Long-term Effects of Fetal Alcohol Spectrum Disorders on Dentate Gyrus Synaptic Plasticity

by

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BA, University of North Carolina at Wilmington, 2004
MA, University of Delaware, 2009

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the Department of Biology

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University of Victoria

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Supervisory Committee

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Abstract

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Developmental ethanol exposure causes both structural and functional changes in the brain that can result in cognitive and behavioral abnormalities. The hippocampal formation, an area of the brain strongly linked with learning and memory, is particularly vulnerable to the teratogenic effects of ethanol. Research in this thesis focused on uncovering the effects of developmental ethanol exposure on hippocampal function in adulthood, particularly synaptic plasticity (a putative neurobiological mechanism of learning and memory). The first experiment sought to determine the temporal vulnerability of hippocampal synaptic plasticity as a function of exposure to ethanol during a single trimester. Ethanol exposure during the 1st or 3rd trimester equivalent resulted in minor changes in synaptic plasticity in adult offspring. In contrast, ethanol exposure during the 2nd trimester equivalent resulted in a pronounced decrease in long-term potentiation (LTP), indicating that the timing of exposure determines the severity of the deficit. The second experiment was aimed at determining the effects of prenatal ethanol exposure (1st and 2nd trimester equivalent combined) on bidirectional synaptic plasticity. Prenatal ethanol exposure resulted in a profound reduction in LTP but did not affect long-term depression. These findings show that prenatal ethanol exposure creates an imbalance in bidirectional synaptic plasticity. The third experiment sought to determine if prenatal ethanol exposure alters the affect of acute ethanol exposure in adulthood on synaptic plasticity. Acute exposure to ethanol in adulthood attenuated LTP in control offspring. Conversely, the magnitude of LTP was not affected by acute ethanol application in prenatal ethanol offspring. These results suggest that prenatal ethanol...
exposure alters the physiological response to ethanol in adulthood. Together, the results from the experiments undertaken in this thesis demonstrate long-lasting alterations in synaptic plasticity as the result of developmental ethanol exposure. Furthermore, these results allude to a malfunction of neural circuits within the hippocampal formation, perhaps relating to the learning and memory deficits observed in individuals with fetal alcohol spectrum disorders.
Table of Contents

Supervisory Committee ................................................................. ii
Abstract ......................................................................................... iii
Table of Contents ........................................................................ v
List of Tables ................................................................................ vii
List of Figures ................................................................................ viii
List of Abbreviations ....................................................................... ix
Acknowledgments ........................................................................... xi
Dedication ......................................................................................... xii

1. Introduction .................................................................................. 1
   1.1 Fetal Alcohol Spectrum Disorders ............................................. 1
      1.1.1 Ethanol .............................................................................. 1
      1.1.2 Fetal Alcohol Spectrum Disorders Defined ......................... 2
      1.1.3 Cause for a Spectrum ......................................................... 3
      1.1.4 Central Nervous System Damage in Fetal Alcohol Spectrum Disorders .... 6
   1.2 Dentate Gyrus ........................................................................... 7
      1.2.1 Sub-structure of the Hippocampal Formation ....................... 7
      1.2.2 Anatomy and Connectivity of the Dentate Gyrus .................. 9
      1.2.3 Dentate Gyrus: The Gateway to All Things Awesome .......... 11
      1.2.4 Learning and Memory ....................................................... 12
      1.2.5 Synaptic Plasticity ............................................................ 14
   1.3 Fetal Alcohol Spectrum Disorders and the Hippocampal Formation .... 25
      1.3.1 Human Studies .................................................................. 26
      1.3.2 Animal Models ................................................................. 28
   1.4 Summary and Objectives .......................................................... 37

2. Methods ....................................................................................... 39
   2.1 Animals .................................................................................. 39
   2.2 Breeding .................................................................................. 39
   2.3 Administration of the Liquid Ethanol Diet – Modeling FASD ........ 40
   2.4 Postnatal Ethanol Exposure ...................................................... 41
   2.5 Blood Ethanol Concentrations ................................................... 42
   2.6 Electrophysiology .................................................................... 42
      2.6.1 Slice Preparation .............................................................. 42
      2.6.2 Recordings ....................................................................... 43
      2.6.3 Conditioning Stimulus Protocols ....................................... 44
   2.7 Data and Statistical Analysis ..................................................... 44

3. Fetal Alcohol Spectrum Disorders and Abnormal Synaptic Plasticity in the Adult Dentate Gyrus of Male and Female Offspring ......................................................... 46
   3.1 Impact of the Timing of Exposure on Long-term Potentiation in the Adult Dentate Gyrus ......................................................................................... 46
      3.1.1 Methods .............................................................................. 46
      3.1.2 Findings ............................................................................ 47
      3.1.3 Discussion ........................................................................ 49
   3.2 Effects of Prenatal Ethanol Exposure during both the 1\textsuperscript{st} and 2\textsuperscript{nd} Trimester Equivalent on Bidirectional Synaptic Plasticity in the Adult Dentate Gyrus ........................................................................ 73
List of Tables

Table 1 Fetal Alcohol Spectrum Disorders ................................................................. 2
Table 2 Literature Summary of FASD and Hippocampal Synaptic Plasticity In Vitro .... 36
Table 3 Literature Summary of FASD and Hippocampal Synaptic Plasticity, In Vivo .... 37
Table 4 Summary of Maternal and Offspring Parameters ........................................... 61
Table 5 Summary of Post-tetanic and Long-term Potentiation Results. ..................... 65
Table 6 Summary of Maternal and Offspring Parameters ........................................... 76
Table 7 Summary of Potentiation and Depression Data ............................................. 83
Table 8 Summary of Maternal and Offspring Parameters ........................................... 93
Table 9 Summary of Post-tetanic and Long-term Potentiation Data for Male Offspring ................................................................................................................................. 103
Table 10 Summary of Post-tetanic and Long-term Potentiation Data for Female Offspring ................................................................................................................................. 103
Table 11 Conversion Table of BEC and Ethanol Concentrations Used ...................... 143
List of Figures

Figure 1 Location of the Hippocampal Formation .......................................................... 8
Figure 2 Hippocampal Tri-synaptic Circuit ................................................................... 9
Figure 3 Dentate Granule Cell Connectivity .................................................................. 12
Figure 4 Basic cellular mechanisms mediating long-term potentiation and depression .. 22
Figure 5 In vitro Electrophysiology .............................................................................. 43
Figure 6 Experimental Timeline .................................................................................. 48
Figure 7 Synaptic Transmission in the Medial Perforant Path Following Ethanol Exposure During the 1st Trimester Equivalent .............................................................. 50
Figure 8 Effect of 1st Trimester Equivalent Ethanol Exposure on PTP and LTP in All Offspring ..................................................................................................................... 52
Figure 9 Synaptic Transmission in the Medial Perforant Path Following Ethanol Exposure During the 2nd Trimester Equivalent .............................................................. 54
Figure 10 Effect of 2nd Trimester Equivalent Ethanol Exposure on PT and LTP in All Offspring ..................................................................................................................... 56
Figure 11 Synaptic Transmission in the Medial Perforant Path Following Ethanol Exposure During the 3rd Trimester Equivalent .............................................................. 58
Figure 12 Effect of 3rd Trimester Equivalent Ethanol Exposure on PTP and LTP in All Offspring ..................................................................................................................... 59
Figure 13 Effects of Developmental Ethanol Exposure on Post-tetanic Potentiation in the Young Adult DG ................................................................................................. 62
Figure 14 Effects of Developmental Ethanol Exposure on Long-term Potentiation in the Young Adult DG ................................................................................................. 64
Figure 15 Experimental Timeline .................................................................................. 75
Figure 16 Input/Output function ..................................................................................... 77
Figure 17 Paired Pulse Plasticity ..................................................................................... 77
Figure 18 Effects of Prenatal Ethanol Exposure on CS Induced Plasticity ................. 79
Figure 19 Effect of Receptor Antagonists on the Induction of PTD and LTD in AL Males. ............................................................................................................................... 81
Figure 20 Effects of Prenatal Ethanol Exposure on Low Frequency Stimulation Induced Plasticity ..................................................................................................................... 82
Figure 21 Interaction Between Prenatal Treatment and CS ........................................ 94
Figure 22 Interaction Between Prenatal Treatment and Acute Ethanol Exposure .... 95
Figure 23 Effects of Acute Ethanol Exposure on Post-tetanic Potentiation .......... 97
Figure 24 Interaction Between Prenatal Treatment and CS ........................................ 98
Figure 25 Interaction Between Prenatal Treatment and Acute Ethanol Exposure .... 99
Figure 26 Interaction Between Acute Ethanol Exposure and CS ......................... 100
Figure 27 Theta Burst Stimulation Induced Plasticity Following Acute Ethanol Exposure ....................................................................................................................... 101
Figure 28 High Frequency Stimulation Induced Plasticity Following Acute Ethanol Exposure ....................................................................................................................... 102
Figure 29 BMI Dependent Potentiation ...................................................................... 145
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADH</td>
<td>alcohol dehydrogenase</td>
</tr>
<tr>
<td>AMPA</td>
<td>α-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid</td>
</tr>
<tr>
<td>AP</td>
<td>action potential</td>
</tr>
<tr>
<td>AP5</td>
<td>α-animo-5-phosphonopentanoic acid</td>
</tr>
<tr>
<td>APV</td>
<td>α-animo-5-phosphonopentanoic acid</td>
</tr>
<tr>
<td>ARBD</td>
<td>alcohol related birth defects</td>
</tr>
<tr>
<td>ARND</td>
<td>alcohol related neurodevelopment disorder</td>
</tr>
<tr>
<td>BDNF</td>
<td>brain derived neurotrophic factor</td>
</tr>
<tr>
<td>BMI</td>
<td>bicuculline methiodide</td>
</tr>
<tr>
<td>CA</td>
<td>cornu ammonis</td>
</tr>
<tr>
<td>Ca2+</td>
<td>calcium</td>
</tr>
<tr>
<td>CaM</td>
<td>calmodulin</td>
</tr>
<tr>
<td>CaMKII</td>
<td>calcium/calmodulin-dependent protein kinases II</td>
</tr>
<tr>
<td>CF</td>
<td>chloride</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPP</td>
<td>3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid</td>
</tr>
<tr>
<td>CIEE</td>
<td>chronic intermittent ethanol exposure</td>
</tr>
<tr>
<td>CS</td>
<td>conditioning stimulus/stimuli</td>
</tr>
<tr>
<td>DG</td>
<td>dentate gyrus</td>
</tr>
<tr>
<td>EC</td>
<td>entorhinal cortex</td>
</tr>
<tr>
<td>EDC</td>
<td>ethanol derived calories</td>
</tr>
<tr>
<td>EE</td>
<td>ethanol exposure</td>
</tr>
<tr>
<td>EE3</td>
<td>ethanol exposure during the 3\textsuperscript{rd} trimester equivalent</td>
</tr>
<tr>
<td>EPSC</td>
<td>excitatory postsynaptic current</td>
</tr>
<tr>
<td>EPSP</td>
<td>excitatory postsynaptic potentiation</td>
</tr>
<tr>
<td>ERK</td>
<td>extracellular signal-regulated kinase</td>
</tr>
<tr>
<td>EtOH</td>
<td>ethanol</td>
</tr>
<tr>
<td>FAS</td>
<td>fetal alcohol syndrome</td>
</tr>
<tr>
<td>FASD</td>
<td>fetal alcohol spectrum disorder</td>
</tr>
<tr>
<td>fEPSP</td>
<td>field excitatory postsynaptic potentiation</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GAD</td>
<td>glutamate decarboxylase</td>
</tr>
<tr>
<td>GD</td>
<td>gestational day</td>
</tr>
<tr>
<td>HFS</td>
<td>high frequency stimulation</td>
</tr>
<tr>
<td>IEG</td>
<td>immediate early genes</td>
</tr>
<tr>
<td>I/O</td>
<td>input/output</td>
</tr>
<tr>
<td>IP3</td>
<td>inositol trisphosphate</td>
</tr>
<tr>
<td>IPSC</td>
<td>inhibitory postsynaptic current</td>
</tr>
<tr>
<td>IQ</td>
<td>intelligence quotient</td>
</tr>
<tr>
<td>IR</td>
<td>input resistance</td>
</tr>
<tr>
<td>K+</td>
<td>potassium</td>
</tr>
<tr>
<td>LFS</td>
<td>low frequency stimulation</td>
</tr>
<tr>
<td>LPP</td>
<td>lateral perforant path</td>
</tr>
<tr>
<td>LTD</td>
<td>long-term depression</td>
</tr>
<tr>
<td>LTP</td>
<td>long-term potentiation</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>Mg2+</td>
<td>magnesium</td>
</tr>
<tr>
<td>mGluR</td>
<td>metabotropic glutamate receptor</td>
</tr>
<tr>
<td>ML</td>
<td>molecular layer</td>
</tr>
<tr>
<td>MPP</td>
<td>medial perforant path</td>
</tr>
<tr>
<td>Na+</td>
<td>sodium</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-Methyl-D-aspartate</td>
</tr>
<tr>
<td>NSC</td>
<td>neural stem cell</td>
</tr>
<tr>
<td>PD</td>
<td>postnatal day</td>
</tr>
<tr>
<td>pFAS</td>
<td>partial fetal alcohol syndrom</td>
</tr>
<tr>
<td>PKA</td>
<td>cAMP-dependent protein kinase A</td>
</tr>
<tr>
<td>PKC</td>
<td>protein kinase C</td>
</tr>
<tr>
<td>PLC</td>
<td>phosphoinositide-specific phospholipase C</td>
</tr>
<tr>
<td>PNEE</td>
<td>prenatal ethanol exposure</td>
</tr>
<tr>
<td>PNEE\textsubscript{1}</td>
<td>prenatal ethanol exposure during the 1\textsuperscript{st} trimester equivalent</td>
</tr>
<tr>
<td>PNEE\textsubscript{1,2}</td>
<td>prenatal ethanol exposure during the 1\textsuperscript{st} and 2\textsuperscript{nd} trimester equivalent</td>
</tr>
<tr>
<td>PNEE\textsubscript{2}</td>
<td>prenatal ethanol exposure during the 2\textsuperscript{nd} trimester equivalent</td>
</tr>
<tr>
<td>PP</td>
<td>paired pulse</td>
</tr>
<tr>
<td>PPP</td>
<td>protein phosphatase</td>
</tr>
<tr>
<td>PTD</td>
<td>post-tetanic depression</td>
</tr>
<tr>
<td>PTP</td>
<td>post-tetanic potentiation</td>
</tr>
<tr>
<td>RMP</td>
<td>resting membrane potential</td>
</tr>
<tr>
<td>ROS</td>
<td>reactive oxygen species</td>
</tr>
</tbody>
</table>
SI – sham intubated
TBS – theta burst stimulation
TM – transmembrane
VGCC – voltage gated calcium channel
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First off, I would like to acknowledge my fantastic graduate advisor and mentor, Dr. Christie. Without his extraordinary support, guidance and scientific knowledge I would not have been able to accomplish the work put forth these past few years. I want to also thank Dr. Christie for showing me the magnificence of knowledge and restoring my faith in scientific research.

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- ONTD for the much needed distractions.
- Staff at the Spiral Cafe for making the tastiest caffeinated drinks.
- Rowland Institute for providing me with the opportunity to write outside of our apartment.
I dedicate this work to my husband, Brett, and to my parents for their invaluable support.
1. Introduction

It is well established that the hippocampal formation plays an important role in memory formation (Squire 1992). The study of synaptic plasticity in the hippocampal formation is of fundamental importance to neuroscience research. Synaptic plasticity, first discovered in the hippocampal formation, is a candidate cellular mechanism of learning and memory processes (Bliss & Collingridge 1993). Numerous studies have demonstrated the hippocampal formation’s vulnerability to the neurotoxic effects of ethanol, both structurally and behaviorally (Berman & Hannigan 2000). The aim of this thesis is to provide a better understanding of how fetal alcohol spectrum disorders (FASDs) affect the function of the hippocampal dentate gyrus, through measures of synaptic plasticity. This section provides a general introduction to FASDs followed by an overview of the hippocampal formation, focusing on the dentate gyrus. It will end with a survey of the literature on the known effects of fetal alcohol spectrum disorders on the hippocampus and rationale for the research efforts of this thesis.

1.1 Fetal Alcohol Spectrum Disorders

1.1.1 Ethanol

Alcohol is a family of organic chemical compounds that contain one or more hydroxyl (–OH) groups attached to a carbon. Of the many types of alcohols, only one, ethanol, is consumed on a regular basis by humans. Ethanol is classified as a psychoactive substance and depressant drug that also acts as a teratogen.

The World Health Organization recently reported that 55% of all adults have consumed ethanol (2011). This makes ethanol the most widely used psychoactive substance in the world (McKenzie et al 2011). Furthermore, ethanol is the world’s third largest risk factor for disease, contributing to at least 60 different types of diseases/disorders (Organization 2011). One of the most prevalent disorders, FASDs, is a developmental disorder resulting from maternal consumption of ethanol during pregnancy.
1.1.2 Fetal Alcohol Spectrum Disorders Defined

When ethanol is consumed by a pregnant female it is distributed throughout the body of both the mother and fetus. Because ethanol is both lipid and water soluble it freely passes through the placental membrane and spreads to all tissues and organs of the fetus. Thus ethanol disrupts development and sets the stage for a myriad of lifelong struggles. Out of every 100 births, an estimated two to five children are born each day with an ethanol related disorder (May et al 2009), making it the leading preventable cause of intellectual disabilities and behavioral problems in the United States (Bailey & Sokol 2008). Furthermore, these unfortunate individuals will suffer their entire lives as these disabilities only get worse with age (Streissguth et al 1994). Disorders resulting from developmental ethanol exposure are classified under the umbrella term fetal alcohol spectrum disorders (FASDs). These disorders include fetal alcohol syndrome (FAS), partial fetal alcohol syndrome (PFAS), alcohol related birth defects (ARBD), and alcohol related neurodevelopment disorder (ARND) (Stratton et al 1996) (Table 1). Disorders within the spectrum are characterized with some or all of the following problems: facial dysmorphology, growth deficits, physical abnormalities, and/or central nervous system (CNS) damage.

Table 1 Fetal Alcohol Spectrum Disorders

<table>
<thead>
<tr>
<th>Conditions Covered by the term Fetal Alcohol Spectrum Disorder (FASD)</th>
<th>Acronym</th>
<th>Characteristics</th>
</tr>
</thead>
</table>
| Fetal Alcohol Syndrome | FAS | • Facial dysmorphology  
• Growth deficits  
• Physical abnormalities  
• Central nervous system damage |
| Partial Fetal Alcohol Syndrome | PFAS | • Some facial dysmorphology, growth deficits, and physical abnormalities  
• Central nervous system damage |
| Alcohol Related Birth Defects | ARBD | • Some physical abnormalities  
• Central nervous system damage  
* Previously referred to as Fetal Alcohol Effects (FAE) |
| Alcohol Related Neurodevelopmental Disorder | ARND | • Central nervous system damage  
* Previously referred to as Fetal Alcohol Effects (FAE) |
1.1.3 Cause for a Spectrum

The exact causal factor for why a spectrum of adverse effects results from developmental ethanol exposure is unknown; it may be that there is no single source. We are aware of a number of risk factors that can be linked to particular clinical diagnosis within the spectrum of ethanol related disorders. The type and severity of the disorder largely depends on the dose and developmental timing of exposure. Furthermore, the diversity of FASD is in large part due to maternal factors. Each of these factors is discussed below.

1.1.3.1 Dose

Epidemiological studies by May and colleagues (May et al 2009; May et al 2004) indicate that mothers who give birth to FASD children typically binge drink. In women, binge drinking is defined as consuming four or more drinks in approximately a two hour time span, whereas in men it is five or more drinks (Wechsler & Nelson 2001). For the common woman, a blood ethanol concentration (BEC) of 80 mg/dl (i.e. 0.08 %; see appendix for conversion table) is reached through binge drinking (Gunzerath et al 2004). However, the more severe forms of FASD are correlated with slightly higher maternal BEC level (FAS: ~200 mg/dl; PFAS: ~125 mg/dl), corresponding to three or more drinks per occasion (May et al 2000; May et al 2009; May et al 2008; May et al 2011). The other types of FASDs, ARND and ARBD, are correlated with lower consumption levels that are spread out over long periods of time (May et al 2006). Consumption levels that do not result in a childhood diagnosis of an ethanol related disorder have still been shown to affect development (Jacobson & Jacobson 1994). These children have lower overall cognitive and behavioral skills and their IQ levels fall below average (Jacobson & Jacobson 1994; Streissguth et al 1989). Animal models of FASD have also demonstrated that the type and extent of the damage largely depends on the frequency and timing of exposure (Maier et al 1996; Maier & West 2001).

1.1.3.2 Frequency and Timing

Although many advances have been made in our understanding of the detrimental effects ethanol has on a developing fetus, ethanol consumption during pregnancy is fairly common. In a recent interview of women in the United States of America, 30.3%
reported drinking at some point during their pregnancy. Even more alarming, 8.3% of these women reported binge drinking; binge drinking was defined at four or more drinks on any one occasion. Within the first trimester of pregnancy, 22.5% of women reported drinking. Following the first trimester, consumption rates declined to 7.9% during the third trimester (Ethen et al 2009).

Studies examining drinking patterns in Italy report that pregnant women typically drink low to moderate levels daily (consumption with meals is common) (May et al 2006). This resulted in the occurrence of more cases of PFAS to that of FAS. In South Africa where binge like consumption is more common on the weekends (throughout pregnancy) there is a much higher rate of FAS to that of PFAS (May et al 2000; May et al 2008). Thus, in the more severe instances of FASDs the regular occurrence of heavy drinking throughout pregnancy is typical (Abel 1998).

The timing of exposure will also affect the outcome of fetal development. Over the nine months of development each structure and organ in the fetal body develops at its own rate. Over this time, each of these structures/organs development can be broken up into stages and critical periods. The characteristic facial features of FAS are associated with high ethanol consumption during the sixth to ninth week of gestation (1st Trimester) (Sulik 2005). Whereas, the resulting behavioral and cognitive deficits depend on the timing of their associated neural systems development but generally occur from consumption during the second and third trimester (Guerri et al 2009).

1.1.3.3 Maternal Factors

There is increasing evidence that environmental and genetic factors influence the various outcomes reported in FASD. Older women tend to have children with higher degrees of damage related to ethanol exposure than those born to younger women (Jacobson et al 1998). Likewise, the more children a woman has, the higher the degree of damage will ensue in the offspring (Jacobson et al 1998).

Nutritional status of a pregnant mother will also determine the level of ethanol toxicity to the fetus. Numerous studies have reported nutrient deficiencies in pregnant drinking mothers. These women usually have a lower intake of omega-3 fatty acids, riboflavin, calcium, zinc, and vitamin B (Keen et al 2010; May et al 2004; Tamura et al
In addition, ethanol disrupts the supply of nutrients to the developing fetus (Dreosti 1993; Halsted et al 2002). The interactions of ethanol and nutritional level may also explain why higher than normal rates of FASD are found in South African populations, where there are reports of major malnutrition (May et al 2004).

The ability to metabolize ethanol and the time it takes to clear it out of the body plays a large part in the severity of damage caused by ethanol. Analysis of gene variants in the enzymes that metabolize ethanol such as alcohol dehydrogenase (ADH) has indicated that alterations in the ability to metabolize ethanol contribute to the outcome of FASD. The reduced presence of ADH1B2 and ADH1B3 but not ADH1B1 in mothers that have given birth to children with FASD suggests that these ADH variants may be key for the protection from the teratogenic effects of ethanol (Jacobson et al 2006; Khaole et al 2004). Interestingly, individuals with higher levels of ADH1B2 and ADH1B3 are also at a reduced risk for ethanol abuse and alcoholism (May et al 2011).

Other studies have demonstrated genetic influences on the susceptibility of acquiring FASD. By comparing the effects of developmental ethanol exposure in different species of animals and different animal strains within a species, studies have shown that slight alterations in the genetic code can influence the severity of FASD (Green et al 2007; Maier & West 2001; West & Goodlett 1990). Furthermore, epigenetic mechanisms also influence the outcome of FASD. Ethanol has been shown to alter DNA methylation (Kim & Shukla 2006; Shukla et al 2008), modify histones (Shukla et al 2008), and disrupt amino acids critical for normal cell function (Halsted et al 2002) in both drinking mothers and the developing fetus.

In addition to maternal factors, there are also reports of paternal influences. Offspring fathered by chronic ethanol consumers have increased perinatal mortality and display signs of germ cell (spermatozoon and oocyte) defects (Stockard 1913). Interestingly, these degenerate conditions were passed to subsequent generations (Stockard 1913). Furthermore, paternal ethanol consumption has also been shown to influence birth weight and litter size in rodent models of FASD (Abel 2004; Mankes et al 1982). Thus, the severity of FASD can be determined by both maternal and paternal factors such as genetics, age, and nutritional status (Guerri et al 2009; May et al 2011).
Among women who report the consumption of ethanol during pregnancy, strong predictors of consumption included pre-pregnancy binge drinking, continued use of cigarettes throughout pregnancy, ethnicity, and the pregnancy being unintentional. There are many other predictors that correlate with consumption of ethanol while pregnant. These include (but are not limited to) initial age of regular ethanol consumption (May et al. 2005), socioeconomic status (Abel & Hannigan 1995), religion (May et al. 2008), history of depression (Rubio et al. 2008), and social relationships (May et al. 2005; May et al. 2008).

1.1.4 Central Nervous System Damage in Fetal Alcohol Spectrum Disorders

Ethanol exposure during in utero development can lead to various birth defects (ranging from mild to severe) in the CNS, craniofacial structure, or other organ systems. The range of effects associated with fetal ethanol exposure is referred to collectively as FASDs. The most serious of these disorders, FAS, is diagnosed by the presence of growth deficits, facial dysmorphology, and CNS abnormalities due to heavy maternal ethanol consumption during pregnancy (Jones & Smith 1973; Jones et al. 1973). Children exposed prenatally to ethanol who do not meet the diagnostic criteria for FAS still manifest considerable physical abnormalities, CNS damage and growth defects (Coles 1994; Hamilton et al. 2003; Maier et al. 1996; Mattson et al. 2001). Upon examination of all disorders in the spectrum, CNS damage is the one recurring characteristic (Riley & McGee 2005). Thus, FASD is classified as a neurodevelopmental disorder in which early alterations in brain development result in CNS dysfunction and symptomatology that persists throughout one’s life (Sampson et al. 1997).

FASD results in an overall reduction in size and shape of the head and brain (Archibald et al. 2001; Jones & Smith 1973; Jones et al. 1973; Mattson & Riley 1998; O’Leary-Moore et al. 2011). This may be in part due to a reduction in the volume of the cranial and cerebral vault (Archibald et al. 2001; Autti-Rämö et al. 2002; Swayze et al. 1997). Furthermore, these reductions can also be the result of alterations in both grey and white matter density (Archibald et al. 2001; Sowell et al. 2008; Sowell et al. 2002). Interestingly, specific regions of the brain are more vulnerable to the effects of ethanol than others. This is largely dependent on location of these regions relative to the
ventricular system, where ethanol accumulates, and their time course of development. These areas include the cerebellum, corpus callosum, prefrontal cortex, and hippocampal formation (Archibald et al 2001; Autti-Rämö et al 2002; Nuñez et al 2011; Riley & McGee 2005).

The following section provides an introduction to the hippocampal formation, particularly the dentate gyrus (the focus of this thesis work), and will be followed by an in depth look at how developmental ethanol exposure affects the hippocampal formation.

1.2 Dentate Gyrus

1.2.1 Sub-structure of the Hippocampal Formation

The hippocampal formation is a sub-cortical CNS structure found in the mammalian telencephalon. The hippocampal formation is part of the limbic system located along the mid-line surrounding the lateral ventricle. In human and nonhuman primates, the hippocampal formation lies horizontally on the medial surface of the brain in the temporal lobe. However, in rats the hippocampal formation is ventrally oriented and expands from an area near the septal nuclei down into the temporal lobe (Figure 1). This change in position is most likely due to the increased development of the cerebral cortex and corpus callosum in higher order mammals. However, the general structure and connectivity of the hippocampal formation is well preserved across mammal species.

