (p55)
NM_001033225	*Pnrc1*	proline-rich nuclear receptor coactivator 1
NM_009086	*Polr1b*	polymerase (RNA) I polypeptide B
NM_201371	*Prmt8*	protein arginine N-methyltransferase 8
NM_025682	*Pspc1*	paraspeckle protein 1
NM_009025	*Rasa3*	RAS p21 protein activator 3
NM_026446	*Rgs19*	regulator of G-protein signaling 19
NM_133982	*Rpp25*	ribonuclease P/MRP 25 subunit
NM_172604	*Scara3*	scavenger receptor class A, member 3
NM_009127	*Scd1*	stearoyl-Coenzyme A desaturase 1
NM_001013370	*Sesn1*	sestrin 1
NM_009167	*Shc3*	src homology 2 domain-containing transforming protein C3

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_023596	*Slc29a3*	solute carrier family 29 (nucleoside transporters), member 3
NM_008539	*Smad1*	SMAD family member 1
NM_033218	*Srebf2*	sterol regulatory element binding factor 2
NM_026155	*Ssr3*	signal sequence receptor, gamma
NM_012028	*St6galnac5*	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 5
NM_177344	*Tmem203*	transmembrane protein 203
NM_197996	*Tspan15*	tetraspanin 15
NM_028341	*Ttc39c*	tetratricopeptide repeat domain 39C
NM_009462	*Usp10*	ubiquitin specific peptidase 10
NM_138592	*Usp39*	ubiquitin specific peptidase 39
NM_001039385	*Vgf*	VGF nerve growth factor inducible
NM_153391	*Wdr19*	WD repeat domain 19
NM_001115130	*Zbtb44*	zinc finger and BTB domain containing 44
High Cell Death Males Only		
NM_011920	*Abcg2*	ATP-binding cassette, sub-family G (WHITE), member 2
NM_001276719	*Ackr2*	atypical chemokine receptor 2
NM_001048008	*Agtpbp1*	ATP/GTP binding protein 1
NM_001035532	*Akap2*	A kinase (PRKA) anchor protein 2
NM_009658	*Akr1b3*	aldo-keto reductase family 1, member B3 (aldose reductase)
NM_026316	*Aldh3b1*	aldehyde dehydrogenase 3 family, member B1
NM_001013814	*Amt*	aminomethyltransferase
NM_028390	*Anln*	anillin, actin binding protein
NM_001253813	*Ano6*	anoctamin 6
NM_177583	*Aph1b*	aph1 homolog B, gamma secretase subunit
NM_026674	*Aph1c*	aph1 homolog C, gamma secretase subunit
NM_175105	*Aqp11*	aquaporin 11
NM_001025102	*Arl14ep*	ADP-ribosylation factor-like 14 effector protein
NM_026402	*Atg3*	autophagy related 3
NM_025272	*Atp6v0e*	ATPase, H+ transporting, lysosomal V0 subunit E
NM_009125	*Atxn2*	ataxin 2
NM_001122993	*B3galt5*	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 5
NM_026116	*Bbs2*	Bardet-Biedl syndrome 2 (human)
NM_173404	*Bmp3*	bone morphogenetic protein 3
NM_001136064	*Bscl2*	Berardinelli-Seip congenital lipodystrophy 2 (seipin)
NM_007569	*Btg1*	B cell translocation gene 1, anti-proliferative
NM_001017985	*C2cd3*	C2 calcium-dependent domain containing 3
NM_001252533	*Cacnb2*	calcium channel, voltage-dependent, beta 2 subunit
NM_001081557	*Camta1*	calmodulin binding transcription activator 1
NM_025821	*Carhsp1*	calcium regulated heat stable protein 1

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_001271353	*Cbs*	cystathionine beta-synthase
NM_011337	*Ccl3*	chemokine (C-C motif) ligand 3
NM_009875	*Cdkn1b*	cyclin-dependent kinase inhibitor 1B
NM_001159364	*Cep97*	centrosomal protein 97
NM_025844	*Chordc1*	cysteine and histidine-rich domain (CHORD)-containing, zinc-binding protein 1
NM_001177770	*Clcc1*	chloride channel CLIC-like 1
NM_172621	*Clic5*	chloride intracellular channel 5
NM_001033175	*Cln6*	ceroid-lipofuscinosis, neuronal 6
NM_001081158	*Cluh*	clustered mitochondria (cluA/CLU1) homolog
NM_026977	*Cnppd1*	cyclin Pas1/PHO80 domain containing 1
NM_013499	*Cr1l*	complement component (3b/4b) receptor 1-like
NM_133239	*Crb1*	crumbs family member 1, photoreceptor morphogenesis associated
NM_011804	*Creg1*	cellular repressor of E1A-stimulated genes 1
NM_007791	*Csrp1*	cysteine and glycine-rich protein 1
NM_009984	*Ctsl*	cathepsin L
NM_027545	*Cwf19l2*	CWF19-like 2, cell cycle control (S. pombe)
NM_027816	*Cyp2u1*	cytochrome P450, family 2, subfamily u, polypeptide 1
NM_001165980	*Dcaf17*	DDB1 and CUL4 associated factor 17
NM_029974	*Dcst1*	DC-STAMP domain containing 1
NM_029083	*Ddit4*	DNA-damage-inducible transcript 4
NM_001282055	*Ddx46*	DEAD (Asp-Glu-Ala-Asp) box polypeptide 46
NM_172477	*Dennd2a*	DENN/MADD domain containing 2A
NM_001037937	*Deptor*	DEP domain containing MTOR-interacting protein
NM_001037938	*Dhrs4*	dehydrogenase/reductase (SDR family) member 4
NM_178704	*Dpy19l3*	dpy-19-like 3 (C. elegans)
NM_001085390	*Dusp5*	dual specificity phosphatase 5
NM_001159375	*Eif4a1*	eukaryotic translation initiation factor 4A1
NM_145941	*Eif4g1*	eukaryotic translation initiation factor 4, gamma 1
NM_175522	*Elfn1*	leucine rich repeat and fibronectin type III, extracellular 1
NM_134065	*Epdr1*	ependymin related protein 1 (zebrafish)
NM_029250	*Etnk1*	ethanolamine kinase 1
NM_025626	*Fam107b*	family with sequence similarity 107, member B
NM_001113283	*Fam214a*	family with sequence similarity 214, member A
NM_001206335	*Fam234a*	family with sequence similarity 234, member A
NM_172591	*Fcho2*	FCH domain only 2
NM_019740	*Foxo3*	forkhead box O3
NM_025799	*Fuca2*	fucosidase, alpha-L- 2, plasma
NM_008065	*Gabpa*	GA repeat binding protein, alpha
NM_008079	*Galc*	galactosylceramidase

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_001081421	*Galnt16*	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 16
NM_001081151	*Gan*	giant axonal neuropathy
NM_001113560	*Glo1*	glyoxalase 1
NM_027227	*Glod5*	glyoxalase domain containing 5
NM_001035122	*Golm1*	golgi membrane protein 1
NM_001195774	*Gprc5b*	G protein-coupled receptor, family C, group 5, member B
NM_001177874	*Gps1*	G protein pathway suppressor 1
NM_030022	*Grifin*	galectin-related inter-fiber protein
NM_001177656	*Grin1*	glutamate receptor, ionotropic, NMDA1 (zeta 1)
NM_001172117	*Hck*	hemopoietic cell kinase
NM_001162950	*Hif3a*	hypoxia inducible factor 3, alpha subunit
NM_025812	*Hmg20a*	high mobility group 20A
NM_016957	*Hmgn2*	high mobility group nucleosomal binding domain 2
NM_016805	*Hnrnpu*	heterogeneous nuclear ribonucleoprotein U
NM_024255	*Hsdl2*	hydroxysteroid dehydrogenase like 2
NM_008300	*Hspa4*	heat shock protein 4
NM_001101605	*Ifit1bl1*	interferon induced protein with tetratricpeptide repeats 1B like 1
NM_008360	*Il18*	interleukin 18
NM_023579	*Ipo5*	importin 5
NM_013565	*Itga3*	integrin alpha 3
NM_177290	*Itgb8*	integrin beta 8
NM_026200	*Kcnv1*	potassium channel, subfamily V, member 1
NM_001159864	*Kctd18*	potassium channel tetramerisation domain containing 18
NM_172898	*Kirrel2*	kin of IRRE like 2 (Drosophila)
NM_010636	*Klf12*	Kruppel-like factor 12
NM_010637	*Klf4*	Kruppel-like factor 4 (gut)
NM_001161800	*Klhl7*	kelch-like 7
NM_029999	*Lbh*	limb-bud and heart
NM_001113545	*Lima1*	LIM domain and actin binding 1
NM_013860	*Limd1*	LIM domains containing 1
NM_175271	*Lpar4*	lysophosphatidic acid receptor 4
NM_207206	*Lpcat4*	lysophosphatidylcholine acyltransferase 4
NM_001146048	*Lrrc1*	leucine rich repeat containing 1
NM_028838	*Lrrc2*	leucine rich repeat containing 2
NM_178005	*Lrrtm2*	leucine rich repeat transmembrane neuronal 2
NM_181470	*Ltv1*	LTV1 ribosome biogenesis factor
NM_053201	*Magee1*	melanoma antigen, family E, 1
NM_027920	*March8*	membrane-associated ring finger (C3HC4) 8
NM_008566	*Mcm5*	minichromosome maintenance complex component 5

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_001252094	*Mettl20*	electron transfer flavoprotein beta subunit lysine methyltransferase
NM_019721	*Mettl3*	methyltransferase like 3
NM_001243584	*Mif4gd*	MIF4G domain containing
NM_001285487	*Mknk1*	MAP kinase-interacting serine/threonine kinase 1
NM_020042	*Mocs1*	molybdenum cofactor synthesis 1
NM_001159288	*Morc2a*	microrchidia 2A
NM_001101482	*Mrap2*	melanocortin 2 receptor accessory protein 2
NM_025878	*Mrps18b*	mitochondrial ribosomal protein S18B
NM_013759	*Msrb1*	methionine sulfoxide reductase B1
NM_172722	*Naa25*	N(alpha)-acetyltransferase 25, NatB auxiliary subunit
NM_008669	*Naga*	N-acetyl galactosaminidase, alpha
NM_178728	*Napepld*	N-acyl phosphatidylethanolamine phospholipase D
NM_023239	*Ndnl2*	necdin-like 2
NM_001163592	*Nhsl1*	NHS-like 1
NM_028024	*Nkiras2*	NFKB inhibitor interacting Ras-like protein 2
NM_010342	*Npbwr1*	neuropeptides B/W receptor 1
NM_198326	*Nsfl1c*	NSFL1 (p97) cofactor (p47)
NM_026497	*Nudt12*	nudix (nucleoside diphosphate linked moiety X)-type motif 12
NM_146317	*Olfr725*	olfactory receptor 725
NM_019409	*Omg*	oligodendrocyte myelin glycoprotein
NM_145517	*Ormdl1*	ORM1-like 1 (S. cerevisiae)
NM_145460	*Oxnad1*	oxidoreductase NAD-binding domain containing 1
NM_028944	*P4htm*	prolyl 4-hydroxylase, transmembrane (endoplasmic reticulum)
NM_027032	*Pacrg*	PARK2 co-regulated
NM_025823	*Pcyox1*	prenylcysteine oxidase 1
NM_008803	*Pde8a*	phosphodiesterase 8A
NM_011057	*Pdgfb*	platelet derived growth factor, B polypeptide
NM_133667	*Pdk2*	pyruvate dehydrogenase kinase, isoenzyme 2
NM_013743	*Pdk4*	pyruvate dehydrogenase kinase, isoenzyme 4
NM_001163314	*Pgap1*	post-GPI attachment to proteins 1
NM_001109690	*Phf21a*	PHD finger protein 21A
NM_053191	*Pi15*	peptidase inhibitor 15
NM_001081456	*Plcd4*	phospholipase C, delta 4
NM_019588	*Plce1*	phospholipase C, epsilon 1
NM_001164056	*Pld1*	phospholipase D1
NM_007408	*Plin2*	perilipin 2
NM_133931	*Pot1a*	protection of telomeres 1A
NM_023200	*Ppp1r7*	protein phosphatase 1, regulatory (inhibitor) subunit 7
NM_031869	*Prkab1*	protein kinase, AMP-activated, beta 1 non-catalytic subunit
NM_001252458	*Prkd2*	protein kinase D2

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_207232	*Ptpdc1*	protein tyrosine phosphatase domain containing 1
NM_001163565	*Ptpn5*	protein tyrosine phosphatase, non-receptor type 5
NM_153781	*Pygb*	brain glycogen phosphorylase
NM_001024945	*Qsox1*	quiescin Q6 sulfhydryl oxidase 1
NM_009000	*Rab24*	RAB24, member RAS oncogene family
NM_175122	*Rab39b*	RAB39B, member RAS oncogene family
NM_019491	*Rala*	v-ral simian leukemia viral oncogene A (ras related)
NM_178045	*Rassf4*	Ras association (RalGDS/AF-6) domain family member 4
NM_018750	*Rassf5*	Ras association (RalGDS/AF-6) domain family member 5
NM_015754	*Rbbp9*	retinoblastoma binding protein 9
NM_001286653	*Rcan2*	regulator of calcineurin 2
NM_001163512	*Rgs12*	regulator of G-protein signaling 12
NM_007483	*Rhob*	ras homolog family member B
NM_001163354	*Rhot1*	ras homolog family member T1
NM_030259	*Rilpl2*	Rab interacting lysosomal protein-like 2
NM_001163310	*Rit1*	Ras-like without CAAX 1
NM_024288	*Rmnd5a*	required for meiotic nuclear division 5 homolog A
NM_001163461	*Rpap2*	RNA polymerase II associated protein 2
NM_145620	*Rrp9*	RRP9, small subunit (SSU) processome component, homolog (yeast)
NM_001256073	*Scgb1b3*	secretoglobin, family 1B, member 3
NM_172938	*Scml4*	sex comb on midleg-like 4 (Drosophila)
NM_018732	*Scn3a*	sodium channel, voltage-gated, type III, alpha
NM_001098227	*Sdcbp*	syndecan binding protein
NM_011342	*Sec22b*	SEC22 homolog B, vesicle trafficking protein
NM_001253386	*Serinc2*	serine incorporator 2
NM_009252	*Serpina3n*	serine (or cysteine) peptidase inhibitor, clade A, member 3N
NM_021286	*Sez6*	seizure related gene 6
NM_001040459	*Shroom4*	shroom family member 4
NM_011734	*Siae*	sialic acid acetylesterase
NM_001110350	*Sin3a*	transcriptional regulator, SIN3A (yeast)
NM_172152	*Slc24a4*	solute carrier family 24 (sodium/potassium/calcium exchanger), member 4
NM_007854	*Slc29a2*	solute carrier family 29 (nucleoside transporters), member 2
NM_001164639	*Slk*	STE20-like kinase
NM_183316	*Snapc5*	small nuclear RNA activating complex, polypeptide 5
NM_009238	*Sox4*	SRY (sex determining region Y)-box 4
NM_023220	*Sppl2a*	signal peptide peptidase like 2A
NM_001081037	*Srgap1*	SLIT-ROBO Rho GTPase activating protein 1
NM_011374	*St8sia1*	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1
NM_013666	*St8sia5*	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 5

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_024222	*Stt3b*	STT3, subunit of the oligosaccharyltransferase complex, homolog B (S. cerevisiae)
NM_011505	*Stxbp4*	syntaxin binding protein 4
NM_025617	*Tceanc2*	transcription elongation factor A (SII) N-terminal and central domain containing 2
NM_146008	*Tcp11l2*	t-complex 11 (mouse) like 2
NM_001184706	*Tfdp2*	transcription factor Dp 2
NM_001289550	*Tgfb1i1*	transforming growth factor beta 1 induced transcript 1
NM_009381	*Thrsp*	thyroid hormone responsive
NM_001081145	*Tigd2*	tigger transposable element derived 2
NM_172664	*Tlk1*	tousled-like kinase 1
NM_011604	*Tlr6*	toll-like receptor 6
NM_027992	*Tmem106b*	transmembrane protein 106B
NM_172049	*Tmem18*	transmembrane protein 18
NM_027935	*Tmem50a*	transmembrane protein 50A
NM_001164792	*Tpbg*	trophoblast glycoprotein
NM_001170855	*Trim36*	tripartite motif-containing 36
NM_001170912	*Trim66*	tripartite motif-containing 66
NM_173378	*Trp53bp2*	transformation related protein 53 binding protein 2
NM_001199105	*Trp53inp1*	transformation related protein 53 inducible nuclear protein 1
NM_009447	*Tuba4a*	tubulin, alpha 4A
NM_146116	*Tubb4b*	tubulin, beta 4B class IVB
NM_023053	*Twsg1*	twisted gastrulation BMP signaling modulator 1
NM_001083319	*Ubp1*	upstream binding protein 1
NM_026573	*Upf3b*	UPF3 regulator of nonsense transcripts homolog B (yeast)
NM_146216	*Vac14*	Vac14 homolog (S. cerevisiae)
NM_001025250	*Vegfa*	vascular endothelial growth factor A
NM_011728	*Xpa*	xeroderma pigmentosum, complementation group A
NM_011916	*Xrn1*	5'-3' exoribonuclease 1
NM_023249	*Ypel1*	yippee-like 1 (Drosophila)
NM_001005341	*Ypel2*	yippee-like 2 (Drosophila)
NM_178404	*Zc3h6*	zinc finger CCCH type containing 6
NM_001007460	*Zdhhc23*	zinc finger, DHHC domain containing 23
NM_001113399	*Zfp385b*	zinc finger protein 385B
NM_175480	*Zfp612*	zinc finger protein 612
NM_172738	*Zfp954*	zinc finger protein 954
NM_027335	*2210016F16Rik*	RIKEN cDNA 2210016F16 gene
NR_030708	*6820431F20Rik*	cadherin 11 pseudogene
High Cell Death Females Only		
NM_013454	*Abca1*	ATP-binding cassette, sub-family A (ABC1), member 1

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_009338	*Acat2*	acetyl-Coenzyme A acetyltransferase 2
NM_153151	*Acat3*	acetyl-Coenzyme A acetyltransferase 3
NM_133222	*Adgrl4*	adhesion G protein-coupled receptor L4
NM_001289656	*Agbl3*	ATP/GTP binding protein-like 3
NM_009642	*Agtrap*	angiotensin II, type I receptor-associated protein
NM_021299	*Ak3*	adenylate kinase 3
NM_001042541	*Akap1*	A kinase (PRKA) anchor protein 1
NM_175667	*Ankef1*	ankyrin repeat and EF-hand domain containing 1
NM_133237	*Apcdd1*	adenomatosis polyposis coli down-regulated 1
NM_001198911	*Arhgef2*	rho/rac guanine nucleotide exchange factor (GEF) 2
NM_009713	*Arsa*	arylsulfatase A
NM_026855	*Arv1*	ARV1 homolog, fatty acid homeostasis modulator
NM_020025	*B3galt2*	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2
NM_019835	*B4galt5*	UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 5
NM_001001182	*Baz2b*	bromodomain adjacent to zinc finger domain, 2B
NM_199195	*Bckdhb*	branched chain ketoacid dehydrogenase E1, beta polypeptide
NM_007574	*C1qc*	complement component 1, q subcomponent, C chain
NM_001146287	*Cables1*	CDK5 and Abl enzyme substrate 1
NM_001159319	*Cacnb1*	calcium channel, voltage-dependent, beta 1 subunit
NM_009786	*Cacybp*	calcyclin binding protein
NM_011796	*Capn10*	calpain 10(Capn10)
NM_001284503	*Cask*	calcium/calmodulin-dependent serine protein kinase (MAGUK family)
NM_001109873	*Cbfa2t3*	core-binding factor, runt domain, alpha subunit 2, translocated to, 3 (human)
NM_007625	*Cbx4*	chromobox 4
NM_001111060	*Cd59a*	CD59a antigen
NM_009872	*Cdk5r2*	cyclin-dependent kinase 5, regulatory subunit 2 (p39)
NM_001033443	*Cdkl4*	cyclin-dependent kinase-like 4
NM_007692	*Chkb*	choline kinase beta
NM_021350	*Chml*	choroideremia-like
NM_001289429	*Cipc*	CLOCK interacting protein, circadian
NM_016856	*Cpsf2*	cleavage and polyadenylation specific factor 2
NM_031251	*Ctns*	cystinosis, nephropathic
NM_007801	*Ctsh*	cathepsin H
NM_177662	*Ctso*	cathepsin O
NM_001012477	*Cxcl12*	chemokine (C-X-C motif) ligand 12
NM_001045525	*Cyb5d1*	cytochrome b5 domain containing 1
NM_001008231	*Daam2*	dishevelled associated activator of morphogenesis 2
NM_153555	*Dcaf8*	DDB1 and CUL4 associated factor 8
NM_013932	*Ddx25*	DEAD (Asp-Glu-Ala-Asp) box polypeptide 25

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_001039514	*Dhps*	deoxyhypusine synthase
NM_028136	*Dhx36*	DEAH (Asp-Glu-Ala-His) box polypeptide 36
NM_001105667	*Dtymk*	deoxythymidylate kinase
NM_026932	*Ebna1bp2*	EBNA1 binding protein 2
NM_172698	*Efcab14*	EF-hand calcium binding domain 14
NM_001111277	*Eif2b3*	eukaryotic translation initiation factor 2B, subunit 3
NM_018749	*Eif3d*	eukaryotic translation initiation factor 3, subunit D
NM_001286411	*Elf1*	E74-like factor 1
NM_175101	*Emc3*	ER membrane protein complex subunit 3
NM_001146348	*Eng*	endoglin
NM_175353	*Exoc6*	exocyst complex component 6
NM_029007	*Fam84a*	family with sequence similarity 84, member A
NM_001004147	*Fbll1*	fibrillarin-like 1
NM_001081243	*Filip1*	filamin A interacting protein 1
NM_001159573	*Fip1l1*	FIP1 like 1 (S. cerevisiae)
NM_134080	*Flnb*	filamin, beta
NM_172673	*Frmd5*	FERM domain containing 5
NM_173739	*Galnt18*	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 18
NM_010288	*Gja1*	gap junction protein, alpha 1
NM_001038015	*Gnpda2*	glucosamine-6-phosphate deaminase 2
NM_175193	*Golim4*	golgi integral membrane protein 4
NM_177366	*Gpr157*	G protein-coupled receptor 157
NM_181850	*Grm3*	glutamate receptor, metabotropic 3
NM_008175	*Grn*	granulin
NM_008229	*Hdac2*	histone deacetylase 2
NM_027382	*Hdac8*	histone deacetylase 8
NM_175189	*Hepacam*	hepatocyte cell adhesion molecule
NM_145942	*Hmgcs1*	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1
NM_019752	*Htra2*	HtrA serine peptidase 2
NM_029573	*Idh3a*	isocitrate dehydrogenase 3 (NAD+) alpha
NM_010518	*Igfbp5*	insulin-like growth factor binding protein 5
NM_010551	*Il16*	interleukin 16
NM_172471	*Itih5*	inter-alpha (globulin) inhibitor H5
NM_001289437	*Kansl2*	KAT8 regulatory NSL complex subunit 2
NM_001039347	*Kcnd3*	potassium voltage-gated channel, Shal-related family, member 3
NM_032397	*Kcnn1*	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 1
NM_001145779	*Kif2a*	kinesin family member 2A
NM_178357	*Klf11*	Kruppel-like factor 11
NM_011803	*Klf6*	Kruppel-like factor 6

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_008379	*Kpnb1*	karyopherin (importin) beta 1
NM_021284	*Kras*	Kirsten rat sarcoma viral oncogene homolog
NM_173012	*Letm2*	leucine zipper-EF-hand containing transmembrane protein 2
NM_001199043	*Lgals8*	lectin, galactose binding, soluble 8
NM_001083125	*Lhx6*	LIM homeobox protein 6
NM_011698	*Lin7b*	lin-7 homolog B (C. elegans)
NM_145376	*Lpcat1*	lysophosphatidylcholine acyltransferase 1
NM_001145952	*Lpp*	LIM domain containing preferred translocation partner in lipoma
NM_028233	*Lrpprc*	leucine-rich PPR-motif containing
NM_001164036	*Ly6e*	lymphocyte antigen 6 complex, locus E
NM_001163628	*Lyrm5*	LYR motif containing 5
NM_015806	*Mapk6*	mitogen-activated protein kinase 6
NM_175439	*Mars2*	methionine-tRNA synthetase 2 (mitochondrial)
NM_028372	*Mblac2*	metallo-beta-lactamase domain containing 2
NM_008563	*Mcm3*	minichromosome maintenance complex component 3
NM_172457	*Mob3a*	MOB kinase activator 3A
NM_026483	*Mphosph10*	M-phase phosphoprotein 10 (U3 small nucleolar ribonucleoprotein)
NM_026490	*Mrpl19*	mitochondrial ribosomal protein L19
NM_177092	*Msrb3*	methionine sulfoxide reductase B3
NM_023556	*Mvk*	mevalonate kinase
NM_053089	*Naa15*	N(alpha)-acetyltransferase 15, NatA auxiliary subunit
NM_001081475	*Nasp*	nuclear autoantigenic sperm protein (histone-binding)
NM_001114085	*Nde1*	nudE neurodevelopment protein 1
NM_001170591	*Nfu1*	NFU1 iron-sulfur cluster scaffold
NM_010914	*Nfyb*	nuclear transcription factor-Y beta
NM_001163610	*Nhsl2*	NHS-like 2
NM_001164472	*Nip7*	NIP7, nucleolar pre-rRNA processing protein
NM_001271397	*Nol8*	nucleolar protein 8
NM_018868	*Nop58*	NOP58 ribonucleoprotein
NM_028749	*Npl*	N-acetylneuraminate pyruvate lyase
NM_008723	*Npm3*	nucleoplasmin 3
NM_173788	*Npr2*	natriuretic peptide receptor 2
NM_027289	*Nt5dc2*	5'-nucleotidase domain containing 2
NM_013745	*Nufip1*	nuclear fragile X mental retardation protein interacting protein 1
NM_001190179	*Nup35*	nucleoporin 35
NM_008751	*Nxph1*	neurexophilin 1
NM_001252326	*Pan2*	PAN2 poly(A) specific ribonuclease subunit
NM_027924	*Pdgfd*	platelet-derived growth factor, D polypeptide
NM_008831	*Phb*	prohibitin
NM_008884	*Pml*	promyelocytic leukemia

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_148932	*Pom121*	nuclear pore membrane protein 121
NM_177782	*Prex1*	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1
NM_145925	*Pttg1ip*	pituitary tumor-transforming 1 interacting protein
NM_029391	*Rab4b*	RAB4B, member RAS oncogene family
NM_177644	*Rasal2*	RAS protein activator like 2
NM_027526	*Rasgef1a*	RasGEF domain family, member 1A
NM_001204931	*Reep6*	receptor accessory protein 6
NM_024233	*Rexo2*	RNA exonuclease 2
NM_144528	*Rnf126*	ring finger protein 126
NM_001042556	*Rpf2*	ribosome production factor 2 homolog
NM_009121	*Sat1*	spermidine/spermine N1-acetyl transferase 1
NM_198021	*Scyl2*	SCY1-like 2 (S. cerevisiae)
NM_001161845	*Sgk1*	serum/glucocorticoid regulated kinase 1
NM_001168525	*Sgms1*	sphingomyelin synthase 1
NM_029612	*Slamf9*	SLAM family member 9
NM_013612	*Slc11a1*	solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1
NM_153150	*Slc25a1*	solute carrier family 25 (mitochondrial carrier citrate transporter), member 1
NM_178934	*Slc2a12*	solute carrier family 2 (facilitated glucose transporter), member 12
NM_001177627	*Slc2a6*	solute carrier family 2 (facilitated glucose transporter), member 6
NM_172653	*Slc39a10*	solute carrier family 39 (zinc transporter), member 10
NM_001012305	*Slc39a12*	solute carrier family 39 (zinc transporter), member 12
NM_029643	*Slc52a2*	solute carrier protein 52, member 2
NM_009320	*Slc6a6*	solute carrier family 6 (neurotransmitter transporter, taurine), member 6
NM_001007567	*Slc7a6os*	solute carrier family 7, member 6 opposite strand
NM_134133	*Smim3*	small integral membrane protein 3
NM_001177833	*Smox*	spermine oxidase
NM_144918	*Smyd5*	SET and MYND domain containing 5
NM_175483	*Snx33*	sorting nexin 33
NM_001190156	*Snx7*	sorting nexin 7
NM_009236	*Sox18*	SRY (sex determining region Y)-box 18
NM_013663	*Srsf3*	serine/arginine-rich splicing factor 3
NM_001001326	*St5*	suppression of tumorigenicity 5
NM_001252505	*St6gal1*	beta galactoside alpha 2,6 sialyltransferase 1
NM_001159745	*St8sia4*	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4
NM_013515	*Stom*	stomatin
NM_175367	*Ston2*	stonin 2
NM_001102423	*Stx16*	syntaxin 16
NM_133670	*Sult1a1*	sulfotransferase family 1A, phenol-preferring, member 1

Table 2-5. Continued.

Accession	Gene Symbol	Gene Name
NM_001164495	*Syn3*	synapsin III
NM_178385	*Tbcc*	tubulin-specific chaperone C
NM_001289603	*Tfcp2*	transcription factor CP2
NM_009368	*Tgfb3*	transforming growth factor, beta 3
NM_016897	*Timm23*	translocase of inner mitochondrial membrane 23
NM_026708	*Tlcd1*	TLC domain containing 1
NM_016928	*Tlr5*	toll-like receptor 5
NM_133211	*Tlr7*	toll-like receptor 7
NM_028766	*Tmem43*	transmembrane protein 43
NM_009415	*Tpi1*	triosephosphate isomerase 1
NM_001099792	*Trmt61a*	tRNA methyltransferase 61A
NM_001282086	*Trpc6*	transient receptor potential cation channel, subfamily C, member 6
NM_001177751	*Tsc22d1*	TSC22 domain family, member 1
NM_001145162	*Ube2ql1*	ubiquitin-conjugating enzyme E2Q family-like 1
NM_153131	*Unc5a*	unc-5 netrin receptor A
NM_013933	*Vapa*	vesicle-associated membrane protein, associated protein A
NM_001164314	*Wars*	tryptophanyl-tRNA synthetase
NM_001172152	*Wdr17*	WD repeat domain 17
NM_173181	*Zc2hc1a*	zinc finger, C2HC-type containing 1A
NM_175513	*Zfp804a*	zinc finger protein 804A
NM_001081005	*1500012F01Rik*	zinc finger, NFX1-type containing 1, antisense RNA 1

Table 2-6. Significant ethanol-induced gene expression changes that were unique to low cell death strains.

