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PRENATAL ALCOHOL EXPOSURE ALTERS HISTAMINE H₃ RECEPTOR MODULATION OF GLUTAMATE RELEASE AND LONG-TERM POTENTIATION IN THE RAT DENTATE GYRUS

by

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DISSERTATION

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Com carinho, para minha esposa Érica

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Abstract

Children with Fetal Alcohol Spectrum Disorder exhibit long lasting behavioral impairments, such as learning disabilities, problems in executive functioning and memory deficits. Currently, there are no rationally designed, clinically available pharmacological tools to circumvent prenatal alcohol-induced cognitive deficits. Recently, using a rat model of voluntary drinking during pregnancy, Savage and collaborators (2010) showed that the inverse agonist of histamine H₃ receptors reverses fetal ethanol-induced deficits in learning and memory, without further increasing cognition in control rats. These differential effects and the notion that H₃ receptors reside in presynaptic nerve terminals

to inhibit neurotransmitter release, led to the hypothesis that *fetal alcohol exposure elevates histamine H_3 receptor-mediated depression of glutamate release from perforant path nerve terminals in the dentate gyrus, and that this effect may contribute to the observed long-term potentiation (LTP) deficit in these animals.* In the present study, this hypothesis was examined using a combination of electrophysiological and radiohistochemical approaches.

Long-Evans rat dams voluntarily consumed between 2.40 and 2.82 g/kg/day of 5% sweetened ethanol solution throughout gestation. This level of consumption produced a mean peak serum ethanol concentration of 84 mg/dL. No differences in offspring birthweight or litter size were observed. Prenatal alcohol exposure did not significantly affect electrophysiological raw measures of granule cell responsiveness or baseline probability of glutamate release in the dentate gyrus, but significantly impaired coupling of population spikes-to-fEPSP slopes and LTP of perforant path-to-granule cell synapses. Prenatal alcohol-induced LTP deficit was reversed by systemic injection of the inverse agonist of H_3 receptors ABT-239 *in-vivo,* without further improvement in control rats. Conversely, agonism of these receptors by systemic injection of methimepip mimicked the LTP deficit in control offspring both *in-vivo* and *in-vitro,* without further decreasing LTP in prenatal alcohol exposed rats. Measurement of methimepip-stimulated binding of [^{35}S]-GTPγS in the dentate gyrus in prenatal alcohol exposed rats suggested an increased H_3 receptor-effector coupling in these animals. This increased receptor-effector coupling resulted in heightened agonist inhibition of glutamate release *in-vitro,* but not *in-vivo.* No differences were found in the density of histamine H_3 receptors in the dentate gyrus.

Taken together, these results suggest that heightened H_3 receptor-mediated modulation of glutamate release in the dentate gyrus of fetal-ethanol exposed rats is a possible contributing mechanism for LTP deficit observed in these animals. It is expected that these experiments will provide insights into the mechanisms by which prenatal ethanol exposure affects hippocampal-dependent learning and memory. A better understanding of this phenomenon may provide targets for therapeutic intervention in children with alcohol-related neurodevelopmental disorders.

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1. Introduction

1.1 Overview of fetal alcohol spectrum disorder

The discovery of teratogen effects of the antiemetic drug thalidomide in the 1960s gave a strong impulse to research on birth defects caused by other drugs, including alcohol[1]. In 1973, a seminal series of articles was published in *the Lancet*, depicting a number of malformations related to alcohol consumption during pregnancy and first introducing the term *fetal alcohol syndrome* (FAS) (Jones and Smith, 1973; Jones et al., 1973). Many more studies and case reports were published after the guidelines for diagnosing fetal alcohol syndrome were set up by Jones and Smith (Hanson et al., 1976; Kaminski et al., 1976; Mulvihill et al., 1976; Pierog et al., 1977; Streissguth, 1976) including some studies using animal models (Bauer-Moffett and Altman, 1977; Chernoff, 1977; Henderson and Schenker, 1977; Randall et al., 1977). It was established early on that the degree of alcohol induced teratogenicity varied according to the dosage administered (Chernoff, 1977; Coles et al., 2010; Randall and Taylor, 1979; Reyes et al., 1989; Testa et al., 2003). Thus, a continuum of subtle to gross malformations is related to the degree and pattern of fetal alcohol exposure. More recently, the term *fetal alcohol effects* (FAE) was created to describe less extreme disorders after prenatal alcohol exposure, while the term *fetal alcohol spectrum disorder* (FASD) encompasses the full range of outcomes observed in individuals exposed to prenatal alcohol, including the most extreme forms of FAS (Niccols, 2007; Riley and McGee, 2005). Epidemiologically, FASD accounts for the majority of identifiable causes of mental retardation in the United States. Estimates

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1 The term alcohol refers to the chemical substance ethyl alcohol, or ethanol. These terms are used interchangeably throughout the manuscript.

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of prevalence for FAS vary from 1 to 7 per 1000 live births, while FASD estimates range from 10 to 40 per 1000 live births (reviewed by Niccols, 2007). However, due to the lack of appropriate diagnostic tools, the actual number children with subtle FASD may be much greater.

Perhaps one of the most intriguing effects observed in humans and animals exposed to alcohol during development is the long lasting impaired ability to learn and retain information. Prenatal ethanol-induced cognitive deficits have been studied under a variety of paradigms. In humans, prenatal ethanol exposure results in impairments in executive functioning (Pei et al., 2011), language (Wyper and Rasmussen, 2011), sensory processing and adaptive behavior (Carr et al., 2010), social cognition and facial emotion processing ability (Greenbaum et al., 2009), math performance (Coles et al., 2009), visual memory (Rasmussen et al., 2009), verbal learning and spatial recall performance (Willoughby et al., 2008), processing of complex information (Aragon et al., 2008), spatial memory (Uecker and Nadel, 1998) and working memory (Malisza et al., 2005). These deficits are associated with altered activation patterns in areas of the brain associated with working memory (Malisza et al., 2005), anomalies in the microstructure of the corpus callosum (Wozniak et al., 2009), disrupted white matter tracts (Lebel et al., 2008), thinning of cortical areas (Zhou et al., 2011) and reduced size of the thalamus, globus pallidus and hippocampus (Nardelli et al., 2011). Many of the prenatal alcohol effects observed in humans have been detected in animal models: Specifically to rats, developmental exposure to alcohol impairs spatial memory (Gianoulakis, 1990; Kim et al., 1997; Reyes et al., 1989), social memory (Kelly et al., 2009), delay and trace fear conditioning (Hunt et al., 2009) and working memory (Girard et al., 2000). These effects

are associated with alterations in macro and microstructure of the brain (Smith and Davies, 1990), receptor density (Galindo et al., 2004; Samudio-Ruiz et al., 2010; Savage et al., 1991) and signaling mechanisms (Allan et al., 1997; Nio et al., 1991).

The importance of the hippocampus in spatial, episodic and declarative memories has led to a thorough investigation of the effects of prenatal alcohol exposure in this brain region. It was found that effects such as reduced neuronal number and density (Barnes and Walker, 1981), abnormal axon branching (West and Pierce, 1984) and decreased dendritic arborization (Abel et al., 1983) contribute to altered hippocampal function in different paradigms of prenatal ethanol exposure that may, in turn, be the underlying cause of the hippocampal-dependent behavioral deficits. As stated above, the induction of developmental malformations varies in severity according to the level of alcohol exposure. This statement is also true for the hippocampal formation and its subregions, given that a lower regimen of prenatal alcohol exposure, for example, failed to promote hippocampal cell loss (Barnes and Walker, 1981). In addition, not all areas of the hippocampus are affected in the same way (Barnes and Walker, 1981; Samudio-Ruiz et al., 2010; Samudio-Ruiz et al., 2009). Interestingly, dentate gyrus function seems to be particularly vulnerable to low and moderate levels of exposure (Samudio-Ruiz et al., 2010; Samudio-Ruiz et al., 2009; Sutherland et al., 1997). This high vulnerability, as well as its key role in cognitive processing, makes the dentate gyrus a valuable region for the study of the effects of low and moderate levels of alcohol exposure in the developing brain.

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1.2 Effects of moderate prenatal alcohol exposure on dentate gyrus function

1.2.1 Development and anatomical organization of the dentate gyrus

The development of the mammalian brain is a dynamic process that takes place from early stages of embryonic development into adulthood. During this process, a series of events such as cell proliferation, migration, differentiation, synaptic integration and programmed cell death take place in order to establish all brain structures, connections and functional capabilities of the adult nervous system (Kim and Sun, 2011). In the rat, the dentate gyrus starts developing along with the hippocampus during the second and third weeks of gestation. Around gestational days 17 and 18, granule cells start migrating from the hippocampal neuroepithelium into the location of the primordial dentate gyrus, giving rise to the outermost portion of the granule cell layer. This first migration wave is followed by a second wave of cells that settle in the core region of the dentate that will form the hilus (Altman and Bayer, 1990). During the first week after birth, the dentate gyrus undergoes a growth spurt that results in a massive increase in the size and number of cells. The period of cell proliferation in the dentate gyrus of rats peaks on postnatal days 10 to 12 and then subsides. Meanwhile, these cells also undergo a process of synaptic formation and stabilization (Guerri, 1998). Lastly, cells that do not form sufficient connections are cleared from the dentate gyrus via programmed cell death (Kim and Sun, 2011).

In adult rats, the dentate gyrus is the subregion of the hippocampus that receives the majority of cortical inputs originating from the entorhinal cortex (Figure 1.1). It is characterized by its highly organized laminar conformation and by its mostly unidirectional synapses described by Ramon y Cajal in 1893. Morphologically, the

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dentate gyrus is composed of three distinct layers. The outermost layer, named the molecular layer, is largely devoid of cell bodies. This layer is composed primarily of axons originating from layer II of the entorhinal cortex and projecting into the molecular layer via the perforant path, and by dendrites of granule cells that extend from the granule cell layer. The granule cell layer, as its name suggests, is composed of densely packed somas of granule cells and by a disperse population of pyramidal basket cells.
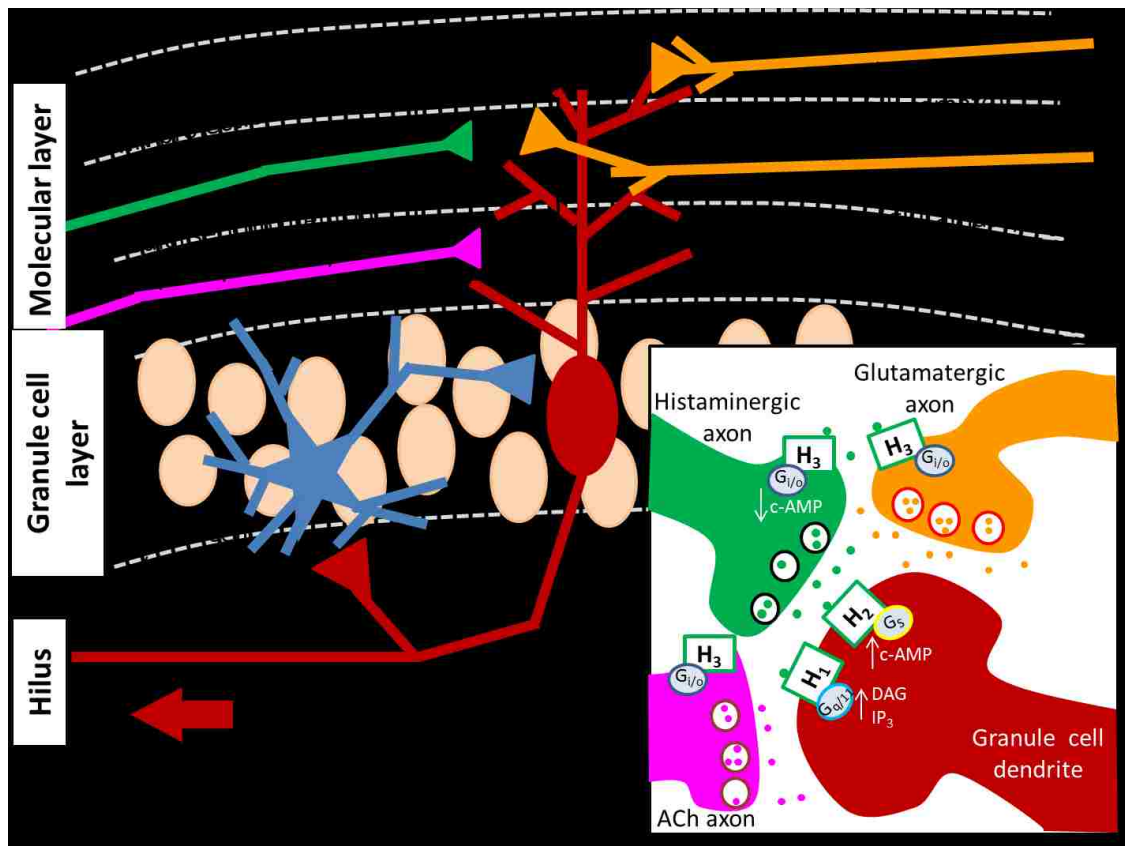
Figure 1.1 Interactions between histaminergic, glutamatergic, GABAergic and acetylcholinergic in the dentate gyrus

Granule cell dendrites in the molecular layer (maroon) receive glutamatergic innervation from the entorhinal cortex via the perforant path (orange), histaminergic innervation from the tuberomammillary nucleus (TMN, green), acetylcholinergic innervation from the medial septum (pink) and from GABAergic basket cells in located the interface between the granule cell layer and hilus. Inset: detail of histaminergic receptors postsynaptic (H_1 and H_2) and presynaptic location (H_3). Histamine H_1 receptors couple to $G_{q/11}$ proteins leading to DAG and IP_3 generation in the granule cell. Histamine H_2 receptors couple to G_s leading to increased levels of c-AMP. Both receptors contribute to excitation of the granule cells. Histamine H_3 receptors, on the other hand, couple to $G_{i/o}$ proteins in presynaptic nerve terminals, leading to reduced c-AMP and inhibition of neurotransmitter release.

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Immediately below the granule cell layer lies the polymorphic layer or hilus, characterized by mossy fibers that originate from granule cells and project to the CA3 region of the hippocampus (Scharfman, 2007). These three layers are folded either into a V-shape dorsally or a U-shape ventrally, and run alongside the entire extent of the hippocampus. Because of this peculiar fold-conformation, the molecular layer may be subdivided in two parts: the suprapyramidal layer, located between the CA1 and CA3 regions, and separated by the hippocampal fissure; and the infrapyramidal layer, located on the opposite side, bordering the lateral ventricle. The intersection of these two layers is called the crest (Amaral et al., 2007).