The hippocampal formation is composed of six structures; the dentate gyrus (DG), hippocampus [cornu ammonis (CA) 3, CA2, CA1]), subiculum, presubiculum, parasubiculum, and entorhinal cortex (EC) (Amaral & Lavenex 2006). However, the hippocampal formation is typically referred to as the “tri-synaptic circuit” (Andersen et al 1971). The tri-synaptic name derives from the intrinsic connective circuit of the DG, CA3, and CA1. Cortical input coming from the EC synapses onto cells in the DG. From the DG, information is sent onto the CA3. Then, the CA3 transfers this information onto the CA1 (Andersen et al 1971) (Figure 2).
Figure 1 Location of the Hippocampal Formation.
The hippocampal formation (*pink*) sits below the surface of the neocortex with a linear shape and horizontal orientation in the human brain (*left*), but is more c-shaped and vertically oriented in the rat brain (*right*).

One of the unique features of the tri-synaptic circuit is that the connections are primarily unidirectional (Amaral & Lavenex 2006). The input carried by the perforant path from the EC to the DG provides the hippocampal formation with its primary source of cortical information. Because the DG is the recipient of this information, it is believed that the first stage of information processing that ultimately leads to memory formation occurs in the DG (Andersen et al 1971, Peng, 2005 #198; Peng & Houser 2005). The DG can be thought of as the gateway into the hippocampus, eventually passing information along to the CA3.
Figure 2 Hippocampal Tri-synaptic Circuit.

Synapse 1: The medial and lateral entorhinal cortex (gray) sends projection to the dentate gyrus (light purple) via the perforant path (green). Synapse 2: Granule cells of the dentate gyrus send projections to the CA3 (light blue) through the mossy fibers (dark purple). Synapse 3: CA3 pyramidal cells send projections to the CA1 (light red) by way of the Schaffer collaterals (dark blue). CA: cornu ammonis; DG: dentate gyrus; LEC: lateral entorhinal cortex; MEC: medial entorhinal cortex; PaS: parasubiculum; PrS: presubiculum; Sub: subiculum.

1.2.2 Anatomy and Connectivity of the Dentate Gyrus

The DG is a three layered structure, visually identified by its V/U shape and can be divided into subareas known as the suprapyramidal blade, infrapyramidal blade, and the crest. The suprapyramidal blade is the portion of the dentate that is located between the CA3 and CA1 and the infrapyramidal blade is located opposite of this. The crest is the area in which the two blades join, thus creating the characteristic V/U shape.

The molecular layer, granule cell layer, and hilus (also referred to as the polymorphic layer) make up the three layers of the DG. The molecular layer is the outermost layer and the hilus is the inner cellular region of the DG. In between these two layers lies the
granule cell layer. Each layer of the DG is unique in both its cellular content and connectivity.

The principle cell of the DG is the granule cell. Granule cells reside in the granule cell layer of the DG, have elliptical shaped bodies, and are tightly packed in together (Rapp & Gallagher 1996). Arising from their cell bodies is a characteristic cone-shaped tree of spinney apical dendrites (Amaral & Lavenex 2006) that extend outward into the molecular layer (Desmond & Levy 1985). It is here in the molecular layer that the DG receives its major excitatory cortical input by way of the perforant path.

Within the granule cell layer, pyramidal basket cells and a heterogeneous population of interneurons can also be found. Pyramidal basket cells reside at the border between the granule cell layer and the hilus. Based on their immunoreactivity for glutamate decarboxylase, the synthesizing enzyme for the inhibitory neurotransmitter γ-aminobutyric acid (GABA) (Ribak 1992), these cells are classified as inhibitory interneurons. Basil dendrites of pyramidal basket cells receive excitatory input from dentate granule cells (Acsády et al 1998). Pyramidal basket cell axons form pericellular plexuses that surround and synapse onto the bodies of granule cells (Sik et al 1997). This distinctive network of connectivity between granule cells and pyramidal basket cells results in a very powerful form of feedback inhibition (Figure 3).

The DG also has a feedfoward inhibition component (Mott et al 1997). In the outer edge of the molecular layer, inhibitory interneurons known as molecular layer perforant path-associated (MOPP) cells dwell (Han et al 1993). Both their axons and dendrites extend throughout the molecular layer. Thus MOPP cells receive excitatory input from the perforant path and provide inhibition to granule cells. Axo-axonic cells are located at border of the molecular and granule layers and are presumably inhibitory (Soriano et al 1989b).

Within the hilus layer, numerous cell types exist. Associational/commissural projections originating from neurons in the hilus terminate in the ipsilateral and contralateral inner molecular layer (Laurberg & Sørensen 1981)(Figure 3). The mossy cells from which these projections arise are immunoreactive for glutamate, thus suggesting that the associational/commissural projections are excitatory (Soriano et al 1994). In addition, these mossy cells receive excitatory input from granule cells (Seress et
al 1989). The hilus also contains hilar perforant path-associated (HIPP) cells, accounting for 16% of the GABAergic cells in the DG (Boyett & Buckmaster 2001). Other cells found within the hilus and other layers of the DG include hilar commissural-associational pathway-associated (HICAP) cells, interneuron specific cells, and those that have not been well characterized or named.

The perforant projections from the EC terminate mainly on the dendritic tree of granule cells and provide excitatory glutamatergic input (Scharfman 2007). The perforant pathway is divided into the lateral and medial perforant paths, corresponding to their region of origin in the EC (Hunsaker et al 2007). The lateral and medial perforant paths innervate the molecular layer in a laminar fashion (Figures 2 and 3). From the outside in, the molecular layer is divided into the lateral, medial, and inner layers. The lateral and medial layers receive input from the lateral and medial perforant pathways, respectively.

Because of the complex network of connectivity within the DG, granule cells are characterized by low levels of excitability (Coulter & Carlson 2007). In addition to receiving excitatory inputs from the EC and mossy cells, granule cells receive inhibition from GABAergic interneurons. This intricate network provides granule cells with both feedforward and feedback inhibition. Through this powerful inhibition along with other intrinsic factors, there is a low level of excitability seen in granule cells.

1.2.3 Dentate Gyrus: The Gateway to All Things Awesome

Based on its location and connectivity the DG functions as the gateway to the hippocampus. The EC is the primary cortical input to the rest of the hippocampal formation, whereby layer II of the EC projects to the molecular layer of the DG by way of the perforant path. The granule cells of the DG then send information onto the stratum lucidum of the CA3 via mossy fibers. Early studies where the perforant pathway was stimulated with either short or long duration stimuli showed that the DG selectively blocks or facilitates the signal, respectively (Alger & Teyler 1976; Andersen et al 1966). EC seizure induction through chemical or electrical stimulation resulted in a propagation of seizure related metabolic changes and alterations in hippocampal related behaviours. Weak EC seizures resulted in increased metabolic changes in both the EC and DG, whereas moderate seizures lead to metabolic changes in the EC, DG, CA3, and CA1
(Collins et al 1983), suggesting that the DG controls seizure spread to hippocampus. Similar results have been obtained by monitoring c-fos protein expression and regional electrical activity after seizure induction. Both c-fos expression and regional activity were shown to exist on a temporal scale, being that expression and activity were first seen in the EC, then in the DG, then in the CA3, and then CA1 (Lothman et al 1992; Peng & Houser 2005; Walther et al 1986). These studies demonstrate ways in which the DG acts as the gateway into the hippocampus and reveal the importance of the DG in the overall function of the hippocampal formation.

![Figure 3 Dentate Granule Cell Connectivity.](image)

Granule cells (purple) are the principle neurons of the dentate gyrus. These cells receive excitatory input from entorhinal afferents (green) and mossy cells (blue) from the contralateral hilus. Interneurons residing in the dentate gyrus regulate granule cell activity through feedforward (MOPP cells, yellow) and feedback (pyramidal basket cells, red) inhibition. Circle represents excitation; square represents inhibition.

1.2.4 Learning and Memory

The temporal lobe has emerged as a leading candidate for the locus of learning and memory in the CNS (Squire & Zola-Morgan 1991). Patients who have undergone
bilateral lesions of portions of the temporal lobes (including the hippocampal formation) for the treatment of epilepsy display memory deficits following surgery. The most famous case, that of H.M., was first reported by Scoville and Milner in (1957). Through a series of studies they showed that memory deficits relating to the removal of both the right and left medial temporal lobes were specific to the formation of new long-term memories, specifically those of a declarative nature.

Two main theories for hippocampal function have been postulated: 1) Declarative memory theory and 2) Cognitive map theory (reviewed in (Morris 2007; Purves 2008; Wechsler & Nelson 2001)). Declarative memory is defined as memory for everyday facts and events that can be consciously recalled. According to the declarative memory theory (also referred to the episodic memory theory) the hippocampal formation is part of the medial temporal lobe memory system (Squire & Zola-Morgan 1991) and its primary role is in the formation (Vargha-Khadem et al 1997) and initial storage/consolidation of declarative memories (McNaughton et al 2003; O'Reilly & Norman 2002). Cognitive map theory states that the hippocampal formation organizes and stores stimuli with respect to a spatial framework (i.e. cognitive map) (O'Keefe & Conway 1978; O'Keefe & Recce 1993) and is used for spatial navigation (Fyhn et al 2004; Hafting et al 2005; Taube et al 1990). Furthermore, the hippocampal formation contributes to spatial processing by making associations based on timing and between complex relationships. Importantly, these two theories are not necessarily mutually exclusive, as both suggest that the hippocampal formation is involved in the processing and initial storage of information.

The hippocampal formation may not be a unitary structure and different functions may be related to differences in anatomical structure and inputs. Furthermore, there is a heterogeneous distribution of function along the septotemporal axis of the hippocampal formation (reviewed in (Bannerman et al 2004). It is believed that septal (dorsal) portions of the rat hippocampal formation are involved in declarative memory and sensory information processing, whereas the temporal hippocampal formation is involved in emotional information processing and regulation of stress responses (Fanselow & Dong 2010; Hunsaker et al 2007).
1.2.4.1 Role of the Dentate Gyrus

Sub-region analysis of the hippocampal formation advocates a heterogeneous distribution of function. One of the hypothesized roles of the DG is that of pattern separation in spatial memory tasks. Rodents with lesions of the DG show impairments in spatial location tasks involving short spatial separations of choices (Gilbert et al 2001). Additionally, tests of metric spatial manipulation show that rats with DG lesions are impaired at recognizing displaced objects (Goodrich-Hunsaker et al 2005; Hunsaker & Kesner 2008). The existence of place fields within the DG also indicates the DG involvement in pattern separation (Jung & McNaughton 1993). Place cells are a population of granule cells that fire only when a rodent is in a given environment (Eichenbaum et al 1989; Muller & Kubie 1987). When an environment is modified, pattern separation is expressed as changes in pattern activity of place cells (Leutgeb et al 2007). These findings suggest that the DG contributes to pattern separation and recognition of an environment. In addition to pattern separation, the DG may also play a significant role in other hippocampal functions.

Rolls and Kesner (2006) suggest that the DG, particularly the dentate/mossy fiber system, plays an important role in the encoding of new information. To support this notion, it has been shown that rodents with DG lesions are impaired in acquisition (i.e. learning) of spatial tasks. Following DG lesions, rats display impairments in the learning of the Morris water maze task (Sutherland et al 1983; Xavier et al 1999) and are impaired in contextual fear conditioning acquisition (Lee & Kesner 2004). Additionally, disruption of granule cell input into the CA3 results in an impairment of spatial learning, but spares consolidation and memory retrieval (Lassalle et al 2000). These studies suggest that the DG processes/prepares spatial information for the CA3.

1.2.5 Synaptic Plasticity

In the 1940’s, Canadian psychologist Donald Hebb proposed a theory postulating the basic mechanism for learning and memory formation, now known as synaptic plasticity (Hebb 1949). Synaptic plasticity is the modification of the effectiveness of synaptic transmission at preexisting synapses. Hebb proposed that information storage in the brain occurs “When an axon of cell A is near enough to excite cell B and repeatedly
or persistently takes part in firing it, some growth process or metabolic change takes place in one or both cells such that A’s efficiency, as one of the cells firing B is increased.” (Hebb 1949) This idea of activity-dependent strengthening of synaptic connections was first experimentally observed in the hippocampal DG of rabbits by Timothy Bliss and Terje Lomo (Bliss & Lømo 1973). Ever since, synaptic plasticity has been the subject of intense neuroscientific investigation.

Many forms of activity-dependent synaptic plasticity exist, distinguished by their pharmacology, temporal dependence, directionality, and locus of induction (pre or postsynaptic). Plasticity lasting from milliseconds to minutes is known as short-term plasticity, whereas plasticity lasting on the order of hours to days is referred to as long-term plasticity.

Before we go any further, a brief introduction into basic synaptic transmission is warranted.

1.2.5.1 Synaptic Transmission

When EC neurons fire they excite dentate granule cells, in essence passing along information in the form of synaptic transmission. EC pyramidal cell axons (medial and lateral perforant pathways) come in close contact with the dendrites of DG granule cells and form synapses within the DG molecular layer. The axon terminal of the EC cell contains synaptic vesicles that are filled with the neurotransmitter glutamate and upon arrival of an action potential the vesicle fuses with the presynaptic membrane and releases glutamate into the synaptic cleft. Glutamate travels through this cleft over to the postsynaptic cell where it binds to and activates receptors located on the plasma membrane of spines protruding from the granule cell dendrites. Activation of postsynaptic glutamate receptors results in the net flux of positive ions into the cell and the generation of an excitatory postsynaptic potential (EPSP).

EPSPs are typically the result of activation of two types of glutamate receptors. Binding of glutamate to α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) receptors results in the opening of the channel pore and the net flow of positive ions (sodium, Na⁺) into the cell. AMPA receptor activation thus leads to rapid depolarization of the postsynaptic cell (i.e. granule cell) and is a large contributor to the EPSP. In
addition, N-methyl-D-aspartate (NMDA) receptors contribute to the EPSP, by regulating the duration of synaptic transmission. Activation of NMDA receptors requires the binding of both glycine and glutamate. However, sufficient depolarization of the granule cell is also needed to release the NMDA receptor magnesium (Mg$^{2+}$) block and allow the channel to open. NMDA receptors are selective for cations and activation results in an influx of Na$^+$ and calcium (Ca$^{2+}$) ions. The movement of ions across the cell membrane results in a change in the membrane potential (i.e. EPSP) that can be detected with an extracellular electrode.

1.2.5.2 Short-term Plasticity

Short-term plasticity is typically the result of a change in presynaptic function (Mochida et al 2008). These presynaptic changes last on the order of milliseconds to minutes and are thought to be related to changes in transmitter release. Two mechanisms can account for these changes in transmitter release (reviewed in (Malenka & Siegelbaum 2003): 1) A presynaptic action potential can induce a change in the amplitude of the transient rise in intracellular Ca$^{2+}$; 2) Modulation of the synaptic vesicle cycle. Paired pulse plasticity and post-tetanic plasticity are forms of pre-synaptic plasticity that will be recorded and evaluated in this thesis.

1.2.5.2.1 Paired Pulse Plasticity

When two stimuli (in our case current pulses) are delivered to the presynaptic cell with a short inter-stimulus interval (5-500 msec), the synaptic response (i.e. EPSP) to the second stimulus changes relative to the first stimulus. This form of short-term plasticity is known as paired pulse (PP) plasticity and can be expressed as either facilitation or depression of the synaptic response. PP plasticity is the result of a change in presynaptic transmitter release and is triggered by the biochemical processes occurring within the presynaptic axon terminal. The display of facilitation or depression largely depends on the initial state of the synapse and the transmitter release probability of the cell (Dobrunz & Stevens 1997).

Paired pulse facilitation (PPF) is characterized by an enhancement of the synaptic response (i.e. transmitter release) to the second stimulus. PPF involves the increase in transmitter release to the second stimulus (as compared to the first) and is likely the result
of Ca\(^{2+}\) influx following the first stimulus (Malenka & Siegelbaum 2003). PPF occurs in synapses that have a low probability of transmitter release. In contrast, synapses that display PP depression (PPD) are said to have a high probability of release. PPD is thought to result from either a temporary depletion of docked vesicles (Liley & North 1953) or from a temporary decrease in release probability of the releasable pool (Wu & Borst 1999). Additionally, some evidence suggests that PPD may result from the inactivation of presynaptic Ca\(^{2+}\) currents (Forsythe et al 1998).

1.2.5.2.2 Post-tetanic Potentiation/Depression

Other forms of short-term plasticity can be observed following a conditioning stimulus (CS). CS are designed to induce numerous and prolonged trains of action potentials in the presynaptic cell. Following a CS, post-tetanic potentiation (PTP) or depression (PTD) of the EPSP can be seen and last for several minutes. PTP is caused by a slow rise of intracellular Ca\(^{2+}\) in the presynaptic terminal that persists following the CS (reviewed in (Zucker 1989). It has been suggested that mitochondria plays an integral role in PTP of synaptic transmission through the regulation of intracellular Ca\(^{2+}\) (Tang & Zucker 1997). Similar to PPD, PTD is thought to result from the transient depletion of the readily releasable pool or the inactivation of Ca\(^{2+}\) channels.

Although the molecular mechanisms behind PTP/D are not fully understood, recent research has shed light on a few candidates. One such mechanism involves the phosphorylation of synapsin I by calcium/calmodulin-dependent protein kinase II (CaMKII) (Greengard et al 1993) in PTP. However, the phosphorylation of both synapsin I and II by cAMP-dependent protein kinase A (PKA) and CaMKII can also regulate PTP. Deletion of both synapsin I and II lead to an increase in PTD, further demonstrating the role these proteins play in PTP/D (Rosahl et al 1995). The small GTP-binding protein rab3a also leads to an increase in PTD (Geppert et al 1994).

Because short-term plasticity acts on a short time scale, it is thought to play a role in information processing. Recent evidence demonstrates short-term plasticity’s contribution to the optimization of information transfer through specific firing patterns of corresponding neurons (Rotman et al 2011). Other studies have shown that transmission of information is dependent on the modification of release probability by short-term
plasticity (Zador 1998). Within the DG, short-term plasticity, through its short-term modifications of sensory information, is likely to contribute to the formation of short-term memories (Mongillo et al 2008).

1.2.5.3 Long-term Plasticity

Long-term plasticity is defined as an activity dependent long-lasting change in synaptic efficacy. Following a CS, synaptic transmission can either be increased (LTP) or decreased (LTD) over time, known as bidirectional synaptic plasticity. The directional change in synaptic efficacy is determined by the level of Ca$^{2+}$ influx into the postsynaptic cell and the resulting activity. Several properties of bidirectional synaptic plasticity make it a candidate mechanism for the establishment of stable memories.

1.2.5.3.1 Long-term Potentiation

NMDA mediated LTP is a “classic” and well characterized form of LTP. Upon repetitive presynaptic activation and the resulting release of glutamate, the postsynaptic cell is sufficiently depolarized and the NMDA Mg$^{2+}$ block is released (Wigström et al 1986). Removal of the Mg$^{2+}$ block allows Na$^{+}$ and Ca$^{2+}$ to flow into the cell through the NMDA receptor (Frank et al 1989). The ensuing rapid rise in intracellular Ca$^{2+}$ is necessary for the induction of LTP (Lynch et al 1983; Malenka et al 1989). The translation of the Ca$^{2+}$ entry into LTP is believed to occur through the activation of intracellular cascades that eventually lead to the enhancement of AMPA receptor mediated currents (Derkach et al 1999) and the insertion of additional AMPA receptors into the plasma membrane (Lu et al 2001; Shi et al 1999).

Typically, LTP is broken down into stages based on the temporal order of expressed mechanisms. However, these phases are not exclusive from each other and may overlap (Sweatt 2010). Potentiation of EPSPs lasting on order of 5-20 minutes post CS is known as short-term potentiation (STP) (Malenka 1994). STP is the result of NMDA receptor activation and the rapid increase in postsynaptic Ca$^{2+}$. The induction of STP is NMDA dependent, but independent of protein kinase activity (Sweatt 2010). Volianskis and Jensen (2003) also provide evidence that STP involves regulation of presynaptic activity by showing that the decay of STP depends on the rate of stimulation.
The early phase of LTP (E-LTP) sets in around 20-30 minutes post induction and can last for 2-3 hours. E-LTP is mediated by activation of various kinases, including CaMKII and protein kinase C (PKC). As Ca$^{2+}$ enters the postsynaptic cell it binds to calmodulin (CaM). As binding increases, CaMKII undergoes autophosphorylation, resulting in CaMKII activation even after Ca$^{2+}$ levels have subsided (Malenka et al 1989). Further neuronal activity translocates CaMKII to the postsynaptic density, where CaMKII phosphorylates plasma membrane bound AMPA receptors; thereby enhancing AMPA mediated currents and the insertion of additional AMPA receptors into the postsynaptic membrane (Malenka & Bear 2004; Zamanillo et al 1999). Interestingly, blockade of CaMKII prevents LTP in the lateral perforant path, but causes only small reductions in LTP in the medial perforant path (Zhang et al 2005). PKC’s role in E-LTP may be similar to that of CaMKII, given that activation of PKC leads to the phosphorylation of AMPA receptors at the same phosphorylation site (serine-831) as does CaMKII (Malenka et al 1989; Malinow et al 1988) (Figure 4).

Long-lasting LTP (late phase of LTP, L-LTP) requires protein synthesis at synaptic and/or dendritic sites (Winder et al 1999). L-LTP can persist for hours, days, weeks, up to a year (reviewed in (Lynch 2004). Mitogen activated protein kinase (MAPK) pathways are activated during LTP, indicated by phosphorylation of extracellular regulated kinase 1/2 (ERK1/2) (Klann & Sweatt 2008). Translocation of phosphorylated ERK1/2 to the nucleus, leads to the activation of transcription factor CREB and CRE-mediated gene expression (Abraham & Williams 2008; Davis et al 2000; Lynch 2004; Silva 2003). Activation of these signalling cascades leads to the regulation of transcription and protein synthesis in the nucleus (Kandel 2001). Although there is experiential evidence indicating that specific mRNAs and proteins are involved in L-LTP, it remains to be determined which ones are necessary (Vickers & Wyllie 2007). Stimulation paradigms that induce L-LTP also strongly induce the expression of immediate early genes (IEGs) including c-fos, Arc, and brain-derived neurotrophic factor (BDNF), indicating that IEGs are among proteins synthesized for maintenance of L-LTP (Castrén et al 1993; Cole et al 1989; Lyford et al 1995).

In addition to postsynaptic changes, there is evidence for both presynaptic and trans-synaptic modifications in the induction and maintenance of LTP. Presynaptic
changes, such as transmitter release probability, are thought to be regulated by the retrograde messenger nitric oxide released from the postsynaptic cell (Lu et al 1999). Trans-synaptic changes occurring in the synaptic cleft involve cell adhesion molecules, specifically increases in PSA-NCAM after CS application (Guiraudie-Capraz et al 2010).

Of important note, synaptic plasticity has been reported in the human DG (Beck et al 2000). Through a series of studies, it was shown that activity-dependent NMDA-LTP was readily induced in the perforant path DG synapse and that LTP properties were similar to those in the rodent (Beck et al 2000).

1.2.5.3.1 Experimental Induction of LTP

Experimentally, LTP is commonly induced by the application of a CS. Within the hippocampal formation, many early studies utilized high frequency stimulation (HFS) (Bliss & Lømo 1973; Collingridge et al 1983; Lovinger et al 1990). HFS commonly consists of repeated pulses/stimuli given at 100 Hz. This results in repetitive presynaptic activation and release of glutamate allowing for sufficient depolarization of the postsynaptic cell and removal of the Mg$^{2+}$ (Coan & Collingridge 1985). However, such closely spaced stimulations do not allow the postsynaptic membrane to return to resting potentials. This results in the temporal summation of non-NMDA potentials, leading to depolarization that eventually reaches the action potential threshold (Sweatt 2010). The resulting action potential generates a back-propagating action potential into the dendrites and depolarizes the NMDA receptor. Consequently, this is an artificial means of evoking the NMDA component of synaptic transmission (Herron et al 1986). Additionally, HFS has not been shown to occur physiologically in the behaving animal (Sweatt 2010). Taken together, HFS may not be the optimal paradigm for studying the mechanism underlying LTP (Morrisett & Swartzwelder 1993). Nevertheless, HFS provides a reliable means of inducing LTP and is still used today, mainly in in vitro recordings (Chen et al 2001; Van Praag et al 1999).

The “optimal” frequency for the induction of signal propagation from the DG to the CA3 has been reported to fall between 5 and 10 Hz (Andersen et al 1966; Mott & Lewis 1992). This frequency lies within that of theta oscillations (Vanderwolf 1969; Winson 1974), suggesting that the DG is tuned to theta-like oscillations (Hsu 2007 Bol
163, Chapter 32). When a rat explores a new environment or is learning, theta oscillations are prominent in limbic structures including the hippocampal formation (Buzsáki 2002; 2005; Klausberger et al 2003; Winson 1978). Stimulation patterns that mimic theta frequency, such as theta burst stimulation (TBS), have been successfully implemented in the induction of synaptic plasticity *in vivo* and *in vitro* (Bawin et al 1984; Larson et al 1986; Pavlides et al 1988). TBS typically consists of pulses, trains, or bursts of stimulation given at 200 ms intervals (i.e. 5 Hz). Although the 200 ms interval provides plenty of time for the postsynaptic membrane to return to resting potentials, temporal summation is still taking place (Sweatt 2010). This summation is believed to result from an increase of synaptic disinhibition mediated through activation of GABA$_B$ receptors located on presynaptic cells (Brucato et al 1996; Nathan et al 1990; Nathan & Lambert 1991; Stäubli et al 1999). Following disinhibition, increased excitability ensues, NMDA receptors are activated, Ca$^{2+}$ flows into the postsynaptic cell, and LTP is induced (Sweatt 2010). Apart from initial induction mechanisms, there is a tacit assumption that different patterns of LTP activation all engage the same basic mechanism (Steward et al 2007).

Within the slice preparation (i.e. *in vitro*) the induction of NMDA-LTP within the DG requires pharmacological disinhibition (Colino & Malenka 1993; Motro et al 1996) see Appendix B). In the absence of disinhibition, small to no LTP can be obtained (Hanse & Gustafsson 1992; Nguyen & Kandel 1996; Wigström & Gustafsson 1983). Granule cells have a high resting membrane potential (Williamson et al 1993) and GABA receptor mediated inhibition (Mody 2005). GABA$_A$ receptors contribute to both phasic (synaptically located) and tonic inhibition (extrasynaptically located) within the hippocampal formation. Unlike GABA$_B$ receptors, GABA$_A$ are responsible for mediating fast inhibition resulting from the influx of chloride into the cell. In the *in vitro* hippocampal slice preparation, the systems regulating inhibition are severed (originating from the medial septum) and enhanced inhibition cannot be overcome by repeated stimulation. Correspondingly, an early study by Wigstrom and Gustafsson suggested that the successful induction of LTP in the DG required inhibition of the GABA$_A$ receptor (1983). Since then, it has become common practice to block GABA inhibition with a GABA$_A$ receptor antagonist such as bicuculline methiodide (BMI) or picrotoxin.
Figure 4 Basic cellular mechanisms mediating long-term potentiation and depression.

Depiction of post-synaptic mechanisms associated with changes in synaptic efficacy. Representative EPSP traces are presented below each model. The size, either measured by the initial slope or by the amplitude, of the EPSP is indicative of synaptic transmission. **Left:** During basal/baseline synaptic transmission, glutamate (black circles) binds to both AMPA and NMDA receptors on the postsynaptic neuron. Sodium (Na\(^+\)) enters the neuron through AMPA receptors, while ion flow through the NMDA receptor is blocked by magnesium (Mg\(^{2+}\)) (grey circle).