Accession	Gene Symbol	Gene Name
Both Low Cell Death Males and Low Cell Death Females		
NM_007481	*Arf6*	ADP-ribosylation factor 6
NM_183294	*Cdkl1*	cyclin-dependent kinase-like 1 (CDC2-related kinase)
NM_153409	*Csrnp3*	cysteine-serine-rich nuclear protein 3
NM_011932	*Dapp1*	dual adaptor for phosphotyrosine and 3-phosphoinositides 1
NM_172594	*Dhx29*	DEAH (Asp-Glu-Ala-His) box polypeptide 29
NM_133659	*Erg*	avian erythroblastosis virus E-26 (v-ets) oncogene related
NM_182808	*Fam19a1*	family with sequence similarity 19, member A1
NM_001081126	*Gpr161*	G protein-coupled receptor 16
NM_153419	*Grwd1*	glutamate-rich WD repeat containing 1
NM_010638	*Klf9*	Kruppel-like factor 9
NM_022565	*Ndst4*	N-deacetylase/N-sulfotransferase (heparin glucosaminyl) 4
NM_001048168	*Nfyc*	nuclear transcription factor-Y gamma
NM_134025	*Pex12*	peroxisomal biogenesis factor 12
NM_025443	*Pno1*	partner of NOB1 homolog
NM_183173	*Sowaha*	sosondowah ankyrin repeat domain family member A
NM_001040426	*Thsd4*	thrombospondin, type I, domain containing 4
NM_144953	*1700019D03Rik*	RIKEN cDNA 1700019D03 gene
Low Cell Death Males Only		
NM_198111	*Akap6*	A kinase (PRKA) anchor protein 6
NM_001277188	*Ano4*	anoctamin 4
NM_001042558	*Apaf1*	apoptotic peptidase activating factor 1
NM_007492	*Arx*	aristaless related homeobox
NM_179203	*Atad3a*	ATPase family, AAA domain containing 3A
NM_030235	*Avl9*	AVL9 homolog (S. cerevisiase)
NM_009747	*Bdkrb2*	bradykinin receptor, beta 2
NM_001277216	*Bmpr1b*	bone morphogenetic protein receptor, type 1B
NM_001199301	*Cacng5*	calcium channel, voltage-dependent, gamma subunit 5
NM_001285463	*Carf*	calcium response factor
NM_009831	*Ccng1*	cyclin G1
NM_001289915	*Cd83*	CD83 antigen
NM_009856	*Cd83*	CD83 antigen
NM_138585	*Cherp*	calcium homeostasis endoplasmic reticulum protein
NM_023215	*Chtop*	chromatin target of PRMT1
NM_009963	*Cry2*	cryptochrome 2 (photolyase-like)
NM_028868	*Cxxc1*	CXXC finger 1 (PHD domain)
NR_002854	*Dlx1as*	distal-less homeobox 1, antisense
NM_138669	*Eif4a3*	eukaryotic translation initiation factor 4A3

Table 2-6. Continued.

Accession	Gene Symbol	Gene Name
NM_001242423	*Fam105a*	family with sequence similarity 105, member A
NM_010197	*Fgf1*	fibroblast growth factor 1
NM_001077698	*Fmnl1*	formin-like 1
NM_177059	*Fstl4*	follistatin-like 4
NM_030719	*Gatsl2*	GATS protein-like 2
NM_008105	*Gcnt2*	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme
NM_023587	*Hacd2*	3-hydroxyacyl-CoA dehydratase 2
NM_198937	*Hn1l*	hematological and neurological expressed 1-like
NM_001081212	*Irs2*	insulin receptor substrate 2
NM_080465	*Kcnn2*	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 2
NM_026214	*Kctd4*	potassium channel tetramerisation domain containing 4
NM_029274	*Kmt2b*	lysine (K)-specific methyltransferase 2B
NM_138593	*Larp7*	La ribonucleoprotein domain family, member 7
NM_001029850	*Magi1*	membrane associated guanylate kinase, WW and PDZ domain containing 1
NM_001085373	*Mcc*	mutated in colorectal cancers
NM_023431	*Mum1*	melanoma associated antigen (mutated) 1
NM_001177965	*Naa10*	N(alpha)-acetyltransferase 10, NatA catalytic subunit
NM_021362	*Pappa*	pregnancy-associated plasma protein A
NM_029078	*Pcf11*	PCF11 cleavage and polyadenylation factor subunit
NM_028376	*Pfn4*	profilin family, member 4
NM_008891	*Pnn*	pinin
NM_001164082	*Polr3d*	polymerase (RNA) III (DNA directed) polypeptide D
NM_001081214	*Pprc1*	peroxisome proliferative activated receptor, gamma, coactivator-related 1
NM_133783	*Ptges2*	prostaglandin E synthase 2
NM_019933	*Ptpn4*	protein tyrosine phosphatase, non-receptor type 4
NM_001042499	*Rabl3*	RAB, member RAS oncogene family-like 3
NM_001136227	*Rtkn*	rhotekin
NM_009136	*Scrg1*	scrapie responsive gene 1
NM_027135	*Sec24d*	Sec24 related gene family, member D (S. cerevisiae)
NM_001142809	*Slc6a8*	solute carrier family 6 (neurotransmitter transporter, creatine), member 8
NM_177909	*Slc9a9*	solute carrier family 9 (sodium/hydrogen exchanger), member 9
NM_133854	*Snapin*	SNAP-associated protein
NM_029688	*Srxn1*	sulfiredoxin 1 homolog (S. cerevisiae)
NM_018803	*Syt10*	synaptotagmin X
NM_172476	*Tmc7*	transmembrane channel-like gene family 7
NM_177412	*Tmcc1*	transmembrane and coiled coil domains 1
NM_011607	*Tnc*	tenascin C
NM_198102	*Tra2a*	transformer 2 alpha homolog (Drosophila)

Table 2-6. Continued.

Accession	Gene Symbol	Gene Name
NM_178110	*Trim62*	tripartite motif-containing 62
NM_001142580	*Vipas39*	VPS33B interacting protein, apical-basolateral polarity regulator, spe-39 homolog
NM_175639	*Wdr43*	WD repeat domain 43
NM_178398	*Wipi2*	WD repeat domain, phosphoinositide interacting 2
NM_025347	*Ypel3*	yippee-like 3 (Drosophila)
NM_001033324	*Zbtb16*	zinc finger and BTB domain containing 16
NM_011981	*Zfp260*	zinc finger protein 260
NM_027264	*Zfp715*	zinc finger protein 715
NM_011763	*Zfp9*	zinc finger protein 9
NM_027251	*2010107G23Rik*	RIKEN cDNA 2010107G23 gene
NM_001113550	*4833420G17Rik*	RIKEN cDNA 4833420G17 gene
NM_001123370	*9030025P20Rik*	RIKEN cDNA 9030025P20 gene
Low Cell Death Females Only		
NM_001287180	*Atf4*	activating transcription factor 4
NM_001081304	*Atf6*	activating transcription factor 6
NM_018808	*Dnajb1*	DnaJ heat shock protein family (Hsp40) member B1
NM_172400	*Dnajc8*	DnaJ heat shock protein family (Hsp40) member C8
NM_013503	*Drd5*	dopamine receptor D5
NM_145537	*Edem2*	ER degradation enhancer, mannosidase alpha-like 2
NM_030565	*Fam20c*	family with sequence similarity 20, member C
NM_178213	*Hist2h2ab*	histone cluster 2, H2ab
NM_001013758	*Lingo3*	leucine rich repeat and Ig domain containing 3
NM_001166635	*Mid1ip1*	Mid1 interacting protein 1 (gastrulation specific G12-like (zebrafish))
NM_001163387	*Nlgn1*	neuroligin 1
NM_011865	*Pcbp1*	poly(rC) binding protein 1
NM_007531	*Phb2*	prohibitin 2
NM_001141981	*Rbm43*	RNA binding motif protein 43
NM_001252547	*Sh2d3c*	SH2 domain containing 3C
NM_001167983	*Sipa1l1*	signal-induced proliferation-associated 1 like 1
NM_001110240	*Slc24a2*	solute carrier family 24 (sodium/potassium/calcium exchanger), member 2
NM_133697	*Smim14*	small integral membrane protein 14
NM_009186	*Tra2b*	transformer 2 beta homolog (Drosophila)
NM_029979	*Trim35*	tripartite motif-containing 35
NM_001163769	*U2af1*	U2 small nuclear ribonucleoprotein auxiliary factor (U2AF) 1
NM_001111078	*Uhrf1*	ubiquitin-like, containing PHD and RING finger domains, 1
NM_145940	*Wipi1*	WD repeat domain, phosphoinositide interacting 1
NM_144546	*Zfp119a*	zinc finger protein 119a

Table 2-7. Sex-specific differential gene expression changes after exposure to ethanol in all strains.

Accession	Gene Symbol	Gene Name
Males Only		
NM_009705	*Arg2*	arginase type II
NM_175251	*Arid2*	AT rich interactive domain 2 (ARID, RFX-like)
BC011101	*Armcx3*	armadillo repeat containing, X-linked 3
NM_027870	*Armcx3*	armadillo repeat containing, X-linked 3
NM_001162485	*Arrdc1*	arrestin domain containing 1
NM_007631	*Ccnd1*	cyclin D1
NM_153384	*Clrn1*	clarin 1
NM_001290183	*Ddit3*	DNA-damage inducible transcript 3
NM_007837	*Ddit3*	DNA-damage inducible transcript 3
NM_025926	*Dnajb4*	DnaJ heat shock protein family (Hsp40) member B4
NM_010121	*Eif2ak3*	eukaryotic translation initiation factor 2 alpha kinase 3
NM_001168620	*Enpp5*	ectonucleotide pyrophosphatase/phosphodiesterase 5
NM_001163567	*Fam102b*	family with sequence similarity 102, member B
NM_080433	*Fezf2*	Fez family zinc finger 2
NM_175490	*Gpr75*	G protein-coupled receptor 75
NM_053262	*Hsd17b11*	hydroxysteroid (17-beta) dehydrogenase 11
NM_001162884	*Igsf10*	immunoglobulin superfamily, member 10
NM_001164598	*Irf2bp2*	interferon regulatory factor 2 binding protein 2
NM_030110	*Micu3*	mitochondrial calcium uptake family, member 3
NM_001005863	*Mtus1*	mitochondrial tumor suppressor 1
NM_001111324	*Nedd9*	neural precursor cell expressed, developmentally down-regulated gene 9
NM_146169	*Paip2b*	poly(A) binding protein interacting protein 2B
NM_183028	*Pcmtd1*	protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 1
NM_026383	*Pnrc2*	proline-rich nuclear receptor coactivator 2
NM_133249	*Ppargc1b*	peroxisome proliferative activated receptor, gamma, coactivator 1 beta
NM_027626	*Psd3*	pleckstrin and Sec7 domain containing 3
NM_177698	*Psd3*	pleckstrin and Sec7 domain containing 3
NM_025831	*Pxdc1*	PX domain containing 1
NM_001033172	*Rab11fip2*	RAB11 family interacting protein 2 (class I)
NM_001081549	*Rcan1*	regulator of calcineurin 1
NM_007901	*S1pr1*	sphingosine-1-phosphate receptor 1
NM_001079686	*Syne1*	spectrin repeat containing, nuclear envelope 1
NM_153399	*Syne1*	spectrin repeat containing, nuclear envelope 1
NM_001033304	*5330417C22Rik*	RIKEN cDNA 5330417C22 gene
Females Only		
NM_019764	*Amotl2*	angiomotin-like 2

Table 2-7. Continued.

Accession	Gene Symbol	Gene Name
NM_026965	*Comtd1*	catechol-O-methyltransferase domain containing 1
NM_013562	*Ifrd1*	interferon-related developmental regulator 1
NM_001024526	*Larp4*	La ribonucleoprotein domain family, member 4
NM_133807	*Lrrc59*	leucine rich repeat containing 59
NM_011840	*Map2k5*	mitogen-activated protein kinase kinase 5
NM_008808	*Pdgfa*	platelet derived growth factor, alpha
NM_030064	*Phf23*	PHD finger protein 23

Table 2-8. Ethanol-induced gene expression changes that were significant in all four groups: high cell death males (HCD-M), low cell death males (LCD-M), high cell death females (HCD-F), and low cell death females (LCD-F).

Accession	Gene Symbol	Gene Name
NM_030210	*Aacs*	acetoacetyl-CoA synthetase
NM_013851	*Abca8b*	ATP-binding cassette, sub-family A (ABC1), member 8b
NM_178162	*Agfg2*	ArfGAP with FG repeats 2
NM_001172205	*Arid5a*	AT rich interactive domain 5A (MRF1-like)
NM_023598	*Arid5b*	AT rich interactive domain 5B (MRF1-like)
NM_001042591	*Arrdc3*	arrestin domain containing 3
NM_001042592	*Arrdc4*	arrestin domain containing 4
NM_026217	*Atg12*	autophagy related 12
NM_016847	*Avpr1a*	arginine vasopressin receptor 1A
NM_001284410	*Bcl2l11*	BCL2-like 11 (apoptosis facilitator)
NM_007570	*Btg2*	B cell translocation gene 2, anti-proliferative
NM_009770	*Btg3*	B cell translocation gene 3
NM_177716	*Ccdc184*	coiled-coil domain containing 184
NM_001081345	*Chd2*	chromodomain helicase DNA binding protein 2
NM_001252525	*Cpeb1*	cytoplasmic polyadenylation element binding protein 1
NM_001110850	*Crem*	cAMP responsive element modulator
NM_007762	*Crhr1*	corticotropin releasing hormone receptor 1
NM_016748	*Ctps*	cytidine 5'-triphosphate synthase
NM_028979	*Cyp2j9*	cytochrome P450, family 2, subfamily j, polypeptide 9
NM_020010	*Cyp51*	cytochrome P450, family 51
NM_001252457	*Ddx39b*	DEAD (Asp-Glu-Ala-Asp) box polypeptide 39B
NM_053272	*Dhcr24*	24-dehydrocholesterol reductase
NM_001285807	*Dtna*	dystrobrevin alpha
NM_013642	*Dusp1*	dual specificity phosphatase 1
NM_001048054	*Dusp16*	dual specificity phosphatase 16
NM_008748	*Dusp8*	dual specificity phosphatase 8
NM_130450	*Elovl6*	ELOVL family member 6, elongation of long chain fatty acids (yeast)
NM_183187	*Fam107a*	family with sequence similarity 107, member A
NM_178908	*Fam26e*	family with sequence similarity 26, member E
NM_175104	*Fam53c*	family with sequence similarity 53, member C
NM_010191	*Fdft1*	farnesyl diphosphate farnesyl transferase 1
NM_001253751	*Fdps*	farnesyl diphosphate synthetase
NM_001164259	*Fgfrl1*	fibroblast growth factor receptor-like 1
NM_001159706	*Folh1*	folate hydrolase 1
NM_029102	*Glt8d2*	glycosyltransferase 8 domain containing 2
NM_008149	*Gpam*	glycerol-3-phosphate acyltransferase, mitochondrial
NM_027518	*Gpr137c*	G protein-coupled receptor 137C
NM_145066	*Gpr85*	G protein-coupled receptor 85

Table 2-8. Continued.

Accession	Gene Symbol	Gene Name
NM_144835	*Heatr1*	HEAT repeat containing 1
NM_008255	*Hmgcr*	3-hydroxy-3-methylglutaryl-Coenzyme A reductase
NM_008256	*Hmgcs2*	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2
NM_007545	*Hrk*	harakiri, BCL2 interacting protein (contains only BH3 domain)
NM_010476	*Hsd17b7*	hydroxysteroid (17-beta) dehydrogenase 7
NM_019564	*Htra1*	HtrA serine peptidase 1
NM_015790	*Icosl*	icos ligand
NM_145360	*Idi1*	isopentenyl-diphosphate delta isomerase
NM_172439	*Inpp5j*	inositol polyphosphate 5-phosphatase J
NM_153526	*Insig1*	insulin induced gene 1
NM_010591	*Jun*	jun proto-oncogene
NM_001286944	*Jund*	jun D proto-oncogene
NM_001081134	*Kcng1*	potassium voltage-gated channel, subfamily G, member 1
NM_078477	*Klf16*	Kruppel-like factor 16
NM_029436	*Klhl24*	kelch-like 24
NM_001252658	*Ldlr*	low density lipoprotein receptor
NM_001252055	*Ly6c1*	lymphocyte antigen 6 complex, locus C1
NM_011841	*Mapk7*	mitogen-activated protein kinase 7
NM_175341	*Mbnl2*	muscleblind-like 2
NM_023799	*Mgea5*	meningioma expressed antigen 5 (hyaluronidase)
NM_021565	*Midn*	midnolin
NM_025436	*Msmo1*	methylsterol monoxygenase 1
NM_013602	*Mt1*	metallothionein 1
NM_008630	*Mt2*	metallothionein 2
NM_138656	*Mvd*	mevalonate (diphospho) decarboxylase
NM_001008542	*Mxi1*	MAX interactor 1, dimerization protein
NM_001145959	*Ndrg2*	N-myc downstream regulated gene 2
NM_026742	*Ndufaf4*	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, assembly factor 4
NM_028995	*Nipal3*	NIPA-like domain containing 3
NM_138747	*Nop2*	NOP2 nucleolar protein
NM_024193	*Nop56*	NOP56 ribonucleoprotein
NM_010941	*Nsdhl*	NAD(P) dependent steroid dehydrogenase-like
NM_001134791	*Osbpl9*	oxysterol binding protein-like 9
NM_153594	*Pcmtd2*	protein-L-isoaspartate (D-aspartate) O-methyltransferase domain containing 2
NM_178149	*Pik3ip1*	phosphoinositide-3-kinase interacting protein 1
NM_001024955	*Pik3r1*	phosphatidylinositol 3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)
NM_152813	*Plcd3*	phospholipase C, delta 3
NM_001033253	*Plekhg1*	pleckstrin homology domain containing, family G (with RhoGef domain) member

Table 2-8. Continued.

Accession	Gene Symbol	Gene Name
NM_013807	*Plk3*	polo-like kinase 3
NM_029494	*Rab30*	RAB30, member RAS oncogene family
NM_001038621	*Rabgap1l*	RAB GTPase activating protein 1-like
NM_001099624	*Rapgef2*	Rap guanine nucleotide exchange factor (GEF) 2
NM_001252494	*Rapgef6*	Rap guanine nucleotide exchange factor (GEF) 6
NM_028234	*Rbm33*	RNA binding motif protein 33
NM_001166553	*Rnf145*	ring finger protein 145
NM_146244	*Rps6kl1*	ribosomal protein S6 kinase-like 1
NM_009129	*Scg2*	secretogranin II
NM_011521	*Sdc4*	syndecan 4
NM_030261	*Sesn3*	sestrin 3
NM_027921	*Slc16a14*	solute carrier family 16 (monocarboxylic acid transporters), member 14
NM_001159593	*Slc20a1*	solute carrier family 20, member 1
NM_011400	*Slc2a1*	solute carrier family 2 (facilitated glucose transporter), member 1
NM_001081263	*Slc44a5*	solute carrier family 44, member 5
NM_001038643	*Slco3a1*	solute carrier organic anion transporter family, member 3a1
NM_001252481	*Smad2*	SMAD family member 2
NM_001025428	*Spag9*	sperm associated antigen 9
NM_182927	*Spred3*	sprouty-related, EVH1 domain containing 3
NM_011898	*Spry4*	sprouty homolog 4 (Drosophila)
NM_009270	*Sqle*	squalene epoxidase
NM_009272	*Srm*	spermidine synthase
NM_016795	*Srpk1*	serine/arginine-rich protein specific kinase 1
NM_011358	*Srsf2*	serine/arginine-rich splicing factor 2
NM_133774	*Stard4*	StAR-related lipid transfer (START) domain containing 4
NM_019675	*Stmn4*	stathmin-like 4
NM_001040085	*Sytl2*	synaptotagmin-like 2
NM_174989	*Ticam1*	toll-like receptor adaptor molecule 1
NM_001048267	*Tnpo1*	transportin 1
NM_001109748	*Tomm40*	translocase of outer mitochondrial membrane 40 homolog (yeast)
NM_001009935	*Txnip*	thioredoxin interacting protein
NM_001169576	*Ube2h*	ubiquitin-conjugating enzyme E2H
NM_030724	*Uck2*	uridine-cytidine kinase 2
NM_145967	*Vstm2a*	V-set and transmembrane domain containing 2A
NM_001005342	*Ypel4*	yippee-like 4 (Drosophila)
NM_027166	*Ypel5*	yippee-like 5 (Drosophila)
NM_001110309	*Zfp426*	zinc finger protein 426
NR_045177	*2900055J20Rik*	RIKEN cDNA 2900055J20 gene
NM_001033273	*5031439G07Rik*	RIKEN cDNA 5031439G07 gene

Discussion

This study was designed to better understand the molecular mechanisms underlying genetic variation in susceptibility to the teratogenic effects of alcohol. The BXD RI strains are a useful resource to examine genetic variation in animal models of developmental alcohol exposure. Previous studies have shown differential vulnerability to several developmental phenotypes and malformations in the BXD mice after exposure to developmental alcohol (Downing, Balderrama-Durbin, et al., 2012; Goldowitz et al., 2014). For example, a study found differential susceptibility to ethanol-induced skeletal and soft-tissue malformations in the BXD strains after exposure to prenatal ethanol (Downing, Balderrama-Durbin, et al., 2012). By using the BXD strains and subsequent quantitative trait locus (QTL) mapping, they were able to identify genomic regions and candidate genes mediating genetic variation to the teratogenic effects of ethanol. Similarly, a previous study from our lab examined fourteen BXD strains and the two parental, B6 and D2 strains and identified differential susceptibility to cell death in the hippocampus after exposure to postnatal ethanol (Goldowitz et al., 2014). Out of the sixteen strains examined, we identified three BXD strains that showed high susceptibility to ethanol-induced cell death and three BXD strains that showed low vulnerability after exposure to neonatal ethanol (Goldowitz et al., 2014). Subsequent QTL mapping of ethanol-induced hippocampal cell death phenotype identified a small genomic region and possible candidate genes (Goldowitz et al., 2014). While these previous studies in the BXD strains were able to identify genetic variation in ethanol teratogenic phenotypes and identify presumed candidate genes mediating these responses, further studies are needed to examine strain differences in gene expression changes after exposure to developmental alcohol. Therefore, in the present study, we examined ethanol-induced gene expression changes in the BXD strains that showed differential vulnerability to hippocampal cell death after exposure to neonatal ethanol. Additionally, as a recent study has reported sex-specific hippocampal gene expression changes after exposure to developmental alcohol, we extended our analysis, examining both males and females separately, to identify sex-specific differences within or between strains that show differential vulnerability to ethanol-induced cell death in the hippocampus (Lunde-Young et al., 2019).

We identified gene expression changes after postnatal ethanol exposure in all BXD and parental strains with little overlap between males and females in the same strain. However, there were limited sex x treatment interactions in our analysis and further enrichment analysis failed to identify any significant gene ontology categories. In contrast, strain x treatment and strain x sex x treatment analysis revealed numerous ethanol-induced gene expression changes showed strain differences. Enrichment analysis revealed over-represented categories in our differentially expressed genes list included a number of apoptosis pathways. Genes that were involved in cell death pathways and also showed high fold changes after ethanol exposure included *Bcl2l11*, a member of the BCL-2 family that has been shown to act as an apoptotic activator (Luo & Rubinsztein, 2013), *Jun*, the transcription factor that has been shown to induce apoptosis, among its many functions (Bossy-Wetzel, Bakiri, & Yaniv, 1997), and *Txnip*, a member of the alpha arrestin family whose upregulation has been linked to inflammasome activation and apoptosis (**Figure 2-7A-C**) (Ye et al., 2017). Because of their large ethanol-induced gene

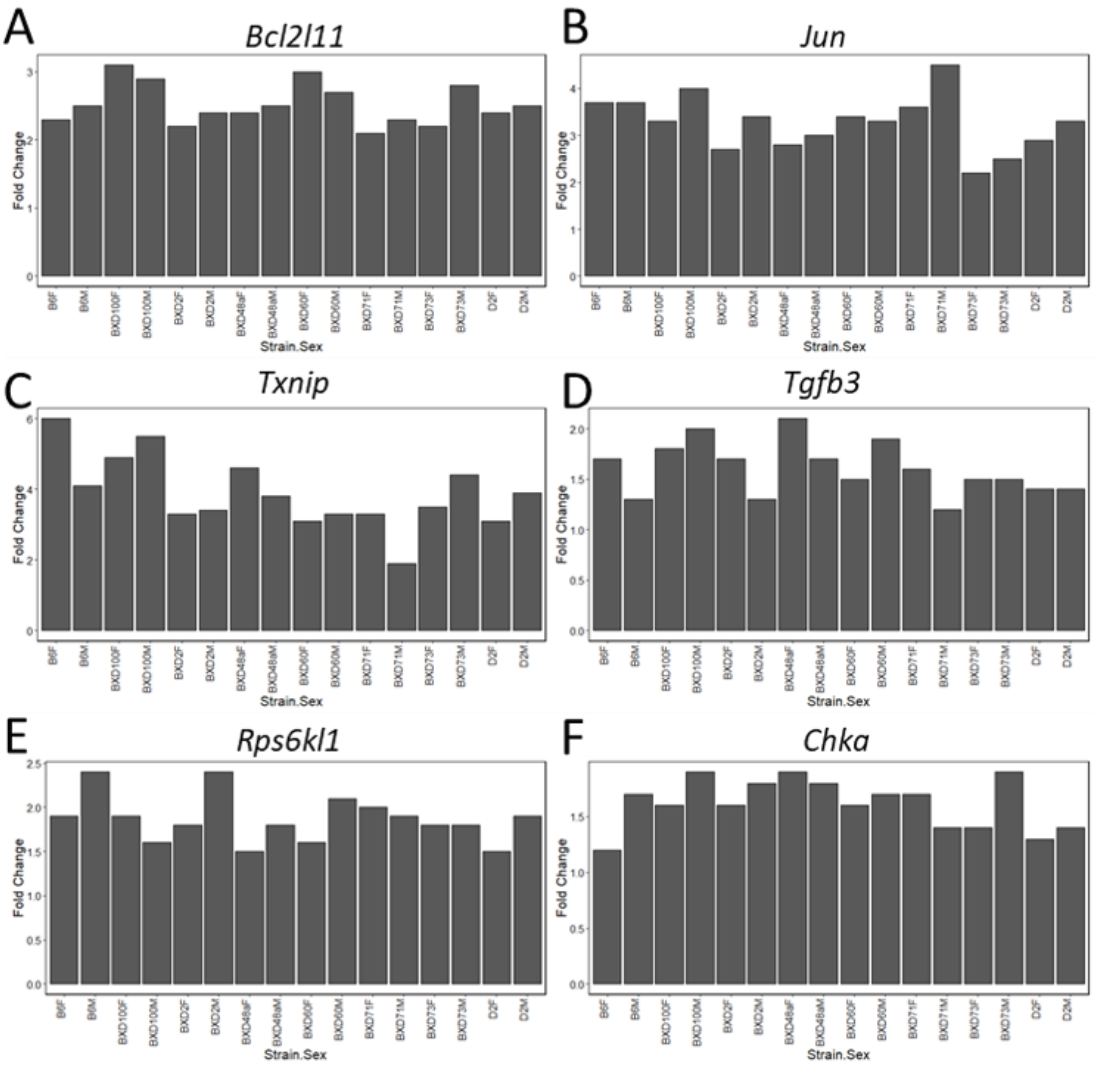

Figure 2-7. Strain differences for differentially expressed genes in the hippocampus after postnatal ethanol exposure.

Differential ethanol-induced expression changes of (A) *Bcl2l11*, (B) *Jun*, (C) *Txnip*, (D) *Tgfb3*, (E) *Rps6kl1*, and (F) *Chka*. in both males and females of all strains examined: B6, BXD100, BXD2, BXD48a, BXD60, BXD71, BXD73, and D2.

expression, varied responses among strains, and previous links to cell death and apoptosis, these genes were further analyzed using free tools available on genenetwork.org. For each gene, correlations between hippocampal gene expression from the accessible "Hippocampus Consortium M430v02 (Jun06) PDNN" and previously published behavioral phenotypes in the BXD strains were calculated. All three genes were found to be significantly correlated with a number of behavioral phenotypes including anxiety, activity, learning and memory, and ethanol behaviors such as drinking in the dark. For learning and memory behaviors, *Bcl2l11* and *Jun* were correlated with a number of contextual fear conditioning and spatial memory (such as the Y-maze) phenotypes, while *Txnip* was correlated with multiple reversal leaning phenotypes. *Txnip* and *Jun* were also correlated with hippocampal morphology including hippocampus mossy fiber pathway volume. Though the most interesting phenotype correlations were the large number of apoptosis and neurogenesis phenotypes that were correlated with *Bcl2l11* and *Jun*. *Bcl2l11* was correlated with cell production and cell death in neurons as well as apoptosis and neurogenesis during development. *Jun* in particular was found to be correlated with a number of cell death traits during development including cell death in the brainstem following prenatal ethanol exposure. As genes with polymorphisms between the parental strains are more likely to be involved in the regulation of differential phenotypes, we used the variant browser on www.GeneNetwork.org to identify the presence of any polymorphisms including nonsynonymous single nucleotide polymorphism (SNP). We identified both *Bcl2l11* and *Jun* contained SNPs between the parental strains while information for the D2 strain was not available for *Txnip*. These results identify *Bcl2l11*, *Jun*, and *Txnip* as excellent candidates involved in the differential cell death phenotype see in the BXD RI and parental strains.

There were also a number of growth factors that have been linked to either apoptosis or neuroprotection that were significantly differentially expressed among the strains after ethanol exposure including *Vgf*, a gene expressed in a subpopulation of neuroendocrine cells that upregulated by nerve growth factor (Kury, Schroeter, & Jander, 2004; Takeuchi et al., 2018), *Egr3*, an immediate-early growth response gene induced by mitogenic stimulation (H. Yang, Dong, Zhou, & Li, 2021), *Fgf1*, a member of the fibroblast growth factor family involved in cell migration and proliferation (Liu et al., 2018; Pirou et al., 2017; Rodriguez-Enfedaque et al., 2009), *Fgfr3*, a member of the fibroblast growth factor receptor family involved in mitogenesis and differentiation (X. Liao et al., 2018; Okada et al., 2019), and *Tgfb3*, a member of the transforming growth factor-beta superfamily involved in recruitment and activation of transcription factors that regulate gene expression (Ke, Mei, Wong, & Lo, 2019; Yuan et al., 2019). Interestingly, *Tgfb3* was identified within the significant QTL for our ethanol induced-hippocampal cell death from our previous study (**Figure 2-7D**) (Goldowitz et al., 2014). Another gene that was within the previously identified QTL and was differentially expressed in BXD strains after exposure to postnatal ethanol was *Rps6kl1*, a member of the ribosomal S6 kinase family involved in protein synthesis, cell growth, and cell proliferation (**Figure 2-7E**). Additional analysis found *Tgfb3* was significantly correlated with developmental apoptosis and neurogenesis as well as behaviors involving learning and memory and ethanol responses. *Rps6kl1* was correlated with less relevant phenotypes though it was correlated with hippocampal mossy fiber pathway volume and cell proliferation through

Tgfb2 stimulation. *Chka*, a catalyzing enzyme in the choline metabolism pathway, was also differentially expressed in BXD strains after developmental ethanol exposure. *Chka* has recently been linked to apoptotic pathways (Raikundalia, Sa'Dom, Few, & Too, 2021; Rizzo et al., 2021). This is of particular interest as choline supplementation has been shown to alleviate postnatal growth restriction and alterations in cognitive functioning in humans exposed to prenatal alcohol (Jacobson et al., 2018). In animal models, choline supplementation has also been shown to decrease FASD-like behaviors as well as modulate inflammation and apoptosis (Bottom, Abbott, & Huffman, 2020; King et al., 2019). Correlational analysis showed *Chka* was significantly correlated with numerous hippocampal phenotypes including volume of the dentate gyrus and volume of CA3/CA4. *Chka* was also correlated with cell death after exposure to prenatal ethanol as well as cell proliferation through Tgfb2 stimulation. Additional analysis also showed that *Chka* has non-synonymous SNPs between the parental B6 and D2 strains.

The parental B6 and D2 strains have shown differential vulnerability to developmental alcohol exposure including ethanol-induced malformations and apoptotic responses (Chen et al., 2011; Downing et al., 2009). In most studies, the B6 strain has shown to be more susceptible to ethanol-induced developmental abnormalities while the D2 strain has shown to be relatively resistant (Boehm et al., 1997; Downing et al., 2009). Previous studies have used this differential vulnerability in B6 and D2 strains to compare gene expression changes that are present in one strain and not the other to identify genes that might mediate this differential response (Downing, Balderrama-Durbin, et al., 2012; Lossie et al., 2014). Similarly, in the present study we took advantage of BXD strains that showed differential cell death in the hippocampus after exposure to postnatal ethanol. We compared the differential gene expression changes in the BXD strains that showed high susceptibility to ethanol-induced cell death to see which genes were expressed in all three HCD strains. Likewise, we compared ethanol-induced gene expression changes in the BXD strains that were resistant to cell death in the hippocampus after postnatal ethanol exposure in all three LCD strains. Due to the large sex-specific changes within each strain we analyzed the males and females separately in this analysis.