The most abundant neuronal cell type in the dentate gyrus is the granule cell. These cells receive virtually all their excitatory inputs primarily from the entorhinal cortex. Their axons project into the polymorphic layer and follow a distinctive path into the CA3 region of the hippocampus. Collectively, these glutamatergic fibers, described by Ramon y Cajal as "mossy" because of the appearance that results from their numerous varicosities, provide the majority of the excitatory output from the dentate gyrus (Gaarskjaer, 1981). Granule cells have secondary axons that branch-out from mossy fibers and re-enter the granule cell layer, providing an important regulatory feedback into the pyramidal basket cells (Acsady et al., 1998). The pyramidal basket cells are inhibitory interneurons critical in regulating activity of granule cells. Activation of pyramidal basket cells results in feedback inhibition of granule cells (Ribak et al., 1978; Sloviter, 1991). While the majority of pyramidal basket cells are located in the interface of the granule cell layer and the polymorphic layer, some may be found deep in the polymorphic layer (Ribak and Seress, 1983). The third important neuronal cell group in

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the dentate gyrus comprises the mossy cells. These are excitatory neurons whose cell bodies reside in the polymorphic layer (Soriano and Frotscher, 1994). The majority of their dendrites project deep within the polymorphic layer, with a few occasionally crossing the granule cell layer into the molecular layer (Amaral, 1978). In turn, axons originating from the mossy cells project back to the inner third of the molecular layer, either ipsi- or contralaterally. In rats, those fibers that cross to the dentate gyrus located in the contralateral hemisphere give rise to the associational commissure, innervating both granule cells and pyramidal basket cells (Scharfman, 1995). Lastly, there is a group that comprises neurons that do not fall into any of the previous categories. These cells give rise to axons that usually follow the branching of the pyramidal basket cells. Most of these neurons are also GABAergic, but may also express other neuroactive substances, such as somatostatin (Acsady et al., 2000). The specific role and origin of these cells are not yet completely understood.

As mentioned above, the majority of extrinsic innervation to the dentate gyrus is comprised of perforant path fibers that originate in the entorhinal cortex. However, other regions of the brain also send projections to the dentate gyrus, and these afferents play an important role in regulating activity of the dentate gyrus. In addition to the entorhinal cortex, the molecular layer receives a minor excitatory innervation from the presubiculum and parasubiculum (Kohler, 1985). Moreover, the interface of the granule cell layer with the polymorphic layer receives important cholinergic and GABAergic inputs from cells from the medial septum nucleus and the diagonal band of Broca, whose axons project through the septum (Amaral and Kurz, 1985). These fibers make synapses onto specific cell populations: cholinergic fibers preferentially innervate dendritic spines in granule

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cells, while GABAergic fibers localize predominantly in the proximity of pyramidal basket cells (Lubke et al., 1997). The polymorphic layer also receives inputs from noradrenergic, dopaminergic and serotonergic fibers projecting respectively from the locus coeruleus, ventral tegmental area and raphe nuclei (Loughlin et al., 1986; Swanson, 1982; Vertes et al., 1999). Altogether, these neurotransmitter systems are capable of exerting important effects on processes associated with cognition (Gonzalez-Burgos and Feria-Velasco, 2008; Tully and Bolshakov, 2010).

Another important source of input into the dentate gyrus are excitatory fibers originating from the supramammillary region of the hypothalamus, and terminating predominantly in the granule cell layer, but occasionally spilling over to the interface with the molecular and polymorphic layers (Magloczky et al., 1994). Similarly, histaminergic fibers project from the tuberomammillary nucleus in the hypothalamus and diffusely innervate the granule cell layer (reviewed by Haas et al., 2008). The specific role of these histaminergic inputs will be discussed in further detail throughout this manuscript.

1.2.2 Long-term potentiation in the dentate gyrus
Because of its simple and highly organized synaptic architecture, readily accessible excitatory fibers and distinct neuronal populations, the dentate gyrus offers uniquely favorable conditions for the study of synaptic plasticity. In fact, the pioneering work by Bliss and Lomo introducing the expression "long-term potentiation" (LTP) was conducted in the dentate gyrus of anesthetized rabbits (Bliss and Lomo, 1973).

The expression "synaptic plasticity" is used to explain the capacity that neurons have to adapt to different situations and respond to changes in the firing patterns that they receive

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from other neurons. This expression is commonly modified to describe specific forms of plasticity according to their (i) direction, (ii) magnitude and (iii) duration: in this perspective, plasticity expressed as increased neuronal activity is commonly referred as potentiation. Conversely, plasticity that is expressed as diminished neuronal activity is referred to as depression. Regarding the magnitude of plasticity, these phenomena may be described as maximal or submaximal. Lastly, regarding the duration, synaptic plasticity can be named short-term or long-term. Therefore, a combination of terms, such as "short term potentiation" or "long term depression" is employed to describe specific entities with different fundamental characteristics, including in the mechanistic level.

Numerous studies have used different induction protocols to successfully elicit various forms of synaptic plasticity in the dentate gyrus. Long term depression, for example, is often achieved by stimulating the perforant path fibers with low-frequency pulses during a relatively long period of time (i.e.: Christie and Abraham, 1994), while LTP, on the other hand, may be achieved via high frequency stimulation of perforant path fibers (i.e.: Bliss and Lomo, 1973; Sutherland et al., 1997) or via application of drugs that activate metabotropic glutamate receptors (O'Leary and O'Connor, 1997). Fine tuning of the intended magnitude, either submaximal or maximal, is accomplished by altering the intensity or the number of times a particular induction protocol is repeated (i.e.: Sutherland et al., 1997). It is important to note that each of these protocols may recruit different mechanisms. Therefore, one has to be careful when comparing results across different studies.

Because long term potentiation is affected by prenatal alcohol exposure, it is useful to understand the basic principles that govern it. LTP in the dentate gyrus shares many of

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principles and mechanisms observed in other brain regions. Induction of LTP requires activation of pre- and/or postsynaptic mechanisms that allow entry of calcium into the cell. This calcium influx occurs through two distinct and independent mechanisms that use either N-methyl-D-aspartate (NMDA) receptors or voltage-gated calcium channels (VGCC). In NMDA-dependent LTP, the activation of 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid (AMPA) receptors provides sufficient depolarization in order to remove the magnesium blockade of NMDA receptors (Collingridge, 2003). Conversely, in NMDA-independent LTP, the initial depolarization results in L-type VGCC opening, also allowing for calcium influx (Grover and Teyler, 1990). Since these mechanisms develop more or less simultaneously, it has to be taken into consideration that, unless specifically noted, the majority of induction protocols published in the literature (including those used further in this manuscript) rely on both NMDA-dependent and VGCC-dependent mechanisms, with various degrees of weight towards one or another (Blundon and Zakharenko, 2008).

After the induction phase, LTP evolves via two temporal-spatially distinct and independent mechanisms: an "early-LTP" phase (or LTP1) and a "late-LTP" phase (or LTP2 and LTP3). Early LTP is predominantly postsynaptic and involves activation of calcium-triggered protein kinases, phosphorylation of proteins, redistribution of AMPA receptors, enlargement of dendritic spines and recruitment of silent synapses (Malenka and Bear, 2004). Early-LTP develops within seconds to minutes after the induction phase and lasts only about 30 to 60 minutes (Malenka and Bear, 2004; Raymond, 2007). This initial early-LTP is gradually substituted by late-LTP, a mechanism predominantly presynaptic (Blundon and Zakharenko, 2008). It is hypothesized that protein synthesis is

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not required for the expression of postsynaptic LTP (early-LTP or LTP1), but is critical for the sustenance of presynaptic LTP (late-LTP or LTP2 and LTP3) (Blundon and Zakharenko, 2008; Malenka and Bear, 2004; Raymond, 2007). Therefore, modulation of presynaptic mechanisms that inhibit signaling cascades that converge into modulation of protein synthesis, such as activation of presynaptically located $G_{i/o}$ coupled receptors, could impair the expression of late LTP.

1.2.3 Prenatal alcohol effects in dentate gyrus function and LTP

In the late 1990's, Sutherland and collaborators found that prenatal alcohol administration through a liquid diet that yielded moderate blood alcohol concentration in pregnant rats resulted in decreased LTP in the dentate gyrus (Sutherland et al., 1997). These authors observed no changes in gross measures of teratogenicity, such as increased pup mortality or reduced weight at birth. Moreover, no changes were found in baseline measures of granule cell responsiveness, suggesting that moderate prenatal alcohol exposure does not alter synaptic strength and number. Both control and prenatal alcohol exposed offspring exhibited potentiation of granule cell responses to electrical stimulation induced by a submaximal tetanization protocol. However, in prenatal alcohol exposed offspring these responses quickly decayed back to baseline levels, suggesting an effect reminiscent of late-LTP or LTP2/3 impairment.

In follow-up studies, a similar prenatal alcohol exposure paradigm resulted in reduced activity-dependent phosphorylation of growth associated protein 43 (GAP-43), a protein localized to presynaptic terminals and involved with neurotransmitter release (Perrone-Bizzozero et al., 1998). It was later established that prenatal alcohol also reduced

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activity-dependent D-aspartate release from hippocampal slices, a marker for glutamate release (Savage et al., 1998), as well as the number and function of presynaptic metabotropic glutamate receptors type 5 (mGluR$_5$) in the dentate gyrus (Galindo et al., 2004). Taken together, these results underlie that functional deficit in the dentate gyrus may be an important player in prenatal ethanol induced cognitive impairment.

More recently, using a mouse model of limited access moderate drinking during pregnancy, Brady and collaborators observed a similar prenatal alcohol-induced LTP deficit in dentate gyrus slices as that observed by Sutherland and collaborators *in-vivo* (Brady et al., submitted for publication). Prenatal alcohol exposure in mice also results in decreased dentate gyrus neurogenesis (Choi et al., 2005), altered NMDA receptor distribution (Samudio-Ruiz et al., 2009) and trafficking (Brady et al., submitted for publication). Clearly, prenatal alcohol exposure results in a constellation of pre- and postsynaptic changes that, together, may play an important role in modulating behavior in these animals (Farr et al., 1988; Martin et al., 1992; Savage et al., 1991; Savage et al., 1989).

Studies hypothesize that dentate gyrus functional deficits result in behavioral impairment in a variety of memory paradigms, especially when the task is challenging enough to elicit subtle alterations (Clelland et al., 2009; Lee and Solivan, 2010). Interestingly, in a simpler version of the Morris water maze, low to moderate prenatal ethanol regimen failed to induce behavioral differences between control and ethanol-exposed offspring. Only when the behavioral task was made more challenging (i.e.: by increasing the time between training and recalling) that a significant impairment in the ethanol-exposed

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group surfaced (Hunt et al., 2009). Thus, restoring the deficits in dentate gyrus function caused by prenatal ethanol might be a reasonable goal for therapeutic intervention.

<u>1.2.4 Strategies for restoring prenatal alcohol-induced plasticity and behavioral deficit</u>

In recent years, there has been a renewed interest in the development of drugs capable of reversing cognitive deficits in major psychiatric disorders where cognition is impaired, such as Parkinson's disease, Alzheimer's disease and schizophrenia (Brioni et al., 2011; Passani and Blandina, 2011). The study of therapeutic interventions in FASD, however, has focused predominantly on behavioral support and prevention (Kodituwakku, 2009). Although these approaches are of critical importance, one has to acknowledge that the development of pharmacotherapeutic tools would be of great utility in treating FASD-associated cognitive deficits.

At least three different mechanisms of action have been proposed for improving cognitive capacity via enhancement of synaptic plasticity: (i) by positively modulating postsynaptic receptors involved with synaptic plasticity, such as AMPA or NMDA glutamate receptors, or by amplifying intracellular signaling cascades that are triggered with activation of the aforementioned receptors, (ii) by increasing the concentration and duration of neurotransmitter available in the synaptic cleft, or (iii) by up-regulating neurotransmitter release from the presynaptic button and presynaptic mechanisms involved in the expression of synaptic plasticity. Promising results have been shown on reversing the cognitive deficits induced by prenatal ethanol. First, Vaglenova and collaborators (2008) showed that sub-chronic injection of aniracetam (50 mg/kg i.p. daily, postnatal days 18-27), a positive allosteric modulator of glutamatergic AMPA

receptors, had a beneficial effect on memory impairment and synaptic plasticity deficit caused by intra-gastric alcohol administration [4/g/kg/day, blood alcohol concentration (BAC) of ~184 mg/dL] throughout pregnancy (Vaglenova et al., 2008). Second, the phosphodiesterase type 1 inhibitor vinpocetine (20 mg/kg i.p. every other day during behavioral testing, postnatal days 25-29) successfully restored spatial learning in rats in mice exposed to alcohol (5 g/kg i.p. BAC of ~239 mg/dL) on postnatal days 4, 6, 8 and 10 (Filgueiras et al., 2010), as well as neuronal plasticity in the visual cortex of ferrets in a binge-like model of prenatal alcohol exposure (3.5 g/kg i.p. , BAC of ~250 mg/dL) during the third trimester equivalent (Medina et al., 2006).   Third, choline supplementation (100 mg/kg s.c. daily from postnatal days 4-30) mitigates prenatal ethanol-induced (5.25 mg/kg/day orally, BAC of ~334 mg/dL) alterations in the cholinergic system (i.e.: Monk et al., 2012), an effect that may explain the beneficial effects of choline supplementation in prenatal ethanol-induced memory impairment (Thomas et al., 2010).   Finally, injection of the presynaptically acting histamine H_3 receptor inverse agonist ABT-239 dose dependently (acute injection of $0.1 - 3$ mg/kg i.p. before training sessions) reversed fetal ethanol induced memory deficit in a model of voluntary ethanol drinking (average consumption of 2.82 g/kg/day, BAC of ~84 mg/dL) during gestation in rats (Savage et al., 2010).   Combined, these studies indicate that reversal of prenatal alcohol-induced plasticity deficit is not only a theoretical possibility, but achievable at least in animal models.

1.3 The histaminergic system

The study by Savage and collaborators in 2010 raised a series of interesting questions about the regulation of cognition by the histaminergic system in rats prenatally exposed

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to alcohol (Savage et al., 2010). First, these authors observed that prenatal alcohol-induced deficits in dentate gyrus-sensitive memory tasks are reversed by the histamine H_3 inverse agonist ABT-239 in a dose-dependent manner. Second, it was observed that ABT-239 injection did not improve memory retention in control offspring, even though the memory tasks were challenging enough that improvement of memory performance in these animals may have been expected. These differential effects led to the question of whether or not prenatal alcohol exposure causes intrinsic changes in the mechanisms through which histamine modulates learning and memory. Some of these mechanisms are discussed further in the following section.