**Middle:** Long-term Potentiation (LTP) elicited by robust simulation is the result of the depolarization of the postsynaptic cell, removal of the magnesium block, and flow of both Na\(^+\) and calcium (Ca\(^{2+}\)) into the postsynaptic neuron. The increase in calcium activates calcium/calmodulin-dependent protein kinase (CaMKII) and triggers intracellular events which eventually lead to AMPA receptors insertion into the membrane (top) and an increase in postsynaptic efficacy or strength, as shown through an enhancement of the EPSP (bottom).

**Right:** Long-term depression (LTD; NMDA-dependent form shown) is the result of low levels of calcium entering the postsynaptic cell through NMDA receptors. These low levels activate calcineurin leading to the activation of protein phosphatase (PP1) and eventually AMPA receptor endocytosis. LTD is typically induced through low frequency stimulation and results in a decrease in synaptic efficacy, as shown through a decrease in the EPSP (bottom).

1.2.5.3.2 Long-term Depression
After LTP had been observed in the DG, it was suggested that in order for synaptic strengthening to be thought of as a mechanism for information processing, other forms of plasticity must exist that selectively weaken specific sets of synapses. In 1992, homosynaptic LTD was discovered in the CA1 and is now believed to be the counterpart to LTP in learning processes and memory formation (Dudek & Bear 1992; Mulkey & Malenka 1992). LTD is operationally defined as a long-lasting decrease in synaptic strength following application of a CS to presynaptic fibers.

The application of a CS, typically a low frequency activation, results in a modest increase in intracellular Ca\(^{2+}\) levels over a period of time (3-30 minutes) (Yang et al 1999). There are two prominent forms of LTD in the hippocampal formation, dependent on the activation of either NMDA receptors or metabotropic glutamate receptors (mGluR) (Dudek & Bear 1992; Mulkey & Malenka 1992; O'Mara et al 1995). In NMDA-dependent LTD, CaM detects Ca\(^{2+}\) entry through NMDA receptors and activates calcineurin (also known as protein phosphatase 2B). Calcineurin in turn activates downstream pathways including the activation of protein phosphatase 1 (PP1) (Mulkey & Malenka 1992). PP1 acts by dephosphorylating various targets, including AMPA receptors in the postsynaptic membrane thereby reducing transmission through AMPA receptors and leading to AMPA receptor internalization (Collingridge et al 2004). Additionally, the neuronal Ca\(^{2+}\) sensor protein hippocalcin is activated by modest levels of Ca\(^{2+}\) and through downstream mechanisms results in the removal of AMPA receptors from the plasma membrane (Palmer et al 2005) (Figure 4).

In contrast, mGluR-dependent forms of LTD rely on the activation of phosphoinositide-specific phospholipase C (PLC). PLC activates inositol trisphosphate (IP3) and PKC. IP3 elicits the release of Ca\(^{2+}\) from intracellular stores and PKC initiates endocytosis of AMPA receptors (Oliet et al 1997). In addition, it has been proposed that Ca\(^{2+}\) influx through voltage-gated Ca\(^{2+}\) channels (Christie et al 1996) or release from intracellular stores contributes to LTD (Nishiyama et al 2000).

1.2.5.4 Candidate Mechanism for Learning and Memory Formation

It has been proposed that learning and memory occurs in the CNS through changes in synaptic weights (Bear & Linden 2003) and that bidirectional synaptic
plasticity is needed for the regulation of these weights (Deco & Rolls 2005; Rolls & Kesner 2006). These bidirectional changes, if rapidly induced and long lasting, make an ideal model for the mechanisms underlying fast acquisition of information and formation of long-term memories. This would suggest that both LTP and LTD are needed for the processing of information and the formation of memories.

Several properties of NMDA-LTP make it the most studied form of synaptic plasticity. Not only has it been observed in practically all excitatory synapses in the CNS, but it is also readily inducible in hippocampal slices (Malenka & Siegelbaum 2003). Furthermore, LTP is believed to underlie hippocampal dependent learning and memory formation. The physiological properties of synaptic plasticity make it an ideal candidate for the learning and memory processes (reviewed in (Bliss & Collingridge 1993; Malenka 2003; Shors & Matzel 1997). First, LTP can be evoked in all hippocampal excitatory pathways. Second, LTP expression is input specific, meaning that only those synapses that had been stimulated were potentiated. The storage capacity for information is increased when plasticity is regulated at individual synapses, thus LTP being synapse specific is suggestive of greater storage capacity (Escobar & Derrick 2007). Third, LTP is associative, meaning weak input coinciding with strong input on another synapse of the same postsynaptic cell can be potentiated and result in LTP of the weak synapse. This property demonstrates the ability of associated synapses to relate patterns of activity and is indicative of classical conditioning. Fourth, LTP can be induced quickly and persist for long periods of time, similar to that of memories. Lastly, LTP (and LTD) can be artificially induced in vivo with stimulations that mimic physiological firing patterns that are associated with hippocampal theta rhythms (Stäubli & Lynch 1987). Together, these assets provide evidence for LTP as a candidate mechanism for learning and memory formation.

Experimental data also provides compelling evidence for LTP’s involvement in hippocampal function. Numerous studies have reported correlations between LTP and performance on hippocampal dependent tasks (Barnes & McNaughton 1985; Chapman et al 1999; Korol et al 1993; Lipp & Wolfer 1998). Additionally, it has been shown that while an animal is exploring a novel environment or avoidance learning there is an associated increase in hippocampal fEPSPs (Green et al 1990; Whitlock et al 2006).
Furthermore, the application of NMDA receptor antagonists not only blocks the induction of LTP, but also alters hippocampal dependent behaviors (Bannerman et al 1995; Morris 1989). Genetic manipulations have also been used to both alter LTP and behavioral performance (Grant et al 1992; Hinds et al 1998; Migaud et al 1998). Furthermore, loss of LTP is associated with a decrease in the stability of place fields (Agnihotri et al 2004; Rotenberg et al 2000), suggesting the role of LTP in spatial memory formation.

The role of LTD in learning and memory processes has been demonstrated through both behavioral and genetic approaches. Detection of novel objects within an environment results in the induction of LTD in the CA1 (Manahan-Vaughan & Braunewell 1999). Furthermore, enhancement of LTD through the application of an NMDA co-agonist can enhance reversal learning of novel target location (Duffy et al 2007). Selective knockout of the NR2B subunit of NMDA receptors results in the abolishment of LTD in the CA1 and impairment in numerous hippocampal dependent tasks (Brigman et al 2010). LTD in the CA1 of freely moving rats was prevented by NR2B antagonists and these same antagonists impaired spatial memory consolidation (Ge et al 2010). These studies support a functional requirement of LTD in spatial learning and memory formation.

1.3 Fetal Alcohol Spectrum Disorders and the Hippocampal Formation

The most common and profound effects of prenatal ethanol exposure are on the development of the CNS. During pregnancy the fetal brain develops from a single pseudostratified cell layer beginning around day 18, to a functioning nervous system by birth. More than 100 billion neurons are born during this time period, forming thousands upon thousands of connections with each other and shaping the brain. Therefore the teratogenic effects of ethanol present in the CNS are not surprising (Guerri et al 2009). Generally, structures positioned along the midline are more susceptible to ethanol’s toxicity than other regions (Swayze et al 1997). These areas include the cerebellum, corpus callosum, prefrontal cortex, and hippocampal formation (Archibald et al 2001; 1

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1 New nomenclature for ligand-gated ion channel subunits has been implemented by the committee of International Union of Basic and Clinical Pharmacology Collingridge GL, Olsen RW, Peters J, Spedding M. 2009. A nomenclature for ligand-gated ion channels. *Neuropharmacology* 56:2-5. However, within this thesis the old nomenclature is used. NR2B = GluN2B; NR1 = GluN1; NR2A = GluN2A.
Autti-Rämö et al 2002; Nuñez et al 2011; Riley & McGee 2005). The remainder of this introduction will concentrate on the changes within and related to the hippocampal formation.

Both human/clinical and animal studies have provided insight into the detrimental effects of ethanol on the hippocampal formation. The next section will summarize the clinical literature and then review the use of animal models of FASD and provide an in-depth examination of the changes observed in hippocampal synaptic plasticity following developmental ethanol exposure.

1.3.1 Human Studies

Many children who have been exposed to ethanol during development exhibit CNS abnormalities and behavioral and cognitive deficits (Hamilton et al 2003; Streissguth et al 1990), which increase in severity throughout life, as more complex tasks are taken on (Streissguth et al 1994; Willford et al 2004).

Increased volume of grey matter in the medial temporal lobe (home to the hippocampal formation) is typically observed in children who were exposed to high levels of ethanol in utero (Archibald et al 2001). MRI analyses revealed that children exposed to large amount of ethanol in utero had significantly less activation in the medial and posterior temporal regions during a verbal learning task, suggesting dysfunction of the temporal lobe memory system (Sowell et al 2007). Thus, the increase in grey matter may be due to an alteration in synaptic pruning or myelination (i.e. white matter) during development and lead to the reduction in activation during learning tasks. Examination of metabolite concentration of choline compounds revealed decreases in these levels in the left hippocampal formation along with decreases in overall volume (Astley et al 2009). Interestingly, because choline is typically found in white matter, these results further indicate possible deficits in myelination. Agenesis of the hippocampal commissure has also been shown in patients with PFAS (Bhatara et al 2002). In the same study, there was also a 25% reduction in the cerebral blood flow in the temporal lobes compared to the cerebellum, further indicating alterations in the development of the hippocampal formation and functional deficits due to prenatal ethanol exposure.
Studies examining the direct effects of FASD on the hippocampal formation have shown asymmetry in hippocampal morphology. Riikonen and colleagues revealed a reduction in the volume of the left hippocampal formation in both patients with FAS and other FASD disorders (Riikonen et al 1999; Riikonen et al 2005). Similar reductions in the left hippocampal volume were found in a later study that also showed that the hippocampal formation in individuals with PFAS does not increase in volume with age as it does in controls (Willoughby et al 2008). Furthermore, positive correlations between learning and memory (spatial delayed recall) and hippocampal volume were found in individuals with FASD (Willoughby et al 2008).

Temporal lobe damage in FASD individuals typically manifests as cognitive and behavioral impairments, many of which are associated with hippocampal function. Common behavioral and cognitive problems in children with FASD include learning difficulties, poor abstract thinking, poor adaptability, communication problems, and attention deficit hyperactivity disorder (Koren et al 2003).

An intellectual disability is characterized by having an intelligence quotient (IQ) score below 70, whereas scores with 71-85 are considered to have borderline intellectual functioning (DSM-IV 2000). Children with FAS have IQs estimated in the low 70s, but reports have shown this number to range from 20 to 120 in individuals (Olson et al 1998; Streissguth et al 1991). The mean IQ of those without full blown FAS is on average in the low 80s (Mattson & Riley 1998). Thus the majority of individuals with FASD fall in the borderline intellectual functioning category, with only 25% having IQ scores below 70 (Mattson & Riley 1998). Similarly, damage to the hippocampal formation early in one’s life can results in lower than average IQ (Vargha-Khadem et al 1997).

Developmental ethanol exposure has been associated with deficits in hippocampal-dependent behavioral task performance. Individuals with FASD exhibit deficits on spatial memory tasks and are impaired in place learning (Hamilton et al 2003; Mattson et al 1999; Uecker & Nadel 1996; 1998). Additionally, they have been shown to be impaired in spatial navigation and on episodic memory tasks (Vargha-Khadem et al 1997). Furthermore, when tested on a verbal learning task, individuals with FAS have a difficult time learning the information (Sowell et al 2007). However once learned, they have no problems in remembering the information. Together these studies have begun to
elucidate hippocampal structural abnormalities and associated behavioral difficulties observed in FASD individuals. Although deficits and damage are obvious in the clinical population, animal research provides scientists with the ability to determine the cause and effect of FASD specific to CNS damage.

### 1.3.2 Animal Models

When studying the effects of ethanol on the development of the hippocampal formation in rodent models it is important to control for the difference in the time course of development. Not only is human in utero development much longer than that of a rodent (nine months vs. 22 days), the hippocampal formation also has different developmental properties. In humans development of the hippocampal formation begins during the second half of the 2nd trimester and continues throughout the 3rd trimester (Seress 2007). In rodents, hippocampal development begins during the second half of the gestational period and continues following birth to around postnatal day (PD) 14 (Altman & Bayer 1990a; Bayer & Altman 1975). Thus, gestational days (GD) 1-10 in rodents (mouse and rat specific) are thought to be equivalent to the 1st trimester of human pregnancy in terms of neuronal development, GD 11-22 equivalent to the 2nd trimester, and PD 1-14 equivalent to the 3rd trimester (Dobbing & Sands 1973; 1979). Typically most rodent studies either give ethanol during the prenatal period (GD 1-22) of development or during the postnatal period (GD 4-9) to mimic either a 1st and 2nd trimester exposure or a 3rd trimester exposure, respectively.

In the FASD field, there are numerous rodent models which aim to mimic the human condition. These models are classified by the method in which ethanol is administered and include a liquid diet, oral gavage/intubation, vapor inhalation, artificial rearing, and subcutaneous or intraperitoneal injection. The first four are the more common methods currently used in FASD research. Unfortunately, there is no perfect model, with each being associated with different advantages and disadvantages (reviewed in (Gil-Mohapel et al 2010)). However, animal models have been extremely important in demonstrating the specificity of ethanol’s teratogenic effects and revealing the underlying changes in the CNS (Kelly et al 2009).
Rodent models have become a useful tool for evaluating the teratogenic effects of ethanol on brain structure and function and have provided valuable insight into our understanding of ethanol related disorders. We can reproduce many of the characteristics of FASDs in rodent models. Following developmental ethanol exposure, dsymorphology of facial features such as smaller head size, closely spaced nostrils, and abnormal upper lip have been observed (Godin et al 2010; Sulik 2005; Sulik & Johnston 1983). Typically reductions in body weight (i.e. growth deficits) are detected after developmental ethanol exposure in rodent models (Christie et al 2005; Helfer et al 2009b; Redila et al 2006) and furthermore, both CNS abnormalities and cognitive and behavioral deficits are commonly induced (Barnes & Walker 1981; Christie et al 2005; Godin et al 2010; Helfer et al 2009a; O’Leary-Moore et al 2011).

1.3.2.1 Structural and Behavioral Alterations in the Hippocampal Formation

One of the first studies to examine the effects of developmental ethanol exposure on the hippocampal formation reported a 20% reduction in the number of pyramidal neurons in the adult rat dorsal CA1 and a 3% reduction in granule cells in the adult DG (Barnes & Walker 1981). When examined in younger animals, a reduction of approximately 10% was reported in the CA1 (Perez et al 1991). Other studies in which the exposure lasted for all three trimester equivalents found a 12% reduction in CA1 pyramidal neurons and 11% reduction in DG granule cells in the adult hippocampal formation (Wigal & Amsel 1990). Since these earlier studies, others have gone on to examine trimester specific effects of ethanol exposure. Livy and colleagues (2003) found that exposure between GD 1-20 had no effect on pyramidal or granule cell number in young animals, but that exposure between GD 1-20 and PD 4-10 or PD 4-10 alone decreased these numbers in all regions of the hippocampal formation. These finding are in agreement with Maier and West (2001), but not Tran and Kelly (2003). The later study examined exposure during all three trimester equivalents and found a reduction in CA1 cell numbers but not in the CA3 or DG (Tran & Kelly 2003). Although there seems to be a few inconsistencies in the effect of developmental ethanol exposure on hippocampal cell numbers, it is apparent that ethanol is having an overall negative effect on the hippocampal structure. Following exposure during all three trimester equivalents, both
whole brain and hippocampal weights were reduced (Gibson et al 2000). Recently, through magnetic resonance microscopy researchers found a reduction in the volume of several brain regions of mice that were exposed to ethanol on GD 8, including pronounced reductions in the hippocampal formation (Parnell et al 2009).

Many studies have shown that pyramidal and granule neurons develop abnormally when exposed to ethanol prenatally. CA1 pyramidal neurons have reduced dendritic length and arborization following prenatal ethanol exposure (Davies & Smith 1981; Smith & Davies 1990; Yanni & Lindsley 2000). In addition, there is a 50% reduction (25% when given a water based ethanol diet) in the number of dendritic spines and a decrease in synapse number per innervated dendrite (Clamp & Lindsley 1998; Ferrer et al 1988; Kuge et al 1993; Lindsley & Clarke 2004). In the DG, prenatal ethanol exposure in rats resulted in an abnormal branching of granule cell axons (i.e. mossy fibers) (West & Hamre 1985) and decreased synaptic formations made with the CA3 (Tanaka et al 1991). Following exposure during the 2nd trimester equivalent aberrant mossy fiber terminals were seen in the stratum oriens and pyramidal cell layer of the CA3, where normally mossy fiber terminals are only found in the stratum lucidum of CA3 (Sakata-Haga et al 2003). These finding along with those demonstrating alteration in hippocampal size and cell number suggest that following developmental ethanol exposure this structure may lack the appropriate neural circuitry needed for proper functioning.

Animal studies support and add to the finding in humans demonstrating deficits in hippocampal-dependent learning and memory tasks after developmental ethanol exposure. Deficits in spatial acquisition and place memory in the Morris water maze have been shown to develop after prenatal exposure to ethanol (Blanchard et al 1987; Christie et al 2005; Gabriel et al 2002; Gianoulakis 1990; Iqbal et al 2004; Kim et al 1997; Richardson et al 2002; Westergren et al 1996). These deficits have also been shown in numerous studies utilizing the postnatal (PD4-9) rat model of ethanol exposure (Goodlett & Peterson 1995; Johnson & Goodlett 2002; Pauli et al 1995; Thomas et al 2008; Wozniak et al 2004). Furthermore, these impairments in the Morris water maze are similar to that produced by hippocampal lesions (Morris et al 1982).

Spatial learning deficits have also been demonstrated through the use of T-maze and radial arm maze, where impairments in the acquisition phase of these tasks are
typically seen in ethanol exposed animals (Abel 1982; Nagahara & Handa 1997; Reyes et al 1989; Stone et al 1996; Thomas et al 1996; Thomas et al 1997; Wainwright et al 1990; Zimmerberg et al 1991). In addition, it has been shown that ethanol disrupts other non-spatial tasks, such as contextual fear conditioning and delayed eye blink conditioning that also involve the hippocampal formation (Brown et al 2007; Hamilton et al 2011; Murawski & Stanton 2010). Many of these studies demonstrated behavioral effects that persisted well into adulthood (Gianoulakis 1990; Nagahara & Handa 1997; Stone et al 1996; Westergren et al 1996).

1.3.2.2 Alterations in Synaptic Plasticity

Not only has our knowledge regarding the neuroanatomical and behavioral effects of developmental ethanol exposure been broadened through the use of animal models, but they have provided considerable insight into the effects of ethanol on hippocampal function. The following is broken up this into two parts: i) the effects immediately following developmental ethanol exposure (neonatal period) and ii) the effects observed in adolescence and adulthood.

1.3.2.2.1 Short-term Plasticity: Neonatal Alterations

Recently it was shown that ethanol exposure during the 3rd trimester equivalent alters glutamatergic transmission by reducing AMPA receptor and NMDA receptor mediated EPSPs in the CA1 (Puglia & Valenzuela 2010b). Utilizing a vapor chamber ethanol exposure approach, these authors went on to show that there were no differences in synaptic strength due to exposure, as measured by AMPA and NMDA receptor mediated I/O curves and PP experiments (Puglia & Valenzuela 2010a; b).

The CA3 region of the hippocampus has also been shown to be susceptible to the damaging effects of ethanol during development. Mameli and colleagues (2005) demonstrated that application of ethanol (50 mM) to a slice preparation altered AMPA and NMDA mediated currents and PP ratios (reduced probability of glutamate release) and decreased AMPA and NMDA (only at 75 mM) evoked currents in the CA3. They showed that through inhibition of presynaptic N-type voltage gated Ca$^{2+}$ channels ethanol decreased transmitter release. Unfortunately, short-term effects on synaptic plasticity in the neonatal state have not been tested in the DG area of the hippocampus. Furthermore,
a correlation of these effects *in vivo* is lacking most likely due to the difficulties recording from such small pups.

1.3.2.2 Short-term Plasticity: Long-lasting Alterations

John J. Hablitz was the first to investigate whether alterations in synaptic plasticity occur in adult offspring exposed to ethanol while *in utero* (Hablitz 1986). In this initial report, PP facilitation was examined *in vitro* in the CA1 of PD 40-60 old rats that had been exposed to a liquid diet containing 35% ethanol derived calories (EDC) from GD 3-21. Hablitz reported that the pattern of evoked responses and I/O relationships were not altered by prenatal ethanol, indicating that the capability of fiber recruitment was not impaired. However, the degree of PP facilitation in the ethanol group was enhanced relative to controls over a range of inter-stimulus-intervals from 5-100 ms. These findings suggest that prenatal ethanol exposure results in a lower intrinsic release probability of neurotransmitter, thus inhibiting glutamate release onto CA1 neurons. Following this study, it was shown that PP facilitation in the CA1 was only increased when offspring (PD 90-120) were exposed *in utero* to high (35% EDC), but not low (17.5% EDC) levels of ethanol (Tan et al 1990), indicating that a certain level of intoxication is necessary for short-term presynaptic impairments to persist into adulthood. Furthermore, when exposed to ethanol (vapor inhalation, 35-45 mg/L) during just the 3rd trimester equivalent, there was no effect on CA1 PP facilitation at PD 46-58 (Bellinger et al 1999). These results suggest that the CA1 is more vulnerable to the toxic effects of ethanol during the first two trimesters of pregnancy than the third trimester alone, likely due to the fact that the bulk of the CA1 neuronal development occurs during the first two trimesters.

Interestingly, when tested in an *in vivo* preparation, ethanol exposure during the first two trimesters (Titterness & Christie 2008), all three trimesters (Richardson et al 2002), or the third trimester only (Byrnes et al 2004) had no effect on PP plasticity in the CA1, suggesting physiological differences between *in vitro* versus *in vivo* recordings.

To date, only one study has examined the long-term effects of developmental ethanol exposure on short-term plasticity in the DG. Christie and colleagues (2005) exposed pregnant dams to 6.61% *v/v* ethanol (35.5% EDC) and assessed PP facilitation
in vivo. They found that PP facilitation was not affected by prenatal ethanol exposure when examined in PD 64-78 male offspring, indicating that the involvement of presynaptic Ca\textsuperscript{2+} in vesicular release at least in the short-term was not impaired by developmental ethanol. Additionally, Sutherland and McDonald (1997) demonstrated that basal synaptic transmission in the adult DG was not affected by prenatal ethanol exposure (5% v/v).

1.3.2.2.3 Long-Term Plasticity: Neonatal Alterations

It is thought that synaptic plasticity plays a key role in early development of the brain (synaptogenesis, neurite outgrowth, etc.) and alterations in these mechanisms may contribute to the persistence of neurodevelopmental disorders (Galvan 2010). In rodents it was recently shown that ethanol exposure during the third trimester-equivalent impairs NMDA-dependent and AMPA-mediated fEPSP LTP in the CA1 (Puglia & Valenzuela 2010a; b). Puglia and colleagues showed that acute ethanol exposure directly onto hippocampal slices inhibited AMPA mediated LTP when concentrations were high (80 mM), suggesting that exposure to high levels of ethanol during the third-trimester has the potential to alter the development of the CA1 and thus changes in synaptic plasticity.

Taking a slightly different approach Zucca and Valenzuela (2010) examined the effects of postnatal ethanol exposure on the GABAergic system. BDNF dependent LTP-GABA\textsubscript{A} in CA3 pyramidal neurons was abolished by both short (slice exposure, 5-40 mM) and long (vapor chamber ethanol exposure, 1 or 2g/dl) term ethanol exposure during the third trimester equivalent in rodents. The effects were mediated through an inhibition of L-type voltage gated calcium channels (Zucca & Valenzuela 2010). Taken together, these results show that several hippocampal subregions are impaired by developmental ethanol exposure almost immediately.

1.3.2.2.4 Long-Term Plasticity: Long-term Alterations

Swartzwelder and colleagues were the first to investigate whether developmental ethanol exposure effects synaptic plasticity later in life (Swartzwelder et al 1988). They reported that exposure to a liquid diet containing 3% v/v ethanol (18.8% EDC) from GD 1-22 significantly decreased the magnitude of LTP recorded in the CA1 of young adult males. Since this initial study, numerous groups have examined the effects of
developmental exposure to ethanol on LTP in the CA1 and reported mixed results. While some studies reported a reduction in LTP following prenatal ethanol exposure (Izumi et al 2005; Richardson et al 2002), others reported that LTP was not affected by prenatal ethanol exposure (Bellinger et al 1999; Byrnes et al 2004; Krahl et al 1999; Tan et al 1990). These mixed results are likely due to a number of variables including slight variation in ethanol exposure, ethanol concentration, method of ethanol delivery, testing age, species used and in vivo vs. in vitro recording techniques. Another reason for differences in the long-term effects of ethanol exposure on CA1 LTP may be the use of diverse CS to induce LTP, leading to varied degree of NMDA receptor activation and ultimately AMPA phosphorylation/insertion into membranes. Because LTP in the CA1 is NMDA-dependent, any ethanol-mediated alterations in the function of these receptors can be a likely mechanism through which LTP is persistently altered in developmentally ethanol exposed animals. In line with this, it has been reported that developmental ethanol exposure leads to reductions in NMDA currents and glutamate binding in the adolescent and adult hippocampus (Morrisett et al 1989; Savage et al 1991). Additionally, decreases in mRNA levels of the NR1 subunit of the NMDA receptor in the adult hippocampal formation have been observed (Barbier et al 2008).

To date, there have only been two studies examining the effects of developmental ethanol exposure on LTD in the CA1 area. Following a single injection of ethanol during the third trimester equivalent (PD 0 or PD 7), LTD in the adolescent CA1 was reduced (Izumi et al 2005). However, when exposed to ethanol (6.61% v/v, 25.5% EDC) during the first two trimesters of gestation, CA1 LTD in vivo was not affected when measured in adolescent male and female offspring (Titterness & Christie 2008). These results suggest that select periods of development may be more vulnerable to ethanol than others, or alternatively, that compensatory mechanisms (receptor subunit up-regulation) come into play while offspring reach adolescent age in comparison to being tested at PD 0 or PD 7. Until the work in this thesis, it was not known how developmental ethanol exposure affected LTD in the DG.