We identified marked differences in ethanol-induced gene expression changes between the high cell death strains (HCD) and low cell death strains (LCD). The number of ethanol-induced gene expression changes were much higher in the HCD strains compared to the LCD strains in both sexes. Both HCD males and females showed higher number of ethanol-induced gene expression changes: the HCD-M showed 1.6x more differentially expressed gene changes compared to LCD-M and the number of ethanol-induced gene expression changes in the HCD-F was double those found in the LCD-F. Additionally, enrichment analysis found several apoptosis and cell death pathways that were unique to HCD-M compared to LCD-M. While females in both HCD and LCD strains showed cell death pathways, there were a number of cytokine pathways that were unique to the HCD-F. Enrichment analysis found locomotion categories in both HCD and LCD males. Females showed a number of behavioral categories including associative learning which was found in both HCD and LCD females. Visual learning and locomotion were found in the HCD-F while learning and cognition were found in the LCD-F. These results demonstrate that there are substantial variations in differential

ethanol-induced gene expression and subsequent biological pathways between the HCD and LCD strains.

Our gene expression analysis focused on examining specific differences and similarities in significant gene expression changes between the four groups, HCD-M, HCD-F, LCD-M, and LCD-F. We identified a number of differentially expressed genes that have been previously linked to developmental ethanol exposure in all four groups. Ethanol-induced gene expression changes that were found in male and female HCD strains, but not LCD strains, and that have been previously linked to gene expression changes after developmental alcohol exposure were *Aimp2*, *Apold1*, *Bysl*, *Dnajc13*, *Heg1*, *Iws1*, *Mdn1*, *Pde4b*, and *Rgs19* (Downing, Flink, et al., 2012; Kleiber et al., 2013; Lossie et al., 2014; Theberge et al., 2019). Interestingly, a previous study examining the B6 and D2 strains found *Aimp2*, a gene that functions as a proapoptotic factor, was differentially expressed after prenatal ethanol exposure in the B6 but not D2 strain (Downing, Balderrama-Durbin, et al., 2012). *Dnajc13*, a member of the Dnaj family that associates with heat-shock proteins, has been found in multiple studies on developmental ethanol exposure including a study that showed ethanol-induced expression changes of *Dnajc13* in adult mice exposed to postnatal ethanol (Kleiber et al., 2013; Lossie et al., 2014). This study also identified differential expression of *Heg1* (gene associated with vessel formation and integrity), *Mdn1* (a gene that acts as a nuclear chaperon required for maturation and nuclear export of ribosomal subunits), *Rgs19* (a member of the RGS (regulators of G-protein signaling) family) in adult mice exposed to postnatal ethanol. Finally, a study identified *Pde4b*, a gene involved in signal transductions through its regulation of cyclic nucleotide concentrations in the cell, as a candidate gene for genetic differences in ethanol-induced cell death in the neural tube using BXD mice (Theberge et al., 2019). In the male and female LCD strain, there was only once gene that has been previously linked to differential gene expression after developmental ethanol exposure. *Ndst4*, an enzyme involved in glycosaminoglycan metabolism, was found to be differentially expressed in the hippocampus of fetuses exposed to chronic ethanol during gestation (Mandal et al., 2015).

There were a number of genes that were only differentially expressed in HCD-M group that have been previously linked to gene expression changes after developmental ethanol exposure including *Amt*, *Anln*, *Kirrel2*, *Mknk1*, *Naa25*, *Phf21a*, *Plcd4*, *Rab24*, and *Tgfb1i1* (Downing, Flink, et al., 2012; Kleiber et al., 2013; Lunde-Young et al., 2019). A study examining gene expression in the adult brains of mice exposed to postnatal ethanol found differential expression of *Kirrel2* (a transmembrane protein and member of the immunoglobulin superfamily of cell adhesion molecules), *Mknk1* (a gene involved in response to environmental stress and cytokines), *Rab24* (a member of the Ras-related protein family involved in the regulation of protein trafficking) *Tgfb1i1* (a transcription factor that plays a key role in male sexual differentiation) (Kleiber et al., 2013). This same study also identified differential gene expression of *Amt* (a gene involved in the glycine cleavage system) and *Plcd4* (an enzyme that plays a role in many cellular processes involving intracellular second messengers) in adult mice exposed to early prenatal ethanol, equivalent to the 1st trimester in humans (Kleiber et al., 2013). Strain differences in *Anln* expression, a gene that places a role in cell growth, migration,

and cytokinesis, expression was found in the B6 and D2 strains, and *Naa25* (an enzyme involved in normal cell-cycle progression) and *Phf21a* (a member of an epigenetic complex that mediates repression of neuron-specific genes) were found to be differentially expressed in both strains after ethanol exposure (Downing, Flink, et al., 2012). Genes that were only differentially expressed in the LCD-M group and have been previously linked to developmental alcohol exposure include *Pnn*, *Ptges2*, *Trim62*, *Zfp715*, and *Zfp9* (Downing, Flink, et al., 2012; Kleiber et al., 2013). In the brain of adult mice exposed to prenatal ethanol, *Trim62* (a gene involved in ubiquitin-protein transferase activity) was differentially expressed while *Ptges2* (an enzyme involved in the conversion of prostaglandin H2 to prostaglandin E2) and *Zfp9* (a zinc finger protein) were differentially expressed after postnatal ethanol exposure (Kleiber et al., 2013). Another study showed differentially expression of *Pnn* (a transcriptional activator involved in RNA transport) in B6 and D2 strains and *Zfp715* (a zinc finger protein involved in down-regulation of several chemokine receptors) was upregulated in B6 mice exposed to prenatal ethanol but not D2 mice (Downing, Flink, et al., 2012). Genes that were expressed in both HCD-M and LCD-M, but not females, and have been previously linked to developmental ethanol exposure include *Fezf2*, *Nedd9*, and *Pcmtd1* (Kleiber et al., 2013; Mandal et al., 2015). Differentially expression of *Fezf2*, a transcriptional repressor that controls the development of dendritic arborization and spines in pyramidal neurons and is involved in innate immune system, was found in the hippocampus of mice exposed to chronic ethanol during gestation (Mandal et al., 2015). *Pcmtd1* (a gene involved in protein-L-isoaspartate (D-aspartate) O-methyltransferase activity) was found to be differentially expressed after early prenatal ethanol exposure while postnatal ethanol exposure induced *Nedd4* (a gene involved in ubiquitin proteasome system of protein degradation and plays a critical role in regulation of membrane receptors) expression changes (Kleiber et al., 2013).

There were a number of genes that were differentially expressed in the HCD-F group that have been previously linked to gene expression changes after developmental ethanol exposure including *Ebna1bp2*, *Fip1l1*, *Hdac2*, *Hdac8*, *Letm2*, *Mars2*, *Naa15*, *Phb*, *Rasal2*, and *Trmt61a* (Downing, Flink, et al., 2012; Kleiber et al., 2013; Lunde-Young et al., 2019). Differential expression of *Phb* (a tumor suppressant gene involved in antiproliferative activity) was found in adult mice exposed to prenatal ethanol while *Hdac8* (a histone involved in transcriptional regulation and cell cycle progression), *Letm2* (a transmembrane protein), *Mars2* (a gene that encodes for a mitochondrial methionyl-tRNA synthetase protein) , and *Rasal2* (an activator of Ras superfamily of small GTPases) showed differential expression after exposure to postnatal ethanol (Kleiber et al., 2013). A study using B6 and D2 strains found differential gene expression of *Ebna1bp2* (a gene involved in RNA binding and protein binding), *Fip1l1* (a gene involved in mRNA splicing and transport of mature transcripts to the cytoplasm), *Hdac2* (a member of the histone deacetylase family involved in transcriptional repression), *Naa15* (a gene involved in post-translational protein modification linked to neuronal growth and development), and *Trmt61a* (a gene involved in t-RNA processing) in both strains after prenatal ethanol exposure (Downing, Flink, et al., 2012). Sex-specific hippocampal differential expression of Ebna1bp2 was also found after exposure to chronic prenatal alcohol (Lunde-Young et al., 2020). Although, this study found

Ebna1bp2 was only downregulated males and not females (Lunde-Young et al., 2019). However, our present study found ethanol-induced expression of *Ebna1bp2* was only downregulated in female HCD strains showing contrasting effects of sex between these two studies (Lunde-Young et al., 2019). Although both our study and the previously mentioned study examined gene expression changes in the hippocampus, we used different alcohol exposure paradigms which could explain this difference. Genes that were only differentially expressed in the LCD-F group that have been previously linked to developmental ethanol exposure include *Hist2h2ab*, *Nlgn1*, and *Pcbp1* (Downing, Flink, et al., 2012; Kleiber et al., 2013). Differential expression of *Hist2h2ab* (a member of the histone H2A family) was found in adult mice exposed to prenatal ethanol while *Nlgn1* (a member of a neuronal cell surface protein family involved in the formation and remodeling of neuronal synapses) showed differential expression after exposure to postnatal ethanol (Kleiber et al., 2013). Another study found *Pcbp1* (a gene involved in RNA binding and nucleic acid biding) expression was differentially expressed after exposure to binge-like ethanol during midgestation (Downing, Flink, et al., 2012). There were no genes that were differentially expressed in both HCD-F and LCD-F but not males, that have been previously linked to developmental ethanol exposure. This could be partly due to the fact that not all studies examined female mice.

Out of the 115 genes that were differentially expressed in both sexes and both cell death profiles, six have previously showed to be differentially expressed after developmental ethanol exposure: *Crem, Folh1, Heatr1, Ly6c1, Nop2,* and *Slc2a1* (Downing, Flink, et al., 2012; Kleiber et al., 2013). Differential expression of *Ly6c1* (a gene involved in acetylcholine receptor binding and activity) and *Slc2a1* (a major glucose transporter in the blood-brain barrier) were found in adult mice prenatal exposed to ethanol while *Folh1* (a transmembrane glycoprotein associated with glutamate excitotoxicity) and *Heatr1* (a ribosome biogenesis factor involved in snoRNA binding) showed differential expression after postnatal ethanol exposure (Kleiber et al., 2013). Ethanol-induced differential expression of *Nop2* (a gene involved in methyltransferase activity and associated with cell proliferation) was found in both B6 and D2 mice exposed to prenatal ethanol while differential expression of *Crem* (a transcription factor involved in signal transduction) was strain-specific with differences only shown in B6 mice and not D2 mice (Downing, Flink, et al., 2012). A few of the interesting genes we found to have significant strain effects of ethanol-induced gene expression changes were also significant in all four groups tested including *Bcl2l11*, *Jun*, and *Txnip*.

Overall, our study identified numerous effects of strain on hippocampal gene expression changes after exposure to postnatal ethanol. Within each strain there was little overlap of differential expression between males and females. Though there were few overlapping gene expression changes that were dependent on sex across all strains. We identified numerous strain differences in gene expression changes after ethanol exposure. Many of our top genes that showed differential expression among the strains were found to be previously linked to cell death, apoptosis, and were correlated with a number of behavioral phenotypes involving learning and memory. An advantage of the current study was the comparison between ethanol-induced gene expression changes in strains that showed differential vulnerability of cell death in the hippocampus after postnatal ethanol

exposure. This allowed us to compare gene expression changes after ethanol exposure in multiple strains that have been shown to be highly susceptible to hippocampal cell death and multiple strains that have shown to be resistant to ethanol-induced cell death in the hippocampus. We observed more perturbed effects of ethanol in the high cell death strains compared to the low cell death strains. Future studies are needed to validate ethanol-induced gene expression changes among the strains and further analyze is needed to understand their direct effect on cell death in the hippocampus. The next step in the project is to evaluate the long-term consequences of ethanol-induced differential gene expression and hippocampal cell death in these diverse strains. Behavioral studies examining the effects of postnatal ethanol exposure on learning and memory in BXD strains could progress our understanding of the genetic variation seen in ethanol teratogenicity and its long-term effects on behavior.

CHAPTER 3. EFFECTS OF GENETICS AND SEX ON ANXIETY, ACTIVITY, AND SPATIAL LEARNING AND MEMORY BEHAVIORS FOLLOWING NEONATAL ETHANOL EXPOSURE IN ADOLESCENT BXD RECOMBINANT INBRED STRAINS

Introduction

Ethanol consumption during pregnancy can cause abnormal development and has been shown to be particularly detrimental to the developing brain (Guerri et al., 2009; D. B. Moore, Madorsky, Paiva, & Barrow Heaton, 2004; Riley & McGee, 2005). Children with FASD exhibit long-lasting cognitive impairments such as deficits in learning and memory (Astley et al., 2009; Guerri et al., 2009; Mattson et al., 2019; Mattson, Crocker, & Nguyen, 2011). Additionally, many children and adolescents with FASD also exhibit neurobehavioral abnormalities including hyperactivity, attention deficits, and anxiety (Glass et al., 2014; Hellemans, Verma, et al., 2010; Mattson et al., 2019; Mattson et al., 2011; Mattson & Riley, 2000; Riley & McGee, 2005). Many of these cognitive impairments and behavioral abnormalities are seen in FASD animal models and these models are a useful tool to investigate the underlying mechanisms behind alcohol-induced neurobehavioral alterations (as reviewed in (Chokroborty-Hoque, Alberry, & Singh, 2014; Fontaine, Patten, Sickmann, Helfer, & Christie, 2016; Patten et al., 2014).

Animal models have also been a useful tool for studying the role of genetics in FASD. Numerous studies have examined differential vulnerability to ethanol's teratogenic effects across differing genetic background (Chen et al., 2011; Downing et al., 2009; Goldowitz et al., 2014; Goodlett et al., 1989; M. L. Green et al., 2007; Lossie et al., 2014; Ogawa et al., 2005). Most studies examining multiple strains have focused on malformations and brain abnormalities in fetuses or neonates while fewer studies have examined differential behavioral responses to developmental alcohol exposure. The handful of studies that have focused on differential behavioral responses to perinatal ethanol exposure have used either selectivity bred strains that show differential alcohol-related traits such as alcohol preference or typically studies only comparing two or three strains (Gilliam et al., 1987; Riley et al., 1993; Thomas et al., 2000; Thomas et al., 1998). These studies identified differential behavioral responses to perinatal ethanol exposure to hyperactivity, deficits in motor coordination, and learning and memory (Gilliam et al., 1987; Riley et al., 1993; Thomas et al., 2000; Thomas et al., 1998).

A great tool for studying genetic variation and differential behavioral responses is the BXD recombinant inbred (RI) strains of mice which have been generated by crossing B6 and D2 strains and inbreeding progeny for over 20 generations (Taylor et al., 1999; X. Wang et al., 2016). The BXD strains differ in alcohol responses in adults and show differential vulnerabilities to several malformations and developmental abnormalities after exposure to alcohol during development (Baker et al., 2017; Cook et al., 2015; Crabbe & Belknap, 1992; Downing, Balderrama-Durbin, et al., 2012; Goldowitz et al., 2014; Theberge et al., 2019). Though the behavioral effects of developmental alcohol exposure have yet to be examined in the BXD RI panel.

A number of animal studies have examined the effects of developmental alcohol exposure on activity and anxiety using the elevated plus maze or open field. Many of these studies report hyperactivity and/or anxiety -like behaviors though these results can vary depending on species, level of alcohol exposure, and time of exposure (Cullen, Burne, Lavidis, & Moritz, 2014; Dursun, Jakubowska-Dogru, & Uzbay, 2006; Fish et al., 2016; Ieraci & Herrera, 2007; Kim et al., 2013; Staples, Rosenberg, Allen, Porch, & Savage, 2013; Xu et al., 2018). Learning and memory deficits after exposure to developmental alcohol has been found using several different behavioral paradigms (as reviewed in(Marquardt & Brigman, 2016; Patten et al., 2014). Deficits in working memory have been assessed using the standard Y-maze and impairments in spatial recognition memory have been examined using a modified Y-maze (Basavarajappa, Nagre, Xie, & Subbanna, 2014; Cantacorps et al., 2017; Sarnyai et al., 2000; Subbanna et al., 2013).

In the present study, we examined the effects of developmental ethanol exposure on adolescent behavior using selected BXD RI strains that show differential responses to developmental ethanol exposure (Goldowitz et al., 2014). Previous work identified BXD strains that showed increased vulnerability to ethanol-induced cell death in the hippocampus after exposure to postnatal ethanol while other BXD strains were resistant to these effects (Goldowitz et al., 2014). A follow-up study revealed unique ethanol-induced hippocampal genetic profiles in these BXD strains as well as identified sex-specific gene expression changes after postnatal ethanol exposure (see Chapter 2). Here, we aim to further investigate the long-term effects of developmental alcohol exposure on cognition and behavior in these selected BXD strains and parental B6 and D2 strains. Adolescent animals exposed to postnatal ethanol (equivalent to the third trimester in humans) were tested across a battery of behavioral tests to examine the effects of developmental alcohol exposure on activity, anxiety, working memory, and spatial recognition memory. Males and females were examined separately to address the effect of sex on these behavioral measures as sex-specific behavioral impairments have been found in both humans and FASD animal models (Hellemans, Verma, et al., 2010; Herman et al., 2008; Ieraci & Herrera, 2007; Kelly, Leggett, & Cronise, 2009; May et al., 2017; Sayal et al., 2007; Woods et al., 2018; Xu et al., 2018).

Materials and Methods

Animals for Behavioral Testing

Original breeders were purchased from both Dr. Robert Williams at the University of Tennessee Health Science Center (UTHSC) or Jackson Laboratory (City, State). All treatments and experiments were approved by the Institutional Animal Care and Use Committee at UTHSC. The present study aims to better understand the long-term effects of postnatal ethanol exposure in strains that show differential vulnerability to ethanol-induced cell death in the hippocampus of male and female mice. To test this, mouse strains were examined including, C57BL/6J (B6), DBA/2J (D2), and BXD

recombinant inbred (RI) strains that showed differential susceptibility to ethanol-induced cell death in the developing hippocampus (Goldowitz et al., 2014). BXD48a and BXD100 showed higher susceptibility to ethanol-induced cell death in the hippocampus while BXD60 and BXD71 showed that low vulnerability to ethanol-induced cell death in hippocampus.

Once all strains were acquired, breeding was conducted at UTHSC. Breeders were the products of on-site mating and thus breeders were not affected by excess stressors such as travel and relocation. Mice were maintained on a 12:12 light:dark cycle and given food and water ad libitum. Environmental enrichments (igloo house and paper bedding) were placed in each mouse cage throughout all experiments. Breeding cages were maintained with multiple male and female mice over 60 days of age. Breeders were checked multiple times per week to assess female mice. When female mice appeared pregnant, they were placed alone in a clean cage and monitored daily for pups. Pregnant dams were separated to 1) acclimate dam to new cage and reduce stress 2) control for differences in pup rearing with other adult male and female mice in original breeding cage and 3) to allow for close monitoring of pups without disturbing other breeders. On average dams were placed in cage alone a week prior to birth. The date of birth of recorded as postnatal day 0 (P0). The first litter from each mother was skipped and not used for experiments. Only litters of 4 or more were kept while litters greater than 8 were culled. Ethanol-exposed and control animals were litter matched. If a litter contain more than two males and/or two females the litter mean for each treatment and sex were calculated and used for behavioral analysis.

Ethanol Treatment

Neonatal mice were treated on postnatal day (P) 7 which is a developmental time point during the third trimester-equivalent in humans. For mice, P7 is the middle of the brain growth spurt, a time during which neurons are completing migration, differentiation, establishing connections through synaptogenesis and dendrite arborization and natural programmed cell death is occurring (Alfonso-Loeches & Guerri, 2011; Gil-Mohapel et al., 2010; Marquardt & Brigman, 2016). Pups were brought to a separate testing room in their cage with their mother between 9:00AM and 10:00AM. Pups were then placed in clean cage on a heating pad while they were weighed, dosed, then promptly placed back in their home cage with their mother. Pups were split into either an ethanol or control group (**Figure 3-1**). As in previous studies, ethanol treated animals received 20% ethanol in sterile saline though subcutaneous injection. The total dose of ethanol was 5.0 g/kg split in two 2.5 g/kg doses, given two hours apart while controls received an isovolumetric volume of sterile saline (Goldowitz et al., 2014). This ethanol exposure represents an acute neonatal binge which has been shown to produce BACs of approximately 350 mg/dl in P7 neonatal mice (Goldowitz et al., 2014; Schaffner et al., 2020). Early prenatal and postnatal rodent studies of blood alcohol concentrations found no differences in BAC levels across multiple strains including B6 and D2 mouse strains (Boehm et al., 1997; Goodlett et al., 1989). As parental B6 and D2 strains do not differ in

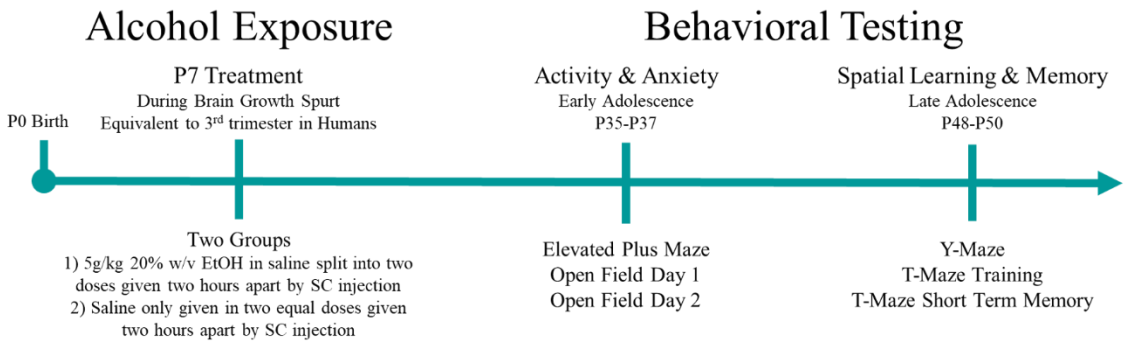

Figure 3-1. Overview of experimental design for the behavioral study.

An overview of the alcohol exposure paradigm (left) and behavioral testing schedule (right). Activity and anxiety were measured during early adolescence (P35-P37) using an elevated plus maze and open field. Spatial learning and memory were measured during late adolescence (P48-P50) using a Y-maze and T-maze.

BACs and because collection of enough blood for BAC is lethal to neonatal pups, additional pups were not produced for this measure.

Pup Identification Methods

At the time of the first injection, pups were toe clipped for identification. The removal of a portion of a digit, i.e. toe clipping, is an unambiguous method to differentiate pups long-term that is not only quick but does not require anesthesia for pups seven days of age (Castelhano-Carlos, Sousa, Ohl, & Baumans, 2010). To reduce pain and discomfort, only hind paws were clipped and only one digit per pup was removed (Dahlborn et al., 2013). The handling of the pup is minimal for this method and less stressful than other move invasive forms of identification in newborn pups (Castelhano-Carlos et al., 2010). However, reading toe clips at the time of behavioral testing can be a stressor to the animal as it involves holding the animal by the tail with its hind paws in the air until the digits are spread out and can be read. Due to the battery of behavioral tests, it was determined reading the toe clip at each test would be an added stressor that could affect behavioral results. Therefore, a second method of identification was also used to minimize stress during behavioral testing. Animals were weaned and separated by sex on postnatal day 28 + 0.86). At this time animals were ear punched which involves placing a small notch along the edge of either ear (Stark & Ostrow, 1991). Ear punching cannot be administered until the animal is three weeks old and therefore this identification method was used in conjunction with toe clipping (The Jackson Laboratory Handbook on Genetically Standardized Mice). Multiple studies found toe clips and ear punches do not have long-term effects as measured by several physiological, developmental, and behavioral tests (Castelhano-Carlos et al., 2010; Dahlborn et al., 2013; Paluch et al., 2014; Schaefer, Asner, Seifert, Burki, & Cinelli, 2010; Taitt & Kendall, 2019; Wever, Geessink, Brouwer, Tillema, & Ritskes-Hoitinga, 2017).

Behavioral Testing Procedure and Schedule

The elevated plus maze (EPM) and open field (OF) were used to examine anxiety and activity and the Y-Maze and T-maze were used to examine spatial learning and memory (**Figure 3-1**). All mice were examined in all behaviors (Fidalgo et al., 2017; Fish et al., 2016). The EPM and OF were conducted during early adolescence (EPM: P35.8 + 1.1; OF1: P36.8 + 1.1; OF2: P37.8 + 1.1). Approximately, two-weeks later animals were tested in the Y-Maze and T-maze during late adolescence (Y-Maze: P49.1 + 1.3; T-maze: P50.1 + 1.3).

All behavioral testing was performed in the Behavioral Core of the Neuroscience Institute at the University of Tennessee Health Science Center. Animals were tested between 11:00AM and 5:00PM. Animals were brought to holding room one hour before testing to acclimate to environment. A white noise machine was used to control for outside sound. Temperature was at an average of 74°F. Dim lighting was used in the

holding room before all behaviors. Likewise, dim lighting was kept in the behavioral testing room for the open field, Y-Maze, and T-maze to reduce stress caused by bright lighting. In contrast brighter lighting conditions were used to induce a more stressful environment during testing in the elevated plus maze. Each apparatus was cleaned with 70% ethanol after each animal was tested. Animals were placed in clean cages after testing and returned to the animal room. All behavior was tracked and recorded using ANY-maze Software version 4.99z (Stoelting Co., Wood Dale Illinois, United States). For all behaviors, the number of entries is defined as eighty-five percent of the animal's body, i.e., all four paws, to enter the zone, while their exit out of a zone requires seventy percent of the animal's body to leave the zone (Any-maze Manual, Stoelting). The number of animals used for each strain, sex, and treatment can be found in **Appendix B, Table B-1**.

Elevated Plus Maze

The elevated plus maze was used to examine anxiety and locomotor activity as previously described (Bailey and Vrawley, 2009; Xu et al., 2018; Icerai et al., 2020; Fish et al., 2016). Mice were placed near the center of an EPM which is plus-shaped (+) consisting of four arms (30 cm X 6 cm), two of which are open and two of which are enclosed with clear 15 cm walls. The runway was elevated 84 cm from the floor. Animals were tested for 5 mins and tracked using ANY-maze Software. The purpose of this test is to measure anxiety-like behavior and activity in mice. Mice have an aversion to open spaces if they are anxious though non-anxious mice exhibit more exploratory behaviors. Therefore, total distance travelled in the maze as well as number of entries and time spent in each of the open and closed arms of the maze was determined.

Open Field

The open field was used to examine anxiety and locomotor activity as previously described (Bailey & Crawley, 2009; Fish et al., 2016; Ieraci & Herrera, 2007; Xu et al., 2018). Mice were placed in a clear OF (40 cm X 40 cm X 40cm) and allowed to explore the arena for 15 minutes. Animals were tested twice in the OF, each session 24 hours apart. Animals were tracked using ANY-maze Software. For each session analysis was conducted at the following time bins: 0 minute to 5 minutes (Bin 1), 5 minutes to 10 minutes (Bin 2), 10 minutes to 15 minutes (Bin 3), and total 15 minute (Total). Activity in the center was used to measure anxiety as mice typically avoid the center and remain close the edge of maze, an innate behavior referred to as thigmotaxis (Bailey & Crawley, 2009). Activity measures in the maze were examined in the whole maze area, center of the maze, and edge of the maze. The following measures were recorded in the entire maze area, center of the maze or edge of the maze: total distance travelled, time spent, and number of entries were evaluated across all time bins on day 1 and day 2.

Y-Maze

The Y-maze was used to examine hippocampal-dependent spatial working memory as previously described (Basavarajappa et al., 2014; Cantacorps et al., 2017; Holcomb et al., 1998; Subbanna et al., 2013). The Y-maze consists of three enclosed arms (12 cm X 5 cm X 5 cm) in the shape of a Y (**Figure 3-2**). To orient the animal to the location of each arm, shapes of various colors were placed on the walls around the Y-maze. Each mouse was placed in the entry arm and allowed to explore freely through the maze for an 8-minute session. Animals were tracked through ANY-maze and the sequence of arms entered was recorded to measure spontaneous alternations. Correct alternation was recorded as three consecutive choices of the three different arms. Spontaneous alternations are calculated by dividing the total number of alternations by the total number of choices minus 2 (Cantacorps et al., 2017; Holcomb et al., 1998; Subbanna et al., 2014). Total distance travelled Y-maze was also examined. Additionally, distance travelled, number of entries, and time spent in each arm was also examined.

T-Maze

The T-maze was used to examine spatial working memory. The behavioral paradigm used was previously described using a Y-maze (Shivakumar, Subbanna, Joshi, & Basavarajappa, 2020; Subbanna et al., 2014). However, since our animals were previously tested in the Y-maze for spontaneous alternations, we did not want any carry over-effects from this previous exposure. Therefore, we used the same protocol as previously described using a modified T-maze to assess spatial recognition memory in our animals (**Figure 3-3**). The T-maze consisted of three arms: one entry arm (50 cm X 10 cm) and two top arms (28 cm X 10 cm). To orient the animal to the location of each arm, shapes of various colors were placed on the walls around the Y-maze. Mice were placed in the entry arm and allowed to freely explore for an 8-minute training session. During the training session, one of the top arms was blocked and the mouse was only able to assess one of the top arms. The location of the blocked arm was randomized. Short-term memory was assessed after a 3-hour interval. During the short-term memory testing trial, both top arms of the T-Maze were opened. Mice were placed into the entry arm and allowed to explore both top arms for 3 minutes. The animal's ability to discriminate between the two top arms was measured by examining time in the novel (previously blocked armed) compared to total time between both the novel and familiar, previously opened arm. The discrimination ratio (novel arm / (novel arm + familiar arm) was used to calculate the time spent between both arms and the number of entries into both arms. Other measures recorded during both the training (T) and short-term memory (STM) sessions included: total distance travelled in the whole maze as well as number of entries and time spent in the entry arm and opened arm. Additionally, during the STM session the following measures were recorded in the novel (previously blocked arm): number of entries, time spent, and latency to enter.

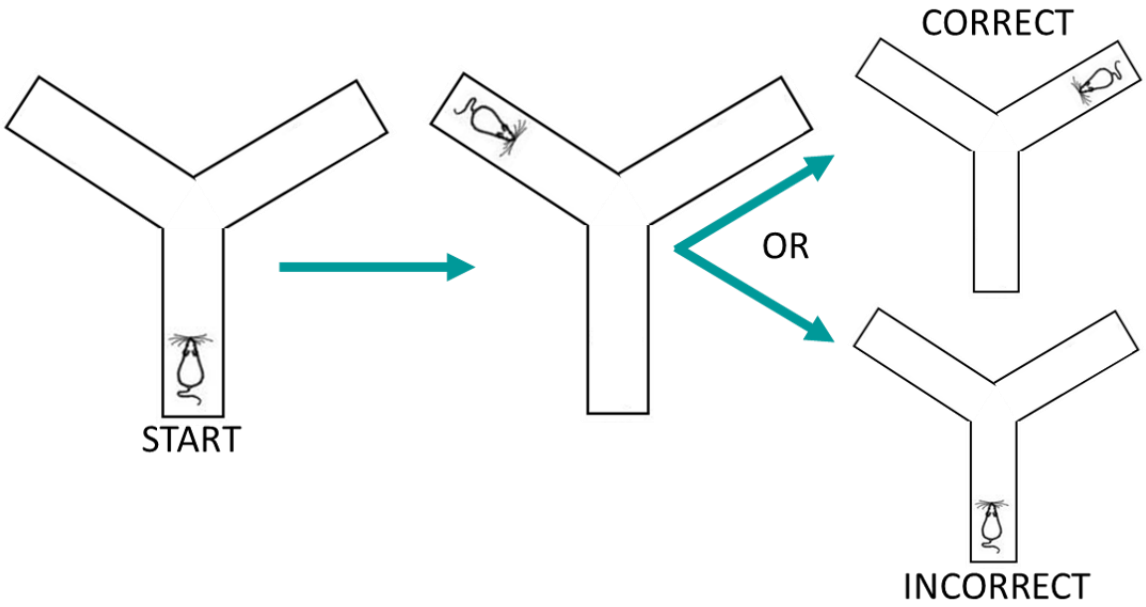

Figure 3-2. Diagram of spontaneous alternations measured in the Y-maze.