1.3.1 Biochemistry and anatomical organization

Histamine is synthesized from histidine through an oxidative decarboxylation catalyzed by histidine-decarboxylase (HDC). Expression of HDC in the brain is mainly neuronal, although other cells, such as mast cells, may synthesize and release histamine in the brain (Panula et al., 1984). Mammalian histaminergic neurons in adults are located exclusively in the tuberomammillary nucleus of the posterior hypothalamus (Wouterlood et al., 1986). Their axons diffusely project to most areas of the brain, including the neocortex, hippocampal formation, amygdala, basal ganglia, thalamus, superior colliculus and cerebellum (Kohler et al., 1985; Takagi et al., 1986). Respectively, injection of retrograde tracers into any of these areas is evenly uptaken by five different clusters of histaminergic neurons (E_{1-5}) in the hypothalamus (Steinbusch, 1991; Takeda et al., 1984). In turn, tuberomammillary histaminergic neurons receive input from the brainstem, infralimbic cortex, lateral septal nucleus, and preoptic region (Ericson et al., 1989; Ericson et al., 1991; Sherin et al., 1998), and can be modulated by a number of

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neurotransmitters, such as adenosine (Oishi et al., 2008), GABA (Sherin et al., 1998; Yum et al., 2008), glutamate (Faucard et al., 2006), orexin/hypocretin (Eriksson et al., 2001), endogenous opioids (Eriksson et al., 2000) and monoamines (Ericson et al., 1989). Histaminergic neurons have a distinct pace-maker firing activity of about 1 Hz, which can be positively modulated during arousal, learning or stress (Stevens et al., 2001; Takahashi et al., 2006) and decreased during sleep and anesthesia (Takahashi et al., 2006). The effects of histamine are terminated by break-down into the inactive molecule tele-methylhistamine by membrane-bound histamine methyltransferase (Barnes and Hough, 2002).

1.3.2 Subtypes of histamine receptor and their signaling mechanisms

Histamine exerts its actions by binding to proteins in the cell membrane known as receptors. To date, four different subtypes of histamine receptors have been characterized, namely H_1, H_2, H_3 and H_4 receptors. These receptors differ primarily on their localization, signaling mechanisms and pharmacology (Figure 1.1, inset).

Histamine H_1 receptors are expressed throughout the entire brain, with high densities in the tuberomammillary nucleus, cortex, hippocampus, striatum and thalamus. Outside the nervous system, H_1 receptors can be found in immune cells where they contribute to allergic reactions. The primary effectors of H_1 receptors are $G\alpha_{q/11}$ proteins. Upon activation of these proteins, there is an increase in phospholipase C activity, generation of inositol tri-phosphate (IP_3) and diacylglycerol (DAG), and consequent mobilization of intracellular calcium via activation of IP_3 receptors. Secondary signaling mechanisms include production of arachidonic acid, cyclic-GMP and nitric oxide. On the cellular and

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behavioral levels, activation of H_1 receptors results in cell excitability, increased firing rate of neurons, wakefulness and arousal. Conversely, antagonism of these receptors results in sedation, decreased reaction time and sleepiness (Haas et al., 2008).

Histamine H_2 receptors were first described in the gastric mucosa where they control secretion of gastric acid, but are also expressed in the brain. High levels are found in the brainstem and hippocampus while lower levels exist in the cerebral cortex, striatum and hypothalamus (Karlstedt et al., 2001; Ruat et al., 1991). Histamine H_2 receptors couple to $G\alpha_s$ proteins, resulting in stimulation of adenylyl cyclase and consequent increase in intracellular levels of cyclic-AMP and PKA activity (Palacios et al., 1978; Wang et al., 1996). Because of its coupling mechanisms, activation of histamine H_2 receptors by a given agonist generally results in excitation of target neurons (Olianas et al., 1984; Wu et al., 2012).

Histamine H_3 receptors differ from histamine H_1 and H_2 receptors by their predominantly presynaptic localization. These receptors are expressed throughout the brain, with high levels in the caudate, medial frontal cortex, hypothalamus and mesolimbic areas, and moderate expression in the dentate gyrus, cortex, thalamus and olfactory bulb (Pillot et al., 2002). Histamine H_3 receptors act as classic auto- and heteroreceptors, coupling to $G\alpha_{i/o}$ proteins and leading to inhibition of adenylyl-cyclase, decreased levels of cyclic-AMP and reduction of neurotransmitter release (Haas et al., 2008). A secondary mechanism of these receptors is the $G\beta\gamma$-protein mediated blockade of the SNARE complex and VGCCs, thus reducing exocytosis and consequent neurotransmitter release through a mechanism independent of adenylyl-cyclase inhibition (Betke et al., 2012; Brown and Reymann, 1995). One interesting characteristic of histamine H_3 receptors is

the ability to interact with G-proteins without being bound by an agonist, a phenomenon described as "constitutive activity", which can be negatively modulated by inverse agonists. The constitutive activity in H_3 receptors is considered to be one of the highest among G-protein coupled receptors (Bakker, 2004) and may have an important role in the physiological regulation of neurotransmitter release. In addition, alternative splicing of the H_3 receptor gene leads to different isoforms. At least six different possible isoforms have been identified in the rat; three of them (rH_{3A}, rH_{3B} and rH_{3C}) are naturally expressed and functional (Bakker, 2004). These isoforms differ in their pharmacololgycal proprieties, signaling mechanisms and constitutive activity. The rH_{3A} isoform couples preferentially to p44/p42 MAPK activation, displays low agonist affinity and higher constitutive activity (Drutel et al., 2001; Morisset et al., 2000). Conversely, rH_{3B} and rH_{3C} isoforms efficiently inhibit adenylyl-cyclase, exhibit higher agonist affinity and relatively lower constitutive activity (Drutel et al., 2001; Morisset et al., 2000). Together, these isoforms add a layer of complexity to histamine H_3 modulation of presynaptic processes.

Histamine H_4 receptors are homologous to histamine H_3 receptors in their signaling mechanism, as they also couple to $G\alpha_{i/o}$ proteins leading to inhibition of adenylyl cyclase. However studies indicate that expression of these receptors is mostly restricted to cells of the immune system. Limited expression is also found in a few neurons of the sensory dorsal root ganglia, horn of the spinal cord, as well as in limited areas of the cortex and thalamus (Strakhova et al., 2009). The role of histamine H_4 receptors in these neurons, however, has remained highly speculative.

1.3.3 Role of the histaminergic system in cognition

The involvement of the histaminergic system in modulation of levels of arousal and sleep/wake cycles generated the question of whether or not this system is involved in learning and memory. Early studies of this subject led to contradictory results, either showing a detrimental effect on retention of a task with histamine injected immediately before learning (Roussinov and Yonkov, 1976) or a facilitatory effect on memory retention (de Almeida and Izquierdo, 1986). Moreover, depletion of brain histamine following the injection of alpha-fluoromethylhistidine, an inhibitor of histamine synthesis, led to either facilitation (Sakai et al., 1998) or impairment (Chen et al., 1999; Kamei et al., 1993) of memory retention, and the results are further complicated when the sedative effects of histamine depletion are considered. For example, lack of endogenous histamine in HDC knock-out mice resulted in increased contextual fear conditioning and LTP in the CA1 region of the hippocampus (Liu et al., 2007). The effects of histamine also seem to be dependent of the region of the brain: Local application of histamine in the ventral hippocampus, for example, impaired retrieval of a conditioned behavior (Alvarez and Banzan, 1996; Alvarez and Banzan, 2001), whereas no effect was observed in the dorsal hippocampus (Alvarez and Banzan, 2001). Therefore, it is clear that histamine alone can exert effects in both directions, either impairing or improving memory, and the receptors that are predominantly being activated will likely determine this direction.

More recently, particular interest has been devoted towards the histamine H_3 receptors role in cognitive functions. These receptors reside predominantly in pre-synaptic nerve terminals (Pillot et al., 2002), and their activation is associated with suppression of

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release of not only histamine (i.e.: Giannoni et al., 2010), but also acetylcholine (i.e.: Bacciottini et al., 2002), GABA (i.e.: Welty and Shoblock, 2009) and glutamate (i.e.: Brown and Haas, 1999), all of which can impact learning and memory. Antagonists and inverse agonists of H_3 receptors also showed pro-cognitive actions in a diversity of animal models (reviewed by Brioni et al., 2011; Esbenshade et al., 2008). For example, modulation of H_3 activity affected learning and memory in hippocampal dependent tasks (Bardgett et al., 2010; Flood et al., 1998; Fox et al., 2005; Medhurst et al., 2007b). Additionally, antagonism of H_3 receptors increased theta oscillations in the hippocampus, a neurophysiologic indicator of cognitive power (Hajos et al., 2008). As mentioned above, systemic injection of the inverse agonist ABT-239 resulted in differential effects in prenatal ethanol induced memory deficit in a challenging version of the Morris water maze and contextual fear conditioning when compared to control offspring (Savage et al., 2010). These observations led to the statement of the hypothesis illustrated below.

2. Rationale, hypothesis and specific aims

2.1 Rationale

Prenatal alcohol exposure during pregnancy is the most common cause of cognitive deficit in the United States. Fetal Alcohol Spectrum Disorder is characterized by lifelong cognitive impairment. The dentate gyrus, a region in the hippocampus that receives the majority of excitatory hippocampal input, is particularly vulnerable to prenatal alcohol exposure. In the dentate gyrus, prenatal alcohol exposure results in decreased synaptic plasticity, in impaired activity-dependent glutamate release and in reduced markers of presynaptic function. These functional deficits may account, in part, for the cognitive impairment observed in children whose mothers consumed alcohol during pregnancy, especially in more challenging cognitive tasks. Therefore, restoring presynaptic function in the dentate gyrus seems to be a reasonable goal for therapeutic intervention.

In a first attempt to restore cognitive deficit in rats prenatally exposed to moderate doses of ethanol, Savage and collaborators observed that inverse agonism of histamine H_3 receptors via intraperitoneal injection of ABT-239 resulted in a dose-dependent improvement of memory in fetal ethanol exposed offspring. These doses of ABT-239, however, did not facilitate memory retention in control offspring, even though the tasks were challenging enough so that facilitation of cognitive processes by ABT-239 may have been expected. The differential effects observed in that study led to the hypothesis of an intrinsic dysregulation of histamine H_3 receptors in rats prenatally exposed to alcohol.

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2.2 Hypothesis

Prenatal exposure to moderate levels of ethanol increases histamine H_3 receptor-effector coupling and H_3-mediated inhibition of glutamate release, contributing to a synaptic plasticity deficit in the dentate gyrus of rats.
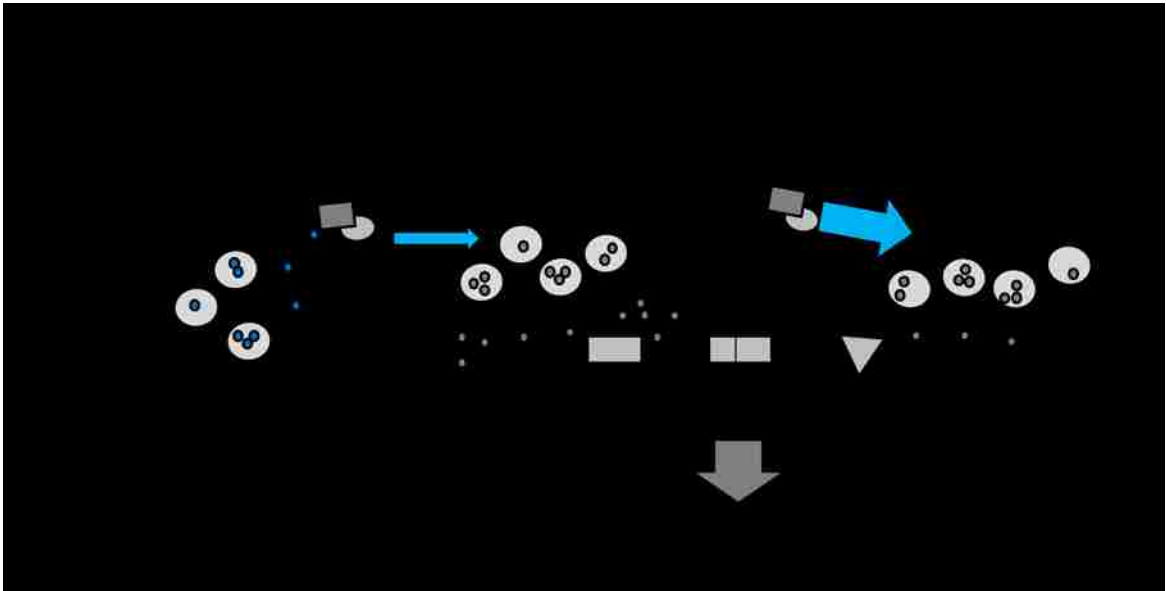
Figure 2.1 Schematic representation of the working hypothesis

In control offspring, activation of histamine H3 receptors results in modulation of glutamate release from presynaptic terminals. Increased receptor-effector coupling in prenatal alcohol exposed offspring leads to an exacerbated decrease of glutamate release, contributing to the LTP deficit observed in these offspring.

2.3 Specific aims

This hypothesis was approached in three specific aims:

2.3.1 Aim 1:

To assay the effects of activation and blockade of histamine H_3 receptors on long-term potentiation elicited by submaximal tetanization of the perforant path-to-dentate gyrus synapses in rats prenatally exposed to ethanol and controls *in vitro* and *in vivo*.

2.3.2 Aim 2:

To indirectly determine histaminergic H_3 modulation of glutamate release in the dentate gyrus of rats prenatally exposed to ethanol and controls by measuring baseline and activity dependent paired-pulse plasticity of glutamatergic evoked responses *in vitro* and *in vivo*.

2.3.3 Aim 3:

To measure histamine H_3 receptor density and receptor-effector in the dentate gyrus of rats prenatally exposed to ethanol and controls using total antagonist binding and agonist-stimulated binding of GTPγS *in vitro*.

2.3.4 Distribution of specific aims across chapters

As part of a hybrid dissertation format, this manuscript comprises three original research articles, either submitted or in process of submission, distributed in the next three chapters. In the first chapter (chapter 3), Aim 1 is investigated using an inverse agonist of histamine H_3 receptors in an *in-vivo* model of prenatal alcohol-induced LTP in the dentate gyrus. The following chapter (chapter 4) expands these observations by investigating the effects of a histamine H_3 agonist in LTP and H_3-mediated inhibition of glutamate release in the dentate gyrus of rats prenatally exposed to alcohol and controls (Aims 1 and 2). Finally, the third chapter (chapter 5) takes advantage of *in vitro* biochemical and electrophysiological techniques to investigate histamine H_3 modulation of LTP, H_3-mediated inhibition of glutamate release, H_3 receptor density and coupling to G-proteins (Aims 1, 2 and 3).

3. Effects of the cognition-enhancing agent ABT-239 on fetal ethanol-induced deficits in dentate gyrus synaptic plasticity

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3.1 Abstract

Prenatal ethanol exposure causes deficits in hippocampal synaptic plasticity and learning. At present, there are no clinically effective pharmacotherapeutic interventions for these deficits. Here, we examined whether the cognition-enhancing agent ABT-239, a histamine H_3 receptor antagonist, could ameliorate fetal ethanol-induced long-term potentiation deficits. Long-Evans rat dams consumed a mean of 2.82 g/kg ethanol during a four-hour period each day. This voluntary drinking pattern produced a mean peak serum ethanol level of 84 mg/dL. Maternal weight gain, offspring litter size and birthweights were not different between ethanol-consuming and control groups. A

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stimulating electrode was implanted in the entorhinal cortical perforant path and a recording electrode in the dorsal dentate gyrus of urethane-anesthetized adult male offspring. Baseline input/output responses were not affected either by prenatal ethanol exposure or by 1 mg/kg ABT-239 administered two hours prior to data collection. No differences were observed between prenatal treatment groups when a ten tetanus train protocol was used to elicit LTP. However, LTP elicited by three tetanizing trains was markedly impaired by prenatal ethanol exposure compared to control. This fetal ethanol-induced LTP deficit was reversed by ABT-239. In contrast, ABT-239 did not enhance LTP in control offspring using the three tetanus train protocol. These results suggest that histamine H_3 receptor antagonists may have utility for treating fetal ethanol-associated synaptic plasticity and learning deficits. Further, the differential effect of ABT-239 in fetal alcohol offspring compared to controls raises questions about the impact of fetal ethanol exposure on histaminergic modulation of excitatory neurotransmission in affected offspring.