Assessment of LTP in the DG of rats exposed prenatally to ethanol (5% v/v) found that LTP was reduced in male offspring well into adulthood (PD 120-150). Christie and colleagues (2005) further demonstrated these effects in the DG of younger adult
males (PD 64-78; 6.61% v/v; 35.5% EDC). When examined at an even younger age (PD 30-35), prenatal ethanol exposure (6.61% v/v; 35.5% EDC) still reduced the magnitude of LTP in the DG in males, but enhanced LTP in females (Titterness & Christie 2010), demonstrating a gender dependent effect of ethanol. Furthermore, the application of 3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), a competitive NMDA receptor antagonist, 90 minutes prior to the application of the CS significantly changed the slope of the fEPSPs during baseline measures in control animals, but not ethanol exposure animals. However, CPP did reduce the magnitude of LTP induced in all animals (Titterness & Christie 2010). These results suggest a dynamic contribution of NMDA receptors to LTP in ethanol exposed animals. In adult males, the application of a ten train CS failed to unmask deficits in LTP following prenatal ethanol exposure (5 % v/v). However, LTP elicited by a three train CS was significantly impaired by prenatal ethanol exposure (Varaschin et al 2010). Thus, LTP is only reduced in ethanol exposed animals when a sub-maximal number of trains are used to induce LTP (Varaschin et al 2010). This suggests that the effects of prenatal ethanol exposure on synaptic plasticity in the DG are of a more subtle nature and may indicate a dysfunction of mGluR₅ receptors, since they are known to play a facilitatory role in the expression of LTP under sub-maximal conditions (Naie & Manahan-Vaughan 2005; Raymond & Redman 2002; Raymond et al 2000; Varaschin et al 2010). These studies demonstrate that prenatal ethanol exposure effects DG plasticity in vivo that persists throughout life. However, the effects of developmental ethanol exposure on LTP in the DG have not been examined in vitro or in adult females until this thesis. There was also a lack of information in regards to the timing of ethanol exposure and the teratogenic effects on DG synaptic plasticity.
### Table 2 Literature Summary of FASD and Hippocampal Synaptic Plasticity In Vitro

<table>
<thead>
<tr>
<th>Hippocampal Subregion</th>
<th>Long-Term Form of Plasticity</th>
<th>Test Age</th>
<th>Alcohol exposure paradigm / BAC</th>
<th>Stimulation Paradigm</th>
<th>Short-term Plasticity</th>
<th>Long-term Plasticity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro, Slices</td>
<td>CA1</td>
<td>PD 40-60</td>
<td>Liquid Diet; GD 3-21; BEC 220 mg/dl</td>
<td>Pulse frequency: 0.2 Hz</td>
<td>PPF: Short IPI ↑ at 5-400 msec</td>
<td>I/O: -</td>
<td>Hablitz 1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PD 50-70</td>
<td>Liquid Diet; GD 1-22; 3.35% v/v; BEC 31 mg/dl</td>
<td>Pulse frequency: 2 min Duration: 0.05 msec Induction: 60 Hz, 10 sec</td>
<td>LTP M ↓</td>
<td>Swartzwelder et al, 1988</td>
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<tr>
<td></td>
<td></td>
<td>PD 90-120</td>
<td>Liquid Diet; GD 8-22; 35% EDC</td>
<td>Pulse frequency: 90 sec Duration: 0.1 msec Induction: 400 Hz, 200</td>
<td>PPF: M ↑ at 10-200 msec</td>
<td>LTP M –</td>
<td>Tan et al., 1990</td>
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<tr>
<td></td>
<td></td>
<td>PD 90-120</td>
<td>Liquid Diet; GD 8-22; 17.5% EDC</td>
<td>Pulse frequency: 90 sec Duration: 0.1 msec Induction: 400 Hz, 200</td>
<td>LTP M –</td>
<td>Tan et al., 1990</td>
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<td></td>
<td></td>
<td>PD 25-32</td>
<td>Oral Intubation; 4 g/kg/day, GD 8-20; BEC 120-180 mg/dl</td>
<td>Pulse frequency: 1 min Duration: 1 msec Induction: 400 Hz, 200</td>
<td>PPF: M – at 10-200 msec</td>
<td>I/O: M ↓</td>
<td>Krahl et al, 1999</td>
</tr>
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<td></td>
<td></td>
<td>PD 25-32</td>
<td>Oral Intubation; 6 g/kg/day, GD 8-20; BEC 200-240 mg/dl</td>
<td>Pulse frequency: 1 min Duration: 1 msec Induction: 400 Hz, 200</td>
<td>LTP M –</td>
<td>Krahl et al, 1999</td>
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<td>PD 63-77</td>
<td>Oral Intubation; 4 g/kg/day, GD 8-20; BEC 120-180 mg/dl</td>
<td>Pulse frequency: 1 min Duration: 1 msec Induction: 400 Hz, 200</td>
<td>PPF: M – at 10-200 msec</td>
<td>I/O: M –</td>
<td>Krahl et al, 1999</td>
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<td>PD 63-77</td>
<td>Oral Intubation; 6 g/kg/day, GD 8-20; BEC 200-240 mg/dl</td>
<td>Pulse frequency: 1 min Duration: 1 msec Induction: 400 Hz, 200</td>
<td>LTP M –</td>
<td>Krahl et al, 1999</td>
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<td>Oral Intubation; 4 g/kg/day, GD 8-20; BEC 120-180 mg/dl</td>
<td>Pulse frequency: 1 min Duration: 1 msec Induction: 400 Hz, 200</td>
<td>LTP M –</td>
<td>Krahl et al, 1999</td>
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<td>PD 46-50</td>
<td>Vapor Inhalation; 35-45 mg/L air, PD 4-9; BEC 351</td>
<td>Pulse frequency: 1 Hz Duration: 2x 100 Hz, 1 sec Induction: 400 Hz, 200</td>
<td>PPF: M – at 50 msec</td>
<td>I/O: M –</td>
<td>Bellinger et al, 1999</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PD 30-32</td>
<td>S.C. Injection; 2.25 g/kg, PD 0; BEC 200 mg/dl</td>
<td>Pulse frequency: 60 sec Duration: 100 Hz, 1sec or 200 Hz, 1 sec Induction: 400 Hz, 200</td>
<td>LTP ↓ for 100 Hz, 1 sec protocol</td>
<td>I/O: M –</td>
<td>Inumi et al, 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PD 30-32</td>
<td>S.C. Injection; 2.25 g/kg, PD 7; BEC 200 mg/dl</td>
<td>Pulse frequency: 60 sec Duration: 100 Hz, 1sec or 200 Hz, 1 sec Induction: 400 Hz, 200</td>
<td>LTP ↓</td>
<td>Inumi et al, 2005</td>
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<tr>
<td></td>
<td></td>
<td>PD 7-9</td>
<td>S.C. Injection; 40 mM</td>
<td>Pulse frequency: 0.033 Hz Duration: 75 usec Induction: 3x 100 Hz, 1 sec</td>
<td>PP: – at 50 msec</td>
<td>LTP ↓</td>
<td>Puglia and Balenzuela, 2010a</td>
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<td></td>
<td>PD 7-9</td>
<td>S.C. Injection; 40 mM</td>
<td>Pulse frequency: 0.033 Hz Duration: 75 usec Induction: 3x 100 Hz, 1 sec</td>
<td>PP: – at 50 msec</td>
<td>LTP ↓</td>
<td>Puglia and Balenzuela, 2010a</td>
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<td>PD 7-9</td>
<td>S.C. Injection; 4.5 g/dl, PD 2-9; 340/4 BEC mg/dl</td>
<td>Pulse frequency: 0.033 Hz Duration: 75 usec Induction: 3x 100 Hz, 1 sec</td>
<td>PP: –</td>
<td>LTP ↓</td>
<td>Puglia and Balenzuela, 2010b</td>
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<td></td>
<td>PD 7-9</td>
<td>S.C. Injection; 2.25 g/kg, PD 0; BEC 200 mg/dl</td>
<td>Pulse frequency: 60 sec Duration: 1 Hz for 15 min Induction: 3x 100 Hz, 1 sec</td>
<td>LTD ↓</td>
<td>Inumi et al, 2005</td>
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<tr>
<td></td>
<td></td>
<td>PD 30-32</td>
<td>S.C. Injection; 2.25 g/kg, PD 7; BEC 200 mg/dl</td>
<td>Pulse frequency: 60 sec Duration: 1 Hz for 15 min Induction: 3x 100 Hz, 1 sec</td>
<td>LTD ↓</td>
<td>Inumi et al, 2005</td>
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<td></td>
<td>PD 3-6</td>
<td>S.C. Injection; 20 pulses at 0.1 Hz, 500 msec</td>
<td>LTP-GABA ↓ in 5-40 mM exposed</td>
<td>Zueca et al, 2010</td>
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<td></td>
<td></td>
<td>PD 4-6</td>
<td>Vapor Inhalation; 1 or 2 g/dl, PD 1 or 2; 6-7 or 40 mM</td>
<td>Induction: 20 pulses at 0.1 Hz, 500 msec</td>
<td>LTP-GABA ↓</td>
<td>Zueca et al, 2010</td>
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<td>PD 45</td>
<td>L.P. Injection; 2.9 g/kg, GD 8; BEC</td>
<td>Pulse frequency: 0.2 Hz Duration: 5-200 Hz, 1 sec</td>
<td>LTP threshold ↑</td>
<td>Gomez et al, 1992</td>
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Table 3 Literature Summary of FASD and Hippocampal Synaptic Plasticity, *In Vivo*

<table>
<thead>
<tr>
<th>Developmental Ethanol Exposure</th>
<th>Hippocampal Subregion</th>
<th>Long-Term Form of Plasticity</th>
<th>Test Age</th>
<th>Alcohol exposure paradigm / BAC</th>
<th>Stimulation Paradigm</th>
<th>Short-term Plasticity</th>
<th>Long-term Plasticity</th>
<th>References</th>
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<tr>
<td></td>
<td>CA1</td>
<td>LTP</td>
<td>PD 40-50</td>
<td>Oral Intubation; 4 g/kg/day; GD 2-67; 30% v/v</td>
<td>Pulse frequency: 60 sec Induction: 3x 100 pulses</td>
<td>1/O: ↓</td>
<td>LTP M ↓; F ↓</td>
<td>Richardson et al., 2002</td>
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<td>LTP</td>
<td>PD 47-57</td>
<td>Oral Intubation; BAC 245 mg/dl</td>
<td>Pulse frequency: 30 sec Induction: 3x 100 pulses</td>
<td>PPF: M ↓; F ↓ at 30-500 msec</td>
<td>LTP M ↓; F ↓</td>
<td>Byrnes et al., 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LTD</td>
<td>PD 30-35</td>
<td>Liquid Diet; GD 1-22; 6.61% v/v; BEC 192 mg/dl</td>
<td>Pulse frequency: 15 sec Duration: 120 usec Induction: 900 pulses, 3</td>
<td>PPF: M ↓; F ↓ at 50 and 100 msec</td>
<td>LTD M ↓; F ↓</td>
<td>Titterness &amp; Christie, 2008</td>
</tr>
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<td></td>
<td></td>
<td>LTP</td>
<td>PD 120-150</td>
<td>Liquid Diet; GD 1-22; 5% v/v; BEC 83.2 mg/dl</td>
<td>Pulse frequency: 30 sec Duration: 100 usec Induction: 10x 400 Hz</td>
<td>1/O response −</td>
<td>LTP M ↓</td>
<td>Sutherland &amp; McDonald, 1997</td>
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<td></td>
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<td>LTP</td>
<td>PD 64-78</td>
<td>Liquid Diet; GD 1-22; 6.61% v/v; BEC 184 mg/dl</td>
<td>Pulse frequency: 15 sec Duration: 120 usec Induction: 5x (10x 5)</td>
<td>PPF: M ↓ at 25 - 100 msec</td>
<td>LTP M ↓</td>
<td>Christie et al., 2005</td>
</tr>
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<td></td>
<td></td>
<td>LTP</td>
<td>PD 30-35</td>
<td>Liquid Diet; GD 1-22; 6.61% v/v; BEC 86.9 mg/dl</td>
<td>Duration: 0.12 msec Induction: 4x (10x 5 Pulses, 400 Hz)</td>
<td>LTP M ↓; F ↑</td>
<td>Titterness &amp; Christie, 2010</td>
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<td>LTD</td>
<td>PD 105-140</td>
<td>Liquid Diet; GD 1-22; 5% v/v; BAC 84 mg/dl</td>
<td>Induction: 3x 400 Hz, 25 msec or 10x 400 Hz, 25 msec</td>
<td>1/O response −</td>
<td>LTP M ↓ with 3x400 Hz protocol</td>
<td>Varaschin et al., 2010</td>
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1.4 Summary and Objectives

FASDs result in lifelong impairments in cognition and behavior. The hippocampal formation is a temporal lobe structure that plays an integral role in learning and memory. The DG functions as a gate regulating EC input into the hippocampus and is important in pattern separation and the encoding of new spatial information. These learning and memory processes are believed to be the result of synaptic plasticity. It is thought that deficits associated with FASDs are manifested through impairments in synaptic plasticity. Therefore, a disruption in synaptic plasticity in the DG may contribute to the behavioral and cognitive deficits observed in both patients and rodent models of FASDs.

This thesis had five experimental objectives aimed at determining the potential mechanisms contributing to behavioral and cognitive deficits associated with FASD. First, to establish critical periods of DG development in relation to developmental ethanol (1st, 2nd, or 3rd trimester equivalent) induced alterations in both short and long-term plasticity. Second, determine the effects of prenatal ethanol exposure (1st and 2nd trimester equivalent) on bidirectional synaptic plasticity in the adult DG. Third,
determine if exposure to ethanol prenatally (1\textsuperscript{st} and 2\textsuperscript{nd} trimester equivalent) results in an alteration of drug (acute ethanol) sensitivity in the adult DG, measured through changes in LTP. Fourth, establish if developmental ethanol exposure differently effects male and females, assessed in the first three objectives. Five, determine if the use of different conditioning stimuli influence the magnitude of evoked LTP, assessed in the first three objectives.
2. Methods

2.1 Animals

Breeding animals were obtained from Charles Rivers and upon arrival were given at least a week to adjust to their new facility before breeding. The majority of experimental animals used in this study were generated in the animal care facilities at the University of Victoria. However, some ad libitum (AL) animals were obtained from Charles Rivers and acclimated to the facility prior to use. All animals were housed in clear polycarbonate cages (46 X 24 X 20 cm) with Carefresh contact bedding (Absorption Corp., Bellingham, WA, USA) in colony rooms kept at a constant temperature of 21°C and maintained on a 12 hour light/dark cycle. All animals were given ad libitum access to food and water, except when being administered ethanol or pair-fed diets. All animal experimentation was approved by the University of Victoria’s Animal Care Committee and was performed in accordance with the guidelines established by the Canadian Council on Animal Care.

2.2 Breeding

Virgin female Sprague-Dawley rats were paired with breeding males in standard cages with the addition of a metal enrichment tube. Pregnancy checks were performed in the early morning via a vaginal swab. Swabbing consisted of gently inserting an eye dropper filled with sterile 0.9 % saline solution into the vaginal opening and collecting a sample. The sample was then visually examined on a microscope slide with an Olympus microscope with a 10x objective (Olympus CX21, Center Valley, PA, USA) for the presence of sperm. The presence of sperm was used to indicate gestation day 1 (GD1). During pregnancy, females were weighed on GD 1, 7, 14 and 21. The day on which females gave birth was indicated as postnatal day 1 (PD1). On PD2/3, litters were culled to 10 pups and both dams and pups were weighed on PD8, PD 15 and PD22. The pups generated were then weaned and group housed according to gender on PD22/23. Due to unknown pregnancies, some maternal data is missing for AL dams.
2.3 Administration of the Liquid Ethanol Diet – Modeling FASD

In this thesis we have chosen to use the liquid diet model for our 1st and 2nd trimester equivalent exposures and the gavage model for our 3rd trimester equivalent exposure.

The prenatal ethanol diets were administered using well-established procedures (Keiver et al 1997; Keiver et al 1996; Titterness & Christie 2008; Titterness & Christie 2010). On GD1, pregnant dams were individually housed and assigned to one of three feeding groups (named for their associated offspring): (i) prenatal ethanol exposed (PNEE), in which dams were given ad libitum access to a liquid diet containing ethanol (35.5% ethanol derived calories; 6.61% v/v) (see individual studies methods sections for specifics on timing of exposure), (ii) pair-fed (PF), consisting of a similar liquid diet as the PNEE group but with an isocaloric substitution of maltose-dextrin for ethanol, (iii) AL, dams given ad libitum access to standard rat chow. All groups had ad libitum access to water throughout gestation. PNEE dams were slowly introduced to the ethanol during the first three days of diet administration by combining 1/3 ethanol diet with 2/3 pair-fed diet on the first day, 2/3 ethanol diet with 1/3 pair-fed diet on the second day, and 3/3 ethanol diet for the remainder of the diet administration period. Following the end of the specified diet administration period, dams were again given ad libitum access to standard rat chow to reduce any further deleterious effects of ethanol exposure on offspring (Weinberg 1989).

Sprague-Dawley rats, like most rodents, have a distaste for ethanol. Because of this, ethanol fed rats generally consume less calories (10-40%) than those given ad libitum access to normal rodent chow (Fisher et al 1997; Rao & Larkin 1987). Therefore, pair-fed animals serve to distinguish the effects of ethanol and undernutrition (i.e. caloric restriction) on development. Therefore, PF dams were offered an equivalent quantity of food in g/kg that matched that consumed by a PNEE dam on the corresponding day of gestation.

Consumption of the liquid diet was tracked daily by weighing each bottle. Bottles were then refilled with freshly prepared diet. This was done in the late afternoon prior to lights out to prevent a shift in the corticosteroid circadian rhythm that has been observed in animals fed a restricted diet (Gallo & Weinberg 1981). The diets were obtained from
Dyets Inc. (PNEE: 710324; PF: 710109; Bethlehem, PA, USA) and prepared by adding distilled water and ethanol (when appropriate). Both diets contained the necessary nutrients needed to provide adequate nutrition to dams, despite the decrease in the total amount of diet consumed when compared to the ad libitum animals (Weinberg 1985).

2.4 Postnatal Ethanol Exposure

Pregnant dams were individually housed and given free access to food and water. On PD 2/3, litters were culled to eight to ten pups and paw marked. On PD4, litters were assigned to one of two conditions: (i) AL, pups received no developmental treatment or (ii) intubation. A split litter design was implemented for the intubated litters such that pups within these litters were randomly assigned to either a sham intubated (SI3) or ethanol exposed (EE3) group.

Intubation treatments were administered three times a day from PD4 to PD9, corresponding to the 3rd trimester-equivalent of human pregnancy (Dobbing & Sands 1979). For each round of intubations, pups were removed from the dam as a litter and kept on a 37°C heating pad. A premeasured length of polyethylene tubing -10 was lubricated with corn oil and gently inserted down the pup’s esophagus into the stomach (Goodlett & Johnson 1997; Helfer et al 2009b). EE3 pups were intubated twice daily, two hours apart, and infused with a milk/ethanol solution containing 11.9% (v/v) ethanol, totalling 5.25g/kg of ethanol a day. This was followed by a milk only intubation, to supplement EE pups with calories that were lost due to a reduction in suckling. Solutions were infused over a 20-second period. SI3 pups received intubations, without infusion of any solution, since milk infusions are known to abnormally accelerate the growth of SI pups (Goodlett et al 1998).

All animals were weighed daily throughout treatment and this was used to calculate the volume of solution to be administered to the EE group. The milk solution was prepared from a base milk formula similar in composition to rat milk (West et al 1984) and supplemented with a specially formulated vitamin mix (Bio-Serv; Frenchtown, NJ, USA). The ethanol/milk solution was prepared daily by dissolving the 95% ethanol in the milk solution.
2.5 Blood Ethanol Concentrations

Blood ethanol concentrations (BECs) provide a measure of the degree of intoxication and are a reliable predictor of the severity of ethanol related brain insult (Bonthius et al 1988; Bonthius & West 1990). To assess peak BECs, blood samples were obtained by way of a tail clip from pregnant dams or postnatal pups, depending on the study. These samples were collected five hours after the presentation of the ethanol diet from PNEE dams and ninety minutes after the last milk/ethanol intubation from EE3 pups. At these times it is believed that the BECs are at their peak, and it is this peak that has been show to be a determining factor in fetal damage (Livy et al 2003; Maier & West 2001; West & Goodlett 1990). The blood was centrifuged 24 hours after collection and plasma was collected and stored at -20°C until assay. BECs were analyzed using an Analox GL-5 Alcohol Analyzer which measures oxygen consumption during oxidation of ethanol (Analox Instruments, Lunenburg, MA, USA).

2.6 Electrophysiology

2.6.1 Slice Preparation

Between PD 50-70, offspring were anesthetized with isoflurane, rapidly decapitated, and their brains removed in oxygenated (95% O2/5% CO2), ice-cold normal artificial cerebral spinal fluid (nACSF). nACSF contained (in mM) 125.0 NaCl, 2.5 KCl, 1.25 NaH2PO4, 25.0 NaHCO3, 2.0 CaCl2, 1.3 MgCl2, and 10.0 dextrose (pH 7.3). Transverse hippocampal slices (350 μm) were generated using a Vibratome Sectioning System 1500 (Ted Pella, Redding, CA, USA). Slices were kept in order using a modified 24-well plate and incubated in continuously oxygenated nACSF and maintained at 30°C. Sections were allowed to rest for a minimum of 1 hour before recordings commenced, to prevent spontaneous firing from interfering with experimentation (Figure 5).

An in vitro preparation was chosen so that the hippocampal structure could be isolated and it could be determined if the alterations in synaptic plasticity are truly due to a defect in the hippocampal formation.
Figure 5 *In vitro* Electrophysiology.

Hippocampal slices were acquired from adult rodents. Following incubation (minimum 1 hour), experiments were conducted in the medial molecular layer of the DG.

### 2.6.2 Recordings

Field recordings were collected in nACSF using an Axon MultiClamp 700B amplifier and Clampex 10.2 software (Molecular Devices, CA, USA). Using an Olympus BX51 microscope and motorized micromanipulators (Siskyou Design, OR, USA), electrodes were placed in the medial molecular layer of the DG, approximately 200 µm apart (Figure 5). Field excitatory postsynaptic potentials (fEPSPs) were elicited by delivering a 120 µs (10-40 µA) current pulse to the medial perforant path by way of a digital stimulus amplifier (Getting Instruments, CA, USA) and a single, concentric bipolar stimulating electrode (FHC, Bowdoin, ME, USA). fEPSPs were recorded using a single glass recording electrode (0.5-1.5 MΩ) filled with nACSF. A modified input/output (I/O) experiment was conducted in which the stimulation magnitude was increased until a maximal response prior to population spike appearance was acquired. Stimulation magnitude was then set to elicit approximately 50% of the maximal response.

A paired-pulse (PP) experiment was conducted using an inter-pulse interval of 50 ms (5x; 15 s between pairings) to assess presynaptic plasticity (McNaughton 1980) and confirm stimulation of the medial perforant path (Hanse & Gustafsson 1992).

Baseline measurements were collected using fEPSPs evoked every 15 seconds. A stable baseline of 20 minutes was required before a conditioning stimulus (CS) could be applied to the slice. A CS was used to induce both short-term and long-term forms of synaptic plasticity. Baseline stimulation parameters were returned to immediately following the conclusion of the CS and fEPSPs were recorded for a minimum of 60
minutes. An I/O experiment was then conducted with increasing stimulation magnitude (30 to 300 µs pulse width; 15 s intervals).

The studies in this thesis focus solely on transmission mediated through the medial perforant path because of its proximity to the granule cell layer, resilience of basil transmission to the application of GABA_A receptor antagonist, and popularity among neuroscientists (Hanse & Gustafsson 1992).

2.6.3 Conditioning Stimulus Protocols

Short and long-term synaptic plasticity were induced through the application of a CS. Post-tetanic potentiation (PTP) and long-term potentiation (LTP) of fEPSPs were induced using one of two CS: (i) high frequency stimulation (HFS) or (ii) theta burst stimulation (TBS). HFS consisted of four trains of 50 pulses at 100 Hz, 30 seconds apart; whereas, TBS consisted of four pulses at 100 Hz followed 200 ms later by another burst of four pulses, occurring five times with a 30 second inter-train interval. Post-tetanic depression (PTD) and long-term depression (LTD) of fEPSPs was induced using a CS of 1800 pulses delivered at 1 Hz over 30 minutes (low-frequency stimulation; LFS1800) or 900 pulses delivered at 1 Hz over 15 minutes (LFS900).

The GABA_A receptor antagonist bicuculline methiodide (BMI; Sigma-Aldrich, Oakville, ON, Canada) was included in the nACSF during the baseline and CS recordings for all LTP experiments. BMI was prepared as a concentrated stock solution and diluted with nACSF to a concentration of 10 µM prior to each recording. The optimal concentration of BMI was determined through a dose response experiment (see Appendix B) and corresponds to concentrations levels reported to fully block GABA evoked currents (Ueno et al 1997).

2.7 Data and Statistical Analysis

All electrophysiological data analysis was conducted with Axon ClampFit 10.2 software (Molecular Devices, CA, USA). The initial slope of the fEPSP was measured and used for all data analysis.

Measured slopes were processed for analysis using Excel 2007 (Microsoft, Redmond, WA, USA). PP ratios were calculated by dividing the slope of the second fEPSP by the first and converting this number into a percent change. I/O curves were
calculated by normalizing recordings to the value of the 5th pulse and reported as a percent change, with the 5th pulse equalling 100%. For all other experiments, recordings were normalized to the average value of the 20 minute baseline and reported as percent change from baseline. For all studies, data was presented as means ± standard error of the mean (SEM).

To examine if PTP or PTD was present, comparison of the average of the last 4 traces (i.e., -1-0 min) prior to the CS with the average of the first 4 traces of the post-conditioning baseline was conducted using student t-tests. To examine if LTP or LTD was present, comparison of the average of the last 20 traces (i.e., -5-0 min) prior to the CS with the average of the last 20 traces of the post-conditioning baseline was conducted using student t-tests with the exception of the BMI study (see Appendix B). PTP and PTD were measured by averaging the first four traces (i.e., first minute) of the post-CS recording, whereas LTP and LTD were measured by averaging the last 20 traces (i.e., 55-60 min) of the post-CS recording.

Comparisons between experimental groups were conducted using a one, two, or three-way analyses of variance (ANOVA), followed by Tukey HSD post hoc tests when appropriate. In the case of I/O analysis, a Repeated measures ANOVA was conducted. Significant main effects and interactions were further analyzed with one way ANOVAs and Tukey HSD post hoc tests. T-tests were also used to compare between groups, when appropriate. Results were processed for statistical analysis using Statistica 7.0 (Statsoft, Inc., Tulsa, OK, USA) and differences were considered significant when p < 0.05.
3. Fetal Alcohol Spectrum Disorders and Abnormal Synaptic Plasticity in the Adult Dentate Gyrus of Male and Female Offspring

3.1 Impact of the Timing of Exposure on Long-term Potentiation in the Adult Dentate Gyrus

Fetal alcohol spectrum disorders (FASDs) are characterized by CNS damage that manifests as behavioral and cognitive deficits. However, these deficits vary in severity between one individual and another. It is believed that these variation are related to the timing of ethanol exposure in utero (Maier et al 1996; Maier & West 2001).

The hippocampal dentate gyrus (DG) is vital for behavioral performance on learning and memory tasks. In both humans and rodents the DG develops over a prolonged period of time (Altman & Bayer 1975; Angevine 1965) and its development is highly susceptible to the teratogenic effects of ethanol. Thus, variations in the severity of behavioral deficits associated with FASD may be connected to DG development. Select periods of DG development may be more susceptible to ethanol’s teratogenic effects because of the temporal and regional appearance of critical developmental processes such as proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis.

The development of the DG starts at an early gestational period and continues through adulthood. In rats, DG development begins between GD 10 to PD 14 (Altman & Bayer 1975; Angevine 1965). Prior to this time point, the embryo develops into three germ layers and the basic layout of the CNS beginning to shape (Ladher & Schoenwolf 2005). The first DG granule cells are generated late into the 1st trimester equivalent at around GD 10 in the dentate neuroepithelium (Altman & Bayer 1975; Angevine 1965). Around GD 14, these cells along with dividing precursors, begin migrating through the dentate migratory stream along the already forming hippocampus proper and form a secondary dentate matrix (Altman & Bayer 1975; Seress 1977). Following further proliferation, these cells migrate into and begin forming the granule cell layer of the DG in an outside-in manner. This layer becomes recognizable around GD21-22 (Altman & Bayer 1990a; b; Bayer 1980). These cells also form a tertiary dentate matrix, which gives rise to the majority of granule cells generated postnatally (Altman & Bayer 1990a). During the first two weeks after birth, known as the brain growth spurt, 85% of the
granule cells are generated (Altman & Bayer 1975; Bayer 1980). The brain growth spurt is characterized by cellular proliferation and differentiation, neuronal migration, axonal growth, and synaptogenesis. Peak generation of hippocampal interneurons occurs between GD 13 and 14 in the DG (Soriano et al 1989a; b). Synaptic fibers from the EC first appear in the DG around GD 18 (Ceranik et al 1999; Super & Soriano 1994), but the number of synapses formed does not reach adult levels until around PD 25 (Crain et al 1973). Within the DG, commissural hippocampal connections form around PD 2 (Bayer & Altman 1987).