Diagram of spontaneous alternations test used to measure spatial learning and memory in animals. The mouse starts in one arm of the maze and travels to another arm. Once in the second arm visited, the mouse has a choice to travel to the previously visited arm or explore the novel unvisited arm. Travelling to the novel arm is considered a correction response while travelling to the previously visited arm is considered an incorrect response.

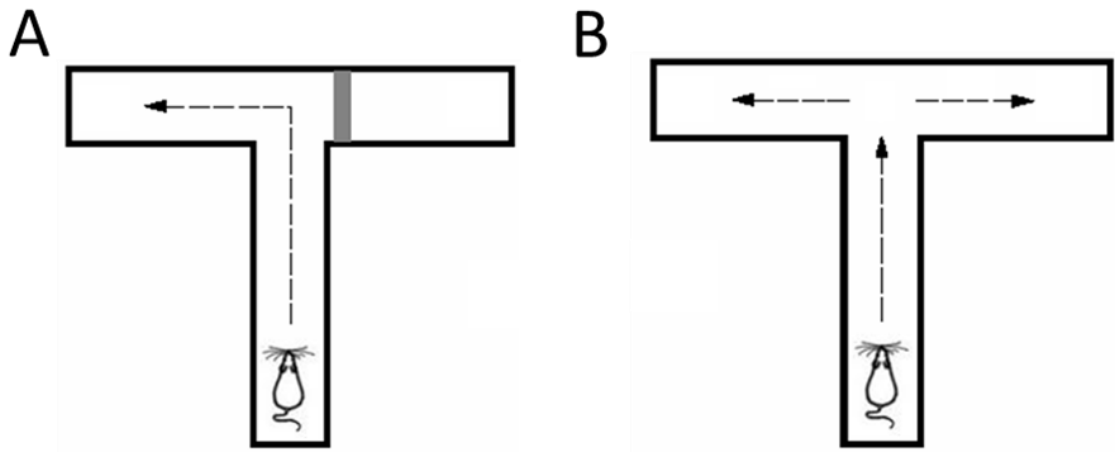

Figure 3-3. Diagram of spatial short-term memory test in the T-maze.

Diagram of spatial short-term memory test using the T-maze. During the (A) training session, mice were placed in the entry arm and allowed to freely explore for an 8-minute session. During this session, one of the top arms was blocked and the mouse was only able to assess one of the top arms. Short-term memory was assessed after a 3-hour interval. For the short-term memory testing session, both top arms of the T-Maze were opened. Mice were placed into the entry arm and allowed to explore both top arms for 3 minutes. The animal's ability to discriminate between the two top arms was measured by examining time in the novel (previously blocked armed) compared to total time between both the novel and familiar, previously opened arm.

Behavioral Analysis

All behavior was exported from ANY-maze and analyzed using the following packages in the R (version 4.1) software environment: plyr package (version 1.8.6), ggplot2 package (version 3.3.3) (Wickham, 2016), and effectsize package (version 0.4.5). The effect of strain, sex, treatment, strain x sex interaction, strain x treatment interaction, sex x treatment interaction, and strain x sex x treatment interaction were examined across the six strains (BXD48a, BXD60, BXD71, BXD100, B6, and D2), two sexes (male and females), and two treatments (control and ethanol) were examined. ANOVAs were used to examine multiple measures in R using the following input: measure.model<-lm(data=Dat,measure~Strain*Sex*Treatment), anova(measure.model). The effect size was calculated using Omega Squared confidence intervals in R using the following input: omega_squared(measure.model, partial = TRUE, ci = 0.09) (Lakens, 2013). Further analysis within each strain was calculated by two-way ANOVAs for effects of sex, treatment, and sex x treatment interactions in GraphPad Prism 7 (GraphPad Software Inc., San Diego, California).

Results

Adolescent Body Weights

Body weight was measured after the animal completed the EPM and again after the Y-maze in all strains and both males and females. Body weights after the EPM showed significant effects of strain ($F_{(5,183)} = 15.96$, $p < 0.001$, $\omega^2 = 0.36$, 90% CI [0.26, 0.43]), sex ($F_{(1,183)} = 74.98$, $p < 0.001$ $\omega^2 = 0.49$, 90% CI [0.41, 0.56]), and an interaction between strain x sex ($F_{(5,183)} = 2.84$, $p < 0.05$ $\omega^2 = 0.08$, 90% CI [0.01, 0.13]). Likewise, weight after the Y-maze also showed significant effects of strain ($F_{(5,186)} = 24.22$, $p < 0.001$ $\omega^2 = 0.27$, 90% CI [0.17, 0.34]), sex ($F_{(1,186)} = 1999.89$, $p < 0.001$ $\omega^2 = 0.26$, 90% CI [0.18, 0.35]), and an interaction between strain x sex ($F_{(5,186)} = 4.53$, $p < 0.05$ $\omega^2 = 0.04$, 90% CI [0.00, 0.08]). There was no significant difference in body weight between control and ethanol animals, nor were there any interactions effects involving treatment.

Elevated Plus Maze

The EPM showed a significant effect of strain on total distance travelled ($F_{(5,195)} = 52.02$ $p < 0.001$, $\omega^2 = 0.54$, 90% CI [0.46, 0.60]; **Figure 3-4**), and number of entries to the open arms ($F_{(5,195)} = 14.90$, $p < 0.001$, $\omega^2 = 0.24$, 90% CI [0.15, 0.31]; **Figure 3-4**), distance travelled in the open arms ($F_{(5,195)} = 53.74$, $p < 0.001$, $\omega^2 = 0.55$, 90% CI [0.47, 0.60]), number of entries into the closed arms ($F_{(5,195)} = 13.21$, $p < 0.001$, $\omega^2 = 0.22$, 90% CI [0.13, 0.29]), and distance travelled in the closed arms ($F_{(5,195)} = 27.21$, $p < 0.001$, $\omega^2 = 0.37$, 90% CI [0.28, 0.44]). There was a significant effect of sex on total distance travelled ($F_{(1,195)} = 4.40$, $p < 0.05$, $\omega^2 = 0.02$, 90% CI [0.00, 0.06]). Specifically, the

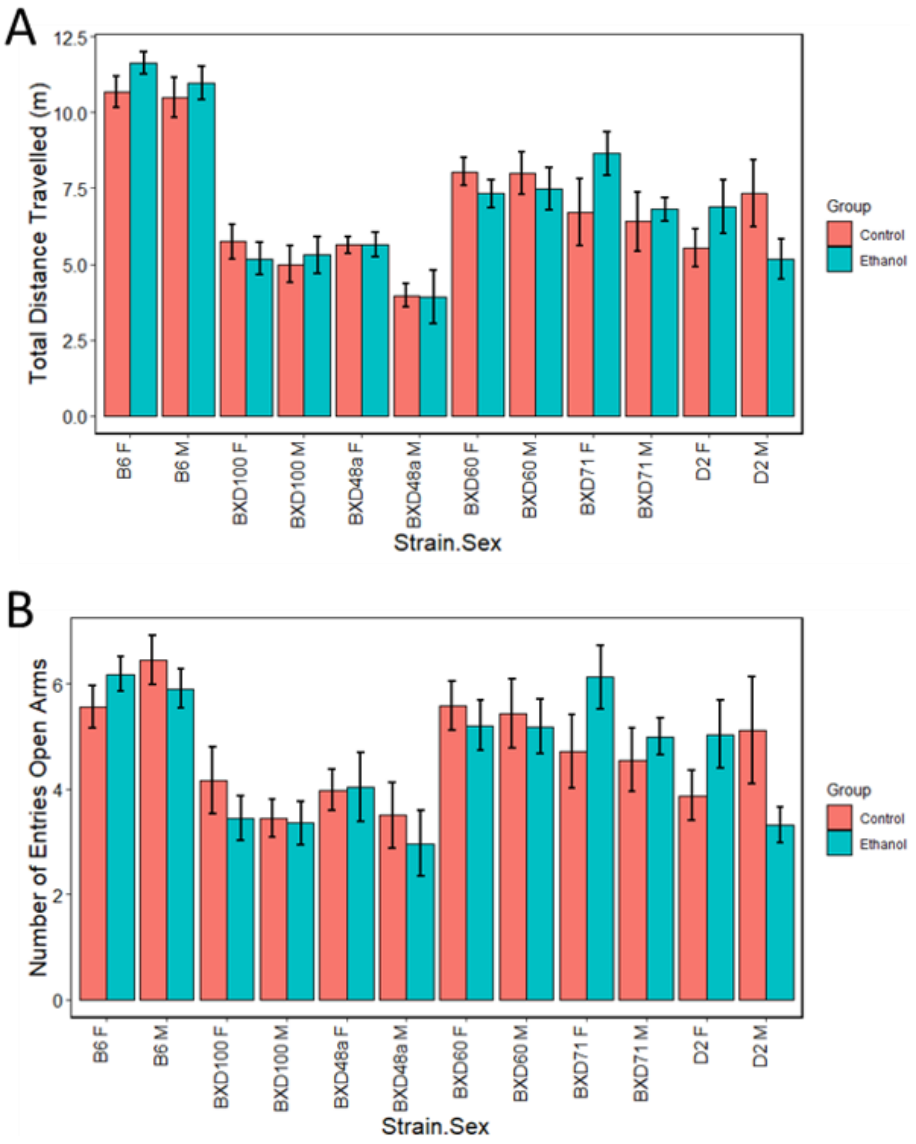

Figure 3-4. Strain and ethanol effects in activity-related and anxiety-like behaviors using the elevated plus maze.

Strain and ethanol effects in (A) total distance travelled were used to measure activity-related behaviors in the elevated plus maze. (B) Number of entries to the open arms of the elevated plus maze were used to measure anxiety-like behavior. Blue bars represent animals exposed to postnatal ethanol while red bars represent non-exposed control animals.

BXD48a strain showed a significant effect of sex ($F_{(1,29)} = 8.73$, $p < 0.01$) and the D2 strain showed a significant sex x treatment interaction ($F_{(1,30)} = 4.20$, $p < 0.05$).

Three-way ANOVA showed no significant difference in EPM measures for treatment or treatment interactions involving treatment. However, there was a trend towards significance for sex x treatment interaction in the number of entries to the open arms ($F_{(1,195)} = 43.62$, $p = 0.059$, $\omega^2 = 0.01$, 90% CI [0.00, 0.05]). Further analysis showed, an significant sex x treatment interaction in the D2 strain ($F_{(1,30)} = 4.67$, $p < 0.05$) and a trend toward significance for treatment in the BXD71 strain ($F_{(1,30)} = 2.78$, $p = 0.10$).

Open Field

OF Day 1. For Day 1 in the OF, an effect of strain was found for multiple measures for the total 15-minute test including: total distance travelled (**Figure 3-5A**; $F_{(5,189)} = 18.23$, $p < 0.001$, $\omega^2 = 0.29$, 90% CI [0.19, 0.36]), time in the center (**Figure 3-5C**; $F_{(5,189)} = 8.34$, $p < 0.001$, $\omega^2 = 0.15$, 90% CI [0.06, 0.21]), time in edge ($F_{(5,189)} = 8.8$, $p < 0.001$, $\omega^2 = 0.16$, 90% CI [0.07, 0.22]), number of entries to the center ($F_{(5,189)} = 10.48$, $p < 0.001$, $\omega^2 = 0.18$, 90% CI [0.09, 0.25]), and number of entries to the edge ($F_{(5,189)} = 10.48$, $p < 0.001$, $\omega^2 = 0.18$, 90% CI [0.09, 0.25]). There were also effects of strain, sex, treatment, strain x sex interaction, strain x treatment interaction, sex x treatment interaction and/or strain x sex x treatment interactions in either the total time or in the one of the three 5-minute bins (**Appendix B, Table B-2**).

Total distance travelled for the total 15-minute test on day 1 was further analyzed within each strain. Results show a trend towards a significant sex x treatment interaction for both the B6 strain ($F_{(1,41)} = 3.84$, $p = 0.056$) and the BXD48a strain ($F_{(1,30)} = 2.76$, $p = 0.10$). The BXD100 strain showed a trend towards a significant effect of treatment ($F_{(1,33)} = 3.01$, $p = 0.09$) for total distance travelled during the 15-minute open field test on day 1. Time in the center for the total 15-minute test on day 1 was further analyzed within each strain. Results showed a significant effect of sex in the BXD48a strain ($F_{(1,30)} = 5.57$, $p < 0.05$). The BXD71 strain showed a significant effect of treatment ($F_{(1,31)} = 5.14$, $p < 0.05$). The BXD100 strain showed a trend towards significant sex x treatment interaction ($F_{(1,33)} = 3.17$, $p = 0.08$) for time in center for the total 15-minute open field test on day 1.

On day 1 there were also several significant effects during the first five minutes of the test (bin 1). Total distance travelled during bin 1 (**Figure 3-6A**) was significant for strain ($F_{(5,189)} = 19.5$, $p < 0.001$, $\omega^2 = 0.30$, 90% CI [0.20, 0.38]). Further analysis found a significant sex x treatment interaction ($F_{(1,41)} = 5.24$, $p < 0.05$) for the B6 strain showing females exposed to postnatal ethanol were more active than non-exposed females while males exposed to postnatal ethanol travelled less than non-exposed males. The BXD100 strain showed a significant effect of sex ($F_{(1,33)} = 4.51$, $p < 0.05$) for total distance travelled during bin 1. The D2 strain showed a significant effect for treatment ($F_{(1,26)} = 6.19$, $p < 0.05$) with both males and females exposed to postnatal ethanol significantly more active compared to non-exposed controls males and females.

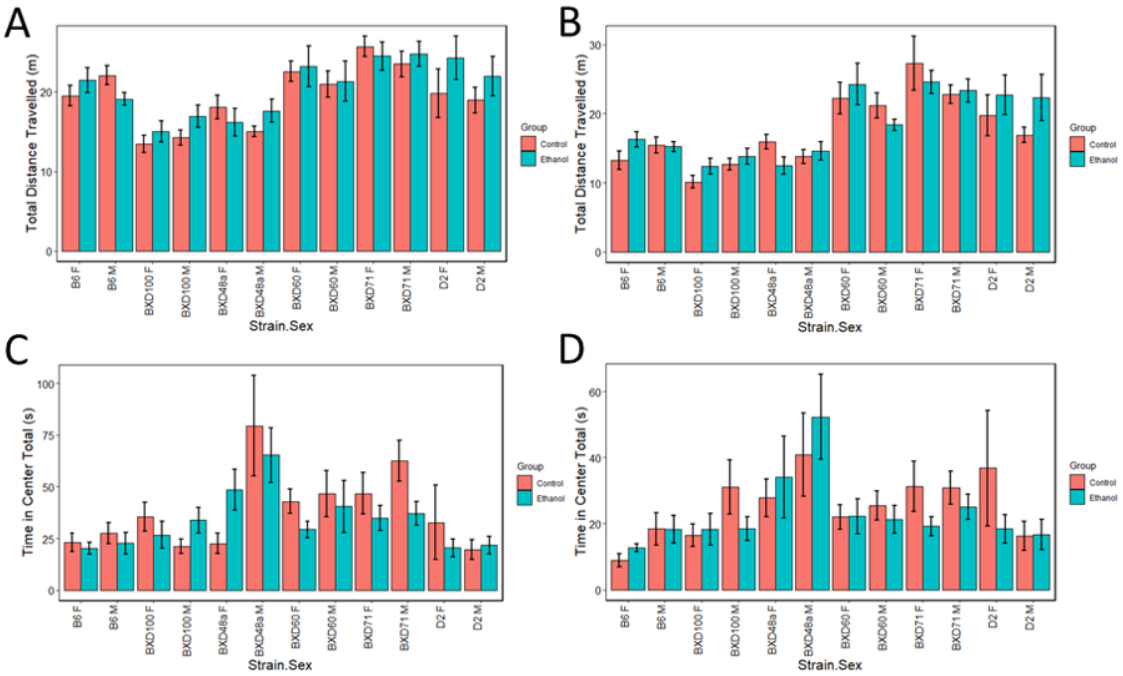

Figure 3-5. Strain and ethanol effects in activity-related and anxiety-like behaviors during the total 15-minute session of the open field test.

Strain and ethanol effects in activity-related (top) and anxiety-like (bottom) behaviors during the total 15-minute session of the open field test on day 1 (left) or day 2 (right). Total distance travelled was used to measure activity-related behaviors on (A) day 1 and (B) day 2. Time in center was used to measure anxiety-like behaviors on (C) day 1 and (D) day 2. Blue bars represent animals exposed to postnatal ethanol while red bars represent non-exposed control animals.

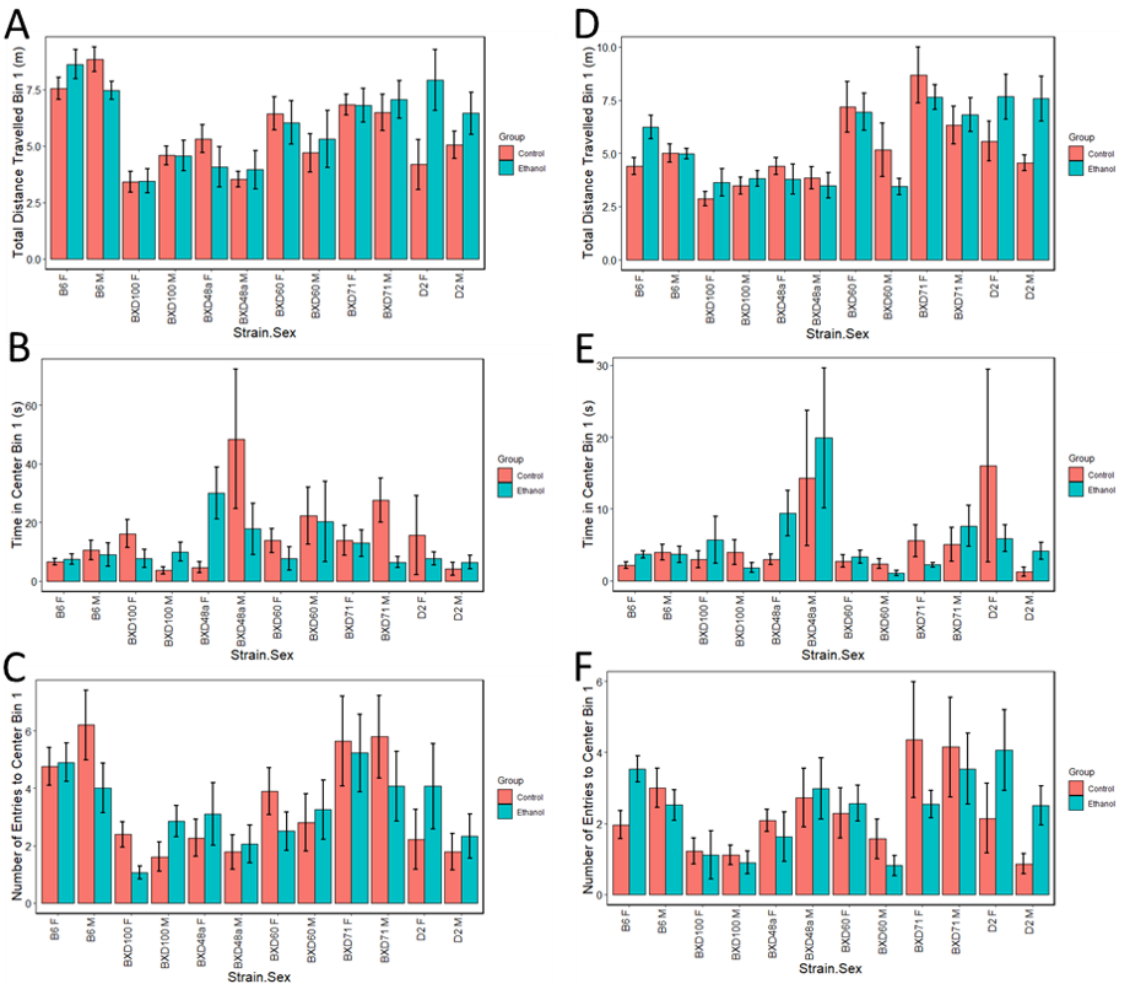

Figure 3-6. Strain and ethanol effects in activity-related and anxiety-like behaviors during the first 5 minutes of the open field test.

Strain and ethanol effects in activity-related (top), and anxiety-like (middle and bottom) behaviors during the first 5 minutes (Bin 1) of the open field on day 1 (left) and day 2 (right). Total distance travelled during bin 1 was used to measure activity-related behaviors on (A) day 1 and (D) day 2. Time in the center of the open field during bin 1 on (B) day 1 and (E) day 2 and number of entries to the center of the open field during bin 1 on (C) day 1 and (F) day 2 were used to measure anxiety-like behaviors. Blue bars represent animals exposed to postnatal ethanol while red bars represent non-exposed control animals.

Time in center of the open field during bin 1 (**Figure 3-6B**) was significant for strain ($F_{(5,189)} = 3.35$, $p < 0.01$, $\omega^2 = 0.05$, 90% CI [0.00, 0.09], and a three-way strain x sex x treatment interaction ($F_{(5,189)} = 3.07$, $p < 0.01$, $\omega^2 = 0.05$, 90% CI [0.00, 0.08]. Further analysis found a significant interaction for sex x treatment interaction ($F_{(1,33)} = 5.01$, $p < 0.05$) for the BXD100 strain showing females exposed to postnatal ethanol spent significantly less time in the center than non-exposed females while males exposed to postnatal ethanol spent more time in the center than non-exposed males. The BXD71 strain had a significant effect for treatment ($F_{(1,31)} = 4.23$, $p < 0.05$) showing males postnatally exposed to alcohol spent significantly less time in the center than non-exposed males. Time in the edge of the open field during bin 1 was significant for strain ($F_{(5,189)} = 6.45$, $p < 0.001$, $\omega^2 = 0.11$, 90% CI [0.04, 0.17] and a two-way strain x treatment interaction ($F_{(5,189)} = 2.65$, $p < 0.05$, $\omega^2 = 0.04$, 90% CI [0.00, 0.07]. There was a trend towards significance for the effect of treatment ($F_{(1,30)} = 4.04$, $p = 0.054$) for time spent in the edge of the open field in the BXD48a strain.

Number of entries to the open field during bin 1 (**Figure 3-6C**) was significant for strain ($F_{(5,189)} = 9.20$, $p < 0.001$, $\omega^2 = 0.16$, 90% CI [0.07, 0.23]). Further analysis revealed a significant two-way sex x treatment interaction ($F_{(1,33)} = 7.00$, $p < 0.01$) for the BXD100 strain showing females exposed to postnatal ethanol decreased the number of entries to the center of the open field compared to non-exposed controls while males exposed to postnatal ethanol increased the number of entries to the center compared to non-exposed males.

OF Day 2. For Day 2 in the OF, an effect of strain was found for multiple measures for the total 15-minute test including: total distance travelled (**Figure 3-5B**; $F_{(5,189)} = 30.42$, $p < 0.001$, $\omega^2 = 0.41$, 90% CI [0.31, 0.48]), time in the center (**Figure 3-5D**; $F_{(5,189)} = 6.60$, $p < 0.001$, $\omega^2 = 0.12$, 90% CI [0.04, 0.17]), time in edge ($F_{(5,189)} = 34.42$, $p < 0.001$, $\omega^2 = 0.44$, 90% CI [0.35, 0.51]), number of entries to the center ($F_{(5,189)} = 11.05$, $p < 0.001$, $\omega^2 = 0.19$, 90% CI [0.10, 0.26]), and number of entries to the edge ($F_{(5,189)} = 13.05$, $p < 0.001$, $\omega^2 = 0.22$, 90% CI [0.13, 0.29]). There were also effects of strain, sex, treatment, strain x sex interaction, strain x treatment interaction, and/or sex x treatment interaction among either the total time or among the one of the three 5-minute bins (**Appendix B, Table B-3**).

Total distance travelled for the total 15-minute test on day 2 was further analyzed within each strain. Results show a trend towards a significant sex x treatment interaction in the BXD48a strain ($F_{(1,30)} = 3.24$, $p = 0.08$). There was a trend towards significant effect of sex in the BXD60 strain ($F_{(1,30)} = 2.58$, $p = 0.11$). The BXD100 strain showed a trend towards significance effect of sex ($F_{(1,33)} = 3.74$, $p = 0.06$) and treatment ($F_{(1,33)} = 2.77$, $p = 0.10$) for total distance travelled during the 15-minute open field test on day 2. Time in the center for the total 15-minute test on day 2 was further analyzed with each strain. Results show a significant effect of sex in the B6 strain ($F_{(1,41)} = 5.49$, $p < 0.05$). The BXD71 strain showed a trend towards significant effect of treatment ($F_{(1,31)} = 3.32$, $p = 0.08$) for time in the center during the 15-minute open field test on day 2.

On day 2 there were also several significant effects during the first five minutes of the test (bin 1). Total distance travelled during bin 1 (**Figure 3-6D**), was significant for strain ($F_{(5,189)} = 16.68$, $p < 0.001$, $\omega^2 = 0.27$, 90% CI [0.17, 0.34]), sex ($F_{(5,189)} = 7.52$, $p < 0.01$, $\omega^2 = 0.03$, 90% CI [0.00, 0.08]), a two-way strain x sex interaction ($F_{(5,189)} = 2.43$, $p < 0.01$, $\omega^2 = 0.03$, 90% CI [0.00, 0.06]), and a two-way strain x treatment interaction ($F_{(5,189)} = 2.91$, $p < 0.05$, $\omega^2 = 0.04$, 90% CI [0.00, 0.08]). Further analysis revealed a significant effect for treatment ($F_{(1,41)} = 4.70$, $p < 0.05$) and an interaction for sex and treatment ($F_{(1,41)} = 4.44$, $p < 0.05$) for the B6 strain showing females exposed to postnatal ethanol were significantly more active than non-exposed controls while postnatal ethanol-exposure had no effect in B6 males. The BXD60 strain had a significant effect of sex ($F_{(1,28)} = 7.85$, $p < 0.01$) and the BXD71 strain showed a trend towards significant effect of sex ($F_{(1,31)} = 3.13$, $p = 0.08$) on total distance travelled during bin 1. The D2 strain showed a significant effect of treatment ($F_{(1,26)} = 7.46$, $p < 0.01$) with both male and female mice exposed to postnatal ethanol showing increased activity compared to non-exposed controls.

Time in the center of the open field during bin 1 (**Figure 3-6E**) was significant for strain ($F_{(5,189)} = 3.26$, $p < 0.01$, $\omega^2 = 0.05$, 90% CI [0.00, 0.09]), and a two-way sex x strain interaction ($F_{(5,189)} = 2.41$, $p < 0.05$, $\omega^2 = 0.03$, 90% CI [0.00, 0.06]). The BXD60 strain showed a trend towards significant effect of sex ($F_{(1,28)} = 1.10$, $p = 0.08$) for time spent in the open field during bin 1. Time in the edge of the open field during bin 1 was significant for strain ($F_{(5,189)} = 7.10$, $p < 0.001$, $\omega^2 = 0.13$, 90% CI [0.04, 0.06]), treatment ($F_{(5,189)} = 4.37$, $p < 0.05$, $\omega^2 = 0.02$, 90% CI [0.00, 0.06]), a two-way strain x sex interaction ($F_{(5,189)} = 2.56$, $p < 0.05$, $\omega^2 = 0.04$, 90% CI [0.00, 0.07]), and a two-way sex x treatment interaction ($F_{(5,189)} = 4.12$, $p < 0.05$, $\omega^2 = 0.01$, 90% CI [0.00, 0.05]). The BXD60 strain showed a significant effect of sex ($F_{(1,28)} = 4.49$, $p < 0.05$) on time spent in the edge during bin 1. The BXD48a strain showed a trend towards significance for effect of sex ($F_{(1,30)} = 3.92$, $p = 0.057$) as well as treatment ($F_{(1,28)} = 3.79$, $p = 0.06$) in the BXD48a for time spend in the edge of the open field during bin 1.

Number of entries to the open field during bin 1 (**Figure 3-6F**) was significant for strain ($F_{(5,189)} = 6.00$, $p < 0.001$, $\omega^2 = 0.11$, 90% CI [0.03, 0.16]). Further analysis revealed a significant two-way sex x treatment interaction ($F_{(1,41)} = 5.65$, $p < 0.05$) for the B6 strain showing females exposed to postnatal ethanol increased the number of entries to the center of the open field compared to non-exposed controls while postnatal ethanol-exposure had no effect in B6 males. The BXD60 strain showed a significant effect of sex ($F_{(1,28)} = 5.38$, $p < 0.05$) for number of entries to the center. The D2 strain showed a significant effect of treatment ($F_{(1,26)} = 5.12$, $p < 0.05$) with both males and female mice exposed to postnatal ethanol showing reduced number of entries to the center compared to non-exposed controls. The D2 mice also showed a trend towards significance for sex ($F_{(1,26)} = 3.28$, $p = 0.08$).

Y-Maze

Spontaneous Alternations (**Figure 3-7A**) in the Y-maze showed significant effects for strain ($F_{(5,189)} = 13.01$, $p < 0.001$, $\omega^2 = 0.22$, 90% CI [0.12, 0.29]) and a three-way interaction between strain x sex x treatment($F_{(5,189)} = 2.35$, $p < 0.05$, $\omega^2 = 0.03$, 90% CI [0.00, 0.06]). Further analysis within each strain found significant effect of sex x treatment ($F_{(1,34)} = 4.75$, $p < 0.05$) for the BXD100 strain showing females exposed to postnatal ethanol exhibited reduced spontaneous alternations compared to non-exposed control females while postnatal ethanol-exposure had no effect in BXD100 males. There was also a trend towards significance for sex x treatment interaction in the BXD48a strain ($F_{(1,29)} = 3.96$, $p = 0.056$).

A strain effect was also found in total distance travelled (**Figure 3-7B**; $F_{(5,190)} = 43.01$, $p < 0.001$, $\omega^2 = 0.50$, 90% CI [0.41, 0.26]). Further analysis showed a significant sex effect in the BXD71 strain ($F_{(1,31)} = 7.82$, $p < 0.01$) for total distance travelled. There was also a trend toward significance for treatment in the B6 strain ($F_{(1,40)} = 2.99$, $p = 0.09$) and the BXD60 ($F_{(1,27)} = 3.06$, $p = 0.09$) for total distance travelled. There were significant effects for strain found in the number of entries into each arm of the Y-maze (A arm: $F_{(5,190)} = 19.63$, $p < 0.001$, $\omega^2 = 0.30$, 90% CI [0.21, 0.38]; B arm: $F_{(5,190)} = 29.80$, $p < 0.001$, $\omega^2 = 0.40$, 90% CI [0.31, 0.47]; C: $F_{(5,190)} = 27.21$, $p < 0.001$, $\omega^2 = 0.38$, 90% CI [0.28, 0.45]) which can be attributed to the strain effect on activity.