3.2 Introduction

Heavy or binge patterns of drinking during pregnancy can cause profound morphological and neurological aberrations in offspring called Fetal Alcohol Syndrome (FAS) (Jones and Smith, 1973; Lemoine et al., 1968). However, increasing evidence indicates that even moderate drinking during pregnancy can cause subtle, long-term behavioral and cognitive impairments in the absence of the birth defects associated with FAS (see review by Kodituwakku, 2009). These behavioral deficits may not become apparent until the educational years (Hamilton et al., 2003; Jacobson et al., 1998; Streissguth et al., 1990) and may increase in severity as the child matures (Streissguth et al., 1994).

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The mechanisms by which prenatal ethanol exposure causes long-lasting impairments in learning and memory are not well understood. Our previous studies of Sprague-Dawley rats using a 5% ethanol liquid diet paradigm as a model of moderate drinking during pregnancy indicated that prenatal ethanol-exposed offspring exhibit performance deficits on increasingly challenging memory tasks (Sutherland et al., 2000; Weeber et al., 2001a). These memory impairments are, in part, linked to physiological alterations that diminish activity-dependent enhancement of synaptic neurotransmission in hippocampal formation of affected offspring (Savage et al., 1998; Sutherland et al., 1997). Further, decreased positive allosteric modulation of dentate granule cell NMDA receptors (Costa et al., 2000) and mGluR5 receptor-mediated potentiation of glutamate release from perforant path nerve terminals (Galindo et al., 2004) have been implicated as putative neurochemical mechanisms underlying fetal ethanol-induced deficits in synaptic plasticity and learning.

Presynaptic histamine H_3 receptors mediate the inhibition of transmitter release of a number of central nervous system neurotransmitters including histamine (Arrang et al., 1983), serotonin, norepinephrine, dopamine (Schlicker et al., 1988; Schlicker et al., 1993; Schlicker et al., 1989), acetylcholine (Clapham and Kilpatrick, 1992), and glutamate (Brown and Reymann, 1996). Conversely, histamine H_3 receptor antagonists enhance the release of acetylcholine, dopamine (Fox et al., 2005) and glutamate (El-Emawy and Savage, unpublished observations) whereas their impact on serotonin, norepinephrine and GABA release have been less-studied to date. The ability of H_3 receptor antagonists to facilitate acetylcholine, dopamine and glutamate release has generated considerable interest in their therapeutic potential for a variety of neurologic and psychiatric disorders,

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particularly diseases with attendant neurocognitive deficits. Indeed, H_3 receptor antagonists enhance behavioral performance in a variety of rodent learning paradigms (i.e.: Foley et al., 2009; Fox et al., 2005; Fox et al., 2003) and reversed contextual fear conditioning and spatial navigation deficits in fetal ethanol-exposed Long-Evans rats (Savage et al., 2010).

The present study had two experimental objectives. First, we wanted to determine whether the LTP deficits observed previously in Sprague-Dawley rats whose mothers consumed a 5% ethanol liquid diet (Sutherland et al., 1997) also occur in Long-Evans rat offspring whose mothers voluntarily drank 5% ethanol in saccharin water. Long-Evans rats were selected for study based on the preponderance of their use in learning and memory studies (Andrews, 1996). The voluntary drinking paradigm was selected because it has the dual advantage of not requiring multiple diet control groups to control for paired-feeding of liquid diet as well as for allaying concern that the consumption of liquid diets may be stressful (Rasmussen et al., 2000), a potential confound in fetal alcohol exposure studies. The second study objective was to determine whether the cognition enhancing agent ABT-239 [4-(2-{2-[(2R)-2-methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile], a non-imidazole antagonist of H_3 histamine receptors (Esbenshade et al., 2005; Miller et al., 2008) that reverses learning deficits in a variety of models including our current fetal ethanol exposure paradigm (Savage et al., 2010), would also reverse fetal ethanol-induced deficits in dentate gyrus LTP. The pharmacologic rationale for selecting ABT-239 was based on prior observations suggesting that prenatal ethanol exposure diminished activity-dependent potentiation of glutamate release in hippocampal slices (Savage et al., 1998). The 1 mg ABT-239/kg

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dose was selected for our study based on prior observations that it was an optimal test dose for increasing acetylcholine release in vivo and enhancing performance in a variety of behavioral paradigms (Fox et al., 2005) including the reversal of fetal ethanol-induced deficits in contextual fear conditioning and spatial navigation (Savage et al., 2010). Given that histamine H_3 receptor agonists inhibit glutamate release (Brown and Reymann, 1996), we predicted that the histamine H_3 receptor antagonist ABT-239 would attenuate fetal ethanol-induced LTP deficits in the dentate gyrus.

3.3 Methods

3.3.1 Material

All reagents were acquired from Sigma-Alrich Corp. (St. Louis, MO) unless indicated otherwise in parenthetical text.

3.3.2 Voluntary drinking paradigm

All procedures involving the use of live rats were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee. Four-month-old Long-Evans rat breeders (Harlan Industries, Indianapolis, IN) were single-housed in plastic cages at 22° C and kept on a "reverse" 12-hour dark / 12-hour light schedule (lights on from 2100 to 0900 hours) with Harlan Teklad rodent chow and tap water ad libitum. After at least one week of acclimation to the animal facility, all female rats were provided 0.066% saccharin in tap water for four hours each day from 1000 to 1400 hours. The saccharin water contained 0% ethanol on the first and second day, 2.5% ethanol (v/v) on the third and fourth day and 5% ethanol on the fifth day and thereafter. Daily four-hour consumption of ethanol was monitored for at least two weeks and then

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the mean daily ethanol consumption was determined for each female. Females whose mean daily ethanol consumption was greater than one standard deviation from the group mean were removed from the study. Typically, less than 12% of females were removed in a given breeding round. The remainder of the females were assigned to either a saccharin control or 5% ethanol drinking group and matched such that the mean pre-pregnancy ethanol consumption by each group was similar. Subsequently, females were placed with proven male breeders until pregnant, as indicated by the presence of a vaginal plug. Female rats did not consume ethanol during the breeding procedure. Beginning on Gestational Day 1, rat dams were provided saccharin water containing either 0% or 5% ethanol for four hours a day, from 1000 to 1400 hours. The volume of saccharin water provided to the control group was matched to the mean volume of saccharin water consumed by the ethanol group. Daily four-hour ethanol consumption was recorded for each dam. At birth, litters were culled to ten pups each. Offspring were weaned at 28 days of age and group-housed, two males per cage, until used in the in vivo electrophysiology studies.

3.3.3 Maternal serum ethanol levels

A separate set of twelve rat dams was used to determine serum ethanol concentrations. These dams were run through the same voluntary drinking paradigm as described above, except that blood samples were collected at the end of the four-hour ethanol consumption episode on gestational days 15, 17 and 19. Each rat dam was briefly anesthetized with isoflurane and had one hundred μL of whole blood collected from the tail vein. Samples were immediately mixed with 0.2 mL of 6.6% perchloric acid, frozen and stored at -20 °C until assayed. Serum ethanol standards were created by mixing rat whole blood from

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untreated rats with known amounts of ethanol ranging from 0 to 240 mg ethanol/dL and then mixing 100 µL aliquots of each standard with perchloric acid and storing the standards frozen with the samples. Serum ethanol samples were assayed using a modification of the method of Lundquist and colleagues (1959).

3.3.4 *In-vivo* electrophysiology

Male adult rat offspring, 105 to 140 days of age and weighing 370-500 g were anesthetized with urethane (two injections of 0.75 g/kg, 30 min apart). A subset of control and fetal ethanol-exposed offspring received an intraperitoneal injection of 1 mg ABT-239/kg dissolved in isotonic phosphate-buffered saline solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM NaH2PO4 and a pH of 7.4.) 5 min after the first urethane injection (approximately 2 hours prior to the three-train tetanization paradigm). Upon loss of the pedal reflex, a rat was placed into a stereotaxic frame (David Kopf Instruments, Tujunga, CA). Rectal temperature was closely monitored and maintained at 37.3 °C using a temperature controller (World Precision Instruments, Sarasota, FL) throughout the entire surgical and recording procedure. The stereotaxic procedure was conducted as described previously (Sutherland et al., 1997). Briefly, after exposing the skull, five holes were drilled using a dental burr. Three self-tapping screws attached to stainless steel wires and gold Amphenol pins were inserted into the skull; two served as ground and reference signals for the recording circuit and one served as the return component of the stimulating circuit. Recording and stimulating Teflon-coated, stainless steel, unipolar electrodes (114 μm outer diameter; A-M Systems, Carlsborg, WA) were implanted using bregma coordinates (recording electrode: AP -3.5 mm and ML +1.8 mm; stimulating electrode: AP -8.1 mm and ML +4.3 mm) (Paxinos and Watson, 1998).

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Electrodes were then connected to an isolated pulse stimulator (Model 2100; A-M Systems, Carlsborg, WA) and to a differential AC amplifier (Model 1800; A-M Systems, Carlsborg, WA). Recording signals were amplified (1000X), bandpass-filtered (0.1 Hz-10 kHz), and transferred to a personal computer via an analog-to-digital converter (Model BNC-2090; National Instruments, Austin, TX). The electrodes were slowly inserted into the dentate gyrus (recording electrode, DV -3.8 mm from bregma) and entorhinal cortex (stimulating electrode, DV -4.0 mm from bregma). Responses to a guide stimulus (400 μA intensity) were monitored during the electrode descent process.

Once optimal positioning of electrodes and stable baseline responses were achieved, an input/output curve was generated, using current intensities ranging from 50 to 500 μA. The stimulus intensity sufficient to generate 40% of maximal population spike (PS) response (ES40), which averaged about 300 μA, was then used for high frequency tetanization and subsequent test-stimulus recordings. Animals failing to exhibit a PS of at least 4 mV with 500 μA stimulus intensity were discarded from the study (n = 2). Synaptic potentiation was induced by either three or ten trains of high frequency stimulation (HFS) of 400 Hz over 25 ms with 30 s inter-train intervals. After tetanization, responses to the test stimulus were measured every 10 seconds over the next 60 minutes. To minimize random fluctuations, responses obtained over one-minute intervals (6 consecutive responses) were averaged. The first ascending portion of each response curve was used to calculate the fast excitatory post-synaptic potential slope (fEPSP). Population spike amplitude was calculated measuring the difference between the average of the first and second positive deflections to the negative peak.

3.3.5 Data analysis

For each rat, the baseline (pretetanus) responses over ten minutes were averaged, the mean normalized to 100% and the post-tetanus response data transformed by the baseline average. Comparison of the effects of prenatal ethanol exposure after tetanus was performed using one-way repeated measures analysis of variance (ANOVA) on ranks followed by Dunnett's post-hoc comparisons against baseline (100% value). Differences between treatments at discrete time points were examined using a two-tailed Student's t-test. All statistical procedures were performed using SigmaPlot[©], Version 11.0 (Systat Software Inc., San Jose, CA).

3.4 Results

3.4.1 Voluntary drinking paradigm

A summary of the voluntary drinking paradigm data is presented in Table 3.1. Rat dams stably consumed an average of 2.82 ± 0.13 grams of ethanol/kg body weight over the four-hour interval each day during pregnancy (approximately 16 mL of 5% ethanol in 0.066% saccharin water). This pattern and level of ethanol consumption produced a mean maternal serum ethanol concentration of 84.0 ± 5.5 mg/dL during the third week of gestation. Ethanol consumption did not affect maternal weight gain during pregnancy, nor did it produce any significant differences in litter size or offspring birth weight or body weights at time of surgery (Table 3.1).

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Table 3.1 Effects of daily four-hour consumption of 5% ethanol on female dams and their offspring

	Saccharin Control	5% Ethanol
Maternal Weight Gain During Pregnancy	127 ± 3[a](44)	121 ± 4(51)
Daily Four-Hour Consumption of 5% Ethanol	NA	2.82 ± 0.13[b](51)
Maternal Serum Ethanol Concentration	NA	84.0 ± 5.5[c](24)
Litter Size	12.5 ± 0.1[d](41)	12.4 ± 0.3 (42)
Pup Birthweight	6.17 ± 0.13[e](41)	6.13 ± 0.11 (42)
Male Offspring Weights at Surgery	473 ± 10[f](24)	473 ± 10 (21)

a - Mean ± S.E.M. grams increase in body weight from GD 1 through GD 21

b - Mean ± S.E.M. grams ethanol consumed / kg body weight / day

c - Mean ± S.E.M. mg ethanol / dL serum, collected at the end of a four-hour drinking period

d - Mean ± S.E.M. number of live births / litter

e - Mean ± S.E.M. grams pup birthweight

f - Mean ± S.E.M. grams body weight on day of stereotaxic surgery

NA - Not applicable

(N) - Group sample size

3.4.2 Baseline input/output responses

Figure 3.1 depicts input/output curves for fEPSP slope and PS amplitude in control and fetal ethanol-exposed offspring treated with either saline or 1 mg ABT-239/kg. Synaptic responses to stimuli ranging from 50 to 500 µA collected prior to tetanus were not affected by prenatal ethanol exposure (Fig. 3.1A and 3.1B) suggesting that the stimulus-response mechanisms mediating baseline glutamate release and postsynaptic ionotropic