Less detail is known about DG development in humans. However, we are aware that neuroepithelial cell proliferation and migration occurs in humans from 7 to 20 weeks of pregnancy (Suzuki 2007), that DG granule cells begin generating late into the 1st trimester around the 10th or 11th week followed by differentiation and migration (Bayer & Altman 2007), and that the brain growth spurt occurs during the 3rd trimester of human pregnancy (Dobbing & Sands 1979; West et al 1987). Accordingly, the human 1st trimester of pregnancy (0-13 weeks) corresponds to developmental events during GD 1-10 in rats, 2nd trimester (14-27 weeks) corresponds to DG 11-21/22, and the 3rd trimester (28-41 weeks) corresponds to the first two weeks of postnatal development (PD 1-14).

In an effort to determine periods of vulnerability within DG development, we exposed pregnant dams or rat pups to ethanol during each individual trimester and evaluated the long-term effects of ethanol exposure on synaptic plasticity in the adult DG.

### 3.1.1 Methods

To model ethanol exposure during an equivalent human trimester of pregnancy, rat dams or pups were exposed to a liquid diet containing ethanol either during the (Figure 6): (i) 1st trimester equivalent = prenatal ethanol exposure 1 (PNEE1): were given ad libitum access to the ethanol liquid diet from gestation day (GD) 1-11; (ii) 2nd trimester equivalent = prenatal ethanol exposure 2 (PNEE2): pregnant dams were given ad libitum access to the ethanol liquid diet from GD 11-21; or (iii) 3rd trimester equivalent = ethanol exposure 3 (EE3): pups were fed an ethanol liquid diet through intubation from postnatal day (PD) 4-9. Appropriate pair-fed (PF) and ad libitum (AL) animals were also reared. To determine peak blood ethanol concentrations, blood samples
were taken on, GD 9 from PNEE₁ dams, GD 19 from PNEE₂ dams, and from EE₃ pups on PD 4.

Experimental recordings were conducted in early adulthood. A stimulating and recording electrode was placed in the DG medial molecular layer of hippocampal slices. Paired pulse (PP) ratios and input/output curves were recorded. Theta burst stimulation (TBS) or high frequency stimulation (HFS), moderate and robust stimuli respectively, were used to induce post-tetanic (PTP) and long-term potentiation (LTP).

**Figure 6 Experimental Timeline.**

The presence of sperm was used to indicate gestation day 1 (GD1). Rat dams or pups were exposed to a liquid diet containing ethanol either during the: (i) 1st trimester equivalent = prenatal ethanol exposure 1 (PNEE₁); (ii) 2nd trimester equivalent = prenatal ethanol exposure 2 (PNEE₂); or (iii) 3rd trimester equivalent = ethanol exposure 3 (EE₃). Appropriate pair-fed and ad libitum animals were also reared. Experimental recordings were conducted in early adulthood (postnatal day (PD) 50-70).

Means ± SEM were calculated for each group of animals. Overall effects across trimester equivalents were examined with a three-way factorial analysis of variance (ANOVA): developmental treatment (AL, PNEE₁, PF₁, PNEE₂, PF₂, EE₃, SI₃) x gender (male, female) x conditioning stimulus (TBS, HFS). Significant main effects and interactions were further analysed with Tukey HSD post hoc tests. Statistical differences within individual trimesters were examined with a three-way factorial ANOVA: developmental treatment (AL, PNEE/EE, PF/SI) x gender (male, female) x CS (TBS, HFS). Significant main effects and interactions were further analysed with tukey post hoc
tests. When no significant interaction between CS and any of the other factors was obtained, data from both TBS and HFS were pooled together. When no significant interaction between gender and any of the other factors was obtained, data from both males and females were pooled together.

3.1.2 Findings

3.1.2.1 1st Trimester Equivalent

3.1.2.1.1 Maternal and Offspring Parameters

All pregnant dams continued to gain body weight throughout gestation. There was no significant difference in percent weight gain over gestation ($F(2,9) = 3.11, p = 0.094$), in gestation length ($F(2,9) = 2.38, p = 0.148$), or in litter size ($F(2,12) = 0.13, p = 0.880$). The average BEC for PNEE$_1$ dams was $155.20 \pm 9.61$ mg/dl, representing a moderate ethanol exposure.

Offspring were weighed throughout the postnatal period until their experimental use in adulthood. Following birth, weights taken on the litter cull date (PD2/3) were significantly affected by prenatal treatment ($F(2,50) = 31.08, p < 0.001$) and gender ($F(1,50) = 39.91, p < 0.001$), no significant interaction of gender and prenatal treatment was found. At birth, male and female pup weights significantly differed ($p < 0.001$). The birth weight of PNEE$_1$ and PF$_1$ pups were significantly reduced compared to AL (PNEE$_1$ vs. AL: $p < 0.001$; PF$_1$ vs. AL: $p < 0.001$). At time of electrophysiological experimentation, there was a main effect of prenatal treatment ($F(2,53) = 7.277, p = 0.002$), gender ($F(1,53) = 209.53, p < 0.001$), and a significant interaction between treatment and gender ($F(2,53) = 4.58, p = 0.014$). Upon assessment, PNEE$_1$ males weighted significantly less than AL and PF$_1$ males (PNEE$_1$ vs. AL: $p = 0.007$; PNEE$_1$ vs. PF$_1$: $p < 0.001$). All maternal and offspring data are summarized in table 4.

3.1.2.1.2 Input/Output and Paired Pulse Plasticity

To determine whether prenatal ethanol exposure during the 1st trimester equivalent alters neural transmission, we conducted two experiments. First, excitatory synaptic transmission was characterized through I/O function. In all slices, the slope of the fEPSP significantly increased with increasing stimulation (repeated measures
ANOVA: F(8,984) = 1785.56, p < 0.001. PNEE1 had no significant effect on I/O function (F(2,123) = 1.06, p = 0.349), regardless of gender (F(1,123) = 0.22, p = 0.775).

Second, PP plasticity was examined to determine whether ethanol exposure altered presynaptic transmitter release. In both male and female offspring (F(1,123) = 0.15, p = 0.700), PP ratios were not significantly different among treatment groups (F(2,123) = 2.09, p = 0.127) (Figure 7).

![Graphs showing I/O Curve and PP Ratio for male and female offspring](image)

**Figure 7 Synaptic Transmission in the Medial Perforant Path Following Ethanol Exposure During the 1st Trimester Equivalent.**

*Top row:* Input/output (I/O) function and paired pulse (PP) ratios for male offspring. *Bottom row:* I/O function and PP ratios for female offspring. Prenatal treatment did not significantly affect synaptic transmission. Data presented as means ± SEM.

3.1.2.1.3 Post-tetanic and Long-term Potentiation

PTP was measured by averaging fEPSP responses over the first minute after the CS (Figure 8). A significant main effect of prenatal treatment on PTP was found.
(F(2,117) = 8.09, p < 0.001), such that lower levels of PTP were evoked in PF₁ offspring as compared to AL (p < 0.001) and PNEE₁ (p = 0.022). PF₁ treatment resulted in a 25% reduction of PTP from AL levels and a 18% reduction of PTP from PNEE₁. A significant main effect of gender was found (F(1,117) = 6.57, p = 0.012), such that higher levels of PTP were evoked in males than in females (p = 0.003). A main effect of CS on PTP (F(1,117) = 33.08, p < 0.001) showed that HFS evoked higher levels of PTP than did TBS (p < 0.001). However, there were no significant interactions between any of the variables.

LTP was measured by averaging fEPSP responses over 56-60 minutes after the CS (Figure 8). A significant main effect of prenatal treatment on LTP was found (F(2,117) = 7.78, p < 0.001), such that lower levels of LTP were evoked in PF₁ offspring as compared to AL (p < 0.001). PF₁ treatment resulted in a 35% reduction of LTP from AL levels. A significant main effect of gender was found (F(1,117) = 4.17, p = 0.043), such that higher levels of PTP were evoked in males than in females (p = 0.016). A main effect of CS on LTP (F(1,117) = 10.93, p = 0.001) showed that HFS evoked higher levels of LTP than did TBS (p < 0.001). However, there were no significant interactions between any of the variables.
Figure 8 Effect of 1st Trimester Equivalent Ethanol Exposure on PTP and LTP in All Offspring.

Pair-fed (PF) treatment during the 1st trimester equivalent significantly attenuated PTP and LTP in adulthood. Top: fEPSP response to CS in the medial molecular layer of the DG. Bottom Left: PTP following PNEE1. Bottom Right: LTP following PNEE1. ◆ corresponds to significance level p < 0.05 from AL; ★ corresponds to significance level p < 0.05 from PNEE1; + indicates a main effect of gender. Data presented as means ± SEM. No significant interaction between CS and any of the other variables was obtained, thus data from TBS and HFS are graphed together.

3.1.2.2 2nd Trimester Equivalent

3.1.2.2.1 Maternal and Offspring Data

All pregnant dams continued to gain body weight throughout gestation, regardless of diet (F(2,8) =0.04, p = 0.961). Furthermore, there were no significant difference in litter size (F(2,8) = 0.55, p = 0.600) or gestation length (F(2,11) = 1.44, p = 0.280). The average BEC for PNEE2 dams was 142.47 ± 8.36 mg/dl.

Offspring were weighed across the postnatal period until their experimental use in adulthood. Following birth, weights taken on the litter cull date (PD2/3) were
significantly affected by prenatal treatment (F(2,47) = 10.54, p < 0.001) and gender (F(1,47) = 14.34, p < 0.001). There was no significant interaction between prenatal treatment and gender. At birth, PNEE\textsubscript{2} and PF\textsubscript{2} pups weighed significantly less than AL (PNEE\textsubscript{2} vs. AL: p < 0.001; PF\textsubscript{2} vs. AL: p = 0.001). At time of electrophysiological experimentation (young adulthood), there was a main effect of gender (F(1,50) = 113.65, p < 0.001), but not prenatal treatment. However, there was a significant interaction between prenatal treatment and gender (F(2,50) = 5.47, p = 0.007). Upon assessment, males weighed significantly more than females across all prenatal treatment groups (p < 0.001) and PNEE\textsubscript{2} males weighed significantly less than AL males (p = .014). All maternal and offspring data are summarized in table 4.

3.1.2.2.2 Input/Output and Paired Pulse Plasticity

To determine whether prenatal ethanol exposure during the 2\textsuperscript{nd} trimester equivalent alters neural transmission, we conducted two experiments. First, excitatory synaptic transmission was characterized through I/O function. fEPSP responses increased with increasing pulse width in all offspring (repeated measures ANOVA; F(8,1008) = 1318.10, p < 0.001). Neither prenatal treatment nor gender had any significant effect on I/O function. There was no significant interaction between prenatal treatment and gender. Second, to investigate whether PNEE\textsubscript{2} altered glutamate release, PP plasticity was measured. There was no main effect of prenatal treatment or gender on PP, nor was there a significant interaction between the two variables (Figure 9).
Figure 9 Synaptic Transmission in the Medial Perforant Path Following Ethanol Exposure During the 2nd Trimester Equivalent.

Top row: Input/output (I/O) function and paired pulse (PP) ratios for male offspring. Bottom row: I/O function and PP ratios for female offspring. Prenatal treatment did not significantly affect synaptic transmission. Data presented as means ± SEM.

3.2.2.1.3 Post-tetanic and Long-term Potentiation

PTP was measured by averaging fEPSP responses over the first minute after CS (Figure 10). A significant main effect of prenatal treatment on PTP was found (F(2, 122) = 6.52, p = 0.002), such that lower levels of PTP were evoked in PNEE₂ (p = 0.027) and PF₂ (p = 0.006) offspring as compared to AL. PNEE₂ treatment resulted in a 16% reduction from AL and PF₂ treatment resulted in a 18% reduction of PTP from AL. PTP in PNEE₂ and PF₂ offspring did not differ (p = 0.910). A main effect of CS on PTP (F(1, 122) = 52.96, p < 0.001) showed that HFS evoked higher levels of PTP than did TBS (p < 0.001). However, there was no main effect of gender or a significant interaction between any of the variables.
LTP was measured by averaging fEPSP responses over 56-60 minutes after the CS (Figure 10). A significant main effect of prenatal treatment on LTP was found (F(2,122) = 14.74, p < 0.001), such that lower levels of LTP were evoked in PNEE2 (p < 0.001) and PF2 (p < 0.001) offspring as compared to AL. PNEE2 treatment resulted in a 48% reduction of LTP from AL levels and PF2 treatment resulted in a 31% reduction of LTP from AL levels. LTP in PNEE2 and PF2 offspring did not differ (p = 0.107). There was no main effect of gender or CS and there was no significant interaction between any of the variables.
Figure 10 Effect of 2nd Trimester Equivalent Ethanol Exposure on PT and LTP in All Offspring.

Prenatal ethanol exposure and caloric restriction during the 2nd trimester equivalent reduced PTP and LTP in adulthood. *Top:* fEPSP response to CS in the medial molecular layer of the DG. *Bottom Left:* PTP in the changes following PNEE2. *Bottom Right:* LTP in the changes following PNEE2. * corresponds to significance level p < 0.05 from AL. Data presented as means ± SEM. No significant interaction between gender and any of the other variables or between CS and any of the other variables were obtained, thus data from males and females and from TBS and HFS were graphed together.

3.1.2.3 3rd Trimester Equivalent

3.1.2.3.1 Offspring Parameters

Offspring were weighed across the postnatal period until their experimental use in adulthood. Following birth, weight did not differ across any offspring (p > 0.116). Weights were again taken on PD 8 to test whether postnatal treatment affected weight. At this time point, there was a main effect of postnatal treatment (F(2,49) = 5.45, p = 0.007),
revealing that EE\textsubscript{3} offspring weighed significantly less than AL (p = 0.016) and SI\textsubscript{3} (p = 0.012) offspring. There was no main effect of gender and no interaction between the two variables. At the time of electrophysiological experimentation, there was a main effect of gender (F(1,49) = 231.81, p < 0.001) indicating that males weighed more than females when they reached young adulthood. There was also a significant interaction between postnatal treatment and gender (F(2,49) = 3.56, p = 0.036), but no main effect of postnatal treatment. Further analysis showed that male weights did not differ across treatment groups (p > 0.092). Similarly, female weights did not differ across treatment groups (p > 0.791).

The average BEC for male EE\textsubscript{3} offspring was 347.40 ± 21.29 mg/dl and for female EE\textsubscript{3} offspring was 314.70 ± 15.85 mg/dl. These levels are similar to those found in previously published studies (Helfer et al 2009a; Helfer et al 2009b). Although males had slightly higher BECs than females, this difference did not reach significance (t(16) = 1.23, p =0.42). Similar finding have been reported elsewhere (Tran & Kelly 2003). All offspring data are summarized in table 4.

3.1.2.3.2 Input/Output and Paired Pulse Plasticity

To determine whether prenatal ethanol exposure during the 3\textsuperscript{rd} trimester equivalent alters neural transmission, we conducted two experiments. First, excitatory synaptic transmission was characterized through I/O function. fEPSP responses increased with increasing pulse width in all treatment groups (repeated measures ANOVA: F(8,976) = 1563.19, p < 0.001). Neither postnatal treatment nor gender had any significant effect on I/O function. There was no significant interaction between postnatal treatment and gender. Second, to investigate whether EE\textsubscript{3} altered glutamate release, PP plasticity was measured. There was no main effect of postnatal treatment or gender on PP, nor was there a significant interaction between the two variables (Figure 11).
Figure 11 Synaptic Transmission in the Medial Perforant Path Following Ethanol Exposure During the 3rd Trimester Equivalent.

Top row: Input/output (I/O) function and paired pulse (PP) ratios for male offspring. Bottom row: I/O function and PP ratios for female offspring. Postnatal treatment did not significantly affect synaptic transmission. Data presented as means ± SEM.

3.2.2.1.3 Post-tetanic and Long-term Potentiation

PTP was measured by averaging fEPSP responses over the first minute after the CS (Figure 12). A main effect of CS on PTP was found (F(1, 116) = 30.08, p < 0.001). This indicated that HFS evoked higher levels of PTP than did TBS (p < 0.001). There was no main effect of postnatal treatment or gender. Furthermore, there was no significant interaction between any of the variables.

LTP was measured by averaging fEPSP responses over 56-60 minutes after the CS (Figure 12). A significant main effect of CS on LTP was found (F(1,116) = 13.14, p < 0.001), such that HFS evoked higher levels of LTP than did TBS (p < 0.001). There was no main effect of postnatal treatment or CS. However, there was a significant interaction
between postnatal treatment and CS (F(2,116) = 3.19, p = 0.045). Specifically, within SI3 offspring, TBS evoked significantly less LTP than did HFS (p = 0.002). Within AL and EE3 offspring, TBS and HFS induced similar levels of LTP (p > 0.315). TBS evoked similar levels of LTP in all postnatal treatment groups (p > 0.126). Similarly, HFS evoked similar levels of LTP in all postnatal treatment groups (p > 0.376).

![Diagram showing Theta Burst Stimulation and High Frequency Stimulation](image)

**Figure 12** Effect of 3rd Trimester Equivalent Ethanol Exposure on PTP and LTP in All Offspring.

Within sham intubated (SI) offspring, TBS evoked significantly less LTP than did HFS. Top: fEPSP response to CS in the medial molecular layer of the DG. Bottom Left: PTP of following EE3. Bottom Right: LTP following EE3. ★ corresponds to significance level p < 0.05; + indicates a main effect of CS. Data presented as means ± SEM. Combination of male and female data is graphed.

### 3.1.2.4 Trimester Comparison

#### 3.1.2.4.1 Maternal and Offspring Parameters
All pregnant dams continued to gain body weight throughout gestation. There were no significant differences in percent weight gain over gestation (F(4,13) = 38.28, p = 0.083), in gestation length (F(4,13) = 0.97, p = 0.455), or in litter size (F(4,16) = 1.06, p = 0.408) among AL, PNEE\textsubscript{1} and PNEE\textsubscript{2} dams. Similar BEC levels were acquired from PNEE\textsubscript{1} (155.20 ± 9.61 mg/dl) and PNEE\textsubscript{2} (142.47 ± 8.36 mg/dl) dams (t(6) = 0.99, p = 0.356) and these are comparable to BECs acquired from pregnant dams receiving an ethanol diet throughout (see 3.2.2.1).

Offspring were weighed throughout the postnatal period until their experimental use in adulthood. Following birth, weights taken on the litter cull date (PD2/3) were significantly affected by developmental treatment (F(6,103) = 12.44, p < 0.001) and gender (F(1,103) = 13.62, p < 0.001), no significant interaction of gender and developmental treatment was found. At birth, female pups weighed significantly less than males (p < 0.001). The birth weight of all prenatally treated pups were significantly reduced compared to AL (PNEE\textsubscript{1} vs. AL: p = 0.007; PF\textsubscript{1} vs. AL: p < 0.001; PNEE\textsubscript{2} vs. AL: p = 0.006; PF\textsubscript{2} vs. AL: p = 0.029). Considering EE3 and SI3 pups at this time had not received developmental treatment, their weights were not different from AL and were significantly higher than prenatal treated pups (p < 0.01). At the time of electrophysiological experimentation (PD 50-70), there was a main effect of developmental treatment (F(6,104) = 3.92, p = 0.001), gender (F(1,104) = 313.60, p < 0.001), and a significant interaction between treatment and gender (F(6,104) = 3.62, p = 0.002). All female offspring weighed significantly less than male offspring (p < 0.001), a common gender difference at this age (River 2012). Upon assessment of the interaction, PNEE\textsubscript{1} and PNEE\textsubscript{2} males weighted significantly less than AL males (PNEE\textsubscript{1} vs. AL: p = 0.030; PNEE\textsubscript{2} vs. AL: p < 0.025). Furthermore, PNEE\textsubscript{1} males weighed significantly less than PF\textsubscript{1} males (p = 0.001). However, weights did not differ between the three ethanol treated groups. All maternal and offspring data are summarized in table 4.
### Table 4 Summary of Maternal and Offspring Parameters

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### 3.1.2.4.2 Post-tetanic and Long-term Potentiation

To test whether any one trimester is more vulnerable to the long-term effects of developmental ethanol exposure, we compared CS induced PTP and LTP across trimester equivalents. We observed the effects of developmental ethanol exposure on PTP and LTP elicited by TBS or HFS. Both TBS and HFS produced significant PTP and LTP in all offspring. All PTP and LTP data are summarized at the end of the findings section in table 5.

A significant main effect of developmental treatment (F(6,283) = 6.142, p < 0.001) revealed that PTP magnitude was lower in PF1 (p < 0.001) and PF2 (p = 0.028) offspring when compared to AL. Furthermore, PTP levels in SI3 were significantly higher than those in PF1 (p < 0.001) and PF2 (p = 0.008) offspring. A main effect of CS
(F(1,283) = 95.38, p < 0.001) revealed that HFS elicited significantly higher levels of PTP than did TBS (p < 0.001).

There was no main effect of gender found, but there was a significant interaction between developmental treatment and gender (F(6,283) = 2.86, p = 0.010). Subsequent analyses revealed that SI3 females had significantly more PTP than PF1 (p < 0.001) and PF2 (p = 0.045) females. However, this difference was not seen in male offspring.

Measured PTP levels are depicted in Figure 13.

**Figure 13 Effects of Developmental Ethanol Exposure on Post-tetanic Potentiation in the Young Adult DG.**

Pair-fed (PF) feeding in both the 1st and 2nd trimester equivalents resulted in a significant reduction of post-tetanic potentiation (PTP) magnitude compared to ad libitum (AL) controls. Additionally, sham intubation (SI) during the 3rd trimester equivalent resulted in an increase in the magnitude of evoked PTP compared to both PF1 and PF2. PF feeding differentially affected male and female offspring: Left: Within female offspring, the magnitude of evoked PTP in SI3 offspring was 43.39% higher than that in PF1 and 36.51% higher than PF2 offspring. Right: There were no significant differences between PTP between PF/SI male offspring. Data presented as means ± SEM. Dotted line provides a reference to the mean obtained from AL animals, surrounding dashed box represents the SEM. + corresponds to significance level p < 0.05 from PF1 and PF2.

A significant main effect of developmental treatment (F(6,283) = 12.03, p < 0.001) revealed that LTP magnitude was lower in PF1 (p < 0.001), PF2 (p = 0.028), and PNEE2 offspring when compared to AL. The magnitude of evoked LTP in AL offspring was 91% higher than that in PNEE2, 55% higher than PF2 offspring, and 44% higher than
PF2 offspring. The observed reduction in LTP following ethanol exposure during the 2nd trimester equivalent does not relate to the reduction in adult weight observed (r = 0.07, r² = 0.005, p = 0.401). Furthermore, overall assessment of a relationship between adult weight LTP magnitude did not reveal a significant relationship (r = -0.0276, r² < 0.001, p = 0.631).

Comparisons of ethanol treatment across the different trimesters demonstrated trimester specific effects. Specifically, EE3 resulted in an enhancement of LTP compared to both PNEE₁ (p = 0.004) and PNEE₂ (p < 0.001); and significantly lower LTP in PNEE₂ offspring than in PNEE₁ (p = 0.012). The magnitude of evoked LTP in EE₃ offspring was 40% higher than that in PNEE₁ and 125% higher than PF₂ offspring. These results indicate that ethanol exposure during the 2nd trimester equivalent produces the greatest ethanol induced alterations in LTP.

LTP levels in SI₃ offspring were significantly higher than PF₁ offspring (p < 0.001), indicating the developmental processes taking place during the 1st trimester is differentially effected by early life stress than it is during the 3rd trimester equivalent. A main effect of CS (F(1,283) = 30.16, p < 0.001) revealed that HFS elicited significantly higher levels of LTP than did TBS (p < 0.001). There was no main effect of gender or any significant interactions between variables. Measured LTP levels are depicted in figure 14.
Figure 14 Effects of Developmental Ethanol Exposure on Long-term Potentiation in the Young Adult DG.

Prenatal ethanol exposure during the 2\textsuperscript{nd} trimester equivalent (PNEE\textsubscript{2}) and pair-fed (PF) feeding in both the 1\textsuperscript{st} and 2\textsuperscript{nd} trimester equivalents resulted in a significant reduction of long-term potentiation (LTP) compared to ad libitum (AL) controls. The magnitude of evoked LTP in EE\textsubscript{3} offspring was higher than that in PNEE\textsubscript{1} and PF\textsubscript{2} offspring. Additionally, sham intubation (SI) during the 3\textsuperscript{rd} trimester equivalent resulted in an increase in LTP compared to PF\textsubscript{1}. Data presented as means ± SEM. Dotted line provides a reference to the mean obtained from AL animals, surrounding dashed box represents the SEM. • corresponds to significance level $p < 0.05$ from AL; ★ corresponds to significance level $p < 0.05$ from PNEE\textsubscript{1} and PNEE\textsubscript{2}; ♦ corresponds to significance level $p < 0.05$ from PF\textsubscript{1} and PF\textsubscript{2}; + corresponds to significance level $p < 0.05$ from PF\textsubscript{3}. No significant interaction between gender and any of the other variables or between CS and any of the other variables was obtained, thus data from males and females and from TBS and HFS were pooled together.
Table 5 Summary of Post-tetanic and Long-term Potentiation Results.

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<th>Slice Number</th>
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<table>
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<tr>
<td>EE</td>
<td>113.71</td>
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| HFS              |                  |
| AL               | 124.63           |
| PF               | 95.89            |
| PNEE             | 96.32            |
| PF               | 114.45           |
| PNEE             | 123.66           |
| SI               | 141.86           |
| EE               | 130.80           |
3.1.3 Discussion

Given the known effects of prenatal ethanol exposure (1\textsuperscript{st} and 2\textsuperscript{nd} trimester combined) on DG LTP, this study focused on trimester specific effects (i.e. individual trimester exposures). Results indicated that exposure to ethanol restricted to the 2\textsuperscript{nd} trimester equivalent of pregnancy resulted in severe attenuation of LTP in the adult male and female DG, whereas exposure during the 1\textsuperscript{st} or 3\textsuperscript{rd} trimester did not have a significant effect on LTP. Furthermore, caloric restriction during either the 1\textsuperscript{st} or 2\textsuperscript{nd} trimester equivalent reduces both PTP and LTP in the adult DG.

Developmental ethanol exposure reduces LTP in adults, but only when exposure is restricted to the 2\textsuperscript{nd} trimester equivalent. This finding demonstrates that the timing of exposure is a key factor in the teratogenic effects of ethanol and that the DG is particularly vulnerable during the 2\textsuperscript{nd} trimester equivalent. The effect of PNEE\textsubscript{2} on DG LTP is similar to those reported previously where exposure lasted throughout the 1\textsuperscript{st} and 2\textsuperscript{nd} trimester equivalents (Christie et al 2005; Sutherland et al 1997; Varaschin et al 2010). Interestingly, ethanol exposure restricted to the 1\textsuperscript{st} trimester equivalent did not alter LTP, suggesting that the deficits observed in these earlier studies may be the result of exposure during the 2\textsuperscript{nd} trimester. The decreased adult weight in prenatal ethanol exposed males is similar to that seen in nutrition depravation studies (Palmer et al 2008; Shultz et al 1999). However other studies have not shown this decrease following prenatal ethanol exposure (Christie et al 2005). The difference in LTP in PNEE\textsubscript{2} offspring is probably not due to weight reductions since there was no correlation between adult weight and LTP magnitude found. Furthermore, these deficits are not likely to result from long-term deficits in basal neural transmission. The finding of no significant effect of pre or postnatal treatment on I/O function suggests that these developmental insults do not impact basic cellular properties of the dentate granule cell population.

3.1.3.1 2\textsuperscript{nd} Trimester Equivalent Ethanol Exposure and Long-Lasting Effects on the DG

The reduction in LTP in the adult DG following exposure to ethanol during the 2\textsuperscript{nd} trimester corresponds with behavior studies where midgestational ethanol exposure via a liquid diet (35\% EDC) was sufficient to impair radial arm maze acquisition in both adolescent (PD 26) and adult (PD 80) rats (Hall et al 1994). Although further studies are needed to further link memory impairments with altered DG LTP in FASD, these
findings do help pin point possible developmental processes altered by ethanol neurotoxicity.

The mechanism(s) by which developmental ethanol exposure induces long-lasting behavioral and cognitive deficits is not fully understood. However, our findings suggest that the DG is vulnerable to ethanol during the period of development corresponding to the 2nd trimester of human pregnancy. Thus, by examining the developmental processes taking place during this time, we may be able to narrow down the possible targets of ethanol teratogenic effects.