T-Maze

For the training session, total distance travelled (**Figure 3-8A**) showed significant effects of strain ($F_{(5,191)} = 36.31$, $p < 0.001$, $\omega^2 = 0.45$, 90% CI [0.36, 0.52]) and sex ($F_{(1,191)} = 11.13$, $p < 0.01$, $\omega^2 = 0.05$, 90% CI [0.01, 0.10]). Further analysis within each strain found significant effect of sex ($F_{(1,31)} = 5.59$, $p < 0.05$) and a trend towards significance for sex x treatment interaction ($F_{(1,31)} = 2.80$, $p = 0.10$) in the BXD71 strain. The D2 strain showed a trend towards significance for the effect of sex ($F_{(1,28)} = 3.78$, $p = 0.06$) on total distance travelled. For the training session, number of entries to the open arm (**Figure 3-8B**) showed significant effects of strain ($F_{(5,191)} = 29.78$, $p < 0.001$, $\omega^2 = 0.40$, 90% CI [0.31, 0.74]) and sex ($F_{(1,191)} = 9.14$, $p < 0.01$, $\omega^2 = 0.04$, 90% CI [0.01, 0.09]). Further analysis within each strain found significant effect of sex ($F_{(1,28)} = 4.86$, $p < 0.05$) in the D2 strain. The BXD71 strain showed a trend towards significance for the effect of sex ($F_{(1,31)} = 3.78$, $p = 0.06$) and interaction effect between sex and treatment ($F_{(1,31)} = 2.94$, $p = 0.09$).

For the short term memory session, there was also a significant effect of strain ($F_{(5,191)} = 12.70$, $p < 0.001$, $\omega^2 = 0.21$, 90% CI [0.12, 0.28]) and sex ($F_{(1,191)} = 16.42$, $p < 0.01$, $\omega^2 = 0.07$, 90% CI [0.02, 0.13]) for total distance travelled (**Figure 3-8C**) . There was also a significant effect of strain ($F_{(5191)} = 7.70$, $p < 0.001$, $\omega^2 = 0.13$, 90% CI [0.05, 0.20]) and sex ($F_{(1,191)} = 14.40$, $p < 0.001$, $\omega^2 = 0.06$, 90% CI [0.02, 0.12]) for number of entries to the familiar arm (**Figure 3-8D**). The BXD71 strain showed significant effect of sex ($F_{(1,31)} = 4.74$, $p < 0.05$) and a trend towards a significant effect of treatment ($F_{(1,31)} =$

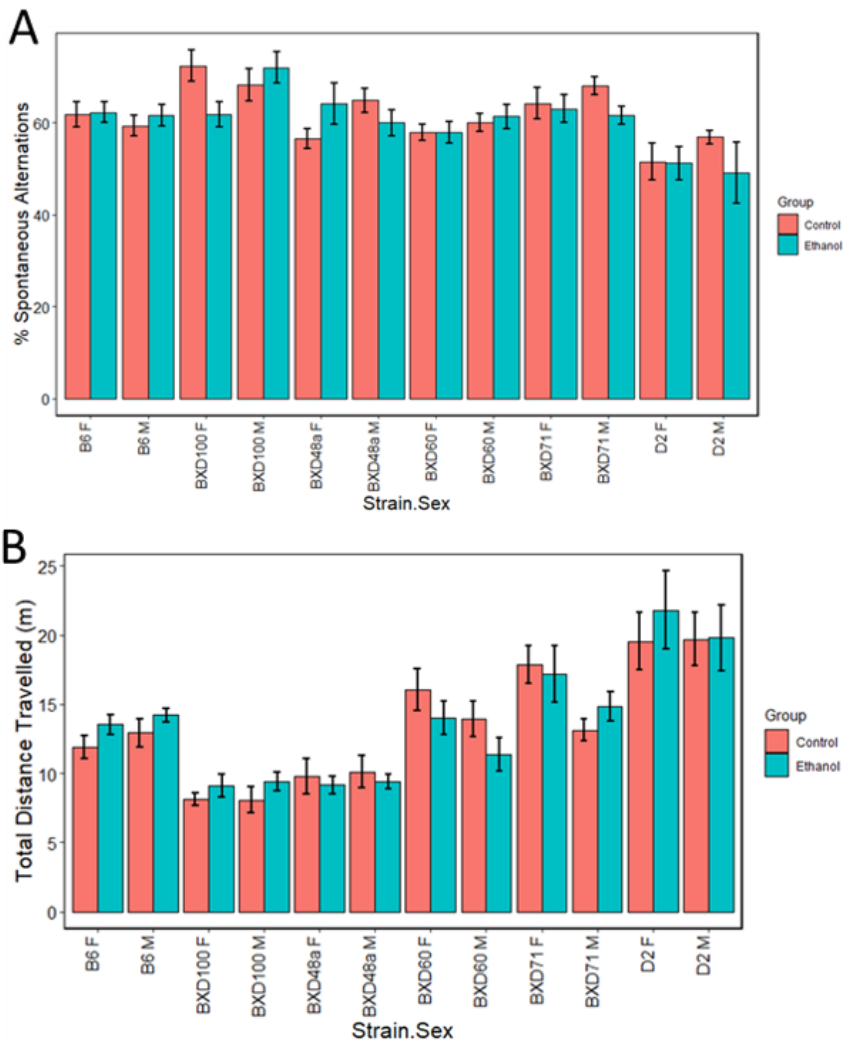

Figure 3-7. Strain and ethanol effects in spontaneous alternations and activity-like behavior using a Y-maze.

Strain and ethanol effects in learning and memory behavior measured by (A) percent of spontaneous alternations in the Y-maze. (B) Total distance travelled was used to measure activity-related behavior. Blue bars represent animals exposed to postnatal ethanol while red bars represent non-exposed control animals. Blue bars represent animals exposed to postnatal ethanol while red bars represent non-exposed control animals.

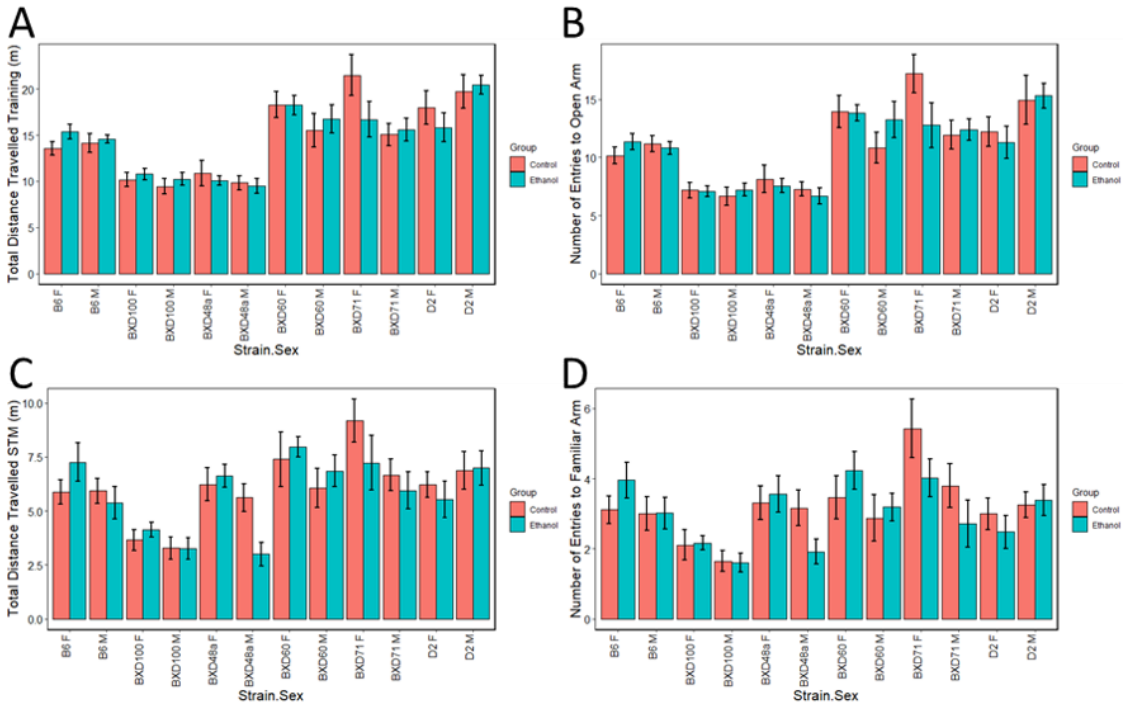

Figure 3-8. Strain and ethanol effects in activity-related and explorative-like behavior using the T-maze.

Strain and ethanol effects in activity-related behaviors measured by total distance travelled during the (A) training session and (C) short-term memory session in the T-maze. Strain and ethanol effects in explorative-like behavior as measure by (B) number of entries to the open arm during the training session and (D) number of entries to the familiar arm during the short-term memory session. Blue bars represent animals exposed to postnatal ethanol while red bars represent non-exposed control animals.

3.42, $p = 0.07$) for number of entries to the familiar arm. There was a trend towards significant effect of sex on the number of entries to the familiar arm the BXD100 strain ($F_{(1,34)} = 2.76$, $p = 0.10$) and BXD48a strain ($F_{(1,28)} = 3.53$, $p = 0.07$).

Number of entries to the novel arm of the T-maze (**Figure 3-9A**) was significant for the effects of strain ($F_{(5,191)} = 11.21$, $p < 0.001$, $\omega^2 = 0.19$, 90% CI [0.10, 0.26]) and sex ($F_{(1,191)} = 10.78$, $p < 0.01$, $\omega^2 = 0.04$, 90% CI [0.01, 0.10]). Further analysis showed a significant effect of sex on the number of entries to the novel arm in the BXD71 strain ($F_{(1,31)} = 5.99$, $p < 0.05$) and the BXD48a strain ($F_{(1,28)} = 5.27$, $p < 0.05$). The BXD48a strain also showed significance for the effect of treatment ($F_{(1,28)} = 4.90$, $p < 0.05$) with both male and female mice exposed to postnatal ethanol showing decreased number of entries to the novel arm compared to non-exposed controls. Latency to enter the novel arm of the T-maze during the short term memory session (**Figure 3-9B**) was significant for the effect of strain ($F_{(5,191)} = 2.33$, $p < 0.05$, $\omega^2 = 0.03$, 90% CI [0.00, 0.06]). Further analysis showed a significant effect for treatment ($F_{(1,34)} = 3.31$, $p < 0.05$) in the BXD100 strain. The BXD48a strain showed a significant effect of sex ($F_{(1,28)} = 5.73$, $p < 0.05$) on latency to enter the novel arm.

Discrimination ratios were calculated to determine the animal's ability to differentiate between the familiar (previously opened arm) and the novel (previously blocked arm). There was a significant effect of strain for time between the two arms (**Figure 3-9C**; $F_{(5,191)} = 2.73$, $p < 0.05$, $\omega^2 = 0.04$, 90% CI [0.00, 0.07]). Further analysis showed a trend towards significance for a sex x treatment interaction ($F_{(1,28)} = 2.59$, $p = 0.10$) in BXD48a strain for time in the novel arm compared to total time in both arms. There was a trend towards significance for treatment ($F_{(1,28)} = 2.69$, $p = 0.10$) in the D2 strain for time in the novel arm compared to total time in both the novel and familiar arm. There was no significant effects or interactions for number of entries between the two arms ($F_{(5,191)} = 1.29$, $p > 0.05$).

Discussion

This study was designed to investigate differential behavioral responses in male and female adolescent mice exposed to postnatal ethanol across selected BXD RI and parental strains. To our knowledge, this is the first paper investigating developmental alcohol exposure on behavior in the BXD RI strains. We show that there is a significant strain difference in almost every behavioral measure in all four tests. Furthermore, we show significant effect of sex for a number of behavioral measures among the strains. Although there were less effects of treatment compared to strain and sex effects, there were several treatment interactions between strain and/sex in our behavioral measures, and several behaviors that showed ethanol-induced behavioral differences within specific strains.

In the elevated plus maze, a significant effect of sex x treatment was found in the D2 parental strain. Postnatal ethanol exposure produced sex-specific responses in the D2 strain with the females showing increased activity and decreased anxiety-related behavior

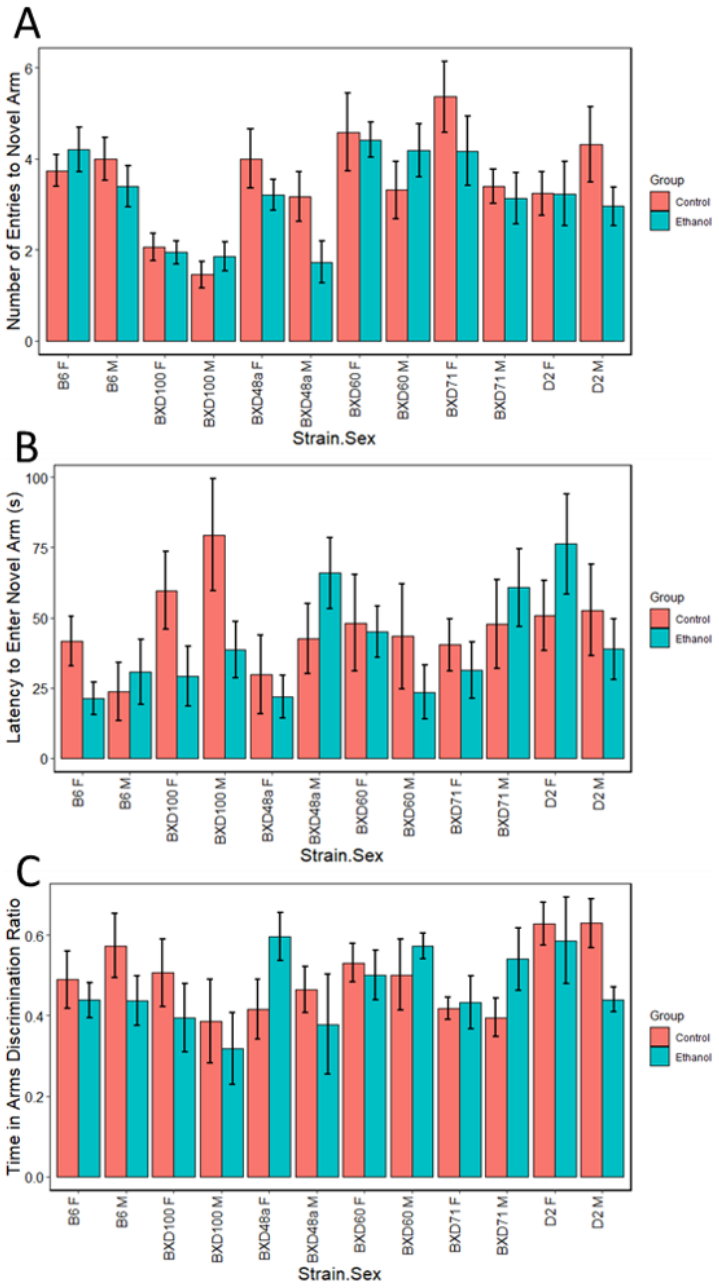

Figure 3-9. Strain and ethanol effects on spatial learning and memory during the short-term memory session in the T-maze.

Strain and ethanol effects on spatial learning and memory measured by (A) number of entries to the novel (previously blocked arm), (B) latency to enter the novel arm, and (C) discrimination ratio of time spent between the novel and familiar arm. during the short term memory session in the T-maze Blue bars represent animals exposed to postnatal ethanol while red bars represent non-exposed control animals.

as measured by total distanced travelled and number of entries into the center, while males showed decreased activity and increased anxiety-like behavior.

There was a three-way strain x sex x treatment interaction for time in the center of the open field on day 1 during the first five minutes. Further analysis found the BXD100 strain showed a significant effect for sex x treatment with females exposed to postnatal ethanol displaying decreased activity measured by total distance travelled and increased anxiety-related behaviors measured by number of entries and time in the center of the maze compared to non-exposed control females. In contrast, BXD100 males displayed opposing effects of ethanol-induced behavior showing increased activity and reduced anxiety-like behaviors. The D2 and BXD71 strains also showed significant effects for treatment on total distance travelled and time in center, respectively. There were several sex and ethanol effects present in open field measures on day 2. The BXD48a strain showed a significant effect for sex x treatment with ethanol-exposed females showing reduced activity during the 15-minute open field test compared to non-exposed females while there was no ethanol-induced change in activity in the males. During the first five minutes of the open field test on day 2, the B6 strain showed an interaction between sex and treatment with increased activity and reduced anxiety-like behaviors seen in females exposed to postnatal ethanol compared to controls while there was an effect of ethanol-exposure in males. The D2 strain showed increased activity and reduced anxiety-related behaviors in both male and females exposed to postnatal ethanol. Additionally, effects of sex were seen in at least one behavioral measure in the elevated plus maze and/or open field across all six BXD and parental strains.

Spontaneous alternations measured in the Y-maze showed a three-way strain x sex x treatment. Further analysis within each strain showed a significant sex x treatment interaction in the BXD100 strain and a trend towards significance in the BXD48a strain. Both of these strains showed more changes in females mice compared to males of the same strain though ethanol exposure decreased spontaneous alternations in BXD100 females compared to controls while ethanol exposure increased spontaneous alternations in BXD48a. The males in both of these strains showed opposite effects of their female counterparts. This result is interesting as both the BXD100 and BXD48a strains have previously been shown to be susceptible to ethanol-induced cell death in the hippocampus compared to the other BXD and parental strains. However, our results showed smaller effects of ethanol on spontaneous alternations compared to another recent behavioral study examining the effects of postnatal ethanol exposure on spatial memory (Subbanna and Basavarajappa, 2014). In addition, the sex-specific effects in opposing directions further complicates the effect of ethanol-exposure on spontaneous alternations in the Y-maze.

Spatial working memory, as measured by discrimination between a familiar and novel arm in the T-maze, showed limited treatment effects. Time spent between each arm measured by a discrimination ratio found an overall effect of strain though further analysis only examined a trend towards significance for a sex x treatment interaction in the BXD48a strain and a trend towards significant effect of treatment in the D2 strain for time in the novel arm compared to time spent in both arms. While there was no treatment

effect for discrimination between the two arms, there was a significant treatment effect in the BXD48a strain and the BXD100 strain, which are the two HCD strains examined in this study. In the BXD48a strain, both males and females exposed to postnatal ethanol exposure showed decreased number of entries to the novel suggesting impaired short-term memory. There was a significant effect of treatment in the BXD100 strain with both males and females exposed to postnatal ethanol showing reduced latency to enter the novel arm which suggests they were not impaired in latency to explore the novel arm.

Developmental alcohol exposure has been shown to affect several behavioral responses including activity, anxiety, and learning and memory though the presence or severity of these behavioral phenotypes vary depending on the level and timing of alcohol exposure as well as the age of behavioral testing (as reviewed in (Chokroborty-Hoque et al., 2014; Marquardt & Brigman, 2016; Patten et al., 2014). While molecular dysfunction and developmental abnormalities such as synaptic activity and apoptosis have been extensively studied in animals exposed to postnatal ethanol (equivalent to the third trimester in humans), behavioral responses to exposure at this developmental timepoint have been understudied. Many behavioral studies have examined the effects of chronic exposure to prenatal ethanol while fewer studies have examined behavioral effects to postnatal alcohol exposure (Marquardt & Brigman, 2016). In addition, many of the studies that do investigate the effects of postnatal ethanol exposure on behavioral responses use a chronic exposure paradigm across multiple postnatal days with even less studies examining the behavioral effects of acute postnatal alcohol exposure. In our current model, we used an acute one-day ethanol exposure paradigm which could explain why we did not see a larger effect of ethanol treatment in adolescent behavior.

We believe our limited effects of postnatal ethanol exposure on behavioral measures were partly due to the overwhelming large effect of strain, followed by sex in many measures. While these results were significant in the large overall analysis, further investigation within each strain revealed effects of treatment and/or sex x treatment interactions for many behavioral measures in almost all strains. The BXD strains used in the current study were selected for their differential vulnerability to hippocampal cell death after exposure to postnatal ethanol (Goldowitz et al., 2014). The BXD100 and BXD48a strains were susceptible to high levels of ethanol induced cell death in the CA1 region of the hippocampus while the BXD60 and BXD71 strains were resistant to ethanol-induced cell death in this region of the hippocampus showing little to no difference compared to control animals (Goldowitz et al., 2014). This previous study also included the B6 and D2 parental strains which showed moderate levels of hippocampal cell death after postnatal ethanol exposure (Goldowitz et al., 2014).

In our present study, the strains that showed the most measures affected by treatment and/or sex x treatment interactions were the B6 and D2 parental strains as well as the BXD100 and BXD48a strains. The B6 and D2 strains showed more effects of treatment on anxiety-like and activity-related behaviors examined in the elevated plus maze and open field. The BXD100 and BXD48a strains showed effects of treatment on anxiety-like and activity-related behaviors in these tests as well, though a smaller number of significant treatment and/or sex x treatment effects were found compared to the

parental strains. Interestingly, the BXD100 and BXD48a strains were the only strains that showed significant effects of treatment and/or sex x treatment effects in behavioral measures associated with learning and memory. For spontaneous alternations in the Y-maze, the BXD100 strain showed a significant sex x treatment effect and the BXD48a strain was almost significant for a sex and treatment interaction ($p = 0.056$) in spontaneous alternations. Greater effects of ethanol-exposure were seen in females of both strains compared to their male counterparts, although the ethanol-exposure showed opposite effects in the BXD100 and BXD48a females. In the T-maze, the BXD48a strain showed a significant effect of treatment with both male and female mice exposed to ethanol showing decreased number of entries to the novel (previously blocked) arm compared to control animals. In the T-maze, the BXD100 strain showed a significant effect of treatment for latency to enter the novel arm during the short-term memory session with both male and female mice exposed to ethanol showing decreased latency to enter the novel arm compared to normal controls. While discrimination between the novel and familiar arm was not significant for treatment interaction in any strain, there was a trend towards significant effect of treatment in the D2 strain ($p = 0.10$) and a trend towards significant sex x treatment interaction in the BXD48a strain ($p = 0.10$). These results show complex relationship between genetic background, sex, and postnatal ethanol-exposure and their effects on adolescent behaviors.

The most significant result in this study is the effect of treatment that was highly strain-dependent across every behavioral measure examined. The second important finding of this study was the presence of sex-specific effects after postnatal ethanol exposure seen in multiple measures across all four behavioral tests. Our behavioral tests did show large individual variability within a strain, sex, and exposure group for many of our measures, especially in ethanol-exposed animals. This large variation could be due to the age of behavioral testing, as adolescent mice tend to show more behavioral variability than adult mice (Brust, Schindler, & Lewejohann, 2015). Future studies could address large variation in behavioral measures by adding more subjects per group and running additional analyses to identify outliers. Although our study found large variability in animal behavior, we were still able to identify multiple behavioral measures effected by acute postnatal ethanol exposure including differences in activity, anxiety, and learning and memory behaviors in the BXD strains and B6 and D2 parental strains. In conclusion, these results support the inclusion of multiple strains and the evaluation of both males and females in behavioral studies examining the effects of developmental alcohol exposure. By evaluating multiple strains and both sexes, we can better understand the effects of genetic background and sex on alcohol-induced neurobehavioral abnormalities.

CHAPTER 4. CONCLUSIONS AND FUTURE DIRECTIONS

Conclusions

Overall, our results demonstrate differential gene expression changes and behavioral responses in mice exposed to postnatal ethanol across the BXD RI mice and parental strains. To our knowledge, this is the first study using the BXD RI strains to examine the effects of genetics and sex on 1) ethanol-induced gene expression changes during development, and 2) adolescent behaviors in mice exposed to postnatal ethanol. Using the BXD RI mice, we were able to identify numerous effects of strain on hippocampal gene expression changes after exposure to postnatal ethanol as well as show strain differences in adolescent behavioral responses after postnatal ethanol exposure.

Differential Ethanol-Induced Gene Expression Changes

We identified a large number of differentially expressed genes in the BXD RI and parental strains that showed significant differential gene expression changes after exposure to postnatal ethanol. Enrichment analysis of differential ethanol-induced gene expression revealed a number of significant over-represented biological categories that were involved in cell death and apoptosis. We identified many genes that met our criteria for differential gene expression after exposure to developmental alcohol, though a few genes were especially interesting as possible candidate genes involved in differential susceptibility to ethanol-induced cell death including *Bcl2l11*, *Jun*, and *Txnip*. While these genes have been previously shown to be related to apoptosis, our study is the first to link these genes to differential gene expression changes after exposure to developmental alcohol exposure, to our knowledge. Another interesting candidate gene involved in differential gene expression changes after exposure to developmental alcohol exposure was *Tgfb3*. This gene is of particular interest because it was previously linked to the significant QTL mediating strain-specific differences in cell death in the hippocampus after postnatal ethanol exposure (Goldowitz et al., 2014). Due to the previous association of Tgfb3 with strain differences in ethanol-induced cell death phenotype as well as its involvement in many relevant biological processes including neuron apoptotic process, cell proliferation, and regulation of transcription, Tgfb3 is an excellent candidate gene. To our knowledge, our findings of differential expression of Tgfb3 after exposure to developmental alcohol are novel.

An advantage of the current study was the comparison of strains that showed differential vulnerability to ethanol-induced cell death in the developing hippocampus. We examined gene expression changes in three BXD strains that showed high vulnerability to cell death in the hippocampus after postnatal ethanol exposure and three strains that were highly resistant to ethanol-induced cell death in the developing hippocampus (Goldowitz et al., 2014). Our goal was to identify ethanol-induced gene expression changes that were present in high cell death (HCD) strains, but not the low cell death (LCD) strains, which might account for the increased susceptibility to ethanol-

induced hippocampal cell death. Likewise, our other goal was to identify gene expression changes after ethanol exposure that were present in the LCD strains, but not the HCD strains, that might account for the resistance to hippocampal cell death after postnatal ethanol exposure. We observed almost double the number of differentially expressed genes after ethanol exposure in the HCD strains compared to the LCD strains. Our enrichment analysis revealed some overlap in the significantly over-represented biological categories between the HCD strains versus LCD strains, though HCD strains showed more categories overall, especially ones pertaining to cell death and brain development.

One of the most interesting findings was that significant ethanol-induced gene expression changes in the HCD and LCD strains were always regulated in the same direction. There were no significant gene expression changes that were regulated in opposing directions in the HCS strains versus the LCS strains. These results suggest more perturbed effects of ethanol in the HCD strains compared to the LCD strains and this is why we see more significant cell death in the hippocampus in the HCD strains than the LCD strains. The results also suggest there may be limited gene expression changes that confer resistance to ethanol-induced cell death in the hippocampus in the LCD strains. This notion that vulnerable strains show more perturbed gene expression changes compared to resistant strains while resistant strains show limited gene expression changes that could account for protection against ethanol's teratogenic effects has been previously proposed in a study that compared ethanol-induced gene expression changes in the vulnerable B6 strain to the resistant D2 strain (Downing, Flink, et al., 2012).

Differential Behavioral Responses in Adolescent Mice Exposed to Developmental Alcohol Exposure

In our behavioral study, our results demonstrate the effects of developmental alcohol exposure on adolescent behavioral responses are highly dependent on strain. While almost all strains showed effects of postnatal ethanol exposure in at the least one measure of behavioral response, the strains that showed the most behavioral alterations after developmental alcohol exposure were the B6 and D2 parental strains as well was the HCD strains BXD100 and BXD48a. In these four strains, we observed many anxiety-like and activity-related behaviors that were significantly affected by postnatal ethanol exposure and in many of these measures there were sex-specific differences within the strain. The LCD strains, BXD60 and BXD71, showed minimal effect of treatment in all behavioral tests. In the BXD60 strain, we did not observe effects of postnatal ethanol exposure on any of our behavioral measures. The BXD71 strain did show significance for treatment effect for a few activity-like and anxiety-related behaviors.

The BXD100 and BXD48a strains were the only strains that showed significant effect of treatment in hippocampal-dependent spatial learning and memory assessments. The treatment effects in the BXD100 and BXD48a were often sex-specific and the direction of the behavioral response after postnatal ethanol exposure did not always indicate impairment in spatial learning. For example, the BXD100 ethanol-exposed

females showed impaired spatial memory in spontaneous alternations in the Y-maze but showed faster exploratory behavior in the novel arm of the T-maze during the short-term memory test indicative of enhanced short-term memory. Similarly, both male and female BXD48a strains exposed to postnatal ethanol showed reduced entries into the novel arm of the T-maze during the short-term T-maze indicative of impaired short-term memory. However, there was a trend towards significant effect of treatment for BXD48a ethanol-exposed females discriminating between the arms of the T-maze indicating enhanced short-term memory compared to non-exposed female controls. These results suggest that there are long-term effects of developmental alcohol exposure on anxiety- and activity-related behaviors and that these effects are highly strain-specific. Furthermore, while the HCD strains did show effects of treatment on learning and memory behaviors the relationship is more complex, not always indicating impairments.

Effects of Sex on Gene Expression and Adolescent Behavior After Postnatal Ethanol Exposure

We observed significant effects of sex on ethanol-exposure on gene expression changes and behavioral responses after exposure to postnatal ethanol exposure. We identified gene expression changes after postnatal ethanol exposure that were highly sex-specific with little overlap in ethanol-induced gene expression changes between males and females in the same strain. In six out of the eight strains examined, males showed more significantly more ethanol-induced gene expression changes. The exceptions were the B6 and BXD71 females that both showed almost double the number of differentially expressed genes compared to the males in the same strain. However, examination of the interaction between sex and treatment in the all BXD and parental strains revealed no significant effect of sex on ethanol-induced gene expression changes. Sex-specific ethanol-induced gene expression changes were limited within each strain and these changes were not carried over across strains. Since there were such robust sex differences within each strain, we analyzed males and females separately for our analysis of ethanol-induced gene expression changes in strains that showed differential vulnerability to cell death in the hippocampus after exposure to postnatal ethanol. When comparing the number of genes that were significantly expressed in all three HCD strains or all three LCD strains, there were more ethanol-induced gene expression changes in the males compared to the females.

We also observed significant effects of sex and interactions between sex and treatment in our behavioral analysis. Every one of the six strains analyzed showed an effect of sex or interaction of sex by treatment in at least one of our behavioral measures. Although more sex-specific behaviors were seen in the anxiety-like and activity-related behaviors. However, effects of sex were often seen within a strain and sex-dependent effects of a behavioral measure were not present in all strains. Likewise, the direction of sex-specific differences were not consistent across all behavioral measures and/or strains. This suggests a complex relationship between developmental ethanol-exposure, sex, and strain on adolescent behaviors.

In our gene expression study, we identified numerous ethanol-induced gene expression changes that were found to be significantly over-represented for a multiple biological categories including in sex differentiation. Previous studies have found the perinatal period is extremely sensitive period for sexual differentiation and each sex has diverse region-specific mechanisms for proper development (as reviewed in (McCarthy & Arnold, 2011; Ratnu, Emami, & Bredy, 2017). During this perinatal period, sex differentiation has been shown in multiple brain regions including the hippocampus (McCarthy & Arnold, 2011). Sexual differentiation pathways have been shown to differentially effect multiple cellular mechanisms in males and females including cell proliferation, cell survival, synaptogenesis, cell death, dendritic braining, and epigenetic modifications (McCarthy & Arnold, 2011). Therefore, if the perinatal period is an extremely sensitive period for sexual differentiation, it stands to reason that exposure to a teratogen such as ethanol during this developmental period could have differential effects in males and females. Our results support this notion showing significant effects for sex in both ethanol-induced gene expression changes in the hippocampus and adolescent behavior after postnatal ethanol exposure, though these effects are highly dependent on strain.

Limitations and Future Studies

While we show significant differential gene expression among BXD RI and parental strains after exposure to postnatal ethanol, there could be other sources of variation that we did not account for in the current study. As briefly mentioned in Chapter 2, we performed a principle component analysis (PCA) to visualize the data and identify other sources of variation that were not accounted for by strain, sex, or treatment (Mulligan et al., 2017). The PCA identified noticeable patterns in our data indicating an unknown source of variation that could be confounding our results. Examples of this possible unknown source of variation could include multiple sample outliers or batch effects. Though we tested 128 samples in our microarray study, our sample size per strain, per sex, per treatment was relatively low at n = 4. Due to our limited sample size per group, we did not exclude any samples from the analysis. Further analysis needs to be conducted to identify the unknown source(s) of variation that could be confounding our results as indicated by the noticeable patterns of data in the PCA.