A. Saline C. ABT-239
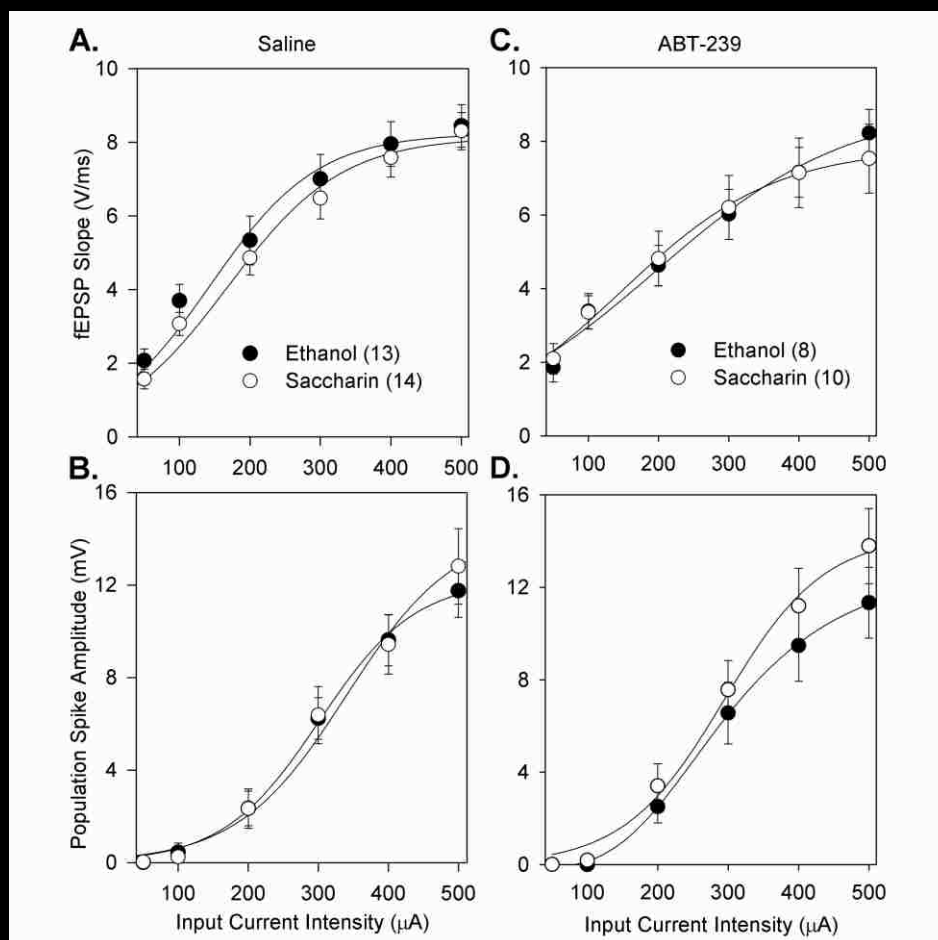
glutamate receptor responsiveness to glutamate are intact in fetal ethanol-exposed offspring. Further, ABT-239 treatment did not affect input/output curves compared to saline-treated offspring in either prenatal treatment group (Fig. 3.1C and 3.1D). Immediately after establishing the I/O curve for each rat, a curve fitting procedure was employed to determine the stimulus intensity that elicited 40% of maximal PS amplitude

(ES40). The ES40 stimulus intensity was then used for both the test stimulus and the high frequency stimuli (HFS) tetanization protocol in subsequent recordings. The mean ES40 values were not different across the four experimental groups (Saccharin-Saline = 305 ± 17 μA; Ethanol-Saline = 323 ± 18 μA; Saccharin-ABT-239 = 281 ± 14 μA; Ethanol-ABT-239 = 290 ± 14 μA).

3.4.3 Synaptic responses to the ten- and three-train tetanizing protocols

Field potential waveform responses obtained from saline-treated rats in the two prenatal treatment groups using the ten-train and three-train tetanus protocols are depicted in Figure 3.2. The response curves are characterized by a rapid ascending fEPSP followed by a negative PS, a typical response of dentate gyrus granule cells upon medial perforant pathway stimulation. Figure 3.3 summarizes the effects of prenatal ethanol exposure on LTP after either the ten-train or three-train tetanizing protocol in the absence of ABT-239 treatment. Application of ten tetanizing trains resulted in LTP in both prenatal exposure groups. fEPSP slope was significantly increased compared to baseline values in controls and ethanol-exposed rats (Fig. 3.3A) throughout the 60 minute post-tetanus recording period. Population spike amplitude was also significantly elevated in both groups compared to baseline (Fig. 3.3B) during the 60 minute post-tetanus period. No statistical differences between control and ethanol exposed offspring were observed.

The three-train tetanizing protocol produced slightly less synaptic potentiation in the control group at time 0 compared to the ten-train protocol. The potentiation of the fEPSP response was significantly greater than the (pretetanus) baseline for up to 48 minutes post-tetanus (Fig. 3.3C), whereas the PS amplitude was significantly greater than baseline

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Field Potential (mV)

A. Saccharin - Ten train protocol
C. Saccharin - Three train protocol
B. Ethanol - Ten train protocol
D. Ethanol - Three train protocol

HFS

Baseline   0 min   60 min
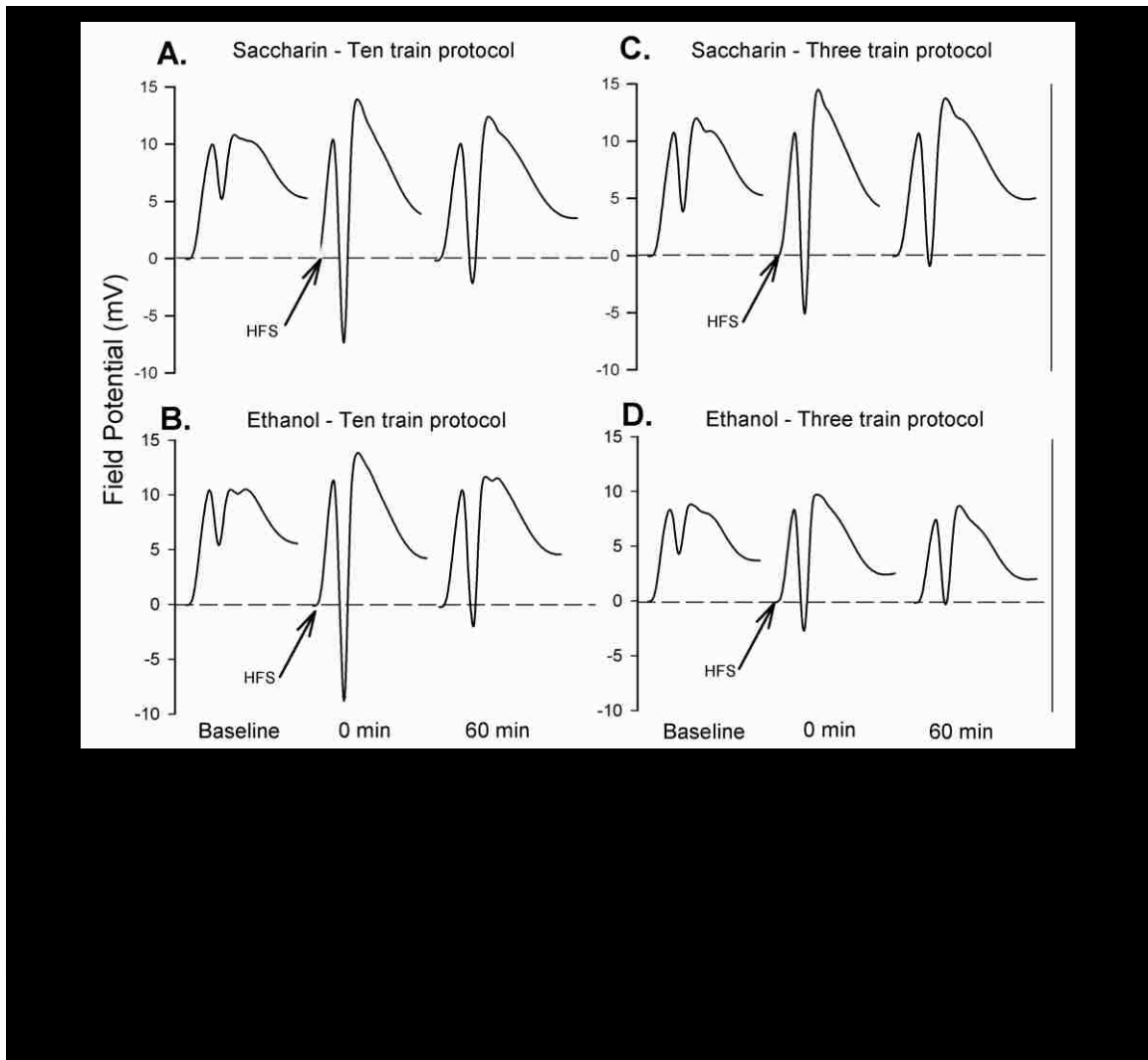
up to 37 minutes post-tetanus (Fig. 3.3D). In striking contrast to the control group, potentiation of the fEPSP response in fetal ethanol-exposed offspring only lasted for the first five minutes after tetanus and then rapidly decayed to the baseline level (Fig. 3.3C). Pair-wise comparisons indicated that fEPSP response in prenatal ethanol-exposed rats was significantly less than control at all time points after the first 2 minutes post-tetanus. While the duration of PS amplitude potentiation was 13 minutes shorter in fetal ethanol-exposed offspring compared to controls (Fig. 3.3D), pair-wise comparisons at each time

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point did not reveal significant differences in PS responses between prenatal treatment groups.
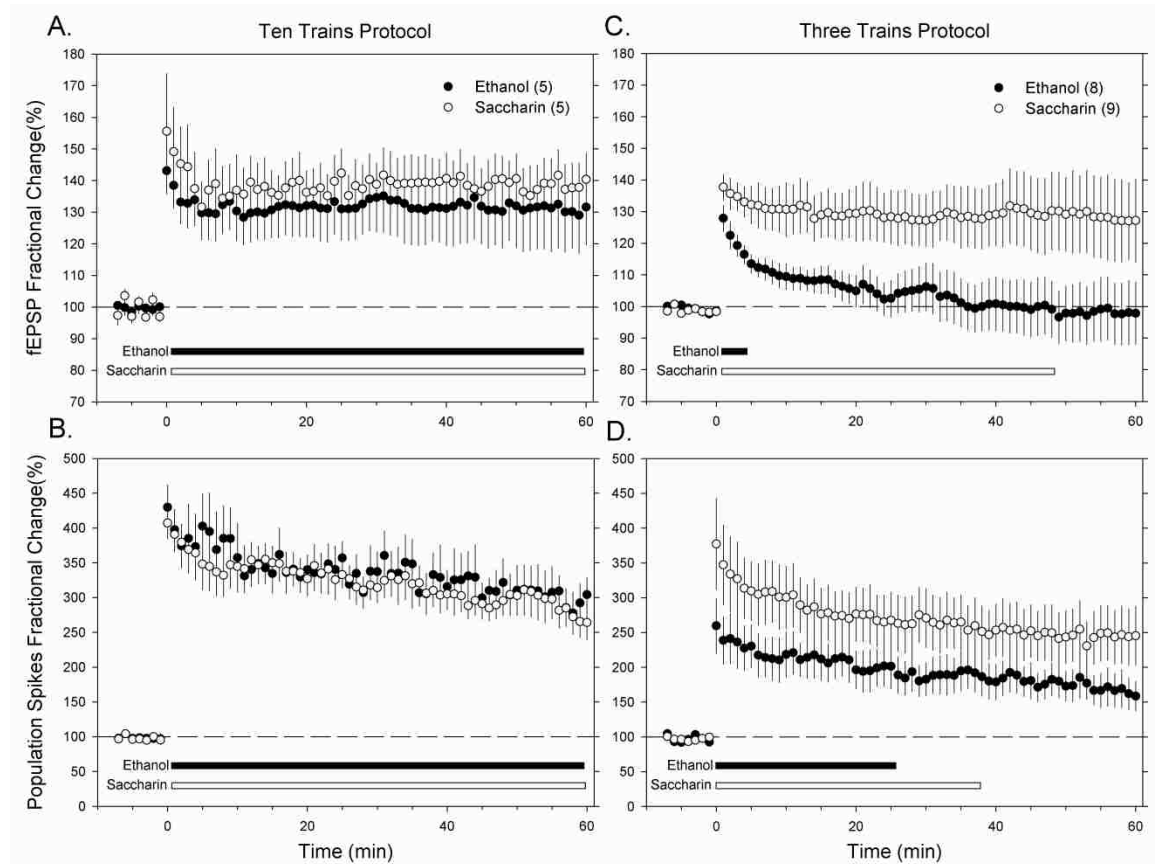
Figure 3.3 Effect of prenatal ethanol exposure on dentate granule cell responses before and after ten or three trains of high frequency stimulation (HFS).

Graphs illustrate fast excitatory postsynaptic potential (fEPSP) responses (3.3A) and population spike (PS) amplitude responses (3.3B) after ten tetanizing trains, and fEPSP responses (3.3C) and PS amplitude responses (3.3D) after three tetanizing trains. Each data point represents the mean ± the S.E.M. responses averaged over one-minute intervals from five to nine rats in each experimental group (sample sizes are noted in parentheses). The horizontal bars at the bottom of each graph denote the duration of time after tetanus that each response was significantly greater than the pretetanus baseline response (one-way repeated measures ANOVA on ranks, Dunnett post-hoc test, $p < 0.05$).

3.4.4 Effects of ABT-239 on synaptic potentiation using the three-train tetanus protocol

The effects of ABT-239 on LTP using the three tetanus train protocol are depicted in Figure 3.4. In control offspring, ABT-239 did not enhance fEPSP slope or PS amplitude

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when compared to saline-treated controls. Indeed, ABT-239 reduced the duration of time after tetanus when the fEPSP response was significantly elevated above baseline (Fig. 4.4A) without altering the potentiation of the PS amplitude response (Fig. 4.4B). However, pair-wise comparisons indicated that the fEPSP responses at each time point post-tetanus were not different between the saline- and ABT-239-treated control rats.
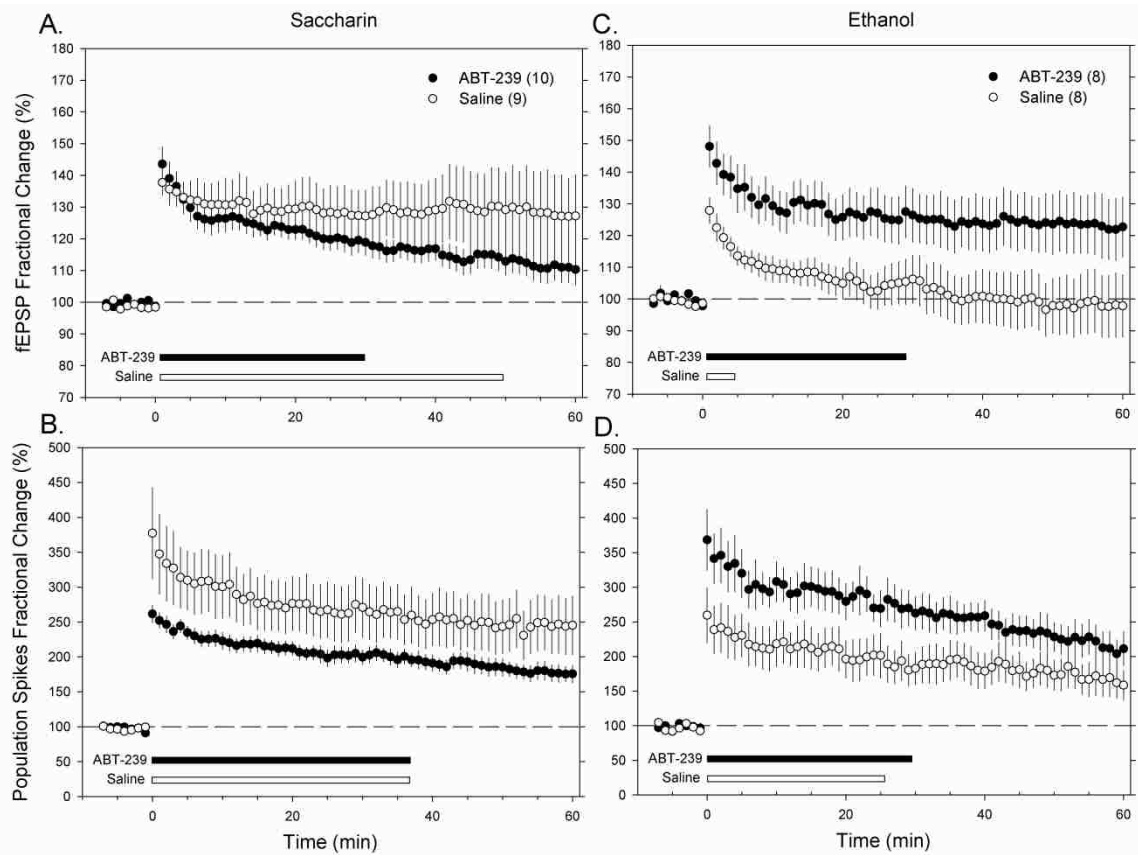
Figure 3.4 Effect of ABT-239 on LTP induced by three tetanizing stimulus trains.