During the 2nd trimester equivalent, generation of granule cells and interneurons takes place, cells migrate to the future site of the DG, and neuronal networks begin to form. Exposure to ethanol during this stage of development has been reported to alter neuron and glial proliferation, migration, and survival in the cortex, brain stem, and hippocampal CA1 (Barnes & Walker 1981; Miller 1995a; b; Rubert et al 2006). Furthermore, ethanol exposure during the second trimester equivalent in rodents leads to intrauterine growth retardation and microcephaly (Kotkoskie & Norton 1988; Middaugh & Boggan 1991). However, limited studies have focused primarily on the DG. Therefore, it is likely that alterations in one or all of these developmental processes lead to long-lasting neurobehavioral disabilities.

Cell proliferation and differentiation along with programmed cell death are important processes in the development of the DG (Georg Kuhn & Blomgren 2011). As early as GD 10, granule cells and their precursors start forming. Peak generation of hippocampal interneurons occurs between GD 13 and 14 in the DG (Soriano et al 1989a; b). One of two studies examining the effects of prenatal ethanol exposure on the DG reported no change in the number of dorsal granule cells in adult rats aged 60 that had been exposed to ethanol prenatally between GD 10 and 21 (Barnes & Walker 1981). Similarly in a later study where ethanol exposure included the late 1st trimester and entire 2nd trimester equivalents (GD 6-21), no alteration in the number of DG granule cells was detected in PD 30-35 rats (Miller 1995b).

In addition to cell genesis, ethanol may also affect differentiation and survival of newly generating cells. Ethanol inhibited differentiation of cultured neural stem cells (NSC) exposed to ethanol (20-100 mM) on GD14 (Tateno et al 2004). When NSC were
exposed to insulin-like growth factor 1 or BDNF in addition to ethanol, differentiation was no longer affected, suggesting that ethanol alters NSC differentiation through inhibition of neurotrophic factor signalling. Interestingly, neuronal survival was not affected in this study (Tateno et al 2004). Developmental ethanol exposure can also alter cell death processes and lead to substantial apoptosis. Through increases in Bax expression and associated processes, exposure to ethanol during the 2nd trimester equivalent in mice promoted increased apoptosis within the developing hippocampal formation (Young et al 2008). Bax is a Bcl-2 family protein involved in the promotion of apoptosis through initiation of mitochondria release of cytochrome c which in turns stimulates caspase 3, 6, 7, and 9 (Cory & Adams 2002). Other studies have also demonstrated increased apoptosis following developmental ethanol exposure through GABA receptor enhancement and NMDA receptor blockade (Ikonomidou et al 2000).

Beginning around GD 14, granule precursor cells start migrating from the dentate neuroepithelium and form the secondary dentate matrix (Altman & Bayer 1975; Angevine 1965; Seress 1977). Proliferating cells within the secondary matrix further migrate, forming the tertiary dentate matrix and parts of the granule cell layer of the DG, which starts to become noticeable right before birth (Altman & Bayer 1990a; Bayer 1980). Abnormalities in neuronal and glial migration have been reported in the cortex, brainstem and cerebellum following prenatal ethanol exposure (Clarren et al 1978; Kumada et al 2006; Miller 1986; 1993). It will be of importance for future studies to examine the effects of developmental ethanol exposure on migration of DG cells and the formation of the proliferative matrixes, as alterations may contribute to the long-term effects of developmental ethanol exposure.

Synaptic fibers from the EC first appear in the DG around GD 18 (Ceranik et al 1999; Super & Soriano 1994). However the number of synapses formed does not reach adult levels until around PD 25 (Crain et al 1973). Any alteration in the EC axonal targeting into the DG may result in reduced synaptic connections or a decrease in fiber number that may make it more difficult to evoke long-lasting potentiation. McNaughton, Douglas, and Goddard (1978) demonstrated that long-lasting enhancement of perforant path synapses requires the coactivity of a large number of perforant path fibers. Interestingly, West and Hamre (1984) showed that prenatal ethanol exposure during GDs
11-21 had no detectable effect on granule cell axon branching, indicating that at least hippocampal mossy fiber development in rats (i.e. axons) is not altered.

Ligand-gated ion channels not only mediate fast excitatory and inhibitory synaptic transmission, but also play an important role in the development of the CNS and have been implicated in the pathophysiology of FASD (Costa et al 2000). NMDA receptors are involved in many developmental processes including proliferation, differentiation, migration, and synapse formation. Following exposure to ethanol between GD 16-21, there was a significant reduction in NMDA sensitive $^{3}$H-glutamate binding sites in the DG (Savage et al 1991; Savage et al 1992). Of importance, this decrease was not observed with exposure during the 1$^{st}$ or 3$^{rd}$ trimester equivalent (Savage et al 1992). Similarly, Diaz 1997 reported that prenatal ethanol exposure between GD 12 to GD 18 resulted in a decrease of $^{3}$H-MK-801 binding in the hippocampus, further supporting the increased teratogenic effects of ethanol during the 2$^{nd}$ trimester equivalent. Nixon and colleagues (2004) showed that the expression of the NR2A subunit of the NMDA receptor was significantly increased in the hippocampus of PD10 rats following exposure to ethanol throughout the 2$^{nd}$ and 3$^{rd}$ trimester equivalents. Alteration in the NMDA receptor complex suggests that PNEE impairs the capacity for excitatory synaptic transmission and may underlie abnormalities in hippocampal development associated with FASDs.

Although LTP was significantly reduced in PNEE2 offspring, LTP levels were also reduced in PF2 offspring. Furthermore, LTP in PNEE2 and PF2 offspring did not differ, suggesting that the reduction in LTP in PNEE2 offspring may also be the partial result of altered prenatal nutrition.

3.1.3.2 Nutrition, Fetal Development, and Synaptic Plasticity

During pregnancy, nutritional status of the mother plays an important role in the fetus’s development. Both undernutrition and malnutrition interfere with the development of the hippocampal formation (Morgane et al 2002). Undernutrition is defined as the availability of all nutrients being required by a species in their diet, but amounts are insufficient; whereas malnutrition is defined as receiving a diet in which one or more essential nutrients is missing or the wrong proportions (Morgane et al 2002).
In the current study, we found that PTP and LTP were negatively affected in our pair-feeding offspring. Although the liquid diet given to PF dams provides adequate nutrition, the quantity of food is limited to correspond to that consumed by PNEE dams. Therefore, PF offspring serve as a model of undernutrition and provide a means of distinguishing the effects of ethanol and caloric restriction on development. However, ethanol consumption not only results in the consumption of less food (Fisher et al. 1997; Rao & Larkin 1987), but also impairs nutrient and vitamin absorption (Gloria et al. 1997; Green 1983) and interferes with nutritional supply to the fetus (Dreosti 1993). Thus, even with the use of a PF group, the nutritional effects that accompany ethanol consumption are difficult to separate from the teratogenic effects of ethanol alone (Dreosti 1993; Fisher et al. 1988; Schenker et al. 1990).

Unlike the time specific alterations observed after developmental ethanol exposure, undernutrition affects development during both the 1st and 2nd trimester equivalent, indicating that undernutrition and ethanol may be affecting development through different mechanisms. These findings are consistent with earlier reports indicating that undernutrition lowers the rate of all brain growth events to the same extent (Smart 1990). Furthermore, the reduction of PTP and LTP following prenatal caloric restriction are in agreement with previously reported findings (Jordan & Clark 1983). Nevertheless, this is the first report to examine the effects of caloric restriction within individual trimesters on LTP in the adult male and female DG.

Similar to our current findings, I/O function and PP plasticity in the adult DG were not affected by prenatal protein malnutrition (Bronzino et al. 1997), indicating normal neural transmission and transmitter release. However, previous studies have reported modulation of paired pulse responses in the DG on PD 15 and 30 but not in adulthood following prenatal protein malnutrition (Bronzino et al. 1999). Interestingly, we did find that PTP was reduced by both 1st and 2nd trimester undernutrition. Following a CS, the initial increase in fEPSP slope is independent of NMDA receptor activation (Volianskis & Jensen 2003) and is typically thought of as a presynaptic process because of the corresponding build up of Ca²⁺ in the core of the presynaptic terminal following the CS (Tang & Zucker 1997). Furthermore, PTP is believed to be dependent on the efflux of Ca²⁺ from mitochondria (Tang & Zucker 1997). Fittingly, following prenatal
malnutrition alterations in mitochondrial function have been observed in the adult rat offspring pancreas, skeletal muscle, liver, and brain (Bennis-Taleb et al 1999; Reusens et al 2008; Selak et al 2003; Wilson et al 2004).

The reduction of LTP following prenatal caloric restriction is in agreement with previously reported findings, further stressing the importance of nutritional status during pregnancy. Large prenatal caloric restriction (50% reduction of control intake) and prenatal protein malnutrition result in the impairment of both the establishment and maintenance of DG-LTP in adult male offspring (Austin et al 1986; Bronzino et al 1997; Jordan & Clark 1983). There is also experimental evidence indicating that prenatal malnourishment alters excitatory glutamatergic activity that may relate to alterations observed in our study and others. Prenatal malnourishment leads to a decrease in glutamate binding in the adult brain (Rotta et al 2003). Furthermore, these authors reported reduced Na\(^+\)-independent \(^3\)H-glutamate binding in cellular membranes, that indicates a decrease in the glutamatergic activity (Rotta et al 2003). These findings implicate dysfunction of the glutamatergic system that may account for deficits in LTP observed in our study and others.

Recently, it has been reported that the number of DG GAD-67-positive interneurons was higher in prenatally malnourished rats at an early age, but by adulthood the number of GAD-67-positive interneurons was not different from that of controls (Díaz-Cintra et al 2007). These findings indicate that prenatal malnutrition slows the development of the DG interneuron network, an important mediator of DG development, thus indicating a mechanism through which prenatal malnutrition alters the development of the DG. It has also been suggested that the decrease in LTP is related to an increase in inhibition following prenatal protein malnutrition (Bronzino et al 1997; Morgane et al 2002; Steiger et al 2003). However, previous reports from our lab did not report alterations in LTP when recorded in adolescent or adult PF offspring in vivo (Christie et al 2005; Titterness & Christie 2010), indicating that this may not be the case following undernutrition. If there was an increase in inhibition in PF offspring, a reduction in LTP would have been observed in these in vivo studies. Additionally, prenatal protein malnutrition selectively alters GABA\(_A\), but not GABA\(_B\), receptor mRNA levels in rat hippocampus and results in the increased sensitivity to the binding of picrotoxin, a
noncompetitive GABA\textsubscript{A} antagonist (Steiger et al 2003 Schweighert 2005). However, this would suggest that the use of BMI, also a GABA\textsubscript{A} antagonist, in our current study would result in an enhancement of LTP in PF animals, opposite of our findings. Interestingly, the observation of reduced LTP in PF offspring in this current study (seen in all three experiments in this thesis) where in vitro recordings were utilized was not found in our previous in vivo recording studies (Christie et al 2005; Titterness & Christie 2010), again implicating alterations in excitation following prenatal undernutrition. Furthermore, these differences highlight the need for caution when comparing results obtained \textit{in vivo} to those \textit{in vitro}. Taken together, more studies are needed to determine the differences between the effects of malnutrition and undernutrition and whether alterations in the GABAergic and/or glutamatergic system result from undernutrition.

In addition to alterations in transmitter systems, neuroanatomical changes related to undernutrition and malnutrition may play a role in deficits observed in LTP. Prenatal protein malnutrition causes alterations in the developmental time course of dentate granule cell, leads to a significant reduction in granule cell density, reduces the complexity of dendritic spines located on granule cells apical dendrites (corresponding to the area of perforant path innervation), and alters hippocampal morphology (Debassio et al 1994; Debassio et al 1996; Dim-Granados et al 1997; Rosoklija et al 2000). Additionally, undernutrition results in the permanent reduction in the number of dentate granule cells synapses (Jordan et al 1982).

Our current work suggests that prenatal undernutrition negatively influences DG synaptic plasticity in later life. We also demonstrate that the timing of caloric restriction does not influence the magnitude of the insult. The reduction in synaptic plasticity observed may underlie deficits in cognitive processes that result from prenatal undernutrition and malnutrition (Datta et al 2000; de Rooij et al 2010; Tonkiss & Galler 1990; Tonkiss et al 1990). Furthermore, the present findings demonstrate the need for a refinement in the liquid diet animal model of FASD, one in which undernutrition, malnutrition, and FASD may be further teased apart.
3.1.3.3 Summary

In summary, the hippocampal DG is vulnerable to ethanol induced alterations in long-term potentiation particularly during the 2nd trimester of pregnancy. The different effects of ethanol at different time points likely results from targeting of different developmental processes. This is not to say that ethanol does not have long-lasting effects on offspring when consumed at other time points during pregnancy, since this study only examined one plastic event in one region of the adult brain. As an example, exposure to ethanol during the 1st trimester has been shown to produce severe facial dysmorphology and interfere with organization of the brain (Kotch & Sulik 1992; Miller 1993). Moreover, prenatal undernutrition is also detrimental to the development of the hippocampal DG, regardless of time. This finding demonstrates the importance of maternal nutritional status on the developing fetus.

3.2 Effects of Prenatal Ethanol Exposure during both the 1st and 2nd Trimester Equivalent on Bidirectional Synaptic Plasticity in the Adult Dentate Gyrus

Young adults with FASD display behavioral and cognitive deficits that are thought to be dependent on the proper functioning of the hippocampal formation. Bidirectional synaptic plasticity is believed to be a cellular mechanism for learning and memory processes (Rolls & Kesner 2006), indicating that both long-term potentiation (LTP) and long-term depression (LTD) are needed for the acquisition of information and formation of long-term memories. Understanding the effects of developmental ethanol exposure on bidirectional synaptic plasticity might help to describe the cause of behavioral and cognitive deficits in FASD.

Long-term effects of prenatal ethanol exposure have been observed in the hippocampal formation through measures of behavioral performance, anatomical assessment, structural plasticity, and select forms of synaptic plasticity. Previous studies using liquid diet animal models of FASD indicated that exposure to ethanol during the 1st and 2nd trimester equivalents (prenatal ethanol exposure) reduces LTP (in vivo recordings) in the adolescent and adult male. To further build on these findings, the present study examined the effects of prenatal ethanol exposure on 1) bidirectional
synaptic plasticity in the adult DG; 2) in both male and female offspring; 3) in an *in vitro* slice preparation. By isolating the hippocampal formation in the *in vitro* slice preparation, it can be determined if hippocampal LTP deficits are truly the result of hippocampal dysfunction. Additionally, it can be determined whether the LTP deficits observed previously in *in vivo* preparations (Christie et al 2005; Sutherland et al 1997; Titterness & Christie 2010; Varaschin et al 2010) can also be detected in an *in vitro* preparation.

### 3.2.1 Methods

To model ethanol exposure during the 1<sup>st</sup> and 2<sup>nd</sup> trimesters of human pregnancy, dams (PNEE<sub>1,2</sub>) were given ad libitum access to an ethanol liquid diet from GD 1-21. To determine peak blood ethanol concentrations, blood samples were taken on GD 15 from ethanol treated dams. Appropriate pair-fed (PF<sub>1,2</sub>) and ad libitum (AL) animals were also reared (Figure 15).

Experimental recordings were conducted in early adulthood (PD 50-70). Stimulating and recording electrodes were placed in the DG medial molecular layer of hippocampal slices. Paired pulse (PP) ratios and input/output curves were recorded. Theta burst stimulation (TBS) or high frequency stimulation (HFS), moderate and robust stimuli respectively, were used to induce post-tetanic (PTP) and long-term potentiation (LTP). Low frequency stimulation consisting of either 900 or 1800 pulses given at a 1 Hz frequency was used to induce post-tetanic (PTD) and long-term depression (LTD).

Means ± SEM were calculated for each group of animals. Statistical differences were examined with a three-way factorial ANOVA: prenatal treatment (AL, PF<sub>1,2</sub>, PNEE<sub>1,2</sub>) x gender (male, female) x CS (TBS, HFS). Significant main effects and interactions were further analysed with Tukey HSD post hoc tests. When no significant interaction between CS and any of the other factors was obtained, data from both HFS and TBD were pooled together. T-tests were also used to compare between groups when appropriate.
The presence of sperm was used to indicate gestation day 1 (GD1). Rat dams or pups were exposed to a liquid diet containing ethanol during the 1st and 2nd trimester equivalents = prenatal ethanol exposure 1,2 (PNEE1,2). Appropriate pair-fed and ad libitum animals were also reared. To determine peak blood ethanol concentrations, blood samples were taken on GD 15. Experimental recordings were conducted in early adulthood (postnatal day (PD) 50-70).

3.2.2 Findings

3.2.2.1 Maternal and Offspring Parameters

All pregnant dams continued to gain body weight throughout gestation, regardless of diet (F(2,17) = 1.86, p = 0.185). Furthermore, there was no significant difference in litter size (F(2,19) = 3.65, p = 0.286) or gestation length (F(2,17) = 0.61, p = 0.089). Average BECs were 147.97 ± 6.17 mg/dl for ethanol treated dams. These BEC levels are similar to those found in previously published studies (Christie et al 2005; Titterness & Christie 2010).

Offspring were weighed across the postnatal period until their experimental use in adulthood. Following birth, weights taken on the litter cull date (PD3/4) were significantly affected by prenatal treatment (F(2,70) = 14.80, p < 0.001) and gender (F(1,70) = 4.24, p < 0.043). PNEE1,2 (p < 0.001) and PF1,2 (p < 0.001) offspring had significantly reduced body weights compared to AL, characteristic of these treatment (Titterness & Christie 2010; Uban et al 2010). Additionally, female offspring weighed significantly less than males (p = 0.036). At the time of electrophysiological experimentation, there was a main effect of gender (F(1,85) = 101.00, p < 0.001) but not prenatal treatment. However, there was a significant interaction between prenatal
treatment and gender (F(2,85) = 3.85, p < 0.025). Upon assessment, AL males weighed more than PF_{1,2} males (p < 0.001) at adulthood (i.e., PD 50-70). Furthermore, male offspring weighed significantly more than female offspring (p < 0.001). Gender differences in weight are common at this age (River 2012). The finding of reduced weight of PF_{1,2} males was surprising, since prenatal caloric restriction typically results in overweight adults (Desai et al 2007). Table 6 summarizes maternal and offspring parameters.

### Table 6 Summary of Maternal and Offspring Parameters.

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</table>

#### 3.2.2.2 Paired Pulse and Input/Output

To determine the effect of PNEE_{1,2} on I/O function, pulse widths between 30 and 300 usec were used to create an I/O curve. These curves were used to determine basal synaptic transmission and slice health. In all slices, the slope of the fEPSP significantly increased with increasing stimulation (F(8,1232) = 1423.21, p < 0.001) (Figure 16). Neither prenatal treatment nor gender had any significant effect on I/O function. There was a significant interaction between prenatal treatment and gender (F(2,154) = 3.489, p = 0.033). However, post hoc analyses did not reveal any significant differences. These results indicate that prenatal ethanol exposure does not have an effect on basal synaptic transmission. To investigate whether PNEE_{1,2} altered glutamate release, PP plasticity was measured. There was no main effect of prenatal treatment or gender on PP, nor was there
a significant interaction between the two variables (Figure 17), indicating prenatal treatment does not affect presynaptic transmitter release in adulthood.

**Figure 16 Input/Output function.**
Input/output curves, showing the fEPSP as a function of pulse width. Prenatal treatment did not affect I/O function. Data presented as means ± SEM. I/O, input/output.

**Figure 17 Paired Pulse Plasticity.**
*Left:* Paired pulse (PP) ratios obtained from the medial molecular layer of the DG. *Top Right:* Sample trace shown for AL offspring. *Middle Right:* Sample traces shown for PF<sub>1,2</sub> offspring. *Bottom Right:* Sample traces shown for PNEE<sub>1,2</sub> offspring. Prenatal treatment did not affect PP plasticity. Data presented as means ± SEM.
3.2.2.3 Post-tetanic and Long-term Potentiation

The following data is summarized at the end of this section in table 7.

Post-tetanic and long-term potentiation (PTP and LTP, respectively) of the fEPSP slope were induced using either theta burst stimulation (TBS; i.e. moderate conditioning stimulus (CS)) or high frequency stimulation (HFS; i.e. robust CS). PTP was measured by averaging fEPSP responses over the first minute after CS, whereas LTP was measured by averaging fEPSP responses at 56-60 minutes post CS.

*PTP*: A significant main effect of CS (F(1,86) = 16.10, p < 0.001) revealed that PTP magnitude was higher following HFS than TBS (p < 0.001). There was no main effect of prenatal treatment or gender and no significant interactions between any of the variables, indicating the prenatal ethanol exposure does not produce long-lasting deficits in short-term plasticity. Measured PTP levels are depicted in figure 18-left.

*LTP*: A significant main effect of prenatal treatment (F(2,86) = 7.40, p = 0.001) revealed that LTP magnitude was lower in PNEE1,2 (p = 0.043) and PF1,2 (p = 0.001) offspring than in AL offspring. Prenatal caloric restriction reduced LTP by 37% of controls, whereas prenatal ethanol exposure reduced LTP by 24% from that of controls. Additionally, there was a main effect of CS (F(1,86) = 16.10, p < 0.001) indicating that LTP levels were higher following HFS than TBS (p = 0.025). There was no significant main effect of gender and no significant interactions were found between any of the variables. Measured LTP levels are depicted in figure 18-right.
Figure 18 Effects of Prenatal Ethanol Exposure on CS Induced Plasticity.

*Top:* fEPSP responses to CS in the medial molecular layer of the DG. *Bottom Left:* Prenatal treatment did not affect PTP levels. *Bottom Right:* Exposure to ethanol and caloric restriction during the 1st and 2nd trimester equivalents significantly attenuated LTP in adulthood. • corresponds to significance level p < 0.05 from AL. Data presented as means ± SEM. No significant interaction between gender and any of the other variables or between CS and any of the other variables was obtained, thus data from males and females and from TBS and HFS were pooled together.

### 3.2.2.4 Post-tetanic and Long-term Depression

In the hippocampal formation, long-term depression (LTD) is typically induced by a low frequency CS made up of 900 pulses at 100 Hz (LFS$_{900}$). However, LFS$_{900}$ failed to induce LTD in the DG of adult AL males (t(16) = 1.09, p = 0.291). This is consistent with previous findings, suggesting that the induction of LTD in the DG is less consistent as rats ages (Trommer et al 1996).
By doubling the number of pulses given but keeping the same frequency as before (LFS_{1800}), we were able to induce LTD in the adult DG. fEPSPs were depressed by 34% sixty minutes after LFS_{1800} (t(26) = 9.57, p < 0.001). In an attempt to characterize LFS_{1800}-mediated LTD, either N-Methyl-D-aspartate (NMDA) receptors or metabotropic glutamate 5 (mGlu5) receptors were pharmacologically blocked. However, LFS_{1800} still induced significant LTD after the application of the NMDA receptor antagonist d-2-amino-5-phosphonovalerate (APV, 50 uM) (t(14) = 5.25, p < 0.001) and after the application of the mGluR5 selective antagonist 2-methyl-6-(phenylethynyl)pyridine hydrochloride (MPEP, 1 uM) (t(18) = 4.73, p < 0.001). Comparison of PTD magnitude across the three groups revealed that the application of MPEP or APV had no effect on the induction of LTD (thus PTD, immediately following LFS_{1800}) (F(2,29) = 2.32, p = 0.116). Similarly, sixty minutes after LFS_{1800}, LTD levels were not significantly different between the groups (F(2,29) = 0.66, p = 0.526) (Figure 19).

It had been previously suggested that the reduction in synaptic efficacy following a prolonged stimulation, one similar to LFS_{1800}, was a result of a rundown of tissue health and not actually LTD (Mockett et al 2002). To test this possibility, I/O function was assessed 1 hour after LFS_{1800} induction. In all slices tested, the slope of the fEPSP significantly increased with increasing stimulation (repeated measures ANOVA; F(8,104) = 149.39, p < 0.001). These data demonstrate that LTD is inducible in the young adult rat DG, although the mechanism of induction is unknown.²

² Characterization of LFS_{1800} evoked LTD was in part completed by Cam Clayton for his Undergraduate Honors Thesis.
Figure 19 Effect of Receptor Antagonists on the Induction of PTD and LTD in AL Males. 

*Top:* LFS\textsubscript{1800} of the medial perforant pathway induced changes following NMDA receptor or mGluR5 antagonists. *Bottom Left:* PTD was not affected by drug treatment. *Bottom Right:* Drug treatment did not affect LTD levels. ● corresponds to significance level \( p < 0.05 \) from control (AL); ★ corresponds to significance level \( p < 0.05 \) from APV. Data presented as means ± SEM.

3.2.2.4.1 LTD Following Prenatal Ethanol Exposure

The effects of PNEE\textsubscript{1,2} on PTD and LTD were assessed using LFS\textsubscript{1800}. PTD was measured by averaging fEPSP responses over the first minute after CS, whereas LTD was measured by averaging fEPSP responses at 56-60 minutes post CS.

The application of LFS\textsubscript{1800} produced PTD and LTD in all slices, regardless of prenatal treatment. Prenatal treatment significantly altered PTD levels (\( F(2,57) = 9.01, p < 0.001 \)) such that prenatal caloric restriction enhanced PTD by 92\% compared to PNEE\textsubscript{1,2} (\( p < 0.001 \)) and by 47\% of AL offspring (\( p = 0.021 \)). Gender also significantly influenced PTD levels (\( F(1,57) = 4.52, p = 0.038 \)), but post hoc analyses failed to reveal significance (Figure 20-left). There was no significant interaction between prenatal treatment and gender.
LFS₁₈₀₀ resulted in similar LTD magnitudes in all prenatal treatment groups (F(2,57) = 1.93, p = 0.154). The was a significant main effect of gender (F(1,57) = 15.52, p < 0.001) such that a higher magnitude of LTD was evoked in male than females (p < 0.001) (Figure 20-right). There was no main effect of prenatal treatment and no significant interaction between prenatal treatment and gender.

Figure 20 Effects of Prenatal Ethanol Exposure on Low Frequency Stimulation Induced Plasticity.

*Top:* LFS₁₈₀₀ of the medial perforant pathway induced changes following prenatal ethanol exposure. *Bottom Left:* PTD was enhanced in PF₁₂ as compared to AL and PNEE₁₂. *Bottom Right:* Prenatal treatment did not affect LTD levels; however there was a main effect of gender present. ● corresponds to significance level p < 0.05 from AL; ★ corresponds to significance level p < 0.05 from PNEE₁₂. + corresponds to significance level p < 0.05. Data presented as means ± SEM. No significant interaction between gender and prenatal treatment was found, thus further analysis was not warranted.
### Table 7 Summary of Potentiation and Depression Data.

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### 3.2.3 Discussion

The major findings of this study include the following: 1) excitatory synaptic transmission and transmitter release were not influenced by either prenatal ethanol exposure or caloric restriction; 2) prenatal ethanol exposure and caloric restriction attenuated LTP in the adult DG; 3) 1 Hz x 1800 pulse stimulation of the medial molecular layer of the DG induced LTD of synaptic transmission in the adult rat DG; 4) prenatal caloric restriction, but not ethanol exposure enhanced PTD; 5) neither prenatal ethanol exposure nor caloric restriction affected LTD, however LTD levels were influenced by gender of the offspring. Taken together, these results indicate that prenatal ethanol
exposure has long lasting effects on some but not all forms of synaptic plasticity within the adult DG of both males and females.