In our gene expression study, we used microarrays which are a hybridization-based technique used to detect predefined RNA sequences withing a sample (Mantione et al., 2014). In contrast, newer next-generation RNA-sequencing (RNA-seq) do not use hybridization and instead use a sequence-based technique that is not reliant on predefined sequence information and therefore can detect novel sequences and splice variants (Mantione et al., 2014). RNA-seq have been shown to have higher detection of low abundance transcripts and higher resolution of differentially expressed genes compared to microarrays (Zhao, Fung-Leung, Bittner, Ngo, & Liu, 2014). However, overall microarrays and RNA-seq are comparable techniques to analyze gene expression changes. Microarrays were chosen for the current study due to 1) cost effectiveness for our large study across eight strains, two sexes, and two treatments, 2) turn-around time

for results, and 3) numerous other microarray expression data is available for the BXD RI strains on GeneNetwork.org. Though future studies on the effects of developmental alcohol exposure should explore the use of RNA-seq.

Future studies are also needed to validate gene expression changes that identified in our present microarray study. Validation is often needed in both microarray and RNA-seq studies and can be achieved through real time quantitative PCR. A limitation of the current study is that we do not know the cell-type that showed differential gene expression. Future studies could extend our current analysis and examine specific gene expression changes within specific cell types such as neurons, astrocytes, and microglia. Future studies can also utilize the publicly accessible tools on GeneNetwork.org to further evaluate top candidate genes. Our study meets the minimum number of strains required to upload our microarray results to the GeneNetwork.org database. Once this is completed, we can further evaluate the ethanol-induced gene expression changes that were differentially expressed among the strains. Gene and phenotype correlations can be evaluated as well as specific gene networks involved in differential ethanol-induced gene expression can be assessed.

In our behavioral studies, while we saw an effect of treatment within strains, our treatment effect on hippocampal learning and memory were not as robust as previous studies have found. While part of this could be strain-specific, we did not observe impaired spatial learning and memory in the B6 strains which has been highly used in behavioral studies assessing the effects of developmental alcohol exposure. An explanation for why we did not have larger ethanol effects on behavioral responses could be due to the type of behavioral tests performed and the age of behavioral testing. For example, behavioral studies in adolescent animals exposed to acute postnatal ethanol have observed learning and memory impairments using more complex behavioral measures such as the Morris water maze, object recognition test, fear conditioning, and radial arm maze (Ieraci & Herrera, 2007; A. F. Wagner & Hunt, 2006; J. L. Wagner, Zhou, & Goodlett, 2014; D. F. Wozniak et al., 2004). Many of these experimental tests also included either a positive component such as a food pellet reward or a negative component such as foot shock or forced water placement (Ieraci & Herrera, 2007; J. L. Wagner et al., 2014; D. F. Wozniak et al., 2004). Also, it is important to note that the behavioral tests used in our current study were highly dependent on activity. Since there were such robust effects of strain on activity levels, this could be overriding some of our ethanol-related effects. Future studies examining differential behavioral responses after exposure to developmental alcohol in these strains should take into account the significant strain effect on activity.

The learning and memory behavioral component in the current study was modeled after a study in B6 mice that showed deficits in spontaneous alternations in the Y-maze and novel arm discrimination in a modified Y-maze in animals exposed to postnatal ethanol (Subbanna et al., 2014). This study administered the same acute ethanol dose on P7 that was used in our current study (Subbanna et al., 2014). There are a few explanations as to why we were unable to replicate these results including 1) the age of

behavioral testing, 2) carryover effects of multiple behavioral tests, and 3) modified T-maze versus modified Y-maze. The first discrepancy in these two studies was the age of behavioral testing. In our current study, we analyzed effects of postnatal ethanol exposure on learning and memory impairments in late adolescent mice (P49-P50) while Subbanna and Basavarajappa (2014) examined adult mice (> P60). While there was only a 10-day difference between our two studies, activity- and anxiety-related behaviors have been shown to differ between late adolescence and early adulthood in B6 mice (Brust et al., 2015). Another possible explanation for why we were unable to detect learning and memory impairments could be due to our multiple battery of testing in the same animals. Subbanna and Basvarajappa used separate animals for each behavioral test while our animals used in our learning and memory experiments had been tested in the elevated plus maze and open field approximately two-weeks prior (Subbanna et al., 2014). Due to our multiple battery of tests, we also modified the spatial recognition memory protocol by using a T-maze instead of the Y-maze since our animals had already been exposed to the Y-maze the previous day and we wanted to avoid any carryover effects (Subbanna et al., 2014). The size and proportions of the arms in the Y-maze and T-maze do differ and this along with the same animals being tested in all behavioral studies could account for discrepancies between our study and that of Subbanna and Basvarjappa (2014). Few other postnatal ethanol studies have tested the same animals in multiple tests though the ones that did used more complex behavior tests such as the Morris water maze and fear conditioning chamber (Ieraci & Herrera, 2007; D. F. Wozniak et al., 2004).

While our current study focused on the hippocampus by examining hippocampal gene expression changes and hippocampal-dependent learning and memory after postnatal ethanol exposure, future studies could also explore other brain regions that have been shown to effected by developmental alcohol exposure. For example, our previous studies also identified differentially susceptibility to ethanol-induced cell death in the cortex of BXD mice exposed to postnatal ethanol (Goldowitz et al., 2014). Interestingly, cell death showed regional specificity within some strains while others showed uniformity in both regions. For example, while the BXD100 strain was identified as a vulnerable strain for cell death in the hippocampus as well as the cortex, the BXD71 strain was highly resistant to cell death in the hippocampus but showed high susceptibility to cell death in layer 2/3 of the cortex (Goldowitz et al., 2014). Ethanol-induced cell death in other brains regions highly involved in cognition, such as the cerebral cortex, could also impair learning and memory.

Overall, our study aimed to better understand genetic variation in ethanol-induced susceptibility to ethanol's teratogenic effects. Our results accomplish this by identifying differential gene expression changes and behavioral responses in animals exposed to postnatal ethanol using the BXD RI mice and parental strains. Additionally, our study identified sex differences in both ethanol-induced gene expression changes adolescent behaviors in mice exposed to postnatal ethanol, though sex-specific effects were highly dependent on strain. To our knowledge, this is the first study using the BXD RI strains to examine the effects of genetics and sex on 1) ethanol-induced gene expression changes during development, and 2) adolescent behaviors in mice exposed to postnatal ethanol.

LIST OF REFERENCES

Abel, E. L., Jacobson, S., & Sherwin, B. T. (1983). In utero alcohol exposure: functional and structural brain damage. *Neurobehav Toxicol Teratol, 5*(3), 363-366.

Ahlers, K. E., Karacay, B., Fuller, L., Bonthius, D. J., & Dailey, M. E. (2015). Transient activation of microglia following acute alcohol exposure in developing mouse neocortex is primarily driven by BAX-dependent neurodegeneration. *Glia, 63*(10), 1694-1713. doi:10.1002/glia.22835

Alfonso-Loeches, S., & Guerri, C. (2011). Molecular and behavioral aspects of the actions of alcohol on the adult and developing brain. *Crit Rev Clin Lab Sci, 48*(1), 19-47. doi:10.3109/10408363.2011.580567

Alvik, A., Aalen, O. O., & Lindemann, R. (2013). Early fetal binge alcohol exposure predicts high behavioral symptom scores in 5.5-year-old children. *Alcohol Clin Exp Res, 37*(11), 1954-1962. doi:10.1111/acer.12182

Amiri, S., Davie, J. R., & Rastegar, M. (2020). Chronic Ethanol Exposure Alters DNA Methylation in Neural Stem Cells: Role of Mouse Strain and Sex. *Mol Neurobiol, 57*(2), 650-667. doi:10.1007/s12035-019-01728-0

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. *Dev Med Child Neurol, 43*(3), 148-154.

Astley, S. J. (2006). Comparison of the 4-digit diagnostic code and the Hoyme diagnostic guidelines for fetal alcohol spectrum disorders. *Pediatrics, 118*(4), 1532-1545. doi:10.1542/peds.2006-0577

Astley, S. J. (2010). Profile of the first 1,400 patients receiving diagnostic evaluations for fetal alcohol spectrum disorder at the Washington State Fetal Alcohol Syndrome Diagnostic & Prevention Network. *Can J Clin Pharmacol, 17*(1), e132-164.

Astley, S. J., Aylward, E. H., Olson, H. C., Kerns, K., Brooks, A., Coggins, T. E., . . . Richards, T. (2009). Functional magnetic resonance imaging outcomes from a comprehensive magnetic resonance study of children with fetal alcohol spectrum disorders. *J Neurodev Disord, 1*(1), 61-80. doi:10.1007/s11689-009-9004-0

Astley, S. J., Magnuson, S. I., Omnell, L. M., & Clarren, S. K. (1999). Fetal alcohol syndrome: changes in craniofacial form with age, cognition, and timing of ethanol exposure in the macaque. *Teratology, 59*(3), 163-172. doi:10.1002/(SICI)1096-9926(199903)59:3<163::AID-TERA8>3.0.CO;2-8

Autti-Ramo, I., Autti, T., Korkman, M., Kettunen, S., Salonen, O., & Valanne, L. (2002). MRI findings in children with school problems who had been exposed prenatally to alcohol. *Dev Med Child Neurol, 44*(2), 98-106. doi:10.1017/s0012162201001748

Bailey, K. R., & Crawley, J. N. (2009). Anxiety-Related Behaviors in Mice. In nd & J. J. Buccafusco (Eds.), *Methods of Behavior Analysis in Neuroscience*. Boca Raton (FL).

Baker, J. A., Li, J., Zhou, D., Yang, M., Cook, M. N., Jones, B. C., . . . Lu, L. (2017). Analyses of differentially expressed genes after exposure to acute stress, acute ethanol, or a combination of both in mice. *Alcohol, 58*, 139-151. doi:10.1016/j.alcohol.2016.08.008

Bakhireva, L. N., Sharkis, J., Shrestha, S., Miranda-Sohrabji, T. J., Williams, S., & Miranda, R. C. (2017). Prevalence of Prenatal Alcohol Exposure in the State of Texas as Assessed by Phosphatidylethanol in Newborn Dried Blood Spot Specimens. *Alcohol Clin Exp Res, 41*(5), 1004-1011. doi:10.1111/acer.13375

Barnes, D. E., & Walker, D. W. (1981). Prenatal ethanol exposure permanently reduces the number of pyramidal neurons in rat hippocampus. *Brain Res, 227*(3), 333-340. doi:10.1016/0165-3806(81)90071-7

Basavarajappa, B. S., Nagre, N. N., Xie, S., & Subbanna, S. (2014). Elevation of endogenous anandamide impairs LTP, learning, and memory through CB1 receptor signaling in mice. *Hippocampus, 24*(7), 808-818. doi:10.1002/hipo.22272

Beery, A. K. (2018). Inclusion of females does not increase variability in rodent research studies. *Curr Opin Behav Sci, 23*, 143-149. doi:10.1016/j.cobeha.2018.06.016

Beery, A. K., & Zucker, I. (2011). Sex bias in neuroscience and biomedical research. *Neurosci Biobehav Rev, 35*(3), 565-572. doi:10.1016/j.neubiorev.2010.07.002

Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N., & Golani, I. (2001). Controlling the false discovery rate in behavior genetics research. *Behav Brain Res, 125*(1-2), 279-284. doi:10.1016/s0166-4328(01)00297-2

Benz, J., Rasmussen, C., & Andrew, G. (2009). Diagnosing fetal alcohol spectrum disorder: History, challenges and future directions. *Paediatr Child Health, 14*(4), 231-237. doi:10.1093/pch/14.4.231

Berman, R. F., & Hannigan, J. H. (2000). Effects of prenatal alcohol exposure on the hippocampus: spatial behavior, electrophysiology, and neuroanatomy. *Hippocampus, 10*(1), 94-110. doi:10.1002/(SICI)1098-1063(2000)10:1<94::AID-HIPO11>3.0.CO;2-T

Berman, R. F., Hannigan, J. H., Sperry, M. A., & Zajac, C. S. (1996). Prenatal alcohol exposure and the effects of environmental enrichment on hippocampal dendritic spine density. *Alcohol, 13*(2), 209-216. doi:10.1016/0741-8329(95)02049-7

Bertrand, J., Floyd, L. L., Weber, M. K., Fetal Alcohol Syndrome Prevention Team, D. o. B. D., Developmental Disabilities, N. C. o. B. D., Developmental Disabilities, C. f. D. C., & Prevention. (2005). Guidelines for identifying and referring persons with fetal alcohol syndrome. *MMWR Recomm Rep, 54*(RR-11), 1-14.

Blanchard, B. A., Riley, E. P., & Hannigan, J. H. (1987). Deficits on a spatial navigation task following prenatal exposure to ethanol. *Neurotoxicol Teratol, 9*(3), 253-258. doi:10.1016/0892-0362(87)90010-9

Boehm, S. L., 2nd, Lundahl, K. R., Caldwell, J., & Gilliam, D. M. (1997). Ethanol teratogenesis in the C57BL/6J, DBA/2J, and A/J inbred mouse strains. *Alcohol, 14*(4), 389-395.

Bonthius, D. J., Goodlett, C. R., & West, J. R. (1988). Blood alcohol concentration and severity of microencephaly in neonatal rats depend on the pattern of alcohol administration. *Alcohol, 5*(3), 209-214. doi:10.1016/0741-8329(88)90054-7

Bonthius, D. J., & West, J. R. (1988). Blood alcohol concentration and microencephaly: a dose-response study in the neonatal rat. *Teratology, 37*(3), 223-231. doi:10.1002/tera.1420370307

Bonthius, D. J., & West, J. R. (1990). Alcohol-induced neuronal loss in developing rats: increased brain damage with binge exposure. *Alcohol Clin Exp Res, 14*(1), 107-118. doi:10.1111/j.1530-0277.1990.tb00455.x

Bonthius, D. J., & West, J. R. (1991). Permanent neuronal deficits in rats exposed to alcohol during the brain growth spurt. *Teratology, 44*(2), 147-163. doi:10.1002/tera.1420440203

Boschen, K. E., & Klintsova, A. Y. (2017). Neurotrophins in the Brain: Interaction With Alcohol Exposure During Development. *Vitam Horm, 104*, 197-242. doi:10.1016/bs.vh.2016.10.008

Bossy-Wetzel, E., Bakiri, L., & Yaniv, M. (1997). Induction of apoptosis by the transcription factor c-Jun. *EMBO J, 16*(7), 1695-1709. doi:10.1093/emboj/16.7.1695

Bottom, R. T., Abbott, C. W., 3rd, & Huffman, K. J. (2020). Rescue of ethanol-induced FASD-like phenotypes via prenatal co-administration of choline. *Neuropharmacology, 168*, 107990. doi:10.1016/j.neuropharm.2020.107990

Brady, M. L., Allan, A. M., & Caldwell, K. K. (2012). A limited access mouse model of prenatal alcohol exposure that produces long-lasting deficits in hippocampal-dependent learning and memory. *Alcohol Clin Exp Res, 36*(3), 457-466. doi:10.1111/j.1530-0277.2011.01644.x

Britton, S. M., & Miller, M. W. (2018). Neuronal Loss in the Developing Cerebral Cortex of Normal and Bax-Deficient Mice: Effects of Ethanol Exposure. *Neuroscience, 369*, 278-291. doi:10.1016/j.neuroscience.2017.11.013

Brust, V., Schindler, P. M., & Lewejohann, L. (2015). Lifetime development of behavioural phenotype in the house mouse (Mus musculus). *Front Zool, 12 Suppl 1*, S17. doi:10.1186/1742-9994-12-S1-S17

Camargo Moreno, M., Mooney, S. M., & Middleton, F. A. (2017). Heterogeneity of p53 dependent genomic responses following ethanol exposure in a developmental mouse model of fetal alcohol spectrum disorder. *PLoS One, 12*(7), e0180873. doi:10.1371/journal.pone.0180873

Cantacorps, L., Alfonso-Loeches, S., Moscoso-Castro, M., Cuitavi, J., Gracia-Rubio, I., Lopez-Arnau, R., . . . Valverde, O. (2017). Maternal alcohol binge drinking induces persistent neuroinflammation associated with myelin damage and behavioural dysfunctions in offspring mice. *Neuropharmacology, 123*, 368-384. doi:10.1016/j.neuropharm.2017.05.034

Castelhano-Carlos, M. J., Sousa, N., Ohl, F., & Baumans, V. (2010). Identification methods in newborn C57BL/6 mice: a developmental and behavioural evaluation. *Lab Anim, 44*(2), 88-103. doi:10.1258/la.2009.009044

Chasnoff, I. J. (1985). Fetal alcohol syndrome in twin pregnancy. *Acta Genet Med Gemellol (Roma), 34*(3-4), 229-232.

Chasnoff, I. J., Wells, A. M., & King, L. (2015). Misdiagnosis and missed diagnoses in foster and adopted children with prenatal alcohol exposure. *Pediatrics, 135*(2), 264-270. doi:10.1542/peds.2014-2171

Chater-Diehl, E. J., Laufer, B. I., Castellani, C. A., Alberry, B. L., & Singh, S. M. (2016). Alteration of Gene Expression, DNA Methylation, and Histone Methylation in Free Radical Scavenging Networks in Adult Mouse Hippocampus following Fetal

Alcohol Exposure. *PLoS One, 11*(5), e0154836. doi:10.1371/journal.pone.0154836
Chen, Y., Ozturk, N. C., Ni, L., Goodlett, C., & Zhou, F. C. (2011). Strain differences in developmental vulnerability to alcohol exposure via embryo culture in mice. *Alcohol Clin Exp Res, 35*(7), 1293-1304. doi:10.1111/j.1530-0277.2011.01465.x
Chesler, E. J., Wang, J., Lu, L., Qu, Y., Manly, K. F., & Williams, R. W. (2003). Genetic correlates of gene expression in recombinant inbred strains: a relational model system to explore neurobehavioral phenotypes. *Neuroinformatics, 1*(4), 343-357. doi:10.1385/NI:1:4:343
Chipman, H., & Tibshirani, R. (2006). Hybrid hierarchical clustering with applications to microarray data. *Biostatistics, 7*(2), 286-301. doi:10.1093/biostatistics/kxj007
Choi, I. Y., Allan, A. M., & Cunningham, L. A. (2005). Moderate fetal alcohol exposure impairs the neurogenic response to an enriched environment in adult mice. *Alcohol Clin Exp Res, 29*(11), 2053-2062. doi:10.1097/01.alc.0000187037.02670.59
Chokroborty-Hoque, A., Alberry, B., & Singh, S. M. (2014). Exploring the complexity of intellectual disability in fetal alcohol spectrum disorders. *Front Pediatr, 2*, 90. doi:10.3389/fped.2014.00090
Christoffel, K. K., & Salafsky, I. (1975). Fetal alcohol syndrome in dizygotic twins. *J Pediatr, 87*(6 Pt 1), 963-967.
Clarren, S. K. (1981). Recognition of fetal alcohol syndrome. *JAMA, 245*(23), 2436-2439.
Clarren, S. K., Alvord, E. C., Jr., Sumi, S. M., Streissguth, A. P., & Smith, D. W. (1978). Brain malformations related to prenatal exposure to ethanol. *J Pediatr, 92*(1), 64-67.
Clayton, J. A. (2016). Studying both sexes: a guiding principle for biomedicine. *FASEB J, 30*(2), 519-524. doi:10.1096/fj.15-279554
Clayton, J. A., & Collins, F. S. (2014). Policy: NIH to balance sex in cell and animal studies. *Nature, 509*(7500), 282-283. doi:10.1038/509282a
Clayton, J. A., & Sullivan, C. (2016). Studying Both Sexes: A New Frontier for Discovery. *Physiologist, 59*(2), 63-65.
Coles, C. D., Brown, R. T., Smith, I. E., Platzman, K. A., Erickson, S., & Falek, A. (1991). Effects of prenatal alcohol exposure at school age. I. Physical and cognitive development. *Neurotoxicol Teratol, 13*(4), 357-367. doi:10.1016/0892-0362(91)90084-a
Cook, M. N., Baker, J. A., Heldt, S. A., Williams, R. W., Hamre, K. M., & Lu, L. (2015). Identification of candidate genes that underlie the QTL on chromosome 1 that mediates genetic differences in stress-ethanol interactions. *Physiol Genomics, 47*(8), 308-317. doi:10.1152/physiolgenomics.00114.2014
Crabbe, J. C., & Belknap, J. K. (1992). Genetic approaches to drug dependence. *Trends Pharmacol Sci, 13*(5), 212-219.
Creeley, C. E., & Olney, J. W. (2013). Drug-Induced Apoptosis: Mechanism by which Alcohol and Many Other Drugs Can Disrupt Brain Development. *Brain Sci, 3*(3), 1153-1181. doi:10.3390/brainsci3031153
Cudd, T. A. (2005). Animal model systems for the study of alcohol teratology. *Exp Biol Med (Maywood), 230*(6), 389-393. doi:10.1177/15353702-0323006-06

Cullen, C. L., Burne, T. H., Lavidis, N. A., & Moritz, K. M. (2014). Low dose prenatal alcohol exposure does not impair spatial learning and memory in two tests in adult and aged rats. *PLoS One, 9*(6), e101482. doi:10.1371/journal.pone.0101482

Dahlborn, K., Bugnon, P., Nevalainen, T., Raspa, M., Verbost, P., & Spangenberg, E. (2013). Report of the Federation of European Laboratory Animal Science Associations Working Group on animal identification. *Lab Anim, 47*(1), 2-11. doi:10.1177/0023677212473290

Das, U. G., Cronk, C. E., Martier, S. S., Simpson, P. M., & McCarver, D. G. (2004). Alcohol dehydrogenase 2*3 affects alterations in offspring facial morphology associated with maternal ethanol intake in pregnancy. *Alcohol Clin Exp Res, 28*(10), 1598-1606. doi:10.1097/01.alc.0000141816.14776.97

Davies, D. L., & Smith, D. E. (1981). A Golgi study of mouse hippocampal CA1 pyramidal neurons following perinatal ethanol exposure. *Neurosci Lett, 26*(1), 49-54. doi:10.1016/0304-3940(81)90424-9

Debelak, K. A., & Smith, S. M. (2000). Avian genetic background modulates the neural crest apoptosis induced by ethanol exposure. *Alcohol Clin Exp Res, 24*(3), 307-314.

Dikranian, K., Ishimaru, M. J., Tenkova, T., Labruyere, J., Qin, Y. Q., Ikonomidou, C., & Olney, J. W. (2001). Apoptosis in the in vivo mammalian forebrain. *Neurobiol Dis, 8*(3), 359-379. doi:10.1006/nbdi.2001.0411

Dobbing, J. (1974). The later growth of the brain and its vulnerability. *Pediatrics, 53*(1), 2-6.

Dobbing, J., & Sands, J. (1979). Comparative aspects of the brain growth spurt. *Early Hum Dev, 3*(1), 79-83. doi:10.1016/0378-3782(79)90022-7

Dodge, N. C., Jacobson, J. L., & Jacobson, S. W. (2014). Protective effects of the alcohol dehydrogenase-ADH1B*3 allele on attention and behavior problems in adolescents exposed to alcohol during pregnancy. *Neurotoxicol Teratol, 41*, 43-50. doi:10.1016/j.ntt.2013.11.003

Downing, C., Balderrama-Durbin, C., Broncucia, H., Gilliam, D., & Johnson, T. E. (2009). Ethanol teratogenesis in five inbred strains of mice. *Alcohol Clin Exp Res, 33*(7), 1238-1245. doi:10.1111/j.1530-0277.2009.00949.x

Downing, C., Balderrama-Durbin, C., Kimball, A., Biers, J., Wright, H., Gilliam, D., & Johnson, T. E. (2012). Quantitative trait locus mapping for ethanol teratogenesis in BXD recombinant inbred mice. *Alcohol Clin Exp Res, 36*(8), 1340-1354. doi:10.1111/j.1530-0277.2012.01754.x

Downing, C., Flink, S., Florez-McClure, M. L., Johnson, T. E., Tabakoff, B., & Kechris, K. J. (2012). Gene expression changes in C57BL/6J and DBA/2J mice following prenatal alcohol exposure. *Alcohol Clin Exp Res, 36*(9), 1519-1529. doi:10.1111/j.1530-0277.2012.01757.x

Driscoll, C. D., Streissguth, A. P., & Riley, E. P. (1990). Prenatal alcohol exposure: comparability of effects in humans and animal models. *Neurotoxicol Teratol, 12*(3), 231-237.

du Plooy, C. P., Malcolm-Smith, S., Adnams, C. M., Stein, D. J., & Donald, K. A. (2016). The Effects of Prenatal Alcohol Exposure on Episodic Memory Functioning: A Systematic Review. *Arch Clin Neuropsychol, 31*(7), 710-726. doi:10.1093/arclin/acw067

Dunty, W. C., Jr., Chen, S. Y., Zucker, R. M., Dehart, D. B., & Sulik, K. K. (2001). Selective vulnerability of embryonic cell populations to ethanol-induced apoptosis: implications for alcohol-related birth defects and neurodevelopmental disorder. *Alcohol Clin Exp Res, 25*(10), 1523-1535.

Dursun, I., Jakubowska-Dogru, E., & Uzbay, T. (2006). Effects of prenatal exposure to alcohol on activity, anxiety, motor coordination, and memory in young adult Wistar rats. *Pharmacol Biochem Behav, 85*(2), 345-355. doi:10.1016/j.pbb.2006.09.001

Eberhart, J. K., & Parnell, S. E. (2016). The Genetics of Fetal Alcohol Spectrum Disorders. *Alcohol Clin Exp Res, 40*(6), 1154-1165. doi:10.1111/acer.13066

Ethen, M. K., Ramadhani, T. A., Scheuerle, A. E., Canfield, M. A., Wyszynski, D. F., Druschel, C. M., . . . National Birth Defects Prevention, S. (2009). Alcohol consumption by women before and during pregnancy. *Matern Child Health J, 13*(2), 274-285. doi:10.1007/s10995-008-0328-2

Farber, N. B., Creeley, C. E., & Olney, J. W. (2010). Alcohol-induced neuroapoptosis in the fetal macaque brain. *Neurobiol Dis, 40*(1), 200-206. doi:10.1016/j.nbd.2010.05.025

Farber, N. B., & Olney, J. W. (2003). Drugs of abuse that cause developing neurons to commit suicide. *Brain Res Dev Brain Res, 147*(1-2), 37-45. doi:10.1016/j.devbrainres.2003.09.009

Ferguson, L. B., Zhang, L., Kircher, D., Wang, S., Mayfield, R. D., Crabbe, J. C., . . . Ponomarev, I. (2019). Dissecting Brain Networks Underlying Alcohol Binge Drinking Using a Systems Genomics Approach. *Mol Neurobiol, 56*(4), 2791-2810. doi:10.1007/s12035-018-1252-0

Ferrer, I., Galofre, F., Lopez-Tejero, D., & Llobera, M. (1988). Morphological recovery of hippocampal pyramidal neurons in the adult rat exposed in utero to ethanol. *Toxicology, 48*(2), 191-197. doi:10.1016/0300-483x(88)90100-x

Fidalgo, S., Skipper, C., Takyi, A., McIver, A., Tsiligkaridis, T., Quadir, A., & Gard, P. R. (2017). Low-dose chronic prenatal alcohol exposure abolishes the pro-cognitive effects of angiotensin IV. *Behav Brain Res, 329*, 140-147. doi:10.1016/j.bbr.2017.04.042

Fish, E. W., Holloway, H. T., Rumple, A., Baker, L. K., Wieczorek, L. A., Moy, S. S., . . . Parnell, S. E. (2016). Acute alcohol exposure during neurulation: Behavioral and brain structural consequences in adolescent C57BL/6J mice. *Behav Brain Res, 311*, 70-80. doi:10.1016/j.bbr.2016.05.004

Flak, A. L., Su, S., Bertrand, J., Denny, C. H., Kesmodel, U. S., & Cogswell, M. E. (2014). The association of mild, moderate, and binge prenatal alcohol exposure and child neuropsychological outcomes: a meta-analysis. *Alcohol Clin Exp Res, 38*(1), 214-226. doi:10.1111/acer.12214

Floyd, R. L., & Sidhu, J. S. (2004). Monitoring prenatal alcohol exposure. *Am J Med Genet C Semin Med Genet, 127C*(1), 3-9. doi:10.1002/ajmg.c.30010

Fontaine, C. J., Patten, A. R., Sickmann, H. M., Helfer, J. L., & Christie, B. R. (2016). Effects of pre-natal alcohol exposure on hippocampal synaptic plasticity: Sex, age and methodological considerations. *Neurosci Biobehav Rev, 64*, 12-34. doi:10.1016/j.neubiorev.2016.02.014

Fryer, S. L., Mattson, S. N., Jernigan, T. L., Archibald, S. L., Jones, K. L., & Riley, E. P. (2012). Caudate volume predicts neurocognitive performance in youth with heavy prenatal alcohol exposure. *Alcohol Clin Exp Res, 36*(11), 1932-1941. doi:10.1111/j.1530-0277.2012.01811.x

Gil-Mohapel, J., Boehme, F., Kainer, L., & Christie, B. R. (2010). Hippocampal cell loss and neurogenesis after fetal alcohol exposure: insights from different rodent models. *Brain Res Rev, 64*(2), 283-303. doi:10.1016/j.brainresrev.2010.04.011

Gilliam, D. M., & Kotch, L. E. (1990). Alcohol-related birth defects in long- and short-sleep mice: postnatal litter mortality. *Alcohol, 7*(6), 483-487. doi:10.1016/0741-8329(90)90036-c

Gilliam, D. M., & Kotch, L. E. (1996). Dose-related growth deficits in LS but not SS mice prenatally exposed to alcohol. *Alcohol, 13*(1), 47-51. doi:10.1016/0741-8329(95)02010-1

Gilliam, D. M., Stilman, A., Dudek, B. C., & Riley, E. P. (1987). Fetal alcohol effects in long- and short-sleep mice: activity, passive avoidance, and in utero ethanol levels. *Neurotoxicol Teratol, 9*(5), 349-357. doi:10.1016/0892-0362(87)90030-4

Glass, L., Graham, D. M., Deweese, B. N., Jones, K. L., Riley, E. P., & Mattson, S. N. (2014). Correspondence of parent report and laboratory measures of inattention and hyperactivity in children with heavy prenatal alcohol exposure. *Neurotoxicol Teratol, 42*, 43-50. doi:10.1016/j.ntt.2014.01.007

Gohlke, J. M., Griffith, W. C., & Faustman, E. M. (2007). Computational models of neocortical neuronogenesis and programmed cell death in the developing mouse, monkey, and human. *Cereb Cortex, 17*(10), 2433-2442. doi:10.1093/cercor/bhl151

Goldowitz, D., Lussier, A. A., Boyle, J. K., Wong, K., Lattimer, S. L., Dubose, C., . . . Hamre, K. M. (2014). Molecular pathways underpinning ethanol-induced neurodegeneration. *Front Genet, 5*, 203. doi:10.3389/fgene.2014.00203

Gonzalez-Burgos, I., Alejandre-Gomez, M., Olvera-Cortes, M. E., Perez-Vega, M. I., Evans, S., & Feria-Velasco, A. (2006). Prenatal-through-postnatal exposure to moderate levels of ethanol leads to damage on the hippocampal CA1 field of juvenile rats: a stereology and Golgi study. *Neurosci Res, 56*(4), 400-408. doi:10.1016/j.neures.2006.08.007

Goodlett, C. R., Gilliam, D. M., Nichols, J. M., & West, J. R. (1989). Genetic influences on brain growth restriction induced by development exposure to alcohol. *Neurotoxicology, 10*(3), 321-334.