Graphs illustrate fEPSP (top panels) and PS responses (bottom panels) in the control (Fig. 3.4A and 3.4B) or fetal ethanol-exposed (Fig. 3.4C and 3.4D) groups treated with either saline or 1 mg ABT-239/kg i.p. approximately two hours prior to tetanization. Each data point represents the mean ± the S.E.M. responses averaged over one-minute intervals from eight to ten rats in each experimental group (sample sizes are noted in parentheses). The horizontal bars at the bottom of each graph denote the duration of time after tetanus that each response was significantly greater than the pretetanus baseline response (one-way repeated measures ANOVA on ranks, Dunnett post-hoc test, $p < 0.05$).

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In contrast to control rats, ABT-239 elevated both fEPSP and PS amplitude responses in prenatal ethanol-exposed rats compared to saline-treated rats (Fig. 3.4C and 3.4D). ABT-239 markedly increased the duration of time that fEPSP responses were elevated over baseline, to a level similar to the ABT-239-treated saccharin control rats. Pair-wise comparisons indicated that the fEPSP responses in ABT-239-treated fetal ethanol rats were significantly greater than saline-treated fetal ethanol rats. Conversely, ABT-239-treated fetal ethanol rats were not significantly different than either the saline- or ABT-239-treated saccharin control rats. ABT-239 also increased PS amplitude responses in fetal ethanol rats (Fig. 3.4D) but the effects were more modest. The period of time when PS amplitude was significantly greater than baseline was increased by just five minutes in ABT-239-treated fetal ethanol rats and the differences between saline- and ABT-239-treated fetal ethanol rats at each post-tetanus time interval were generally not significant.

3.5 Discussion

There are two salient observations from this study. First, intermittent voluntary consumption of moderate quantities of ethanol during pregnancy reduces LTP in fetal ethanol-exposed offspring, but only when a submaximal number of tetanizing trains are employed. When ten tetanizing trains were used to elicit maximal LTP, no differences were observed between groups (Fig. 3.3A and 3.3B). However, the synaptic potentiation elicited by three tetanizing trains was markedly reduced in fetal ethanol-exposed offspring compared to controls (Fig. 3.3C and 3.3D). Thus, the effects of prenatal ethanol exposure on synaptic potentiation are subtle, reminiscent of the subtle learning deficits we have observed in littermates of the offspring used in this study (Savage et al., 2010). The effect of prenatal ethanol exposure on LTP using the three-train tetanizing

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protocol (Fig. 3.3C and 3.3D) is qualitatively similar to that reported by Sutherland et al. (1997). Specifically, the fEPSP response in fetal ethanol-exposed rats rapidly decayed back to baseline levels within five minutes after tetanus (Fig. 3.3C). Population spike responses were also diminished in the fetal ethanol-exposed group (Fig. 3.3D), but to a lesser extent than fEPSP responses. The mechanisms by which prenatal ethanol exposure adversely impacts fEPSP to a greater extent than PS amplitude after submaximal tetanizing stimuli is unknown. A strong correlation between fEPSP slope and PS latency was observed in all treatment groups (data not shown) indicating neither fetal ethanol exposure nor ABT-239 treatment affecting fEPSP/PS coupling. Several authors have reported dissociations between fEPSP slope and PS amplitude after high frequency tetanization (i.e.: Bliss and Gardner-Medwin, 1973; Taube and Schwartzkroin, 1988) suggesting that these are independent parameters and reflect different cellular mechanisms. The early aspects of the fEPSP used for slope measurement are primarily dependent on AMPA receptor activation and activity-dependent increases in fEPSP slope are attributable to increased affinity or number of AMPA receptors responding to glutamate released with the test stimulus. In contrast, the PS reflects the number of granule cells firing action potentials and increases in PS amplitude after tetanus indicate an increase in the number of cells firing action potentials in response to the test stimulus (Bliss and Lomo, 1973; Taube and Schwartzkroin, 1988). Given that fEPSP potentiation was impaired to a greater extent than PS amplitude potentiation, our results suggest that prenatal ethanol exposure may affect the mechanisms responsible for increased postsynaptic AMPA receptor-mediated granule cell depolarizations after tetanus, whereas the recruitment of additional granule cells after tetanus is affected to a lesser extent.

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The differential fEPSP responses in control and fetal ethanol-exposed rat after three trains of tetanic stimulation is reminiscent of results reported by Raymond et al., (2007) describing different forms of LTP distinguished by the magnitude and duration of the LTP response. In this paradigm, four trains of tetanus (LTP2) produces a fEPSP response curve similar to our three-train paradigm in controls (Fig. 3.3C and 3.3D). Their one-train paradigm (LTP1) produces a fEPSP response more robust than the three-train response in our fetal ethanol-exposed rats (Fig. 3.3C). Raymond and colleagues attribute these different levels of LTP to a progressively increasing involvement of different but synergistic mechanisms for increasing postsynaptic cytoplasmic calcium levels. Specifically, LTP1 involves NMDA receptor-mediated increases in calcium and LTP2 includes the synergistic involvement of Group I mGluR receptor-activated signaling cascades that trigger calcium release from endoplasmic stores. The differential LTP effect observed with a three tetanizing train stimulus suggests that a diminished contribution of mGluR receptor-mediated elevations in cytoplasmic calcium may be one factor contributing to the LTP deficit in fetal ethanol-exposed rats. The gradual decay in LTP between five and thirty minutes after tetanus is consistent with the lack of more slowly developing cellular signaling mechanisms important in the maintenance phase of LTP (Reymann and Frey, 2007). In support of this speculation, we have observed significant reductions in mGluR5 receptors in the dentate gyrus of fetal ethanol-exposed rats (Galindo et al., 2004). Studies in other laboratories have demonstrated the facilitatory role of mGluR5 receptor in the expression of LTP, particularly under submaximal tetanizing conditions (i.e.: Naie and Manahan-Vaughan, 2005; Raymond et al., 2000). These data suggest that diminished mGluR5 receptor function in fetal ethanol-

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exposed rats may be critical in the inability of these rats to elicit a "LTP2-type" response after three tetanizing trains, as was observed in the control rats (Fig. 3.3A).

In addition to putative post-synaptic mechanisms for this fetal ethanol-induced LTP deficit, it is important to note that presynaptic mechanisms may also contribute to this deficit. Prenatal ethanol exposure in Sprague-Dawley rats produced a striking reduction in mGluR5 agonist-stimulated phosphorylation of pre-synaptically localized GAP-43 in dentate gyrus slices (Galindo et al., 2004). This deficit was accompanied by a similar deficit in mGluR5 agonist-potentiation of $[^3H]$-D-aspartate release, a marker of glutamate release, from dentate gyrus slices (Galindo et al., 2004). Furthermore, while electrically-evoked $[^3H]$-D-aspartate release in response to a test stimulus was not different between controls and fetal ethanol-exposed rats, the ability of three trains of high frequency stimulations to potentiate evoked $[^3H]$-D-aspartate release from hippocampal slices, a process that develops over the first thirty minutes after tetanus, was significantly reduced in fetal ethanol-exposed rats (Savage et al., 1998). Both the timing and magnitude of these changes suggest that fetal ethanol-induced deficits in activity-dependent elevations in glutamate release could contribute to the results observed in Figure 3.3C. Subsequent studies of entorhinal cortical perforant path nerve terminal function in Long-Evans rats will be required to substantiate this speculation.

The second principal observation in this study is that the LTP deficit observed in fetal ethanol-exposed offspring can be ameliorated by treatment with the histamine H_3 receptor antagonist ABT-239. ABT-239 restored LTP in fetal ethanol-exposed offspring to levels not significantly different than saline-treated control rats (Fig. 3.4). At the 1 mg/kg dose, ABT-239 enhanced synaptic potentiation (Fig. 3.4C and 3.4D) without

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increasing baseline input/output responses (Fig. 3.1C and 3.1D) suggesting that the histamine H_3 receptor system affects the mechanisms of activity-dependent synaptic potentiation at doses that do not affect baseline neurotransmission. While it is tempting to speculate that the primary action of ABT-239 was to directly facilitate glutamate release from perforant path nerve terminals in fetal ethanol-exposed offspring, there are other mechanisms of ABT-239 action that may have contributed to enhanced synaptic potentiation. For example, the inhibition of H_3 receptors on cholinergic nerve terminals facilitates acetylcholine release (Fox et al., 2005), which, in turn, could facilitate glutamatergic neurotransmission. In addition, H_3 receptor antagonist-mediated inhibition of autoreceptors located on histaminergic nerve terminals promotes histamine release (Arrang et al., 1983), which may facilitate excitation of glutamatergic neurons mediated via histamine H_1 and H_2 receptors (Haas, 1984; Manahan-Vaughan et al., 1998). Further, histamine has been reported to have positive allosteric effects at the spermidine site on NMDA receptors (i.e.: Bekkers, 1993). The extent to which each of these other putative mechanisms of ABT-239 action may have contributed to prolonged synaptic potentiation in fetal ethanol-exposed offspring is not known, but it is likely that the manner by which H_3 receptor antagonists enhance synaptic transmission at glutamatergic synapses in the central nervous system is complex.

One of the curious observations from these studies is the fact that LTP was not enhanced by the administration of ABT-239 in saccharin control offspring (Fig. 3.4A and 3.4B). This is notable, in part, because the three-train tetanizing protocol did not maximize the degree of synaptic potentiation possible compared to the ten-train tetanus protocol (Fig. 3.3). Indeed, the data suggest that ABT-239 produced a slight inhibition of synaptic

potentiation at the 1 mg/kg dose (Fig. 3.4A and 3.4B), whereas basic input/output responses prior to the tetanizing protocol were unaffected (Fig. 3.1C and 3.1D). The basis for ABT-239's differential effect on synaptic potentiation between prenatal ethanol-exposed and control offspring is unknown. However, this differential effect raises questions of whether prenatal ethanol exposure has altered histaminergic regulation of glutamatergic transmission in a manner that is amenable to therapeutic intervention. Specifically, does fetal ethanol exposure elevate the inhibitory influence of H_3 receptors on glutamate, and possibly acetylcholine, release in the dentate gyrus? A preliminary study of histamine H_3 agonist-stimulated $[^{35}S]$-GTPγS binding has revealed that histamine H_3 receptor-effector coupling is significantly increased in the dentate gyrus of fetal ethanol-exposed rats (unpublished observations). These results lend credence to the speculation of a fetal alcohol-induced enhancement of histamine H_3 receptor-mediated inhibition of activity-dependent synaptic potentiation. This speculation is currently under investigation in our laboratory.

In summary, we have observed that fetal ethanol-induced LTP deficits are amenable to a cognition-enhancing agent that acts as a H_3 receptor antagonist. The fact that ABT-239 was without effect in unexposed control rat offspring highlights the importance of examining the therapeutic potential of agents in animal models that emulate the clinical disorder in question. Further, the differential effect in fetal ethanol-exposed rats compared to controls also raises suspicion that prenatal ethanol exposure may have altered histaminergic regulation of excitatory neurotransmission in affected brain regions. The mechanistic basis for these alterations is unknown, but may involve fetal alcohol-

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induced alterations in histamine neurotransmission, an area that has not been investigated in this field of research.

4. Differential effects of the histamine H₃ receptor agonist methimepip on dentate granule cell excitability, paired-pulse plasticity and long-term potentiation in prenatal ethanol-exposed rats.

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4.1 Abstract

We have reported that prenatal ethanol-induced deficits in dentate gyrus (DG) long-term potentiation (LTP) *in vivo* are ameliorated by the histamine H₃ receptor inverse agonist ABT-239. ABT-239 did not enhance LTP in control rats, suggesting a heightened H₃ receptor-mediated inhibition of glutamate release in fetal ethanol-exposed offspring. As the modulation of glutamate release is an important feature of LTP, we examined the effect of methimepip, a histamine H₃ receptor agonist, on DG granule cell excitability, glutamate release and LTP in control and prenatal alcohol-exposed (PAE) rats. Long

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Evans rat dams voluntarily consumed either a 0% or 5% ethanol solution four hours daily throughout gestation. Adult offspring were anesthetized with urethane and electrodes implanted into the entorhinal cortex and DG. PAE reduced coupling of excitatory post-synaptic field potentials to population spikes, an effect mimicked in control rats treated with 1 mg/kg methimepip. Methimepip decreased release probability in controls but not PAE offspring. GABAergic feedback inhibition of granule cell responsiveness was not affected by either PAE or methimepip. PAE reduced LTP in DG, another effect mimicked in methimepip-treated control rats. Again, methimepip did not exacerbate the PAE-induced LTP deficit. Thus, while methimepip treatment of control rats mimicked some baseline and activity-dependent deficits observed in saline-treated PAE offspring, methimepip treatment of PAE rats did not exacerbate these deficits. Whether the absence of an added methimepip effect in PAE offspring is a consequence of a "floor effect" for the responses measured or due to differential drug dose responsiveness will require further investigation. Further, more detailed studies of H_3 receptor-mediated responses *in vitro* may provide clearer insights into the role of the H_3 receptor regulation of excitatory transmission at the perforant path - DG synapse in PAE rats.

4.2 Introduction

Learning disabilities are the most common behavioral deficit observed in children whose mothers consumed ethanol during pregnancy. It is estimated that between 2% and 5% of children have fetal alcohol-associated learning deficits (May et al., 2009). Currently, there are no established clinically-effective therapeutic interventions for these deficits (Medina, 2011). Moreover, the development of efficacious treatments for these disabilities will require a clearer understanding of the neurobiological bases of fetal ethanol-induced learning deficits and subsequently, the identification of therapeutic agents whose mechanisms of action would be predicted to have clinical utility.