3.2.3.1 Long-term Potentiation

In this study, no alterations in I/O function or PP plasticity in the adult hippocampal DG were observed, indicating excitatory synaptic transmission and transmitter release were not affected. In agreement with PP plasticity, PTP was not influenced by prenatal treatment. While neither prenatal ethanol treated nor caloric restricted offspring were found to have altered forms of short-term plasticity, they did exhibit reductions in LTP compared to AL offspring. These results are in agreement with those previously obtained through in vivo recordings (Christie et al 2005; Sutherland et al 1997; Titterness & Christie 2010; Varaschin et al 2010). Furthermore, this study demonstrated that not only are adult male offspring affected by prenatal ethanol exposure, female offspring also display deficits in LTP. Interestingly in adolescent females, prenatal ethanol exposure leads to an enhancement of LTP in the DG (Titterness & Christie 2010). Therefore, there may be a difference in the ontogeny of prenatal ethanol effects on LTP when it comes to female and male offspring, one that may relate to sexual maturation (McGivern et al 1993; Sliwowska et al 2008).

Through the isolation of the hippocampal formation in the slice preparation (i.e. in vitro), current findings indicate that ethanol induced deficits, and undernutrition, of LTP are the result of hippocampal dysfunction. Furthermore, since BMI (GABA_A antagonist) is necessary for the induction of LTP in the DG in vitro, the current results reflect long-term dysfunction of the glutamatergic system, most likely NMDA receptors on the postsynaptic membrane (Olney et al 2001; Titterness & Christie 2010). Previous research indicates that prenatal ethanol exposure is associated with long-lasting alterations of NMDA receptor-channel complex in the hippocampal formation. Following prenatal ethanol exposure there is a reduction in the density of NMDA receptors and an alteration in receptor subunit NR2A and NR2B composition (Dim-Granados et al 1997; Hughes et al 1998; Toso et al 2005). Given that the NR2B subunit is important for the maintenance of LTP in the DG (Rosenblum et al 1996; Rostas et al 1996), the reduced number of NR2B subunits may contribute to the decrease in LTP. Prenatal ethanol exposure also
inhibited the actions of the neurosteroids that potentiate NMDA receptor function in neonates (Costa et al 2000), demonstrating reduced sensitivity to excitation. However, it is unclear if these alterations last into adulthood. Furthermore, studies showed that prenatal ethanol exposure resulted in decreased sensitivity to NMDA in the adult rat hippocampus and enhanced Mg$^{2+}$ regulation of the NMDA receptor (Morrisett et al 1992; Morrisett et al 1989). Other studies have shown both a reduction in the number of $^3$H-glutamate binding sites in the hippocampal formation (Farr et al 1988) and reduced NMDA-sensitive $^3$H-glutamate binding following prenatal ethanol exposure (Savage et al 1991). Similarly, Dim 1997 reported that prenatal ethanol exposure resulted in a decrease of $^3$H-MK-801 binding in the hippocampus, further implicating NMDA dysfunction in the alterations of LTP. These data suggest that the reduction in LTP observed in this current study as the result of prenatal ethanol exposure may be the consequence of alterations in the NMDA receptor-channel complex. Further research is needed to characterize the mechanisms mediating these alterations in LTP and directly link them to NMDA receptor dysfunction.

Following activation of NMDA receptors, extracellular signal-regulated kinase 1 and 2 (ERK1/2, of the MAPK family) signalling processes are activated (Ivanov et al 2006; Sweatt 2001; 2004). Recently, Samundio-Ruiz and colleagues (2009) showed that in the adult DG (not CA1), NMDA stimulation of ERK1/2 activation was reduced as a result of prenatal ethanol exposure. Given that ERK1/2 activation plays an important role in hippocampal LTP and is required for select forms of learning and memory (Atkins et al 1998; Blum et al 1999; English & Sweatt 1997; Selcher et al 1999), these finding implicate a potential mechanism through which LTP in our current study is reduced. Interestingly, they also reported that PKA and PKC dependent activation of ERK was not affected by prenatal ethanol exposure, suggesting that other intracellular signalling molecules involved in ERK1/2 activation are affected by prenatal ethanol exposure. Future electrophysiological studies isolating individual glutamate receptors and evaluation of prenatal ethanol exposure effects on their physiological and pharmacological properties are warranted. By demonstrating that deficits in DG LTP in vivo transfer faithfully to in vitro recording, a door has been opened through which the
mechanisms underlying FASD induced deficits in synaptic plasticity and behavior can be further explored.

3.2.3.2 Bicuculline Methiodide (BMI)

Previous studies have reported an upregulation of the β2/3 subunit and alterations in the pharmacological properties of the GABA<sub>A</sub> receptor in the hippocampal formation following prenatal ethanol exposure (Iqbal et al 2004). Although we block GABA<sub>A</sub> receptor transmission with BMI, there is the possibility that it may not bind as readily due to alterations in subunit composition. Alterations in binding would result in increased inhibition and therefore account for the reduction in LTP that we observed. However, it is not currently known if prenatal ethanol exposure alters the binding of BMI. Furthermore, if a reduction in BMI binding is the cause of reduced LTP, we would also expect to see a reduction in PTP, which we do not.

3.2.3.3 In vitro Slice Recordings

These experiments are the first to investigate the long-term effects of prenatal ethanol exposure on synaptic plasticity in the adult DG in an in vitro preparation. The results obtained are in agreement with those previously obtained through in vivo recordings in regards to the attenuation of LTP in PNEE<sub>1,2</sub> offspring (Christie et al 2005; Sutherland et al 1997; Titterness & Christie 2010; Varaschin et al 2010). However, in studies that have included a nutritional control to date there has not been an effect of prenatal caloric restriction on LTP in adult animals (Christie et al 2005; Sutherland et al 1997; Titterness & Christie 2010). Thus, the results seem to indicate that the additional procedures required to generate in vitro recordings may uncover subtle nutritional deficits not apparent in vivo, perhaps masked by the modulatory influences available in the intact brain (i.e. in vivo). In support of this, in experiments performed in vivo in littermates from the present study these deficits in LTP in PF<sub>1,2</sub> animals were not observed (unpublished data). These differences in PF<sub>1,2</sub> LTP highlight the need for further investigation between the differences in in vivo and in vitro methodologies and caution when comparing results obtained in vivo to those in vitro.
3.2.3.4 Possible Mechanisms of Action Underlying FASD

Perhaps not surprisingly, the effects of ethanol on the DG are multifaceted (Matsumoto et al 2007; Santofimia-Castaño et al 2011). Ironically, one of the reasons that ethanol has such complex actions is due to its very simple structure (CH₃CH₂OH). It has both polar and non-polar attributes making it readily accessible to membranes, receptors and different intracellular structures. For instance, ethanol interacts with membrane bound proteins that include excitatory glutamate and inhibitory GABA receptors (Ikonomidou et al 2000). Ethanol acts as an antagonist on NMDA receptors (Abdollah & Brien 1995; Hendricson et al 2004; Mameli et al 2005; Sanna et al 1993; Savage et al 1991) and as an agonist on GABAₓ receptors (Ikonomidou et al 2000; Proctor et al 2006; Sanna et al 2004), thereby affecting synaptic signalling. To further complicate matters ethanol may have subunit specific effects in its ability to alter receptor function (Crews et al 1996; Grobin et al 1998). Because subunit expression varies with age and between brain regions, the overall effect of ethanol on synaptic signalling will vary with development and region of interest. Aside from affecting the postsynaptic density, ethanol can act on voltage-dependent calcium channels whereby presynaptic glutamate release may be attenuated and ultimately synaptic transmission affected (Mameli et al 2005). Ethanol also targets G protein-activated K⁺ channels and serotonin-3 receptors, resulting in a potentiation of their function (Kobayashi et al 1999; McBride et al 2004).

Intracellularly, ethanol can induce oxidative stress by increasing levels of reactive oxygen species that are harmful to the cell (Bondy 1992; Brocardo et al 2011; Montoliu et al 1994). Exposure to ethanol can activate caspase pathways, which may lead to DNA fragmentation and initiation of cell death and neurodegeneration (Franke et al 1997; Olney et al 2002; Pascual et al 2007). Within the nucleus ethanol can interfere with transcription of neurotrophic factors (Minana et al 2000; Wilkemeyer et al 2002). Furthermore, it can alter cellular metabolism related to synaptic signalling. For instance, chronic ethanol exposure markedly reduced the activity of the astrocyte-specific enzyme glutamine synthetase (Norenberg & Martinez-Hernandez 1979) important for neurotransmitter replenishment (Babu et al 1994) and reduced myelination (Deltour et al 1996; Guerri et al 1994; Guerri et al 2001; Rifas et al 1997). The final picture of ethanol’s effect on cellular function is complicated. However, through the use of isolated and
controllable preparations such as in vitro hippocampal recording, ethanol’s effect on individual cellular mechanisms can be described and probed.

3.2.3.5 Long-term Depression

LFS consisting of 900 pulses at 1 Hz is commonly used to evoke LTD (Oliet et al 1997). However, LTD within the hippocampal formation is known to be regulated by age, where young and aged rodents have a lower threshold for LTD than do adults (Errington et al 1995; Kemp et al 2000; Trommer et al 1996; Wei & Xie 1999). Thus the failure to induce LTD with LFS$^{900}$ in the current study is consistent with previous findings. Specifically, in the medial perforant path of the DG, induction of LTD in vitro was less consistent as rats aged (Trommer et al 1996). Interestingly, Sui and colleagues (Sui et al 2000a; Sui et al 2000b) reported that the magnitude of LTD induced in the DG by LFS$^{900}$ significantly increased with age, from PD 17 to PD 63. The differences in the Sui study to those of the current study and previous reports (English & Sweatt 1997; Trommer et al 1996) may be explained by the strain of rat used in these studies. Manahan-Vaughan and colleagues showed that LTD was more easily induced in Wistar strains than in hooded rat strains (Manahan-Vaughan 2000). Fittingly, Sui and colleagues (Sui et al 2000a; Sui et al 2000b) used Wistar rats while all other studies used Sprague-Dawley (English & Sweatt 1997; Trommer et al 1996).

In the current study, novel and robust LTD of the fEPSP in the adult DG that is not resistant to NMDA receptor and mGluR5 antagonists is described. LTD in the adult DG was induced by LFS consisting of 1800 pulses at 1 Hz and was not prevented by the bath application of the NMDA receptor antagonist APV or the mGluR5 antagonist MPEP. The early phase of NMDA-LTD involves the modification of pre-existing proteins and lasts up to 40 minutes post-LFS (Kauderer & Kandel 2000), whereas early mGluR LTD can last up to 2-4 hours post-induction (Neyman & Manahan-Vaughan 2008; Okagami et al 1995). Such findings along with the current ones, indicate that a combination of mechanisms may be involved in LFS$^{1800}$ induced LTD. It is also possible that not just one sources of Ca$^{2+}$ entry is necessary for LTD in the DG. Perhaps multiple sources of Ca$^{2+}$ synergistically operate to induce LTD (Overstreet et al 1997). LFS$^{900}$ induced LTD in the DG has been shown to be sensitive to the NMDA receptor blocker
AP5 (Wei & Xie 1999). However, this has not been replicated in other studies (O'Mara et al 1995; Oliet et al 1997; Poschel & Manahan-Vaughan 2007; Trommer et al 1996). Similarly, mixed results have been reported for the role of mGluR in LFS\textsubscript{900} induced DG LTD (O'Mara et al 1995; Trommer et al 1996). There is some evidence that LFS\textsubscript{900} LTD is dependent on Ca$^{2+}$ influx through T-type (Oliet et al 1997), but not L-type Ca$^{2+}$ channels (Oliet et al 1997; Poschel & Manahan-Vaughan 2007; Trommer et al 1996). Additionally, Ca$^{2+}$ release from intracellular stores has also been suggested to contribute to LTD (Nishiyama et al 2000; Oliet et al 1997). Based on information gathered from LFS\textsubscript{900} LTD studies in the DG, further investigation of the mechanism underlying LFS\textsubscript{1800} induced LTD would include assessment of voltage gated calcium channels, other mGluR receptors, or a combination of receptor antagonists.

In the present study, LFS\textsubscript{1800} induced PTP and LTD were investigated following prenatal ethanol exposure and caloric restriction. In both male and female offspring prenatally exposed to ethanol, neither PTP nor LTD was affected. Conversely, PTD was enhanced in PF\textsubscript{1,2} offspring. However this enhancement of fEPSP depression subsided over time and was not significantly different from other treatment groups 60 minutes post-LFS\textsubscript{1800}. Somewhat similar results were found in the adolescent CA1 following prenatal caloric restriction, where enhancement of the fEPFP 60 minutes after LFS\textsubscript{900} was reported in PF\textsubscript{1,2}, but not PNEE\textsubscript{1,2} offspring (Titterness & Christie 2008). However, the authors did not report whether this enhancement observed in PF\textsubscript{1,2} offspring was observed at early times following LFS\textsubscript{900}.

Two processes are believed to be involved in the expression of PTD, the transient depletion of the readily releasable pool and the inactivation of presynaptic Ca$^{2+}$ channels (Cowan et al 2003). In PF\textsubscript{1,2} offspring, PP plasticity, another measure of the readily releasable pool, was not affected, indicating that alterations in presynaptic transmitter release are not a likely explanation of the observed enhancement of PTD. The finding that PTD but not LTD was blocked by MPEP, suggests that prenatal caloric restriction results in long lasting changes in mGluR5 receptors and possibly their modulation of second messenger systems. Following prenatal protein deprivation, increased glutamate and GABA release, increased density of hippocampal kainite receptors but normal density of NMDA and AMPA receptors, and increased GABA uptake were observed.
(Fiacco et al 2003; Schweigert et al 2005). However, further studies are needed to evaluate whether prenatal caloric restriction induces long-term mGluR abnormalities in the DG.

3.2.3.5 Summary

It has been proposed that learning and memory occurs in the CNS through changes in synaptic weights (Bear & Linden 2003) and that bidirectional synaptic plasticity is needed for the regulation of synaptic strength and to maintain consistency during learning (Rolls & Kesner 2006). In the current study, prenatal ethanol exposure resulted in attenuation of LTP in the DG, but did not affect LTD, illustrating an imbalance of bidirectional plasticity. Since both LTP and LTD are needed for the processing of information and the formation of memories, the observed imbalance may account for the learning and behavioral deficits observed in animal models of, and individuals with, FASD. Uncovering the mechanisms behind changes in hippocampal function, like those in LTP observed in the current study, is of great importance to understanding the teratogenic effects of ethanol.

3.3 Does Prenatal Ethanol Exposure Alter the Sensitivity of the Adult Dentate Gyrus to Acute Ethanol Exposure

The acute consumption of ethanol results in many behavioral and cognitive consequences. Problems in cognitive processing are common at blood ethanol levels of 80 mg/dl (i.e. 17.36 mM ethanol concentration) and higher. At similar concentrations, ethanol modulates both inhibitory and excitatory neural transmission in numerous brain regions (Deitrich et al 1989; Faingold et al 1998; Tsai & Coyle 1998). Enhancement of GABA_A receptor and inhibition of NMDA receptor transmission is thought to contribute to some of the behavioural and cognitive effects of ethanol exposure (Allgaier 2002; Grobin et al 1998; Tsai & Coyle 1998; Woodward 2000). Additionally, addictive behaviors associated with ethanol drinking problems are thought to develop through the inhibition of NMDA receptor transmission (Weitlauf & Woodward 2008).

As an individual with FASD matures, many secondary disabilities begin to emerge, including ethanol or other drug abuse (Koren et al 2003). Prenatal ethanol exposure is a known risk factor for the development of ethanol drinking problems later in
life (Baer et al 2003). Upwards of forty-seven percent of individuals with FASD report ethanol and other drug problems in adulthood (Streissguth et al 2004; Yates et al 1998). Previously it has been shown that prenatal ethanol exposure leads to the tolerance of and preference for ethanol in adulthood (Abel et al 1981; Molina et al 1987; Reyes et al 1993). This increased risk can be a result of alterations in ethanol sensitivity and NMDA mediated plasticity.

To test this hypothesis, hippocampal slices from adult rats prenatally exposed to ethanol were acutely exposed to ethanol. Following this acute exposure, LTP was induced with either high-frequency stimulation (HFS) or theta-burst stimulation (TBS).

### 3.3.1 Methods

Adult male and female Sprague-Dawley rats were socially housed (sexes separated) in standard cages and maintained on a 12-hour light/dark cycle with access to food and water ad libitum. All procedures were approved by the University of Victoria Animal Care Committee and in accordance with the Canada Council on Animal Care.

To model ethanol exposure during the 1st and 2nd trimesters of human pregnancy, dams (PNEE1,2) were given ad libitum access to an ethanol liquid diet from GD 1-21. To determine peak blood ethanol concentrations, blood samples were taken on GD 15 from ethanol treated dams. Appropriate pair-fed (PF1,2) and ad libitum (AL) animals were also reared.

Transverse hippocampal slices were generated as previously described (see 2.6.1). Slices were transferred to recording chambers and superfused with oxygenated normal artificial cerebrospinal fluid (nACSF). Field excitatory postsynaptic potentials (fEPSPs) were evoked and responses recorded. BMI (10 uM) was included in the nACSF during baseline and conditioning stimulus recordings for all experiments. In select experiments, ethanol (20 or 50 mM) was also included in the nACSF during baseline and conditioning stimulus recordings. A stable baseline of 5 minutes was required before the addition of ethanol. Ethanol solutions were prepared by diluting 100% ethanol in nACSF/BMI prior to each experiment. To prevent evaporation of ethanol, solution containers were covered with Parafilm M barrier film throughout experimentation. Following baseline acquisition, either theta burst stimulation (TBS) or high frequency stimulation (HFS) was used to
induce post-tetanic (PTP) and long-term potentiation (LTP). Immediately following the conditioning stimulus, baseline measurements resumed for 1 hour. BMI and ethanol were immediately washed out of the chamber solution after the CS was applied.

Means ± SEM were calculated for each group of animals. Statistical differences were examined with a four-way factorial ANOVA: prenatal treatment (AL, PF₁,₂, PNEE₁,₂) x acute ethanol exposure (0 mM, 20 mM, 50 mM) x gender (male, female) x CS (TBS, HFS). Significant main effects and interactions were further analyzed with Tukey HSD post hoc tests. When no significant interaction between CS and any of the other factors was obtained, data from both HFS and TBD were pooled together.

3.3.2 Findings

3.3.2.1 Maternal and Offspring Parameters

All pregnant dams continued to gain body weight throughout gestation, regardless of diet (F(2,23) = 1.22, p = 0.342). Furthermore, there was no significant difference in litter size (F(2,28) = 0.15, p = 0.863) or gestation length (F(2,22) = 0.73, p = 0.492). Average BECs were 145.32 ± 5.31 mg/dl for ethanol treated dams. These BEC levels are similar to those found in previously published studies (Christie et al 2005; Titterness & Christie 2010)

Offspring were weighed across the postnatal period until their experimental use in adulthood. Following birth, weights taken on the litter cull date (PD3/4) were significantly affected by prenatal treatment (F(2,124) = 28.72, p < 0.001) but not gender (F(1,124) = 1.33, p > 0.251). PNEE₁,₂ (p < 0.001) and PF₁,₂ (p < 0.001) offspring had significantly reduced body weights compared to AL. At the time of electrophysiological experimentation, there was a main effect of prenatal treatment (F(2,137) = 5.98, p = 0.003) and gender (F(1,137) = 241.36, p < 0.001). Additionally, there was a significant interaction between prenatal treatment and gender (F(2,137) = 4.27, p = 0.015). Upon assessment, AL males weighed more than PF₁,₂ males (p < 0.001) at adulthood (i.e., PD 50-70). Additionally, males weighed significantly more than females (p < 0.001), a common gender difference at this age (River 2012). Table 8 summarizes maternal and offspring parameters.
Table 8 Summary of Maternal and Offspring Parameters

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3.3.2.2 Post-tetanic and Long-term Potentiation

To determine how prenatal exposure to ethanol alters the response of the adult DG to acute ethanol exposure, hippocampal slices from all three prenatal treatment groups were exposed to ethanol prior to LTP induction. Following obtainment of a five minute stable baseline in the presence of BMI, either 0 (control), 20, or 50 mM ethanol was added to the perfusion nACSF. Fifteen to 30 minutes later, a CS was applied to induce LTP. At this point, both BMI and ethanol were removed from the nACSF perfusion. PTP was measured by averaging fEPSP responses over the first minute after CS, whereas LTP was measured by averaging fEPSP responses at 56-60 minutes post CS.

**PTP:** Analyses revealed a main effect of prenatal treatment ($F(2,254) = 4.81, p = 0.009$) such that PTP levels in PNEE, 1, 2 offspring were higher than those in PF, 1, 2 ($p = 0.004$). A main effect of acute ethanol exposure ($F(2,254) = 28.80, p < 0.001$) revealed that exposure to 50 mM ethanol causes a significant reduction in PTP as compared to both control ($p < 0.001$) and 20 mM exposure ($p < 0.001$). A main effect of CS ($F(1,254) = 135.26, p < 0.001$) established that HFS induced higher levels of PTP than TBS ($p < 0.001$). There was no main effect of gender ($F(1,254) = 3.56, p = 0.060$), indicating no significant difference of PTP levels between male and female offspring ($p = 0.056$). Additionally, there was a significant interaction between prenatal treatment and CS ($F(2,254) = 4.68, p = 0.010$), prenatal treatment and acute ethanol exposure ($F(4,254) =$...
7.23, p < 0.001), and prenatal treatment, acute ethanol exposure, and gender (F(4,254) = 3.43, p = 0.009). Each interaction is presented below.

The interaction between prenatal treatment and CS indicates that CS differentially affected prenatal treatment groups (Figure 21). Specifically, PTP levels were similar in AL, PF1,2, and PNEE1,2 offspring following TBS (p > 0.868) and PTP levels were significantly lower following TBS compared to HFS in all prenatal treatment groups (p < 0.001). However, HFS evoked significantly different PTP levels in PF1,2 and PNEE1,2 offspring. HFS-PTP magnitude observed in PNEE1,2 offspring was 26% higher than that observed in PF1,2 offspring (p < 0.001). AL HFS-PTP levels did not differ from those in either PNEE1,2 (p = 0.163) or PF1,2 (p = 0.518) offspring.

![Figure 21 Interaction Between Prenatal Treatment and CS.](image)

**Figure 21 Interaction Between Prenatal Treatment and CS.**

HFS-PTP in PNEE1,2 is significantly higher than HFS-PTP in PF1,2. fEPSP response averaged over the first minute after CS is graphed. Each data point represents the mean ± SEM.

The interaction between prenatal treatment and acute ethanol exposure indicates that prenatal treatment groups were affected differently by acute ethanol exposure in adulthood (Figure 22). Specifically, in the absence of acute ethanol, higher PTP levels were evoked in AL offspring compared to PF1,2 (p = 0.003) and PNEE1,2 (p < 0.001). PTP was reduced by 24% in PF1,2 and by 22% in PNEE1,2 compared to AL. PTP levels were similar in all prenatal treatment groups after acute exposure to 20 mM ethanol (p > 0.287). Following acute exposure to 50 mM ethanol, PTP levels in PNEE1,2 were significantly higher than those in AL (48% increase, p = 0.011). PF1,2 PTP levels did not differ from either PNEE1,2 (p = 0.097) or AL (p = 0.999) after acute exposure to 50 mM ethanol. Within AL offspring, PTP levels were significantly reduced following acute
exposure to both 20 mM (28% reduction, \( p < 0.001 \)) and 50 mM (52% reduction, \( p < 0.001 \)) ethanol (compared to control AL); PTP levels were significantly reduced in a dose response manner, being that PTP levels were 33% lower following 50 mM ethanol compared to 20 mM ethanol (\( p = 0.007 \)). Within PF\(_{1,2}\) offspring, PTP levels were significantly reduced following acute exposure to 50 mM ethanol (32% reduction, \( p = 0.005 \)) (compared to no ethanol); PTP levels did not differ between control and 20 mM ethanol (\( p = 0.999 \)) or between 20 mM and 50 mM ethanol (\( p = 0.061 \)). Within PNEE\(_{1,2}\) offspring, PTP levels were not affected by acute ethanol exposure (\( p > 0.898 \)). Therefore, these results demonstrate that prenatal ethanol exposure and caloric restriction modify presynaptic function in the absence of acute ethanol. Furthermore, they indicate that prenatal ethanol exposure alters the responsiveness of the DG to acute ethanol exposure during adulthood.

**Figure 22 Interaction Between Prenatal Treatment and Acute Ethanol Exposure.**

In the absence of acute ethanol exposure, PTP in AL offspring is significantly higher than PTP in PNEE\(_{1,2}\) or PF\(_{1,2}\). Acute ethanol exposure differentially affected PTP in prenatal treatment groups: in AL, PTP was significantly reduced following exposure to 20 and 50 mM ethanol; in PF\(_{1,2}\), only exposure to 50 mM ethanol reduced PTP; in PNEE\(_{1,2}\), PTP was not affected by acute ethanol exposure. Each data point represents the mean ± SEM. fEPSP responses averaged over the first minute after CS.

The prenatal treatment, acute ethanol exposure, and gender interaction signifies that male and female offspring are affected differently by acute ethanol exposure following prenatal treatment (Figure 23). In the absence of ethanol, PTP was similar between males and females, regardless of prenatal treatment (\( p > 0.060 \)). Following acute
exposure to 20 mM ethanol, PTP levels in PNEE_{1,2} males were significantly higher than those in AL males (p = 0.006); all other offspring had similar PTP levels (p > 0.418). After acute exposure to 50 mM ethanol, PTP levels in PNEE_{1,2} females were significantly higher than those in AL males (p = 0.002) and in PF_{1,2} males (p = 0.013). Within AL offspring, PTP levels were differentially affected by acute ethanol exposure in male and females. In males, PTP was significantly lowered following acute exposure to both 20 mM (p < 0.001) and 50 mM (p < 0.001) ethanol; whereas in females PTP was only reduced after acute exposure to 50 mM ethanol (0 mM vs. 20 mM: p = 0.962; 0 mM vs. 50 mM: p < 0.001). Within PF_{1,2} offspring, PTP levels were differentially affected in males and females following acute ethanol exposure. In males, PTP was significantly lowered following acute exposure to 50 mM (p = 0.015; 0 mM vs. 20 mM: p = 0.999); whereas in females PTP levels were not affected by acute ethanol exposure (p > 0.992). Within PNEE_{1,2} offspring, neither males nor females were affected by acute ethanol exposure (p > 0.374). These results demonstrate that within AL and PF_{1,2} offspring, males are more affected by acute ethanol exposure than females. They further show that prenatal ethanol exposure, in both males and females, alters the responsiveness of the DG to acute ethanol exposure during adulthood. PTP data is summarized in tables 9 (male) and 10 (female), found at the end of this section.
**Figure 23 Effects of Acute Ethanol Exposure on Post-tetanic Potentiation.**

*Top:* Depiction of the interaction between prenatal treatment, acute ethanol exposure, and gender. Acute exposure of ethanol does not affect PTP in PNEE<sub>1,2</sub> offspring or and PF<sub>1,2</sub> females. *Bottom Left:* PTP in female offspring. 50 mM ethanol reduces PTP in AL females. *Bottom Right:* PTP in male offspring. 20 mM ethanol reduces PTP in AL males; 50 mM ethanol reduces PTP in AL and PF<sub>1,2</sub> males. Data presented as means ± SEM.

*LTP:* Analyses revealed a main effect of prenatal treatment (F(2,254) = 6.13, p = 0.002) such that LTP levels in PNEE<sub>1,2</sub> offspring were higher than those in PF<sub>1,2</sub> (p < 0.001). A main effect of acute ethanol exposure (F(2,254) = 26.68, p < 0.001) revealed that exposure to ethanol caused a significant reduction in LTP in a dose response manner (0 mM vs. 20 mM: p < 0.001; 0 mM vs. 50 mM: p < 0.001; 20 mM vs. 50 mM: p < 0.001). A main effect of CS (F(1,254) = 46.59, p < 0.001) established that HFS induced higher levels of LTP than TBS (p < 0.001). A main effect of gender (F(1,254) = 8.07, p = 0.005) indicated higher levels of LTP in females compared to males (p = 0.004). In addition to the main effects, there was a significant interaction between prenatal...
treatment and CS (F(2,254) = 4.28, p = 0.015), prenatal treatment and acute ethanol exposure (F(4,254) = 8.73, p < 0.001), and acute ethanol exposure and CS (F(2,254) = 4.83, p = 0.009). Each interaction is presented below.