Goodlett, C. R., Marcussen, B. L., & West, J. R. (1990). A single day of alcohol exposure during the brain growth spurt induces brain weight restriction and cerebellar Purkinje cell loss. *Alcohol, 7*(2), 107-114. doi:10.1016/0741-8329(90)90070-s

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. *Neurobiol Learn Mem, 64*(3), 265-275. doi:10.1006/nlme.1995.0009

Gora-Maslak, G., McClearn, G. E., Crabbe, J. C., Phillips, T. J., Belknap, J. K., & Plomin, R. (1991). Use of recombinant inbred strains to identify quantitative trait loci in psychopharmacology. *Psychopharmacology (Berl), 104*(4), 413-424.

Green, D. R., & Amarante-Mendes, G. P. (1998). The point of no return: mitochondria, caspases, and the commitment to cell death. *Results Probl Cell Differ, 24*, 45-61. doi:10.1007/978-3-540-69185-3_3

Green, D. R., & Reed, J. C. (1998). Mitochondria and apoptosis. *Science, 281*(5381), 1309-1312. doi:10.1126/science.281.5381.1309

Green, M. L., Singh, A. V., Zhang, Y., Nemeth, K. A., Sulik, K. K., & Knudsen, T. B. (2007). Reprogramming of genetic networks during initiation of the Fetal Alcohol Syndrome. *Dev Dyn, 236*(2), 613-631. doi:10.1002/dvdy.21048

Guerri, C. (1998). Neuroanatomical and neurophysiological mechanisms involved in central nervous system dysfunctions induced by prenatal alcohol exposure. *Alcohol Clin Exp Res, 22*(2), 304-312. doi:10.1111/j.1530-0277.1998.tb03653.x

Guerri, C., Bazinet, A., & Riley, E. P. (2009). Foetal Alcohol Spectrum Disorders and alterations in brain and behaviour. *Alcohol Alcohol, 44*(2), 108-114. doi:10.1093/alcalc/agn105

Hamilton, D. A., Kodituwakku, P., Sutherland, R. J., & Savage, D. D. (2003). Children with Fetal Alcohol Syndrome are impaired at place learning but not cued-navigation in a virtual Morris water task. *Behav Brain Res, 143*(1), 85-94. doi:10.1016/s0166-4328(03)00028-7

Hamilton, D. A., Magcalas, C. M., Barto, D., Bird, C. W., Rodriguez, C. I., Fink, B. C., . . . Savage, D. D. (2014). Moderate prenatal alcohol exposure and quantification of social behavior in adult rats. *J Vis Exp*(94). doi:10.3791/52407

Hamilton, G. F., Jablonski, S. A., Schiffino, F. L., St Cyr, S. A., Stanton, M. E., & Klintsova, A. Y. (2014). Exercise and environment as an intervention for neonatal alcohol effects on hippocampal adult neurogenesis and learning. *Neuroscience, 265*, 274-290. doi:10.1016/j.neuroscience.2014.01.061

Hamilton, G. F., Murawski, N. J., St Cyr, S. A., Jablonski, S. A., Schiffino, F. L., Stanton, M. E., & Klintsova, A. Y. (2011). Neonatal alcohol exposure disrupts hippocampal neurogenesis and contextual fear conditioning in adult rats. *Brain Res, 1412*, 88-101. doi:10.1016/j.brainres.2011.07.027

Hannigan, J. H. (1996). What research with animals is telling us about alcohol-related neurodevelopmental disorder. *Pharmacol Biochem Behav, 55*(4), 489-499. doi:10.1016/s0091-3057(96)00251-1

Hard, M. L., Abdolell, M., Robinson, B. H., & Koren, G. (2005). Gene-expression analysis after alcohol exposure in the developing mouse. *J Lab Clin Med, 145*(1), 47-54. doi:10.1016/j.lab.2004.11.011

Heaton, M. B., Moore, D. B., Paiva, M., Madorsky, I., Mayer, J., & Shaw, G. (2003). The role of neurotrophic factors, apoptosis-related proteins, and endogenous antioxidants in the differential temporal vulnerability of neonatal cerebellum to ethanol. *Alcohol Clin Exp Res, 27*(4), 657-669. doi:10.1097/01.ALC.0000060527.55252.71

Hellemans, K. G., Sliwowska, J. H., Verma, P., & Weinberg, J. (2010). Prenatal alcohol exposure: fetal programming and later life vulnerability to stress, depression and anxiety disorders. *Neurosci Biobehav Rev, 34*(6), 791-807. doi:10.1016/j.neubiorev.2009.06.004

Hellemans, K. G., Verma, P., Yoon, E., Yu, W. K., Young, A. H., & Weinberg, J. (2010). Prenatal alcohol exposure and chronic mild stress differentially alter depressive-

and anxiety-like behaviors in male and female offspring. *Alcohol Clin Exp Res, 34*(4), 633-645. doi:10.1111/j.1530-0277.2009.01132.x

Herman, L. E., Acosta, M. C., & Chang, P. N. (2008). Gender and attention deficits in children diagnosed with a Fetal Alcohol Spectrum Disorder. *Can J Clin Pharmacol, 15*(3), e411-419.

Hoff, S. F. (1988). Synaptogenesis in the hippocampal dentate gyrus: effects of in utero ethanol exposure. *Brain Res Bull, 21*(1), 47-54. doi:10.1016/0361-9230(88)90119-0

Holcomb, L., Gordon, M. N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., . . . Duff, K. (1998). Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat Med, 4*(1), 97-100. doi:10.1038/nm0198-097

Hoyme, H. E., Kalberg, W. O., Elliott, A. J., Blankenship, J., Buckley, D., Marais, A. S., . . . May, P. A. (2016). Updated Clinical Guidelines for Diagnosing Fetal Alcohol Spectrum Disorders. *Pediatrics, 138*(2). doi:10.1542/peds.2015-4256

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. *Alcohol, 43*(6), 465-474. doi:10.1016/j.alcohol.2009.08.004

Ieraci, A., & Herrera, D. G. (2007). Single alcohol exposure in early life damages hippocampal stem/progenitor cells and reduces adult neurogenesis. *Neurobiol Dis, 26*(3), 597-605. doi:10.1016/j.nbd.2007.02.011

Ikonomidou, C. (2009). Triggers of apoptosis in the immature brain. *Brain Dev, 31*(7), 488-492. doi:10.1016/j.braindev.2009.02.006

Ikonomidou, C., Bittigau, P., Ishimaru, M. J., Wozniak, D. F., Koch, C., Genz, K., . . . Olney, J. W. (2000). Ethanol-induced apoptotic neurodegeneration and fetal alcohol syndrome. *Science, 287*(5455), 1056-1060.

Jacobson, S. W., Carr, L. G., Croxford, J., Sokol, R. J., Li, T. K., & Jacobson, J. L. (2006). Protective effects of the alcohol dehydrogenase-ADH1B allele in children exposed to alcohol during pregnancy. *J Pediatr, 148*(1), 30-37. doi:10.1016/j.jpeds.2005.08.023

Jacobson, S. W., Carter, R. C., Molteno, C. D., Stanton, M. E., Herbert, J. S., Lindinger, N. M., . . . Jacobson, J. L. (2018). 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. *Alcohol Clin Exp Res, 42*(7), 1327-1341. doi:10.1111/acer.13769

Johnston, M. V., Ishida, A., Ishida, W. N., Matsushita, H. B., Nishimura, A., & Tsuji, M. (2009). Plasticity and injury in the developing brain. *Brain Dev, 31*(1), 1-10. doi:10.1016/j.braindev.2008.03.014

Jones, K. L., & Smith, D. W. (1973). Recognition of the fetal alcohol syndrome in early infancy. *Lancet, 302*(7836), 999-1001. doi:10.1016/s0140-6736(73)91092-1

Jones, K. L., Smith, D. W., Ulleland, C. N., & Streissguth, P. (1973). Pattern of malformation in offspring of chronic alcoholic mothers. *Lancet, 1*(7815), 1267-1271.

Jurgensmeier, J. M., Xie, Z., Deveraux, Q., Ellerby, L., Bredesen, D., & Reed, J. C. (1998). Bax directly induces release of cytochrome c from isolated mitochondria. *Proc Natl Acad Sci U S A, 95*(9), 4997-5002. doi:10.1073/pnas.95.9.4997

Kable, J. A., O'Connor, M. J., Olson, H. C., Paley, B., Mattson, S. N., Anderson, S. M., & Riley, E. P. (2016). Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE): Proposed DSM-5 Diagnosis. *Child Psychiatry Hum Dev, 47*(2), 335-346. doi:10.1007/s10578-015-0566-7

Ke, C. Y., Mei, H. H., Wong, F. H., & Lo, L. J. (2019). IRF6 and TAK1 coordinately promote the activation of HIPK2 to stimulate apoptosis during palate fusion. *Sci Signal, 12*(593). doi:10.1126/scisignal.aav7666

Kelly, S. J., Goodlett, C. R., Hulsether, S. A., & West, J. R. (1988). Impaired spatial navigation in adult female but not adult male rats exposed to alcohol during the brain growth spurt. *Behav Brain Res, 27*(3), 247-257. doi:10.1016/0166-4328(88)90121-0

Kelly, S. J., Leggett, D. C., & Cronise, K. (2009). Sexually dimorphic effects of alcohol exposure during development on the processing of social cues. *Alcohol Alcohol, 44*(6), 555-560. doi:10.1093/alcalc/agp061

Kempermann, G., Jessberger, S., Steiner, B., & Kronenberg, G. (2004). Milestones of neuronal development in the adult hippocampus. *Trends Neurosci, 27*(8), 447-452. doi:10.1016/j.tins.2004.05.013

Kerr, J. F., Wyllie, A. H., & Currie, A. R. (1972). Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer, 26*(4), 239-257. doi:10.1038/bjc.1972.33

Kim, P., Park, J. H., Choi, C. S., Choi, I., Joo, S. H., Kim, M. K., . . . Shin, C. Y. (2013). Effects of ethanol exposure during early pregnancy in hyperactive, inattentive and impulsive behaviors and MeCP2 expression in rodent offspring. *Neurochem Res, 38*(3), 620-631. doi:10.1007/s11064-012-0960-5

King, J. H., Kwan, S. T. C., Yan, J., Jiang, X., Fomin, V. G., Levine, S. P., . . . Caudill, M. A. (2019). Maternal Choline Supplementation Modulates Placental Markers of Inflammation, Angiogenesis, and Apoptosis in a Mouse Model of Placental Insufficiency. *Nutrients, 11*(2). doi:10.3390/nu11020374

Kleiber, M. L., Mantha, K., Stringer, R. L., & Singh, S. M. (2013). Neurodevelopmental alcohol exposure elicits long-term changes to gene expression that alter distinct molecular pathways dependent on timing of exposure. *J Neurodev Disord, 5*(1), 6. doi:10.1186/1866-1955-5-6

Klein, S. L., Schiebinger, L., Stefanick, M. L., Cahill, L., Danska, J., de Vries, G. J., . . . Zucker, I. (2015). Opinion: Sex inclusion in basic research drives discovery. *Proc Natl Acad Sci U S A, 112*(17), 5257-5258. doi:10.1073/pnas.1502843112

Klintsova, A. Y., Hamilton, G. F., & Boschen, K. E. (2012). Long-term consequences of developmental alcohol exposure on brain structure and function: therapeutic benefits of physical activity. *Brain Sci, 3*(1), 1-38. doi:10.3390/brainsci3010001

Klintsova, A. Y., Helfer, J. L., Calizo, L. H., Dong, W. K., Goodlett, C. R., & Greenough, W. T. (2007). Persistent impairment of hippocampal neurogenesis in young adult rats following early postnatal alcohol exposure. *Alcohol Clin Exp Res, 31*(12), 2073-2082. doi:10.1111/j.1530-0277.2007.00528.x

Kluck, R. M., Bossy-Wetzel, E., Green, D. R., & Newmeyer, D. D. (1997). The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science, 275*(5303), 1132-1136. doi:10.1126/science.275.5303.1132

Kotch, L. E., & Sulik, K. K. (1992). Patterns of ethanol-induced cell death in the developing nervous system of mice; neural fold states through the time of anterior neural tube closure. *Int J Dev Neurosci, 10*(4), 273-279.

Kuge, T., Asayama, T., Kakuta, S., Murakami, K., Ishikawa, Y., Kuroda, M., . . . Kishi, K. (1993). Effect of ethanol on the development and maturation of synapses in the rat hippocampus: a quantitative electron-microscopic study. *Environ Res, 62*(1), 99-105. doi:10.1006/enrs.1993.1094

Kury, P., Schroeter, M., & Jander, S. (2004). Transcriptional response to circumscribed cortical brain ischemia: spatiotemporal patterns in ischemic vs. remote non-ischemic cortex. *Eur J Neurosci, 19*(7), 1708-1720. doi:10.1111/j.1460-9568.2004.03226.x

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. *Front Psychol, 4*, 863. doi:10.3389/fpsyg.2013.00863

Lebel, C., Roussotte, F., & Sowell, E. R. (2011). Imaging the impact of prenatal alcohol exposure on the structure of the developing human brain. *Neuropsychol Rev, 21*(2), 102-118. doi:10.1007/s11065-011-9163-0

Leuner, B., & Gould, E. (2010). Structural plasticity and hippocampal function. *Annu Rev Psychol, 61*, 111-140, C111-113. doi:10.1146/annurev.psych.093008.100359

Liao, X., Chen, J., Liu, Y., He, A., Wu, J., Cheng, J., . . . Mei, H. (2018). Knockdown of long noncoding RNA FGFR3- AS1 induces cell proliferation inhibition, apoptosis and motility reduction in bladder cancer. *Cancer Biomark, 21*(2), 277-285. doi:10.3233/CBM-170354

Liao, Y., Wang, J., Jaehnig, E. J., Shi, Z., & Zhang, B. (2019). WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. *Nucleic Acids Res, 47*(W1), W199-W205. doi:10.1093/nar/gkz401

Lindsley, T. A., Comstock, L. L., & Rising, L. J. (2002). Morphologic and neurotoxic effects of ethanol vary with timing of exposure in vitro. *Alcohol, 28*(3), 197-203. doi:10.1016/s0741-8329(02)00279-3

Liu, Q., Yu, W., Zhu, S., Cheng, K., Xu, H., Lv, Y., . . . Wang, K. (2018). Long noncoding RNA GAS5 regulates the proliferation, migration, and invasion of glioma cells by negatively regulating miR-18a-5p. *J Cell Physiol, 234*(1), 757-768. doi:10.1002/jcp.26889

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. *Neurotoxicol Teratol, 25*(4), 447-458. doi:10.1016/s0892-0362(03)00030-8

Lossie, A. C., Muir, W. M., Lo, C. L., Timm, F., Liu, Y., Gray, W., & Zhou, F. C. (2014). Implications of genomic signatures in the differential vulnerability to fetal alcohol exposure in C57BL/6 and DBA/2 mice. *Front Genet, 5*, 173. doi:10.3389/fgene.2014.00173

Lunde-Young, R., Ramirez, J., Naik, V., Orzabal, M., Lee, J., Konganti, K., . . . Ramadoss, J. (2019). Hippocampal transcriptome reveals novel targets of FASD pathogenesis. *Brain Behav, 9*(7), e01334. doi:10.1002/brb3.1334

Luo, S., & Rubinsztein, D. C. (2013). BCL2L11/BIM: a novel molecular link between autophagy and apoptosis. *Autophagy, 9*(1), 104-105. doi:10.4161/auto.22399

Lussier, A. A., Stepien, K. A., Neumann, S. M., Pavlidis, P., Kobor, M. S., & Weinberg, J. (2015). Prenatal alcohol exposure alters steady-state and activated gene expression in the adult rat brain. *Alcohol Clin Exp Res, 39*(2), 251-261. doi:10.1111/acer.12622

Maier, S. E., Chen, W. J., Miller, J. A., & West, J. R. (1997). Fetal alcohol exposure and temporal vulnerability regional differences in alcohol-induced microencephaly as a function of the timing of binge-like alcohol exposure during rat brain development. *Alcohol Clin Exp Res, 21*(8), 1418-1428. doi:10.1111/j.1530-0277.1997.tb04471.x

Maier, S. E., & West, J. R. (2001). Drinking patterns and alcohol-related birth defects. *Alcohol Res Health, 25*(3), 168-174.

Mandal, C., Park, K. S., Jung, K. H., & Chai, Y. G. (2015). Ethanol-related alterations in gene expression patterns in the developing murine hippocampus. *Acta Biochim Biophys Sin (Shanghai), 47*(8), 581-587. doi:10.1093/abbs/gmv050

Mantione, K. J., Kream, R. M., Kuzelova, H., Ptacek, R., Raboch, J., Samuel, J. M., & Stefano, G. B. (2014). Comparing bioinformatic gene expression profiling methods: microarray and RNA-Seq. *Med Sci Monit Basic Res, 20*, 138-142. doi:10.12659/MSMBR.892101

Marjonen, H., Sierra, A., Nyman, A., Rogojin, V., Grohn, O., Linden, A. M., . . . Kaminen-Ahola, N. (2015). Early maternal alcohol consumption alters hippocampal DNA methylation, gene expression and volume in a mouse model. *PLoS One, 10*(5), e0124931. doi:10.1371/journal.pone.0124931

Marquardt, K., & Brigman, J. L. (2016). The impact of prenatal alcohol exposure on social, cognitive and affective behavioral domains: Insights from rodent models. *Alcohol, 51*, 1-15. doi:10.1016/j.alcohol.2015.12.002

Mattson, S. N., Bernes, G. A., & Doyle, L. R. (2019). Fetal Alcohol Spectrum Disorders: A Review of the Neurobehavioral Deficits Associated With Prenatal Alcohol Exposure. *Alcohol Clin Exp Res, 43*(6), 1046-1062. doi:10.1111/acer.14040

Mattson, S. N., Crocker, N., & Nguyen, T. T. (2011). Fetal alcohol spectrum disorders: neuropsychological and behavioral features. *Neuropsychol Rev, 21*(2), 81-101. doi:10.1007/s11065-011-9167-9

Mattson, S. N., & Riley, E. P. (2000). Parent ratings of behavior in children with heavy prenatal alcohol exposure and IQ-matched controls. *Alcohol Clin Exp Res, 24*(2), 226-231.

May, P. A., Baete, A., Russo, J., Elliott, A. J., Blankenship, J., Kalberg, W. O., . . . Hoyme, H. E. (2014). Prevalence and characteristics of fetal alcohol spectrum disorders. *Pediatrics, 134*(5), 855-866. doi:10.1542/peds.2013-3319

May, P. A., Chambers, C. D., Kalberg, W. O., Zellner, J., Feldman, H., Buckley, D., . . . Hoyme, H. E. (2018). Prevalence of Fetal Alcohol Spectrum Disorders in 4 US Communities. *JAMA, 319*(5), 474-482. doi:10.1001/jama.2017.21896

May, P. A., Gossage, J. P., Brooke, L. E., Snell, C. L., Marais, A. S., Hendricks, L. S., . . . Viljoen, D. L. (2005). Maternal risk factors for fetal alcohol syndrome in the Western cape province of South Africa: a population-based study. *Am J Public Health, 95*(7), 1190-1199. doi:10.2105/AJPH.2003.037093

May, P. A., Gossage, J. P., Kalberg, W. O., Robinson, L. K., Buckley, D., Manning, M., & Hoyme, H. E. (2009). Prevalence and epidemiologic characteristics of FASD from various research methods with an emphasis on recent in-school studies. *Dev Disabil Res Rev, 15*(3), 176-192. doi:10.1002/ddrr.68

May, P. A., Tabachnick, B., Hasken, J. M., Marais, A. S., de Vries, M. M., Barnard, R., . . . Hoyme, H. E. (2017). Who is most affected by prenatal alcohol exposure: Boys or girls? *Drug Alcohol Depend, 177*, 258-267. doi:10.1016/j.drugalcdep.2017.04.010

McCarthy, M. M., & Arnold, A. P. (2011). Reframing sexual differentiation of the brain. *Nat Neurosci, 14*(6), 677-683. doi:10.1038/nn.2834

McCarver, D. G., Thomasson, H. R., Martier, S. S., Sokol, R. J., & Li, T. (1997). Alcohol dehydrogenase-2*3 allele protects against alcohol-related birth defects among African Americans. *J Pharmacol Exp Ther, 283*(3), 1095-1101.

Miller, M. W. (1995). Generation of neurons in the rat dentate gyrus and hippocampus: effects of prenatal and postnatal treatment with ethanol. *Alcohol Clin Exp Res, 19*(6), 1500-1509. doi:10.1111/j.1530-0277.1995.tb01014.x

Mooney, S. M., & Miller, M. W. (2001). Effects of prenatal exposure to ethanol on the expression of bcl-2, bax and caspase 3 in the developing rat cerebral cortex and thalamus. *Brain Res, 911*(1), 71-81. doi:10.1016/s0006-8993(01)02718-4

Moore, D. B., Madorsky, I., Paiva, M., & Barrow Heaton, M. (2004). Ethanol exposure alters neurotrophin receptor expression in the rat central nervous system: Effects of neonatal exposure. *J Neurobiol, 60*(1), 114-126. doi:10.1002/neu.20010

Moore, E. M., Glass, L., Infante, M. A., Coles, C. D., Kable, J. A., Jones, K. L., . . . Cifasd. (2021). Cross-Sectional Analysis of Spatial Working Memory Development in Children with Histories of Heavy Prenatal Alcohol Exposure. *Alcohol Clin Exp Res, 45*(1), 215-223. doi:10.1111/acer.14506

Moore, E. M., Migliorini, R., Infante, M. A., & Riley, E. P. (2014). Fetal Alcohol Spectrum Disorders: Recent Neuroimaging Findings. *Curr Dev Disord Rep, 1*(3), 161-172. doi:10.1007/s40474-014-0020-8

Morse, H. C., 3rd, Chused, T. M., Hartley, J. W., Mathieson, B. J., Sharrow, S. O., & Taylor, B. A. (1979). Expression of xenotropic murine leukemia viruses as cell-surface gp70 in genetic crosses between strains DBA/2 and C57BL/6. *J Exp Med, 149*(5), 1183-1196.

Mulligan, M. K., Mozhui, K., Pandey, A. K., Smith, M. L., Gong, S., Ingels, J., . . . Williams, R. W. (2017). Genetic divergence in the transcriptional engram of chronic alcohol abuse: A laser-capture RNA-seq study of the mouse mesocorticolimbic system. *Alcohol, 58*, 61-72. doi:10.1016/j.alcohol.2016.09.001

Nixon, K., & Crews, F. T. (2002). Binge ethanol exposure decreases neurogenesis in adult rat hippocampus. *J Neurochem, 83*(5), 1087-1093. doi:10.1046/j.1471-4159.2002.01214.x

Ogawa, T., Kuwagata, M., Ruiz, J., & Zhou, F. C. (2005). Differential teratogenic effect of alcohol on embryonic development between C57BL/6 and DBA/2 mice: a new

view. *Alcohol Clin Exp Res, 29*(5), 855-863. doi:10.1097/01.alc.0000163495.71181.10

Okada, T., Enkhjargal, B., Travis, Z. D., Ocak, U., Tang, J., Suzuki, H., & Zhang, J. H. (2019). FGF-2 Attenuates Neuronal Apoptosis via FGFR3/PI3k/Akt Signaling Pathway After Subarachnoid Hemorrhage. *Mol Neurobiol, 56*(12), 8203-8219. doi:10.1007/s12035-019-01668-9

Olney, J. W. (2004). Fetal alcohol syndrome at the cellular level. *Addict Biol, 9*(2), 137-149; discussion 151. doi:10.1080/13556210410001717006

Olney, J. W., Tenkova, T., Dikranian, K., Qin, Y. Q., Labruyere, J., & Ikonomidou, C. (2002). Ethanol-induced apoptotic neurodegeneration in the developing C57BL/6 mouse brain. *Brain Res Dev Brain Res, 133*(2), 115-126. doi:10.1016/s0165-3806(02)00279-1

Osterndorff-Kahanek, E. A., Becker, H. C., Lopez, M. F., Farris, S. P., Tiwari, G. R., Nunez, Y. O., . . . Mayfield, R. D. (2015). Chronic ethanol exposure produces time- and brain region-dependent changes in gene coexpression networks. *PLoS One, 10*(3), e0121522. doi:10.1371/journal.pone.0121522

Paluch, L. R., Lieggi, C. C., Dumont, M., Monette, S., Riedel, E. R., & Lipman, N. S. (2014). Developmental and behavioral effects of toe clipping on neonatal and preweanling mice with and without vapocoolant anesthesia. *J Am Assoc Lab Anim Sci, 53*(2), 132-140.

Parnell, S. E., Holloway, H. T., O'Leary-Moore, S. K., Dehart, D. B., Paniaqua, B., Oguz, I., . . . Sulik, K. K. (2013). Magnetic resonance microscopy-based analyses of the neuroanatomical effects of gestational day 9 ethanol exposure in mice. *Neurotoxicol Teratol, 39*, 77-83. doi:10.1016/j.ntt.2013.07.009

Parnell, S. E., O'Leary-Moore, S. K., Godin, E. A., Dehart, D. B., Johnson, B. W., Allan Johnson, G., . . . Sulik, K. K. (2009). Magnetic resonance microscopy defines ethanol-induced brain abnormalities in prenatal mice: effects of acute insult on gestational day 8. *Alcohol Clin Exp Res, 33*(6), 1001-1011. doi:10.1111/j.1530-0277.2009.00921.x

Patten, A. R., Fontaine, C. J., & Christie, B. R. (2014). A comparison of the different animal models of fetal alcohol spectrum disorders and their use in studying complex behaviors. *Front Pediatr, 2*, 93. doi:10.3389/fped.2014.00093

Peadon, E., Rhys-Jones, B., Bower, C., & Elliott, E. J. (2009). Systematic review of interventions for children with Fetal Alcohol Spectrum Disorders. *BMC Pediatr, 9*, 35. doi:10.1186/1471-2431-9-35

Peiffer, J., Majewski, F., Fischbach, H., Bierich, J. R., & Volk, B. (1979). Alcohol embryo- and fetopathy. Neuropathology of 3 children and 3 fetuses. *J Neurol Sci, 41*(2), 125-137. doi:10.1016/0022-510x(79)90033-9

Peirce, J. L., Lu, L., Gu, J., Silver, L. M., & Williams, R. W. (2004). A new set of BXD recombinant inbred lines from advanced intercross populations in mice. *BMC Genet, 5*, 7. doi:10.1186/1471-2156-5-7

Pirou, C., Montazer-Torbati, F., Jah, N., Delmas, E., Lasbleiz, C., Mignotte, B., & Renaud, F. (2017). FGF1 protects neuroblastoma SH-SY5Y cells from p53-dependent apoptosis through an intracrine pathway regulated by FGF1 phosphorylation. *Cell Death Dis, 8*(8), e3023. doi:10.1038/cddis.2017.404

Popova, S., Lange, S., Probst, C., Gmel, G., & Rehm, J. (2018). Global prevalence of alcohol use and binge drinking during pregnancy, and fetal alcohol spectrum disorder. *Biochem Cell Biol, 96*(2), 237-240. doi:10.1139/bcb-2017-0077

Raiha, N. C., Koskinen, M., & Pikkarainen, P. (1967). Developmental changes in alcohol-dehydrogenase activity in rat and guinea-pig liver. *Biochem J, 103*(3), 623-626. doi:10.1042/bj1030623

Raikundalia, S., Sa'Dom, S., Few, L. L., & Too, W. C. S. (2021). MicroRNA-367-3p induces apoptosis and suppresses migration of MCF-7 cells by downregulating the expression of human choline kinase alpha. *Oncol Lett, 21*(3), 183. doi:10.3892/ol.2021.12444

Rajaprakash, M., Chakravarty, M. M., Lerch, J. P., & Rovet, J. (2014). Cortical morphology in children with alcohol-related neurodevelopmental disorder. *Brain Behav, 4*(1), 41-50. doi:10.1002/brb3.191

Ratnu, V. S., Emami, M. R., & Bredy, T. W. (2017). Genetic and epigenetic factors underlying sex differences in the regulation of gene expression in the brain. *J Neurosci Res, 95*(1-2), 301-310. doi:10.1002/jnr.23886

Redila, V. A., Olson, A. K., Swann, S. E., Mohades, G., Webber, A. J., Weinberg, J., & Christie, B. R. (2006). Hippocampal cell proliferation is reduced following prenatal ethanol exposure but can be rescued with voluntary exercise. *Hippocampus, 16*(3), 305-311. doi:10.1002/hipo.20164

Riikonen, R. S. (1994). Difference in susceptibility to teratogenic effects of alcohol in discordant twins exposed to alcohol during the second half of gestation. *Pediatr Neurol, 11*(4), 332-336. doi:10.1016/0887-8994(94)90012-4

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. *Alcohol Clin Exp Res, 17*(6), 1240-1246. doi:10.1111/j.1530-0277.1993.tb05236.x

Riley, E. P., & McGee, C. L. (2005). Fetal alcohol spectrum disorders: an overview with emphasis on changes in brain and behavior. *Exp Biol Med (Maywood), 230*(6), 357-365. doi:10.1177/15353702-0323006-03

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res, 43*(7), e47. doi:10.1093/nar/gkv007

Rizzo, A., Satta, A., Garrone, G., Cavalleri, A., Napoli, A., Raspagliesi, F., . . . Bagnoli, M. (2021). Choline kinase alpha impairment overcomes TRAIL resistance in ovarian cancer cells. *J Exp Clin Cancer Res, 40*(1), 5. doi:10.1186/s13046-020-01794-6

Rodriguez-Enfedaque, A., Bouleau, S., Laurent, M., Courtois, Y., Mignotte, B., Vayssiere, J. L., & Renaud, F. (2009). FGF1 nuclear translocation is required for both its neurotrophic activity and its p53-dependent apoptosis protection. *Biochim Biophys Acta, 1793*(11), 1719-1727. doi:10.1016/j.bbamcr.2009.09.010

Roediger, D. J., Krueger, A. M., de Water, E., Mueller, B. A., Boys, C. A., Hendrickson, T. J., . . . Wozniak, J. R. (2021). Hippocampal subfield abnormalities and memory functioning in children with fetal alcohol Spectrum disorders. *Neurotoxicol Teratol, 83,* 106944. doi:10.1016/j.ntt.2020.106944

Roozen, S., Peters, G. J., Kok, G., Townend, D., Nijhuis, J., & Curfs, L. (2016). Worldwide Prevalence of Fetal Alcohol Spectrum Disorders: A Systematic Literature Review Including Meta-Analysis. *Alcohol Clin Exp Res, 40*(1), 18-32. doi:10.1111/acer.12939

Ruggiero, M. J., Boschen, K. E., Roth, T. L., & Klintsova, A. Y. (2018). Sex Differences in Early Postnatal Microglial Colonization of the Developing Rat Hippocampus Following a Single-Day Alcohol Exposure. *J Neuroimmune Pharmacol, 13*(2), 189-203. doi:10.1007/s11481-017-9774-1

Ryan, S. H., Williams, J. K., & Thomas, J. D. (2008). Choline supplementation attenuates learning deficits associated with neonatal alcohol exposure in the rat: effects of varying the timing of choline administration. *Brain Res, 1237*, 91-100. doi:10.1016/j.brainres.2008.08.048

Sarnyai, Z., Sibille, E. L., Pavlides, C., Fenster, R. J., McEwen, B. S., & Toth, M. (2000). Impaired hippocampal-dependent learning and functional abnormalities in the hippocampus in mice lacking serotonin(1A) receptors. *Proc Natl Acad Sci U S A, 97*(26), 14731-14736. doi:10.1073/pnas.97.26.14731

Sayal, K., Heron, J., Golding, J., & Emond, A. (2007). Prenatal alcohol exposure and gender differences in childhood mental health problems: a longitudinal population-based study. *Pediatrics, 119*(2), e426-434. doi:10.1542/peds.2006-1840

Schaefer, D. C., Asner, I. N., Seifert, B., Burki, K., & Cinelli, P. (2010). Analysis of physiological and behavioural parameters in mice after toe clipping as newborns. *Lab Anim, 44*(1), 7-13. doi:10.1258/la.2009.009020

Schaffner, S. L., Lussier, A. A., Baker, J. A., Goldowitz, D., Hamre, K. M., & Kobor, M. S. (2020). Neonatal Alcohol Exposure in Mice Induces Select Differentiation- and Apoptosis-Related Chromatin Changes Both Independent of and Dependent on Sex. *Front Genet, 11*, 35. doi:10.3389/fgene.2020.00035

Schultz, C., & Engelhardt, M. (2014). Anatomy of the hippocampal formation. *Front Neurol Neurosci, 34*, 6-17. doi:10.1159/000360925

Shivakumar, M., Subbanna, S., Joshi, V., & Basavarajappa, B. S. (2020). Postnatal Ethanol Exposure Activates HDAC-Mediated Histone Deacetylation, Impairs Synaptic Plasticity Gene Expression and Behavior in Mice. *Int J Neuropsychopharmacol, 23*(5), 324-338. doi:10.1093/ijnp/pyaa017

Sickmann, H. M., Patten, A. R., Morch, K., Sawchuk, S., Zhang, C., Parton, R., . . . Christie, B. R. (2014). Prenatal ethanol exposure has sex-specific effects on hippocampal long-term potentiation. *Hippocampus, 24*(1), 54-64. doi:10.1002/hipo.22203

Siler-Marsiglio, K. I., Paiva, M., Madorsky, I., Pan, Q., Shaw, G., & Heaton, M. B. (2005). Functional mechanisms of apoptosis-related proteins in neonatal rat cerebellum are differentially influenced by ethanol at postnatal days 4 and 7. *J Neurosci Res, 81*(5), 632-643. doi:10.1002/jnr.20591

Sliwowska, J. H., Barker, J. M., Barha, C. K., Lan, N., Weinberg, J., & Galea, L. A. (2010). Stress-induced suppression of hippocampal neurogenesis in adult male rats is altered by prenatal ethanol exposure. *Stress, 13*(4), 301-313. doi:10.3109/10253890903531582

Smith, C. C., Guevremont, D., Williams, J. M., & Napper, R. M. (2015). Apoptotic cell death and temporal expression of apoptotic proteins Bcl-2 and Bax in the hippocampus, following binge ethanol in the neonatal rat model. *Alcohol Clin Exp Res, 39*(1), 36-44. doi:10.1111/acer.12606

Sowell, E. R., Thompson, P. M., Mattson, S. N., Tessner, K. D., Jernigan, T. L., Riley, E. P., & Toga, A. W. (2002). Regional brain shape abnormalities persist into adolescence after heavy prenatal alcohol exposure. *Cereb Cortex, 12*(8), 856-865. doi:10.1093/cercor/12.8.856

Staples, M. C., Rosenberg, M. J., Allen, N. A., Porch, M. W., & Savage, D. D. (2013). Impact of combined prenatal ethanol and prenatal stress exposure on anxiety and hippocampal-sensitive learning in adult offspring. *Alcohol Clin Exp Res, 37*(12), 2039-2047. doi:10.1111/acer.12190

Streissguth, A. P., Bookstein, F. L., Barr, H. M., Sampson, P. D., O'Malley, K., & Young, J. K. (2004). Risk factors for adverse life outcomes in fetal alcohol syndrome and fetal alcohol effects. *J Dev Behav Pediatr, 25*(4), 228-238. doi:10.1097/00004703-200408000-00002

Streissguth, A. P., & Dehaene, P. (1993). Fetal alcohol syndrome in twins of alcoholic mothers: concordance of diagnosis and IQ. *Am J Med Genet, 47*(6), 857-861. doi:10.1002/ajmg.1320470612

Streissguth, A. P., Sampson, P. D., Olson, H. C., Bookstein, F. L., Barr, H. M., Scott, M., . . . Mirsky, A. F. (1994). Maternal drinking during pregnancy: attention and short-term memory in 14-year-old offspring--a longitudinal prospective study. *Alcohol Clin Exp Res, 18*(1), 202-218. doi:10.1111/j.1530-0277.1994.tb00904.x

Su, B., Debelak, K. A., Tessmer, L. L., Cartwright, M. M., & Smith, S. M. (2001). Genetic influences on craniofacial outcome in an avian model of prenatal alcohol exposure. *Alcohol Clin Exp Res, 25*(1), 60-69.