We have reported previously that prenatal exposure to relatively low levels of alcohol causes impairments in several learning tasks sensitive to functional damage of the dentate gyrus, particularly when employing more challenging versions of these tasks (Savage et al., 2002; Savage et al., 2010; Sutherland et al., 2000; Weeber et al., 2001b). We have also observed that prenatal alcohol exposure (PAE) causes long-lasting deficits in dentate granule cell long-term potentiation (LTP) (Sutherland et al., 1997; Varaschin et al., 2010) as well as reduced activity-dependent elevations of evoked [^{3}H]-D-aspartate release, a marker of glutamate release (Savage et al., 1998), suggesting a presynaptic mechanism as one component of the LTP deficit in PAE rats. Neurochemical studies of dentate gyrus have shown that metabotropic glutamate receptor subtype 5 (mGluR$_5$)-mediated phosphorylation of presynaptically-localized growth associated protein 43 as well as a mGluR$_5$ receptor-mediated potentiation of evoked [^{3}H]-D-aspartate release is diminished in PAE rats (Galindo et al., 2004), suggesting that mechanisms associated with activity-dependent synaptic vesicle mobilization as one process diminished by PAE.

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These observations led us to examine the question of whether drugs that enhance the release of excitatory neurotransmitters may ameliorate the synaptic plasticity and learning deficits we have observed in PAE rats. The efficacy of a variety of so-called "procognitive agents" whose primary mechanism of action targets nerve terminals are under investigation, including nicotinic cholinergic agonists (Sorensen et al., 2002; Zhang et al., 2008), serotonin $5HT_4$ or $5HT_6$ antagonists (Hirst et al., 2006; Pelkey et al., 2005; Shen et al., 2011) and histamine H_3 receptor antagonists or inverse agonists (Fox et al., 2005; Galici et al., 2009; Medhurst et al., 2007a). Histamine H_3 receptors reside predominantly on nerve terminals (Fujimoto et al., 1991), and inhibit histamine release (Giannoni et al., 2010; Kitbunnadaj et al., 2005), as well as the release of acetylcholine (Bacciottini et al., 2002), GABA (Welty and Shoblock, 2009) and glutamate (Brown and Haas, 1999). Antagonists and inverse agonists of H_3 receptors have procognitive actions in a variety of animal models (Brioni et al., 2011; Esbenshade et al., 2008). There are several ongoing clinical trials examining the cognitive enhancing proprieties of histamine H_3 antagonists and inverse agonists in Alzheimer's disease and schizophrenia (Passani and Blandina, 2011).

Our laboratory recently reported that the histamine H_3 receptor inverse agonist ABT-239 ameliorated PAE-induced deficits in learning (Savage et al., 2010) and reversed a PAE-induced deficit in dentate gyrus LTP (Varaschin et al., 2010), both effects consistent with a presynaptic basis for PAE-induced synaptic plasticity and learning deficits. Curiously, ABT-239 did not enhance learning or LTP in control offspring even though the difficulty of the behavioral tasks and the submaximal LTP stimulus protocol employed may have led one to predict some enhancements in control offspring with ABT-239 treatment.

These differential effects of ABT-239 in control and PAE rats led us to speculate that PAE leads to a heightened H_3 receptor-mediated inhibition of glutamate release at the perforant path nerve terminal which, in turn, would diminish activity-dependent changes in dentate granule cell responsiveness and LTP. We tested this hypothesis by examining the effects of the selective H_3 receptor agonist methimepip on dentate granule cell responses in control and PAE offspring. We predicted that methimepip treatment in controls would emulate the effects of PAE on DG responsiveness and that methimepip would exacerbate the effects of PAE in affected offspring.

4.3 Material and Methods

All reagents were acquired from Sigma-Alrich Corp. (St. Louis, MO) unless indicated otherwise in parenthetical text.

4.3.1 Prenatal Ethanol Exposure Paradigm

Four-month-old Long-Evans rat proven male breeders and two-month-old females (Harlan Industries, Indianapolis, IN) were single-housed in plastic cages at 22° C and kept on a reverse 12-hour dark / 12-hour light schedule (lights on from 2100 to 0900 hours) with Harlan Teklad rodent chow and tap water *ad libitum*. All procedures involving the use of live rats were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.

Breeding procedures were conducted as described previously (Savage et al., 2010). Briefly, after at least one week of acclimation to the animal facility, all female rats were provided 0.066% saccharin in tap water for four hours each day from 1000 to 1400 hours. The saccharin water contained 0% ethanol on the first and second day, 2.5% ethanol (v/v)

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on the third and fourth day and 5% ethanol on the fifth day and thereafter. Daily four-hour consumption of ethanol was monitored for at least two weeks and then the mean daily ethanol consumption was determined for each female. Females whose mean daily ethanol consumption exceeded one standard deviation from the group mean were removed from the study. The remainder of the females were assigned to either a saccharin control or 5% ethanol drinking group and matched such that the mean pre-pregnancy ethanol consumption by each group was similar. Subsequently, females were placed with proven male breeders until pregnant, as indicated by the presence of a vaginal plug. Female rats did not consume ethanol during the breeding procedure. Beginning on Gestational Day 1, rat dams were provided saccharin water containing either 0% or 5% ethanol for four hours a day, from 1000 to 1400 hours. The volume of saccharin water provided to the control group was matched to the mean volume of saccharin water consumed by the ethanol group. Daily four-hour ethanol consumption was recorded for each dam. At birth, litters were culled to ten pups each. Offspring were weaned at 28 days of age and group-housed, two males per cage, until used in the *in vivo* electrophysiology studies.

4.3.2 *In-vivo* Electrophysiology

On a given experiment day, male adult rat offspring, 105 to 140 days of age and weighing 370-500 g were injected with either 1 mg/kg methimepip (Tocris, Ellisville, MO, USA) dissolved in isotonic phosphate-buffered saline solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM NaH_2PO_4; pH = 7.4.) or phosphate-buffered saline alone. Methimepip was selected for use given its relative selectivity as an H_3 receptor agonist along with its ability to cross the blood brain barrier better than other more commonly

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studied H_3 receptor agonists (Kitbunnadaj et al., 2005). The dose of methimepip selected for use was based on Kitbunnadaj et al., (2005) and on pilot studies in our laboratory.

Subsequently, all rats were anesthetized with urethane (two injections of 0.75 g/kg, 30 min apart). Upon loss of the pedal reflex, rats were placed into a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA). Rectal temperature was closely monitored and maintained at 37.3 °C using a temperature controller (World Precision Instruments, Sarasota, FL, USA) throughout the entire surgical and recording procedure. The stereotaxic procedure was conducted as described previously (Sutherland et al., 1997). Briefly, after exposing the skull, five holes were drilled using a dental burr. Three self-tapping screws attached to stainless steel wires and gold Amphenol® pins (A-M Systems, Carlsborg, WA, USA) were inserted into the skull; two served as ground and reference signals for the recording circuit and one served as the return component of the stimulating circuit. Recording and stimulating Teflon-coated, stainless steel, unipolar electrodes (114 μm outer diameter; A-M Systems) were implanted using the following bregma coordinates: recording electrode, AP -3.5 mm and ML +1.8 mm; stimulating electrode, AP -8.1 mm and ML +4.3 mm (Paxinos and Watson, 1998). Electrodes were then connected to an isolated pulse stimulator (Model 2100; A-M Systems) and to a differential AC amplifier (Model 1800; A-M Systems). Recording signals were amplified (1000X), bandpass-filtered (0.1 Hz - 10 kHz), and transferred to a personal computer via an analog-to-digital converter (Models PCI 6221 and BNC-2090; National Instruments, Austin, TX, USA). The electrodes were slowly inserted into the dentate gyrus (recording electrode, DV -3.8 mm from bregma) and entorhinal cortex (stimulating electrode, DV -4.0 mm from bregma) until optimally placed for stimulation of the medial

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perforant path. Field excitatory post-synaptic potential (fEPSP) responses to a guide stimulus (400 µA intensity) were monitored during the electrode descent process. Rats failing to exhibit a population spike (PS) of at least 5 mV of amplitude were discarded from the study.

Baseline input / output responses. Once optimal positioning of electrodes and stable responses were achieved, an input/output curve was generated using current intensities ranging from 25 to 600 µA. The first ascending portion of each response curve was used to calculate the fEPSP slope. PS amplitude was calculated measuring the difference between the average of the first and second positive deflections to the negative peak. fEPSP-to-PS (E-S) coupling was determined by plotting the PS amplitude against the fEPSP slope values obtained during the input/output curves. Linear regression was then used in the resulting graph to infer the slope and x-intercept for each rat.

Paired pulse ratio measures. After collecting input/output measure, the stimulus intensity necessary for the occurrence of a PS was determined for each rat. Then, this stimulus intensity was reduced to 90% of its value. The resulting "subthreshold for PS" stimulus intensity was used for recordings of the fEPSP paired-pulse ratio at interpulse intervals of 30, 40 and 80 milliseconds. Next, the stimulus intensity was increased to 600 µA to elicit a large PS. This "near-maximal PS" stimulus intensity was used to determine the PS paired-pulse ratio at interpulse intervals of 10, 20, 30, 40, 60, 80, 100, 200, 400, 600 and 1000 milliseconds. At each interpulse interval, at least five pairs of pulses were delivered every 30 seconds and averaged to minimize random fluctuations. The paired-pulse ratio was obtained by dividing the fEPSP slope or PS recorded from the second pulse by that of the first pulse.

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Long-term potentiation responses. In a different subset of rats, the stimulus intensity sufficient to generate 40% of maximal PS response (ES_{40}) was determined based on the input/output curve. This stimulus intensity was subsequently used for baseline recording, high frequency tetanus and post-tetanus recordings. Baseline responses to single pulses triggered every 30 s were recorded for 30 min. Synaptic potentiation was induced by a tetanus protocol consisting of three trains of high frequency stimulation of 400 Hz over 25 ms with 30 s inter-train intervals. After tetanus, single pulse responses were measured every 30 seconds over the next 90 minutes. To minimize random fluctuations, responses obtained over two-minute intervals (4 consecutive responses) were averaged. Long-term potentiation was analyzed by averaging the baseline values of the fEPSP slope, normalizing that value to 100% and transforming the post-tetanus response data by the baseline average. For each rat, the post-tetanus average increase from baseline was calculated; these values were then averaged per group and used for statistical analysis.

4.3.3 Statistical Analysis

Group means obtained from each parameter (input/output, E-S coupling, paired-pulse ratio and average post-tetanus fEPSP increase) were analyzed by two-way ANOVA, with the prenatal exposure treatment (0% or 5% ethanol) and drug treatment (saline or methimepip) as independent factors. P values less than 0.05 were deemed significant. Post-hoc analysis, when appropriate, was conducted using the Student-Newman-Keuls method. All data are graphically represented as the mean $\pm$ the standard error of the mean. All statistical procedures were performed using SigmaPlot$^{\circledR}$, Version 11.0 (Systat Software Inc., San Jose, CA).

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4.4 Results

4.4.1 Prenatal Ethanol Exposure Paradigm

Results from voluntary drinking during pregnancy are summarized in Table 1. Pregnant dams stably consumed 2.37 ± 0.09 g/kg/day during the daily four hours period when ethanol was available. In a previous study, similar ethanol consumption resulted in blood alcohol concentration of 84.0 ± 5.5 mg/dL (Savage et al., 2010). No significant changes were observed in dam weight gain during pregnancy, pup birth weight or litter size.

Table 4.1: Mean daily ethanol consumption and impact on maternal weight gain and offspring outcomes

Prenatal Treatment	Daily Ethanol Consumption(*)	Maternal Weight Gain During Pregnancy(‡)	Pup Birth Weight(^)	Litter Size (†)
Control	N/A	84.4 ± 9.4 (12)	8.6 ± 0.8 (11)	10.8 ± 0.8 (11)
PAE	2.40 ± 0.13 (15)	99.3 ± 7.2 (12)	7.9 ± 0.6 (12)	10.4 ± 0.7 (12)

(*) In grams/kg/day, averaged throughout pregnancy.

(‡) Net wet gain between Gestational Days 0 and 20, in grams.

(^) Average pup weight at birth, in grams.

(†) Number of live births per litter.

Data are expressed as the mean ± the standard error of mean. Sample sizes denoted in parenthesis.

4.4.2 Input/Output and E-S Coupling

Electrical stimulation of the entorhinal cortex elicited excitatory post-synaptic field potentials in the dentate gyrus. The fEPSP slope and PS amplitude increased proportionally to the given electrical stimulus intensity (Figure 4.1A). In both PAE and control offspring, the maximal fEPSP slope was approximately 8 V/s at a 600 µA stimulus intensity (Figures 4.1B & 4.1C). Population spikes were first observed at a

mean stimulus intensity of 120 ± 9 µA, with maximal amplitude of 15 ± 1 mV at 600 µA stimulus intensity (Figures 4.1D & 4.1E). Neither PAE nor methimepip treatment significantly reduced input/output measures of fEPSP slope and PS amplitude, suggesting that these raw measures of granule cell responsiveness were not affected by either treatment.
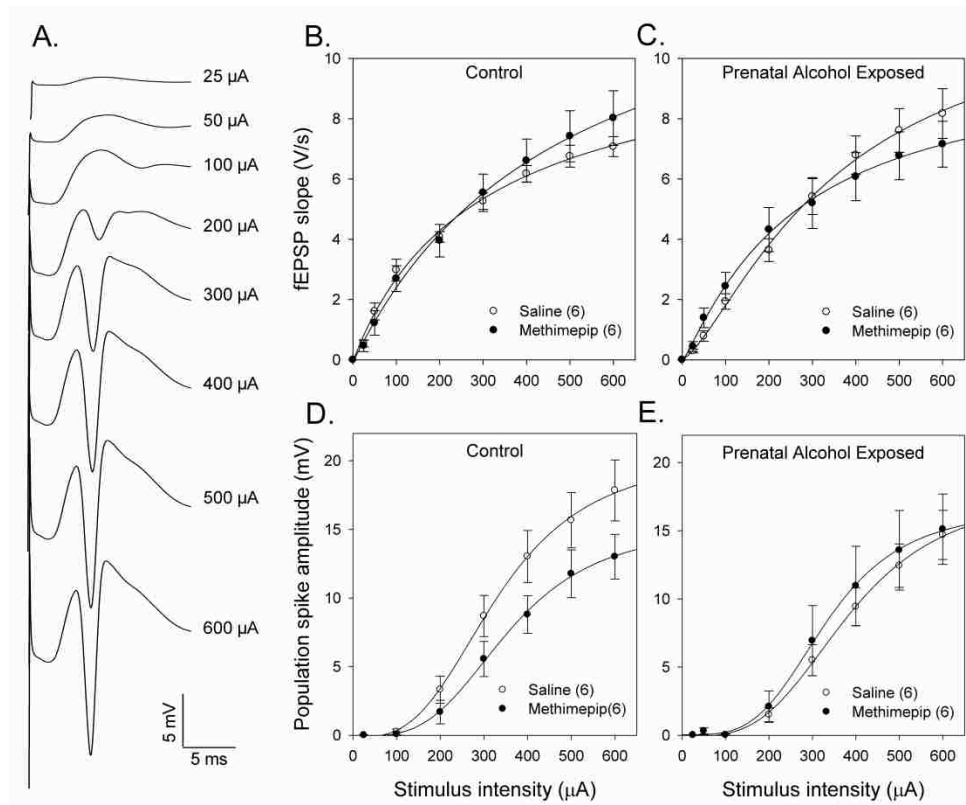
Figure 4.1 Effects of methimepip and PAE on baseline measures of granule cell responsiveness in the dentate gyrus.