The interaction between prenatal treatment and CS indicates that CS differentially affected prenatal treatment groups (Figure 24). Specifically, LTP levels were similar in AL, PF_{1,2}, and PNEE_{1,2} following TBS (p > 0.994). However, HFS-LTP levels in PNEE_{1,2} were higher than those in AL (p < 0.001) and in PF_{1,2} (p < 0.001). HFS-LTP levels did not differ from AL (p = 0.593). Comparisons between CS revealed that HFS evoked greater LTP than did TBS in AL (p = 0.009) and PNEE_{1,2} (p < 0.001) offspring. However, there was no difference between TBS-LTP or HFS-LTP in PF_{1,2} offspring (p = 0.236).

![Figure 24 Interaction Between Prenatal Treatment and CS.](image)

In AL and PNEE_{1,2} offspring, HFS evoked significantly greater than did TBS. Each data point represents the mean ± SEM. fEPSP responses averaged for minutes 56-60 after CS.

The interaction between prenatal treatment and acute ethanol exposure indicates that prenatal treatment groups were affected differentially by acute ethanol in adulthood (Figure 25). In the absence of acute ethanol, LTP was reduced in PNEE_{1,2} (29% reduction, p = 0.036) and PF_{1,2} (43% reduction, p < 0.001) offspring compared to AL. LTP levels did not differ between PF_{1,2} and PNEE_{1,2} (p = 0.801). Following acute exposure to 20 mM ethanol, LTP in PNEE_{1,2} was significantly higher than in AL (p = 0.029). PF_{1,2} LTP levels did not differ from either PNEE_{1,2} (p = 0.396) or AL (p = 0.985) after acute exposure to 20 mM ethanol. After acute exposure to 50 mM ethanol, LTP was not induced in AL slices (12%, t(6) = 1.61, p = 0.158). Therefore, potentiation levels in
PNEE\textsubscript{1,2} were significantly higher than in AL (p = 0.003). Although LTP was induced in PF\textsubscript{1,2} offspring after acute exposure to 50 mM ethanol, potentiation levels did not differ from AL (p = 0.915) or from PNEE\textsubscript{1,2} (p = 0.230). Within AL offspring, LTP levels were significantly reduced following acute exposure to both 20 mM (57\% reduction, p < 0.001) and 50 mM (81\% reduction, p < 0.001) ethanol (compared to control AL); however, LTP levels were not different between 20 mM ethanol and 50 mM ethanol exposure (p = 0.192). Within PF\textsubscript{1,2} offspring, LTP levels were not affected by acute ethanol exposure. Within PNEE\textsubscript{1,2} offspring, PTP levels were not affected by acute ethanol exposure. Therefore, these results demonstrate that acute exposure to ethanol has a negative effect on DG function. Furthermore, these results indicate that both prenatal ethanol exposure and caloric restriction alter the responsiveness of the DG to acute ethanol exposure during adulthood.

![Graph](image)

**Figure 25 Interaction Between Prenatal Treatment and Acute Ethanol Exposure.**
Acute exposure of ethanol does not affect LTP in PNEE\textsubscript{1,2} or and PF\textsubscript{1,2} offspring. 20 mM ethanol reduces LTP in AL offspring; whereas 50 mM ethanol blocks LTP in AL offspring. Each data point represents the mean ± SEM. fEPSP responses averaged for minutes 56-60 after CS.

The interaction between acute ethanol exposure and CS signifies that the different CS evoked dissimilar levels of LTP depending on the level of ethanol present (Figure 26). Both TBS and HFS induced LTP in all slices, regardless of acute ethanol exposure (p < 0.049). In the absence of ethanol, TBS and HFS induced similar levels of LTP (p = 0.677). LTP levels following TBS were significantly lower than those induced by HFS after acute exposure to 20 mM (p < 0.001) and 50 mM ethanol (p < 0.001). In regards to TBS only, LTP was significantly reduced following acute exposure to both 20 mM (p <...
0.001) and 50 mM ethanol (p < 0.001). In contrast, HFS-LTP was not affected by exposure to 20 mM (p = 0.992), but 50 mM ethanol did significantly reduce HFS-LTP by 32% compared to control (p = 0.011). Therefore, these results demonstrate that acute exposure to ethanol has a negative effect on DG function. These results also indicate that TBS-LTP is more sensitive to the negative effects of acute ethanol than is HFS-LTP. Figures 27 and 28 illustrate the time course of LTP in each of the treatment groups. LTP data is summarized in tables 9 (male) and 10 (female), found at the end of this section.

![Graph showing Long-term Potentiation](image)

**Figure 26 Interaction Between Acute Ethanol Exposure and CS.**
Illustration that TBS-LTP is more sensitive to the negative effects of acute ethanol than is HFS-LTP: TBS-LTP was reduced following exposure to 20 and 50 mM ethanol. HFS-LTP was reduced following exposure to 50 mM ethanol. Each data point represents the mean ± SEM. fEPSP responses averaged for minutes 56-60 after CS.
Figure 27 Theta Burst Stimulation Induced Plasticity Following Acute Ethanol Exposure

fEPS responses before and after theta burst stimulation (TBS). TBS given at time 0 minute. Top Row: Female and male AL. Middle Row: Female and male PF1,2. Bottom Row: Female and male PNEE1,2. Each data point represents the mean ± S.E.M of the fEPSP.
Figure 28 High Frequency Stimulation Induced Plasticity Following Acute Ethanol Exposure.

fEPS responses before and after high frequency stimulation (HFS). TBS given at time 0 minute. 

Top Row: Female and male AL. Middle Row: Female and male PF1,2. Bottom Row: Female and male PNEE1,2. Each data point represents the mean ± S.E.M. fEPSP responses averaged over one minute intervals.
Table 9 Summary of Post-tetanic and Long-term Potentiation Data for Male Offspring

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Table 10 Summary of Post-tetanic and Long-term Potentiation Data for Female Offspring

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3.3.2.3. Input/Output Function

To test whether acute ethanol exposure affected slice health, input/output (I/O) function was recorded at the end of each LTP recording. I/O curves were generated from the application of increasing stimulus pulse width. In all slices, the slope of the fEPSP significantly increased with increasing stimulation (repeated measures ANOVA; F(8,1792) = 3314.44, p < 0.001). There was no main effect of prenatal treatment, acute ethanol exposure, gender, or CS. Furthermore, there were no significant interactions.
between any of the variables, demonstrating that the attenuation of LTP by acute application of ethanol is not due to a rundown in the health of the slice.

3.3.3 Discussion

There are three prominent observations from this study. First, in control animals LTP is attenuated by acute ethanol exposure in a dose dependent manner. Second, LTP induced under physiological conditions (TBS) is attenuated to a greater degree by acute ethanol than LTP induced with more robust non-physiologically relevant stimuli (HFS). Third, prenatal ethanol exposure and caloric restriction results in reduced sensitivity to acute ethanol exposure in adulthood. These results demonstrate the negative effects that acute ethanol exposure has on memory related mechanisms and the profound long-term lasting neurological abnormalities associated with prenatal insults.

3.3.3.1 Attenuation of LTP in Control Animals

Acute ethanol exposure attenuated LTP in the adult DG of control male and female rats. This is in agreement with previous studies examining the effects of acute ethanol on DG-LTP in adolescent and awake adult rats (Givens & McMahon 1995; Morrisett & Swartzwelder 1993). We showed that LTP, and PTP, was attenuated following the application of both moderate (20 mM) and high (50 mM) levels of ethanol. Accordingly, the gradual decrease in the degree of LTP in the CA1 by changes in ethanol concentration has been reported by several groups (Izumi et al 2007; Lovinger et al 1990; Sugiura et al 1995). Further supporting this, NMDA-mediated currents were dose dependently attenuated by ethanol when examined in freshly dissociated brain cells (Criswell et al 2003; Dildy & Leslie 1989).

Ethanol acts as both a GABA<sub>A</sub> receptor agonist and NMDA receptor antagonist (Harris et al 1997; Hoffman et al 1992; Ikonomidou et al 2000; Lovinger et al 1989). BMI concentrations similar to those used in this study are capable of blocking currents elicited by GABA and alphaxalone (an anesthetic steroid with a similar binding site as ethanol) (Ueno et al 1997). Thus, in the current study, the presence of BMI not only blocked GABA<sub>A</sub> receptors and isolated the excitatory component of the fEPSP, but also allowed for the investigation of ethanol actions on NMDA receptor. Therefore, the attenuation of LTP following acute ethanol exposure is primarily due to an inhibition of
NMDA receptors; in agreement with previous studies (Hicklin et al 2011; Schummers & Browning 2001). Morrisett and Swartzwelder (1993) reported that the effects of acute ethanol on long-term changes in synaptic strength in the rat DG are largely due to an action at the NMDA receptor-channel complex. Although the mechanism of ethanol induced NMDA receptor inhibition is unclear, recently it has been suggested that ethanol binds at transmembrane (TM) domain 3 of the NR1 subunit and TM4 of the NR2A subunit (Ren et al 2008). Evidence from cortical and hippocampal studies indicate that acute ethanol modulates the phosphorylation and expression of NR1 and NR2 subunits of the NMDA receptor as well as downstream signalling cascades, such as ERK1/2 (Chandler & Sutton 2005; Ferrani-Kile et al 2003; Hicklin et al 2011; Roh et al 2011). Additionally, pharmacological and behavioral studies have shown that ethanol inhibition of NMDA receptors is involved in acute intoxication (Hicklin et al 2011; Wilson et al 1990). Thus, there is considerable evidence to suggest that attenuation of LTP in control animals is a result of ethanol’s inhibitory action on NMDA receptor function, possibly through changes in phosphorylation of specific receptor subunits.

Although the NMDA receptor is a likely candidate for the locus of acute ethanol’s inhibition of LTP, there are other possible sites. Ethanol is known to inhibit L-type Ca\(^{2+}\) channels and AMPA receptor mediated transmission (Dildy-Mayfield & Harris 1992). Although AMPA receptors are inhibited by ethanol to a lesser extent than NMDA receptors, they do account for the majority of the fEPSP (Blake et al 1988; Dildy-Mayfield & Harris 1992). Additionally, evidence suggests that LTP causes a larger increase in the portion of the fEPSP associated with AMPA receptors than that of NMDA receptors (Clark & Collingridge 1995). Thus it is possible that the ethanol mediated decrease in LTP may be partially due to inhibition of AMPA receptors. Whole cell analysis of the change in AMPA mediated currents would provide important information on how acute ethanol exposure affects LTP. On the other hand, acute exposure to ethanol does not seem to affect basal synaptic transmission prior to the induction of LTP nor does it influence the maintenance of LTP (Givens & McMahon 1995). It is also possible that this decrease is due to ethanol inhibition of L-type Ca\(^{2+}\) channels, since they are known to play an important role in the induction (select forms) and maintenance of LTP (Grover et al 2009; Kapur et al 1998; Morgan & Teyler 1999).
Ethanol’s greater suppression of LTP when induced under physiological conditions (TBS) is most likely because this a less powerful induction protocol as compared to HFS. HFS evokes robust levels of LTP through artificial mechanisms and leads to NMDA receptor mediated potentiation of the fEPSP. Conversely, TBS results in the increase of synaptic strength that is typically smaller that that evoked by HFS. Therefore, the more prominent reduction of TBS-LTP following acute ethanol exposure may reflect the strength of the CS. Similarly, Raymond and colleagues (2007; 2008) reported different levels of PTP and LTP following the use of different CS. Within that study, they found that an increase in the number of tetanic trains induced LTP that resulted from not only NMDA receptor mediated Ca\textsuperscript{2+} influx but also activation of group 1 mGluR cascades triggering Ca\textsuperscript{2+} release from internal stores. Thus, the higher magnitude of LTP evoked in the current study by HFS may reflect activation of mGluR. Furthermore, mGluR may not be as greatly affected by acute ethanol exposure as NMDA receptors, also relating to larger HFS-LTP levels observed after the application of acute ethanol exposure.

Memory impairment is common after ethanol consumption and has been observed after acute exposure (Mello 1972; Mello & Mendelson 1972; White et al 2000a; White et al 2000b). Rodent studies have revealed that ethanol consumption impairs spatial and non-spatial learning and memory tasks in a fashion similar to that produced by hippocampal lesions (Boulouard et al 2002; Franke et al 1997; Maren 2001; Melia et al 1996; Morris et al 1982; Sircar et al 2009; White et al 2000a; White et al 2000b). LTP has been proposed as a candidate mechanism for learning and memory formation in the CNS (Bliss & Collingridge 1993). Therefore deficits in DG LTP observed in this study could account for cognitive deficits observed following acute ethanol consumption.

### 3.3.2 Prenatal Ethanol Exposed Offspring are Unaffected by Application of Acute Ethanol

In the absence of acute ethanol, exposure to ethanol throughout the 1\textsuperscript{st} and 2\textsuperscript{nd} trimester equivalent of human pregnancy resulted in a reduction of LTP as compared to AL offspring. These findings are in agreement with those in section 3.2.2.3 of this thesis and those previous observed in vivo (Christie et al 2005; Sutherland et al 1997; Titterness & Christie 2010; Varaschin et al 2010). We also showed that LTP evoked in the adult DG
of PNEE
offspring was not affected by the acute application of ethanol, whereas control rats were negatively affected by acute ethanol exposure. These findings indicate that prenatal ethanol exposure is associated with the reduced pharmacologic effect of acute ethanol.

The cellular and molecular mechanisms of prenatal ethanol exposure’s influence on ethanol neurotoxicity remains unclear. Previous research indicates that prenatal ethanol exposure is associated with long-lasting alterations of NMDA receptors, including altered receptor subunit composition, binding, and receptor function (Costa et al 2000; Dim-Granados et al 1997; Farr et al 1988; Hughes et al 1998; Morrisett et al 1992; Morrisett et al 1989; Toso et al 2005) (see section 3.2.3.1 for a detailed discussion). These results indicate that the reduction in synaptic plasticity seen after prenatal ethanol exposure and the altered pharmacologic effect of acute ethanol may be due to alterations in the NMDA receptor-channel complex. Specifically, alterations in binding affinity and binding site availability suggest that prenatal ethanol exposure may also result in the decreased binding of ethanol to the NMDA receptor in adulthood. However this is speculative and requires investigation. Further study is needed to determine the mechanisms that contribute to the altered sensitivity to acute exposure to ethanol.

Following prenatal ethanol exposure there is an upregulation of the GABA
β2/3 subunit in the hippocampal formation (Iqbal et al 2004). Interestingly, GABA
receptors containing β3 are more sensitive to ethanol than those containing β2 with a threshold at 30 mM (Wallner et al 2003). Additionally, within the hippocampal formation, there is a heightened sensitivity to GABA
receptor positive modulatory influences (alphaxalone, an anesthetic steroid with a similar binding site as ethanol) in adult offspring following prenatal ethanol exposure, indicating long-lasting alterations in neuromodulatory influences on GABA
receptor-mediated inhibitory neurotransmission (Allan et al 1998). However, at concentrations similar to those used in our current study, BMI not only blocks currents elicited by GABA, but it also blocks those elicited by alphaxalone (Ueno et al 1997), suggesting that BMI is also blocking transmission elicited by ethanol. Thus, heightened sensitivity of the GABA
receptor most likely does not contribute to our current findings.
Interestingly, following chronic intermittent ethanol exposure during adolescence, a tolerance to ethanol’s negative effects on behavior and hippocampal function develops. Chronic intermittent ethanol (CIEE) exposure commonly involves the administration of ethanol every 48 hours over a 20 day period. After CIEE between PD 30 and 50, acute application of ethanol resulted in a reduction of the ethanol induced inhibition of hippocampal pyramidal neurons spontaneous firing (Tokunaga et al 2006). Additionally, CIEE during adolescence produced tolerance to both ethanol induced impairments in the spatial memory tasks and ethanol upregulation of allopregnanolone (neuroactive steroid and GABA<sub>A</sub> receptor modulator) in the hippocampal formation (Matthews et al 2002; Silvers et al 2006). However, unlike the long-lasting reduction in ethanol sensitivity observed in PNEE<sub>1,2</sub> offspring, CIEE rats recovered and were again normally affected by acute ethanol exposure twelve days after CIEE (Silvers et al 2006; Tokunaga et al 2006). These findings demonstrate that pre-exposure to ethanol leads to an alteration in the response to later exposure to ethanol.

Although this is the first study to examine the effects of acute ethanol application on synaptic plasticity in prenatal ethanol exposed offspring in adulthood, evidence of prenatal ethanol induced desensitization to ethanol in adulthood has also been shown through other measures. During adulthood, rats prenatally exposed to ethanol exhibited a decreased hypothermic response to intoxicating doses of ethanol (Molina et al 1987). Additionally, prenatal ethanol exposure leads to preference for and increased tolerance of ethanol in adulthood (Abel et al 1981; Molina et al 1987; Reyes et al 1993). As suggested, reduced sensitivity to ethanol (i.e tolerance) may lead to the higher abuse of ethanol (Grobin et al 1998). Thus, the persistent alteration in the pharmacologic effect of acute ethanol on synaptic plasticity in PNEE<sub>1,2</sub> may underlie the high incidence of ethanol drinking problems in individuals with FASD.

3.3.3 Pair-fed Offspring and Acute Ethanol Exposure

We observed that following prenatal caloric restriction (i.e. undernutrition) acute ethanol application had no effect on LTP in adulthood. Correspondingly, previous research has shown altered responses to ethanol in adulthood following undernutrition and malnutrition. Previous research has showed that protein deprivation results in higher
metabolism of ethanol, decreased behavioral impairment, and increased sensitivity to NMDA antagonists (Martin et al 1989; Tonkiss et al 1998; Tonkiss et al 2000). Additionally, there is evidence that prenatal malnutrition alters GABAergic response to ethanol (Borghese et al 1998; Cordoba et al 1997). The current findings illustrate the importance of prenatal nutrition and demonstrate the need for a refinement in the liquid diet animal model of FASD, one in which undernutrition, malnutrition, and FASD may be further teased apart.

3.3.4 Post-tetanic Potentiation

In addition to LTP, PTP observed in AL and PF₁,₂ offspring was also sensitive to acute application of ethanol. Supporting our findings, acute administration of 60 mM ethanol to hippocampal slices resulted in the partial depression of PTP in the CA1 (Tokuda et al 2007). Through a series of experiments, the authors went on to show that the ethanol induced reduction in PTP most likely resulted from inactivation of NMDA receptors or voltage gated Ca²⁺ channels (Tokuda et al 2007). We also found gender differences in the effects of acute ethanol on PTP. In both AL and PF₁,₂ offspring, males had greater ethanol induced reductions in PTP than females. Numerous gender differences in relation to the effects of ethanol on the CNS and body have been previously reported. Women are more susceptible to many of the medial consequences of ethanol use, including cirrhosis of the liver, cardiac disease, neurodegeneration, and cognitive impairment (Fernández-Solà et al 1997; Loft et al 1987; Nixon 1994; Nixon et al 1995; Sohrabji 2002). Conversely, ethanol reduces brain glucose metabolism more in males than females (Wang et al 2003), females are less sensitive to the sedating effects of acute ethanol (Cha et al 2006; Silveri & Spear 2002), and are less sensitive to ethanol induced excitation of GABAₐ mediated inhibitory currents (Cha et al 2006). Therefore, ethanol induced changes in PTP are most likely multifaceted, perhaps resulting from both pre- and post-synaptic alterations. Additionally, these results indicate that acute ethanol produces gender-specific alterations in synaptic plasticity.

3.3.5 Acute Ethanol Concentration

Within this study, the evaluation of acute ethanol exposure on adult synaptic plasticity in the DG was done using two clinically relevant ethanol concentrations, 20 and
50 mM. Intoxication is commonly defined by a BEC greater than 100mg/dl (Rivara et al 1993). 20 mM of ethanol is equivalent to 92.15 mg/dl (i.e. 0.092% blood ethanol content; see Appendix A), slightly lower than intoxicating levels but higher than the legal driving limit in the United States of America and Canada. BECs in the range of 60-100 mg/dl are characterized by pleasure, emotional arousal, numbness of feelings, nausea, and sleepiness. Additional effects include impairments in fine motor skills and vision impairment, owing to functional alterations of the cerebral cortex, forebrain, and cerebellum. 50 mM ethanol corresponds to 230.35 mg/dl (i.e. 0.230% blood ethanol content) and is commonly observed in alcoholics (270mg (Brown & Miller 1993). BECs in the range of 210-300 mg/dl are characterized by aggression, reduced sensation, and depression, low blood pressure, irregular breathing and temperature control, and possibly unconsciousness. Furthermore, functional alterations in the cerebral cortex, forebrain, cerebellum, and brain stem are typically reported. Our finding that both 20 and 50 mM ethanol application attenuates PTP and LTP in the adult hippocampal DG in control animals provides a possible mechanism through which acute ethanol mediates its detrimental effects, as described above.

3.3.5 Summary

In summary, under normal circumstances LTP was attenuated in offspring prenatally exposure to ethanol as compared to controls. However, following acute exposure to ethanol in adulthood, a pronounced reduction in LTP was observed in controls but not in prenatally treated offspring. Further investigation is needed to uncover the implications of the reduced pharmacologic effect of acute ethanol in prenatal ethanol exposed offspring. These findings may relate to the emergence of secondary disabilities common in FASD individuals and could provide insight into the potential treatment of or prevention of these deficits.
4. Conclusion

This thesis provides evidence for long-lasting neurophysiological changes that occur following developmental ethanol exposure. The major findings of this thesis demonstrate that: 1) the 2nd trimester is a critical period of DG development when neural and glial genesis and cell migration occur and it is likely that ethanol’s actions on these processes leads to the long-lasting alterations in synaptic plasticity observed; 2) the long-term effects of prenatal ethanol exposure on synaptic plasticity are specific to long-term potentiation, revealing an imbalance in bidirectional synaptic plasticity; 3) prenatal ethanol exposure alters the sensitivity of the adult DG to acute ethanol application producing a long-lasting tolerance to the inhibitory effects of ethanol; 4) overall, gender played a minor role in mediating the effects developmental ethanol exposure, as there were few differences in synaptic plasticity measures among male and female offspring; 5) both TBS and HFS evoked LTP and did not influence the magnitude of the deficits observed in offspring developmentally exposed to ethanol; TBS-LTP was more sensitive to the inhibitory effects of acute ethanol application; 6) nutritional status is an important regulator of development and undernutrition leads to long-lasting changes in DG function.

Together, the results from the experiments undertaken in this thesis demonstrate long-lasting alterations in synaptic plasticity as the result of developmental ethanol exposure. Furthermore, these results allude to a malfunction of neural circuits within the hippocampal formation, perhaps relating to the learning and memory deficits observed in individuals with fetal alcohol spectrum disorders.
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# Appendix A

## Ethanol Concentrations – Conversion Table

Table 11 Conversion Table of BEC and Ethanol Concentrations Used

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</tr>
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<td>86.82</td>
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<tr>
<td>0.46</td>
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<td>100.00</td>
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</table>
Appendix B
Degree of Disinhibition Required to Produce Consistent Potentiation in the Dentate Gyrus

Granule cells have a high resting membrane potential (Williamson et al 1993) and strong \( \gamma \)-aminobutyric acid (GABA) receptor mediated inhibition (Mody 2005). Thus in the \textit{in vitro} hippocampal slice preparation, induction of long-term potentiation (LTP) in the dentate gyrus (DG) requires the blockade of the GABA\( _A \) receptor. Using the specific GABA\( _A \) receptor antagonist bicuculline methiodide (BMI), the degree of disinhibition required to produce consistent post-tetanic potentiation (PTP) and LTP in the DG following high frequency stimulation (HFS) was examined in the adult rat. We found that a 10 \( \mu \)M BMI concentration was required to produce maximal post-tetanic potentiation and LTP in the rat dentate gyrus. These results reveal the optimal amount of disinhibition necessary to induce long-term potentiation in the rodent DG \textit{in vitro}. Thus in all other potentiation experiments, a BMI concentration of 10 \( \mu \)M was used.
Figure 29 BMI Dependent Potentiation.
Top: HFS of the medial perforant pathway induced changes. Bottom Left: HFS in the presence of BMI induced PTP at all concentration. Bottom Right: HFS in the presence low concentrations of BMI failed to induce LTP. However, in the presence of medium and high concentration of BMI, LTP was induced. Data presented as means ± SEM.

Methods

Adult male Sprague-Dawley rats were socially housed in standard cages and maintained on a 12-hour light/dark cycle with access to food and water ad libitum. All procedures were approved by the University of Victoria Animal Care Committee and in accordance with the Canada Council on Animal Care.

Transverse hippocampal slices were generated as previously described (see 2.6.1). Slices were transferred to recording chambers and superfused with oxygenated normal artificial cerebrospinal fluid (nACSF). Field excitatory postsynaptic potentials (fEPSPs) were evoked and responses recorded. All the following experimental recordings were conducted in the presence of nACSF containing a specified bicuculline methiodide (BMI) concentration. BMI concentrations were categorized as low (0, 1 μM), moderate (2.5, 5
μM), high (7.5, 10) or very high (15, 20 μM). Following baseline acquisition, a HFS was used to induce long-term potentiation (LTP). Immediately following the conditioning stimulus (CS), baseline measurements resumed for 1 hour.

For analysis purposes, recordings were normalized to the average value of the 20 min baseline. Post-tetanic potentiation (PTP) was measured by averaging the first four traces (i.e., first minute) after the conditioning protocol, whereas LTP was measured by averaging the last 40 traces (i.e., 50-60 min) of the post-conditioning baseline.

Test to examine if LTP was present, comparison of the average of the last 40 traces (i.e., -10-0 min) prior to the CS with the average of the last 40 traces of the post-conditioning baseline was conducted using student t-tests. Comparisons between BMI concentrations were conducted using a one-way ANOVA, followed by Tukey post hoc tests as appropriate. Differences were considered significant when $p < 0.05$.

Results

A HFS protocol was applied to the rat DG to examine amounts of in vitro PTP and LTP facilitated under different concentrations of BMI. HFS was able to produce PTP when rat slices were recorded in the both the absence and presence of BMI (Figure 31). A main effect was observed for BMI concentration ($F(7,63) = 11.18, p < 0.000001$). No significant differences in PTP were observed between the low and moderate BMI concentrations ([0 μM] = 30.49 ± 5.23%, n = 8; [1 μM] = 41.33 ± 9.57%, n = 7; [2.5 μM] = 61.80 ± 10.81%, n = 9; [5 μM] = 74.72 ± 6.35%, n = 11, $p > 0.2$). Amongst the high and very high BMI concentrations, only the 10 μM concentration produced significantly more PTP than the low and moderate groups ([10 μM] = 152.99 ± 15.63%, n = 8, $p < 0.0001$ in comparison to: [7.5 μM] = 114.35 ± 11.99%, n = 8; [15 μM] = 99.56 ± 19.40%, n = 11; [20 μM] = 110.61 ± 7.68%, n = 9). A BMI concentration of 10 μM is therefore the lowest concentration that can induce maximal PTP in the rat dentate gyrus.

HFS failed to produce LTP in rat dentate gyrus at low BMI concentrations ([0 μM] = 5.39 ± 7.30%, n = 8, [1 μM] = -0.50 ± 5.38%, n = 7, Figure #). However, LTP could be reliably induced with increased BMI concentrations ([2.5 μM] = 9.62 ± 5.62%, n = 9; [5 μM] = 23.32 ± 7.99%, n = 11; [7.5 μM] = 37.64 ± 7.85%, n = 8; [10 μM] = 61.64 ± 7.71%, n = 8; [15 μM] = 63.96 ± 12.04%, n = 11; [20 μM] = 36.36 ± 9.06%, n = 9). A main effect of BMI concentration was observed with LTP ($F(7,63) = 11.18, p <
Post-hoc analysis demonstrated that BMI concentrations of 10 and 15 µM were able to show significantly more LTP than low and moderate BMI concentrations ($p<0.03$). Therefore, a BMI concentration of 10 µM is the lowest concentration that can induce maximal LTP in the rat dentate gyrus.