Subbanna, S., Nagre, N. N., Shivakumar, M., Umapathy, N. S., Psychoyos, D., & Basavarajappa, B. S. (2014). Ethanol induced acetylation of histone at G9a exon1 and G9a-mediated histone H3 dimethylation leads to neurodegeneration in neonatal mice. *Neuroscience, 258*, 422-432. doi:10.1016/j.neuroscience.2013.11.043

Subbanna, S., Shivakumar, M., Psychoyos, D., Xie, S., & Basavarajappa, B. S. (2013). Anandamide-CB1 receptor signaling contributes to postnatal ethanol-induced neonatal neurodegeneration, adult synaptic, and memory deficits. *J Neurosci, 33*(15), 6350-6366. doi:10.1523/JNEUROSCI.3786-12.2013

Sulik, K. K. (2005). Genesis of alcohol-induced craniofacial dysmorphism. *Exp Biol Med (Maywood), 230*(6), 366-375. doi:10.1177/15353702-0323006-04

Sulik, K. K., Cook, C. S., & Webster, W. S. (1988). Teratogens and craniofacial malformations: relationships to cell death. *Development, 103 Suppl*, 213-231.

Taitt, K. T., & Kendall, L. V. (2019). Physiologic Stress of Ear Punch Identification Compared with Restraint Only in Mice. *J Am Assoc Lab Anim Sci, 58*(4), 438-442. doi:10.30802/AALAS-JAALAS-18-000120

Takeuchi, H., Inagaki, S., Morozumi, W., Nakano, Y., Inoue, Y., Kuse, Y., . . . Shimazawa, M. (2018). VGF nerve growth factor inducible is involved in retinal ganglion cells death induced by optic nerve crush. *Sci Rep, 8*(1), 16443. doi:10.1038/s41598-018-34585-3

Taylor, B. A., Wnek, C., Kotlus, B. S., Roemer, N., MacTaggart, T., & Phillips, S. J. (1999). Genotyping new BXD recombinant inbred mouse strains and comparison of BXD and consensus maps. *Mamm Genome, 10*(4), 335-348. doi:10.1007/s003359900998

Tenkova, T., Young, C., Dikranian, K., Labruyere, J., & Olney, J. W. (2003). Ethanol-induced apoptosis in the developing visual system during synaptogenesis. *Invest Ophthalmol Vis Sci, 44*(7), 2809-2817. doi:10.1167/iovs.02-0982

Terenina, E. E., Cavigelli, S., Mormede, P., Zhao, W., Parks, C., Lu, L., . . . Mulligan, M. K. (2019). Genetic Factors Mediate the Impact of Chronic Stress and Subsequent Response to Novel Acute Stress. *Front Neurosci, 13*, 438. doi:10.3389/fnins.2019.00438

Theberge, E. T., Baker, J. A., Dubose, C., Boyle, J. K., Balce, K., Goldowitz, D., & Hamre, K. M. (2019). Genetic Influences on the Amount of Cell Death in the Neural Tube of BXD Mice Exposed to Acute Ethanol at Midgestation. *Alcohol Clin Exp Res, 43*(3), 439-452. doi:10.1111/acer.13947

Thiel, R., Chahoud, I., Jurgens, M., & Neubert, D. (1993). Time-dependent differences in the development of somites of four different mouse strains. *Teratog Carcinog Mutagen, 13*(6), 247-257. doi:10.1002/tcm.1770130602

Thomas, J. D., Burchette, T. L., Dominguez, H. D., & Riley, E. P. (2000). Neonatal alcohol exposure produces more severe motor coordination deficits in high alcohol sensitive rats compared to low alcohol sensitive rats. *Alcohol, 20*(1), 93-99. doi:10.1016/s0741-8329(99)00080-4

Thomas, J. D., Leany, B. D., & Riley, E. P. (2003). Differential vulnerability to motor deficits in second replicate HAS and LAS rats following neonatal alcohol exposure. *Pharmacol Biochem Behav, 75*(1), 17-24. doi:10.1016/s0091-3057(03)00031-5

Thomas, J. D., Melcer, T., Weinert, S., & Riley, E. P. (1998). Neonatal alcohol exposure produces hyperactivity in high-alcohol-sensitive but not in low-alcohol-sensitive rats. *Alcohol, 16*(3), 237-242. doi:10.1016/s0741-8329(98)00008-1

Titterness, A. K., & Christie, B. R. (2012). Prenatal ethanol exposure enhances NMDAR-dependent long-term potentiation in the adolescent female dentate gyrus. *Hippocampus, 22*(1), 69-81. doi:10.1002/hipo.20849

Tran, T. D., & Kelly, S. J. (2003). Critical periods for ethanol-induced cell loss in the hippocampal formation. *Neurotoxicol Teratol, 25*(5), 519-528. doi:10.1016/s0892-0362(03)00074-6

Uban, K. A., Sliwowska, J. H., Lieblich, S., Ellis, L. A., Yu, W. K., Weinberg, J., & Galea, L. A. (2010). Prenatal alcohol exposure reduces the proportion of newly produced neurons and glia in the dentate gyrus of the hippocampus in female rats. *Horm Behav, 58*(5), 835-843. doi:10.1016/j.yhbeh.2010.08.007

Ullah, N., Naseer, M. I., Ullah, I., Lee, H. Y., Koh, P. O., & Kim, M. O. (2011). Protective effect of pyruvate against ethanol-induced apoptotic neurodegeneration in the developing rat brain. *Neuropharmacology, 61*(8), 1248-1255. doi:10.1016/j.neuropharm.2011.06.031

Valenzuela, C. F., Morton, R. A., Diaz, M. R., & Topper, L. (2012). Does moderate drinking harm the fetal brain? Insights from animal models. *Trends Neurosci, 35*(5), 284-292. doi:10.1016/j.tins.2012.01.006

Viljoen, D. L., Carr, L. G., Foroud, T. M., Brooke, L., Ramsay, M., & Li, T. K. (2001). Alcohol dehydrogenase-2*2 allele is associated with decreased prevalence of fetal alcohol syndrome in the mixed-ancestry population of the Western Cape Province, South Africa. *Alcohol Clin Exp Res, 25*(12), 1719-1722.

Vornholt, E., Drake, J., Mamdani, M., McMichael, G., Taylor, Z. N., Bacanu, S. A., . . . Vladimirov, V. I. (2020). Network preservation reveals shared and unique biological processes associated with chronic alcohol abuse in NAc and PFC. *PLoS One, 15*(12), e0243857. doi:10.1371/journal.pone.0243857

Wagner, A. F., & Hunt, P. S. (2006). Impaired trace fear conditioning following neonatal ethanol: reversal by choline. *Behav Neurosci, 120*(2), 482-487. doi:10.1037/0735-7044.120.2.482

Wagner, J. L., Zhou, F. C., & Goodlett, C. R. (2014). Effects of one- and three-day binge alcohol exposure in neonatal C57BL/6 mice on spatial learning and memory in adolescence and adulthood. *Alcohol, 48*(2), 99-111. doi:10.1016/j.alcohol.2013.12.001

Wang, L., Huang, Y., Jiao, Y., Chen, H., Cao, Y., Bennett, B., . . . Gu, W. (2013). Expression of alcoholism-relevant genes in the liver are differently correlated to different parts of the brain. *Gene Expr, 16*(1), 31-38.

Wang, X., Pandey, A. K., Mulligan, M. K., Williams, E. G., Mozhui, K., Li, Z., . . . Williams, R. W. (2016). Joint mouse-human phenome-wide association to test gene function and disease risk. *Nat Commun, 7*, 10464. doi:10.1038/ncomms10464

Warren, K. R., & Li, T. K. (2005). Genetic polymorphisms: impact on the risk of fetal alcohol spectrum disorders. *Birth Defects Res A Clin Mol Teratol, 73*(4), 195-203. doi:10.1002/bdra.20125

Weinberg, J. (1988). Hyperresponsiveness to stress: differential effects of prenatal ethanol on males and females. *Alcohol Clin Exp Res, 12*(5), 647-652. doi:10.1111/j.1530-0277.1988.tb00258.x

Weinberg, J. (1992). Prenatal ethanol exposure alters adrenocortical response to predictable and unpredictable stressors. *Alcohol, 9*(5), 427-432. doi:10.1016/0741-8329(92)90043-a

Weinberg, J., Sliwowska, J. H., Lan, N., & Hellemans, K. G. (2008). Prenatal alcohol exposure: foetal programming, the hypothalamic-pituitary-adrenal axis and sex differences in outcome. *J Neuroendocrinol, 20*(4), 470-488. doi:10.1111/j.1365-2826.2008.01669.x

West, J. R. (1986). Long-term effects of developmental exposure to alcohol. *Neurotoxicology, 7*(2), 245-256.

West, J. R. (1987). Fetal alcohol-induced brain damage and the problem of determining temporal vulnerability: a review. *Alcohol Drug Res, 7*(5-6), 423-441.

Wever, K. E., Geessink, F. J., Brouwer, M. A. E., Tillema, A., & Ritskes-Hoitinga, M. (2017). A systematic review of discomfort due to toe or ear clipping in laboratory rodents. *Lab Anim, 51*(6), 583-600. doi:10.1177/0023677217705912

Wickham, H. (2016). *ggplot2 : Elegant Graphics for Data Analysis*. In *Use R!*, (pp. 1 online resource (XVI, 260 pages 232 illustrations, 140 illustrations in color). doi:10.1007/978-3-319-24277-4

Wigal, T., & Amsel, A. (1990). Behavioral and neuroanatomical effects of prenatal, postnatal, or combined exposure to ethanol in weanling rats. *Behav Neurosci, 104*(1), 116-126. doi:10.1037//0735-7044.104.1.116

Williams, J. F., Smith, V. C., & Committee On Substance, A. (2015). Fetal Alcohol Spectrum Disorders. *Pediatrics, 136*(5), e1395-1406. doi:10.1542/peds.2015-3113

Willoughby, K. A., Sheard, E. D., Nash, K., & Rovet, J. (2008). Effects of prenatal alcohol exposure on hippocampal volume, verbal learning, and verbal and spatial recall in late childhood. *J Int Neuropsychol Soc, 14*(6), 1022-1033. doi:10.1017/S1355617708081368

Woods, K. J., Thomas, K. G. F., Molteno, C. D., Jacobson, J. L., Jacobson, S. W., & Meintjes, E. M. (2018). Prenatal alcohol exposure affects brain function during place learning in a virtual environment differently in boys and girls. *Brain Behav, 8*(11), e01103. doi:10.1002/brb3.1103

Wozniak, D. F., Hartman, R. E., Boyle, M. P., Vogt, S. K., Brooks, A. R., Tenkova, T., . . . Muglia, L. J. (2004). Apoptotic neurodegeneration induced by ethanol in neonatal mice is associated with profound learning/memory deficits in juveniles followed by progressive functional recovery in adults. *Neurobiol Dis, 17*(3), 403-414. doi:10.1016/j.nbd.2004.08.006

Wozniak, J. R., Riley, E. P., & Charness, M. E. (2019). Clinical presentation, diagnosis, and management of fetal alcohol spectrum disorder. *Lancet Neurol, 18*(8), 760-770. doi:10.1016/S1474-4422(19)30150-4

Xu, W., Hawkey, A. B., Li, H., Dai, L., Brim, H. H., Frank, J. A., . . . Chen, G. (2018). Neonatal Ethanol Exposure Causes Behavioral Deficits in Young Mice. *Alcohol Clin Exp Res, 42*(4), 743-750. doi:10.1111/acer.13598

Yang, H., Dong, Y., Zhou, Y., & Li, H. (2021). Overexpression of miR-874-3p alleviates LPS-induced apoptosis and inflammation in alveolar epithelial cell by targeting EGR3/NF-kappaB. *Acta Biochim Pol, 68*(2), 231-238. doi:10.18388/abp.2020_5523

Yang, Y., Phillips, O. R., Kan, E., Sulik, K. K., Mattson, S. N., Riley, E. P., . . . Sowell, E. R. (2012). Callosal thickness reductions relate to facial dysmorphology in fetal alcohol spectrum disorders. *Alcohol Clin Exp Res, 36*(5), 798-806. doi:10.1111/j.1530-0277.2011.01679.x

Yanni, P. A., & Lindsley, T. A. (2000). Ethanol inhibits development of dendrites and synapses in rat hippocampal pyramidal neuron cultures. *Brain Res Dev Brain Res, 120*(2), 233-243. doi:10.1016/s0165-3806(00)00015-8

Ye, X., Zuo, D., Yu, L., Zhang, L., Tang, J., Cui, C., . . . Cui, G. (2017). ROS/TXNIP pathway contributes to thrombin induced NLRP3 inflammasome activation and cell apoptosis in microglia. *Biochem Biophys Res Commun, 485*(2), 499-505. doi:10.1016/j.bbrc.2017.02.019

Young, C., Klocke, B. J., Tenkova, T., Choi, J., Labruyere, J., Qin, Y. Q., . . . Olney, J. W. (2003). Ethanol-induced neuronal apoptosis in vivo requires BAX in the developing mouse brain. *Cell Death Differ, 10*(10), 1148-1155. doi:10.1038/sj.cdd.4401277

Young, C., Roth, K. A., Klocke, B. J., West, T., Holtzman, D. M., Labruyere, J., . . . Olney, J. W. (2005). Role of caspase-3 in ethanol-induced developmental neurodegeneration. *Neurobiol Dis, 20*(2), 608-614. doi:10.1016/j.nbd.2005.04.014

Yuan, Y., Shen, C., Zhao, S. L., Hu, Y. J., Song, Y., & Zhong, Q. J. (2019). MicroRNA-126 affects cell apoptosis, proliferation, cell cycle and modulates VEGF/TGF-beta levels in pulmonary artery endothelial cells. *Eur Rev Med Pharmacol Sci, 23*(7), 3058-3069. doi:10.26355/eurrev_201904_17588

Zhang, B., & Horvath, S. (2005). A general framework for weighted gene co-expression network analysis. *Stat Appl Genet Mol Biol, 4*, Article17. doi:10.2202/1544-6115.1128

Zhao, S., Fung-Leung, W. P., Bittner, A., Ngo, K., & Liu, X. (2014). Comparison of RNA-Seq and microarray in transcriptome profiling of activated T cells. *PLoS One, 9*(1), e78644. doi:10.1371/journal.pone.0078644

Zhou, F. C., Zhao, Q., Liu, Y., Goodlett, C. R., Liang, T., McClintick, J. N., . . . Li, L. (2011). Alteration of gene expression by alcohol exposure at early neurulation. *BMC Genomics, 12*, 124. doi:10.1186/1471-2164-12-124

Zimmerberg, B., Sukel, H. L., & Stekler, J. D. (1991). Spatial learning of adult rats with fetal alcohol exposure: deficits are sex-dependent. *Behav Brain Res, 42*(1), 49-56. doi:10.1016/s0166-4328(05)80039-7

APPENDIX A. CHAPTER 2 ADDITIONAL INFORMATION

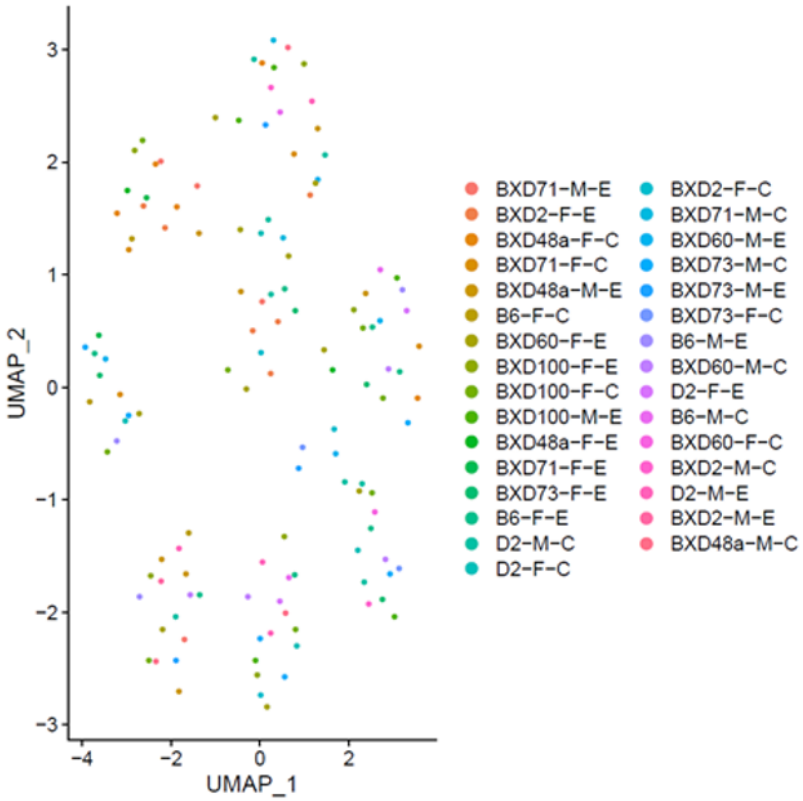

Figure A-1. Principle component analysis (PCA) of samples used for microarray analysis.

APPENDIX B. CHAPTER 3 ADDITIONAL INFORMATION

Table B-1. Animal numbers used for behavioral experiments.

Strain	Sex	Treatment	EPM	OF	Y-Maze	T-Maze
B6	Female	Control	12	12	12	12
B6	Female	Ethanol	12	12	12	12
B6	Male	Control	11	10	11	11
B6	Male	Ethanol	11	11	9	11
BXD100	Female	Control	9	9	9	9
BXD100	Female	Ethanol	9	9	9	9
BXD100	Male	Control	10	9	10	10
BXD100	Male	Ethanol	10	10	10	10
BXD48a	Female	Control	8	8	8	7
BXD48a	Female	Ethanol	8	8	8	8
BXD48a	Male	Control	8	9	8	9
BXD48a	Male	Ethanol	9	9	9	8
BXD60	Female	Control	8	8	8	8
BXD60	Female	Ethanol	8	8	7	8
BXD60	Male	Control	8	8	8	8
BXD60	Male	Ethanol	8	8	8	8
BXD71	Female	Control	7	7	7	7
BXD71	Female	Ethanol	8	8	6	8
BXD71	Male	Control	10	10	10	10
BXD71	Male	Ethanol	11	10	11	10
D2	Female	Control	8	7	8	8
D2	Female	Ethanol	8	7	7	7
D2	Male	Control	9	7	9	9
D2	Male	Ethanol	9	9	9	8

Table B-2. Effects of strain, sex, treatment, and/or interactions in the open field on day 1.

	Bin 1 0 Min - 5 Min			Bin 2 5 Min - 10 Min			Bin 3 10 Min - 15 Min			Total 15 Min		
	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90%CI]**
Day 1 Total Distance Travelled												
Strain	19.50	1.22E-15	0.30 [0.20, 0.38]	16.30	2.30E-13	0.26 [0.17, 0.34]	21.32	2.00E-16	0.32 [0.23, 0.40]	18.23	9.51E-15	0.29 [0.19, 0.36]
Day 1 Distance Travelled in Center												
Strain	7.54	1.78E-06	0.13 [0.05, 0.19]	9.00	1.11E-07	0.16 [0.07, 0.22]	10.55	6.01E-09	0.18 [0.09, 0.25]	10.09	1.41E-08	0.18 [0.09, 0.24]
Day 1 Distance Travelled in Edge												
Strain	20.17	4.24E-16	0.31 [0.21, 0.38]	16.94	7.98E-14	0.27 [0.17, 0.34]	20.05	5.06E-16	0.31 [0.21, 0.38]	19.05	2.51E-15	0.30 [0.20, 0.37]
Treatment				4.35	3.83E-02	0.02 [0.00, 0.06]				4.30	3.95E-02	0.02 [0.00, 0.06]
Day 1 Time in Center												
Strain	3.35	0.006	0.05 [0.00, 0.09]	5.18	0.001	0.09 [0.02, 0.14]	8.31	4.03E-07	0.15 [0.06, 0.21]	8.34	3.81E-07	0.15 [0.06, 0.21]
Sex							6.21	1.36E-02	0.02 [0.00, 0.07]	4.58	3.36E-02	0.02 [0.00, 0.06]
Strain x Sex				3.92	0.002	0.06 [0.00, 0.11]				2.74	0.020	0.04 [0.00, 0.07]
Sex x Treatment				4.06	0.045	0.01 [0.00, 0.05]						
Strain x Sex x Treatment	3.07	0.011	0.05 [0.00, 0.08]									
Day 1 Time in Edge												
Sex							4.80	0.030	0.02 [0.00, 0.06]			
Strain x Sex				3.24	0.008	0.05 [0.00, 0.09]						
Strain x Treatment	2.65	0.024	0.04 [0.00, 0.07]							3.36	0.006	0.05 [0.00, 0.09]
Day 1 Number of Entries to the Center												
Strain	9.20	7.50E-08	0.16 [0.07, 0.23]	7.42	2.25E-06	0.13 [0.05, 0.19]	11.65	8.00E-10	0.2 [0.11, 0.27]	10.48	6.88E-09	0.18 [0.09, 0.25]

	Bin 1 0 Min - 5 Min			Bin 2 5 Min - 10 Min			Bin 3 10 Min - 15 Min			Total 15 Min		
	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**
Day 1 Number of Entries to the Edge												
Strain	11.19	1.83E-09	0.19 [0.10, 0.26]	7.03	4.77E-06	0.12 [0.04, 0.18]	12.49	1.75E-10	0.21 [0.12, 0.28]	9.94	1.87E-08	0.17 [0.08, 0.24]

Table B-3. Effects of strain, sex, treatment, and/or interactions in the open field on day 2.

	Bin 1 0 Min - 5 Min			Bin 2 5 Min - 10 Min			Bin 3 10 Min - 15 Min			Total 15 Min		
	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**
Day 2 Total Distance Travelled												
Strain	16.68	1.23E-13	0.27 [0.17, 0.34]	32.39	2.00E-16	0.42 [0.33, 0.49]	21.20	2.00E-16	0.32 [0.22, 0.39]	30.42	2.00E-16	0.41 [0.31, 0.48]
Sex	7.52	6.70E-03	0.03 [0.00, 0.08]									
Strain x Sex	2.43	3.66E-02	0.03 [0.00, 0.06]									
Strain x Treatment	2.91	1.50E-02	0.04 [0.00, 0.08]									
Day 2 Distance Travelled in Center												
Strain	5.24	0.001	0.09 [0.02, 0.14]	7.80	1.07E-06	0.14 [0.05, 0.20]	7.85	9.67E-07	0.14 [0.06, 0.20]	9.26	6.63E-08	0.16 [0.07, 0.23]
Day 2 Distance Travelled in Edge												
Strain	18.23	9.43E-15	0.29 [0.19, 0.36]	37.34	<2E-16	0.46 [0.37, 0.53]	21.57	2.00E-16	0.33 [0.23, 0.40]	34.42	2.00E-16	0.44 [0.35, 0.51]
Sex	9.14	0.003	0.04 [0.01, 0.09]									
Treatment				6.11	0.014	0.02 [0.00, 0.07]						
Strain x Sex	2.94	0.014	0.04 [0.00, 0.08]									
Strain x Treatment	2.92	0.015	0.04 [0.00, 0.08]									
Day 2 Time in Center												
Strain	3.26	0.008	0.05 [0.00, 0.09]	4.01	0.002	0.07 [0.00, 0.11]	5.40	1.15E-04	0.09 [0.02, 0.15]	6.60	1.10E-05	0.12 [0.04, 0.17]
Sex							9.79	2.03E-03	0.04 [0.01, 0.09]			
Strain x Sex	2.41	0.038	0.03 [0.00, 0.06]									
Day 2 Time in Edge												
Strain	7.10	4.14E-06	0.13 [0.04, 0.19]	4.925	0.001	0.08 [0.02, 0.13]	5.13	1.94E-04	0.09 [0.02, 0.14]	8.50	2.80E-07	0.15 [0.06, 0.21]

	Bin 1 0 Min - 5 Min			Bin 2 5 Min - 10 Min			Bin 3 10 Min - 15 Min			Total 15 Min		
	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**	***F*-Value**	***p*-Value**	**ω^2 [90% CI]**
Day 2 Total Distance Travelled												
Strain	16.68	1.23E-13	0.27 [0.17, 0.34]	32.39	2.00E-16	0.42 [0.33, 0.49]	21.20	2.00E-16	0.32 [0.22, 0.39]	30.42	2.00E-16	0.41 [0.31, 0.48]
Sex	7.52	6.70E-03	0.03 [0.00, 0.08]									
Strain x Sex	2.43	3.66E-02	0.03 [0.00, 0.06]									
Strain x Treatment	2.91	1.50E-02	0.04 [0.00, 0.08]									
Day 2 Distance Travelled in Center												
Strain	5.24	0.001	0.09 [0.02, 0.14]	7.80	1.07E-06	0.14 [0.05, 0.20]	7.85	9.67E-07	0.14 [0.06, 0.20]	9.26	6.63E-08	0.16 [0.07, 0.23]
Day 2 Distance Travelled in Edge												
Strain	18.23	9.43E-15	0.29 [0.19, 0.36]	37.34	<2E-16	0.46 [0.37, 0.53]	21.57	2.00E-16	0.33 [0.23, 0.40]	34.42	2.00E-16	0.44 [0.35, 0.51]
Sex	9.14	0.003	0.04 [0.01, 0.09]									
Sex							8.27	0.004	0.03 [0.00, 0.09]			
Treatment	4.37	0.038	0.02 [0.00, 0.06]									
Strain x Sex	2.56	0.029	0.04 [0.00, 0.07]									
Sex x Treatment	4.12	0.044	0.01 [0.00, 0.05]									
Day 2 Number of Entries to the Center												
Strain	6.01	3.51E-05	0.11 [0.03, 0.16]	9.79	2.45E-08	0.17 [0.08, 0.24]	8.42	3.28E-07	0.15 [0.06, 0.21]	11.05	2.41E-09	0.19 [0.10, 0.26]
Day 2 Number of Entries to the Edge												
Strain	6.93	5.73E-06	0.12 [0.04, 0.18]	11.47	1.11E-09	0.2 [0.10, 0.27]	11.06	2.35E-09	0.19 [0.10, 0.26]	13.05	6.43E-11	0.22 [0.13, 0.29]

VITA

Jessica A. Baker was born in Little Rock Arkansas in 1993 and grew up between Alexandria, Louisiana and Memphis, Tennessee. In 2015, she earned a Bachelor of Science in Neuroscience from Rhodes College in Memphis, Tennessee. As a junior at Rhodes College, she received the University of Tennessee Health Science Center and Rhodes College Neuroscience Institute Research Fellowship. In 2015, she was accepted into Neuroscience Track of the Biomedical Sciences Program at the University of Tennessee Health Science Center. She joined the lab of Dr. Kristin M. Hamre and received an F31 Individual Predoctoral Fellowship from the National Institute on Alcohol Abuse and Alcoholism in 2017. During her graduate career, Jessica was involved in the Graduate Student Executive Council, serving as the Neuroscience Track Representative, Secretary, and finally President. She was able to attend and present at multiple local, national, and international scientific conferences throughout her graduate career. Jessica expects to complete her Doctor of Philosophy in Biomedical Sciences with a concentration in Neuroscience in July of 2021.