Representative traces obtained from stimulus intensities ranging from 25 – 600 µA in a saline-injected control rat (1A). Effects of saline (open circles) or methimepip (filled circles) on fEPSP slope evoked by increasing stimulus intensities in control offspring (1B) and PAE offspring (1C). Effects of saline (open circles) or methimepip (filled circles) on PS amplitude evoked by increasing stimulus intensities in control offspring (1D) and PAE offspring (1E). No statistically significant differences were detected in either treatment group ($P > 0.05$ after two-way ANOVA). Data points represent the mean ± the S.E.M. Sample sizes are denoted in parenthesis.

The effects of prenatal alcohol exposure and methimepip on E-S coupling are illustrated in Figure 4.2. For each individual rat, the PS amplitude was plotted against its respective fEPSP slope and a linear regression was performed. There was a strong correlation between fEPSP slope and PS amplitude, as denoted by regression r^2 values above 0.95 (Figure 4.2A & 4.2B). The slope of this correlation, known as E-S coupling, is suggested as an index of synaptic efficacy and granule cell excitability (Richter-Levin et al., 1991). A two-way ANOVA of group averaged E-S coupling slopes detected a significant interaction between prenatal exposure treatment and drug treatment ($F_{(3,20)} = 21.41$, $P < 0.001$). PAE significantly reduced the E-S coupling slope when compared to control offspring (Figure 4.2C, right-hand side open bar; $P < 0.01$). In addition, methimepip injection in control offspring also reduced the E-S coupling slope (Figure 4.2C, left-hand side black bar; $P < 0.01$), an effect similar to that observed in PAE offspring.
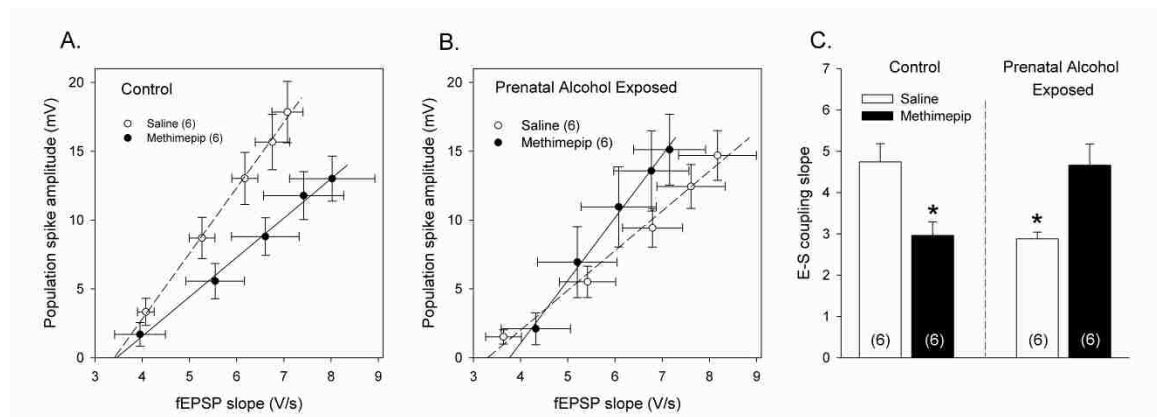
Figure 4.2 Effects of methimepip and PAE on E-S coupling in dentate gyrus.

Effects of saline (open circles) or methimepip (black circles) on PS amplitude responses to a given fEPSP slope in control offspring (2A) or PAE offspring (2B). Lines indicate the average linear regression for saline (dashed) and methimepip-injected (solid) offspring. 2C: Average E-S coupling slope in saline (white bars) or methimepip-injected (black bars) control (left) and PAE offspring (right). Asterisks denote data significantly decreased compared to the saline-injected control group ($P < 0.05$). Data bars represent the mean ± the S.E.M. Sample sizes are denoted in parenthesis.

Surprisingly, methimepip injection in PAE offspring significantly increased the E-S coupling slope when compared to saline-injected PAE offspring (Figure 4.2C, right-hand side black bar; $P < 0.01$).

4.4.3 Paired Pulse Plasticity

Alterations in the fEPSP slope paired pulse ratio usually reflect changes in presynaptic activity related to the probability of neurotransmitter release when pairs of stimuli are given at intervals between 20 and 100 ms (Zucker and Regehr, 2002). To avoid a potential confound of GABAergic inhibition of glutamate release, recordings were performed at stimuli intensities that fail to elicit a PS (Figure 4.3, inset traces). The absence of a PS indicates that granule cells are not firing in response to the fEPSP and, therefore, are unable to activate GABAergic inhibitory circuitry in the dentate gyrus, a potential confounding factor in the assessment of paired pulse ratio as an index of glutamate release. No significant differences were found between saline-injected PAE and saline-injected control offspring (Figure 4.3A-B, open circles, $P > 0.05$). Methimepip injection in control offspring resulted in significant increase of the fEPSP slope paired pulse ratio at pairs of stimuli given at 30 and 40 millisecond intervals, but not at an 80 millisecond interval (Figure 4.3A; $F_{(3,20)} = 5.72$ and $F_{(3,20)} = 6.64$, at 30 and 40 milliseconds intervals respectively; $P < 0.05$). In PAE offspring, methimepip injection did not significantly increase the fEPSP slope paired pulse ratio (Figure 4.3B). In general, however, there was a trend similar to the effect observed in the methimepip-injected control group.
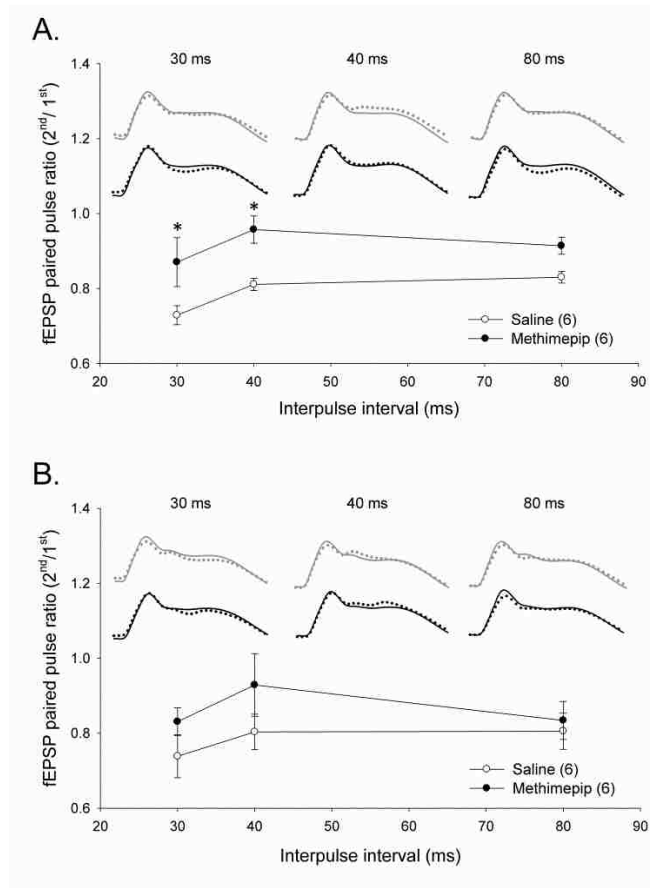
Figure 4.3 Effects of methimepip and PAE on the probability of glutamate release in dentate gyrus.

Effects of saline (open circles) or methimepip (black circles) on the paired pulse ratio of fEPSP slope obtained from control offspring (3A) and PAE offspring (3B) at given interpulse intervals. Asterisks denote data significantly greater than the saline-injected control group ($P < 0.05$). Inset: Representative traces of first (solid line) and second (dotted line) evoked fEPSP responses in saline-injected (gray) or methimepip-injected offspring (black) at given interpulse intervals. Data points represent the mean ± the S.E.M. Sample sizes are denoted in parenthesis.

To assess the effects of methimepip and PAE on GABA release and GABAergic feedback inhibition, the paired-pulse ratio of PS amplitude at stimuli intensities that elicit a large PS were recorded at interpulse intervals varying from 10 to 1000 ms (Jedlicka et al., 2011; Sloviter, 1991). Both in control and PAE offspring, varying the interpulse interval resulted in a curve with three distinct phases: An initial paired pulse inhibition at

interpulse intervals of 10-20 ms, followed by a paired pulse facilitation period at interpulse intervals of 30-100 ms, and then by secondary inhibition at intervals between 200 and 1000 ms (Figure 4.4). Neither PAE nor methimepip altered these parameters under these experimental conditions, suggesting that GABA mediated inhibition of dentate granule cells is not affected using this experimental paradigm.
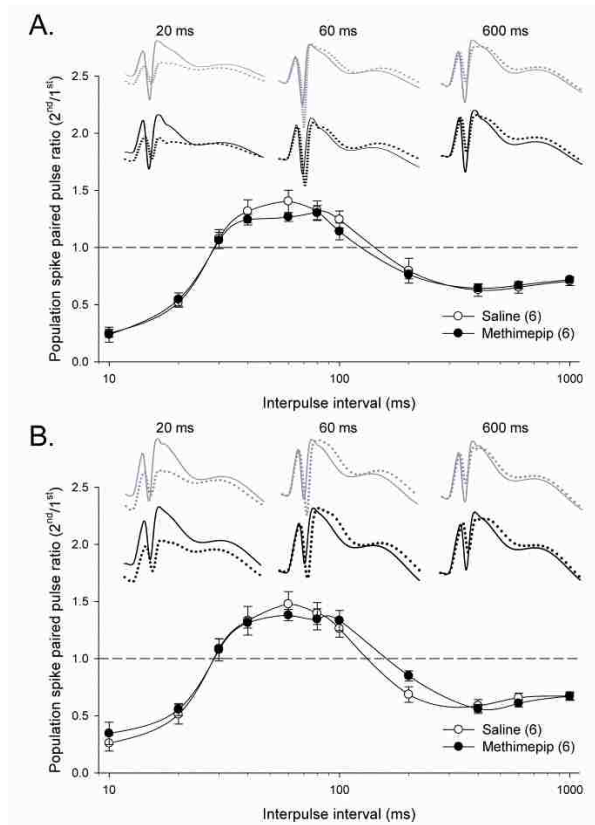
Figure 4.4 Effects of methimepip and PAE on GABAergic inhibition of dentate granule cell responsiveness.

Effects of saline (open circles) or methimepip (black circles) on the paired pulse ratio of the PS amplitude obtained from control offspring (4A) and PAE offspring (4B) at given interpulse intervals. No statistically significant differences were detected in either treatment group. Inset: Representative traces of first (solid line) and second (dotted line) evoked PS amplitude in saline-injected (gray) or methimepip-injected offspring (black) at given interpulse intervals. Data points represent the mean ± the S.E.M. Sample sizes are denoted in parenthesis.

4.4.4 Long-term Potentiation

The effects of prenatal ethanol and methimepip on LTP are depicted in Figure 4.5. In control offspring, high frequency stimulation of the entorhinal cortex resulted in long lasting increase of the fEPSP slope responses in the dentate gyrus (Figure 4.5A, open circles). In this group, the average potentiation from baseline during the 60 minutes recording was of 23.9 ± 2.4 % (Figure 4.5C, left-hand side open bar). As shown previously (Varaschin et al., 2010), PAE significantly reduced the magnitude and duration of LTP (Figure 4.5B, open circles). The PAE-induced LTP deficit was such that the average potentiation in this group was only 16.0 ± 2.6 % above baseline (Figure 4.5C, right-hand side open bar; $F_{(3,20)} = 5.06$, $P < 0.05$). In addition, methimepip injection in
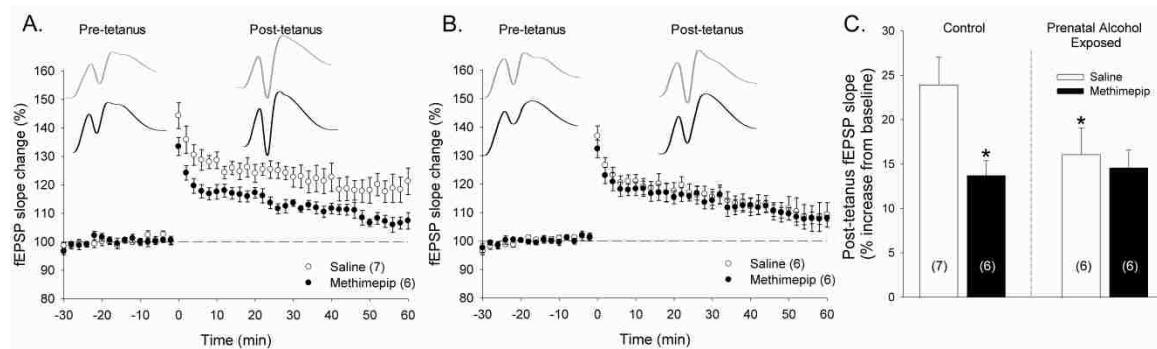
Figure 4.5 Effects of methimepip and PAE on long-term potentiation in dentate gyrus.

Effects of saline (open circles) or methimepip (black circles) on tetanus-induced fEPSP slope increase in control offspring (5A) or PAE offspring (5B). Inset: Representative traces of fEPSP responses of saline (gray) or methimepip injected (black) before and after tetanus. 5C: Average post-tetanus increase from baseline levels in saline (white bars) or methimepip injected (black bars) control (left) and PAE offspring (right). Asterisks denote data significantly decreased compared to the saline-injected control group (P < 0.05). Data bars represent the mean ± the standard error of the mean. Sample sizes are denoted in parenthesis.

control offspring also impaired LTP (Figure 4.5A, black circles). The average tetanus-induced potentiation in methimepip-injected controls was of 13.7 ± 2.6 %, a level similar to that observed in saline-injected PAE offspring (Figure 4.5C, left-hand side black bar, P

< 0.05). LTP in PAE offspring was not further impaired by methimepip injection (Figure 4.5B, black circles). Average potentiation in this group was 14.6 ± 2.6 %, not significantly different from saline-injected PAE offspring (Figure 4.5C, right-hand side black bar, $P > 0.05$).

4.5 Discussion

The salient observations in this study are that the activation of histamine H_3 receptors by the selective agonist methimepip reduces E-S coupling (Figure 4.2), probability of glutamate release (Figure 4.3) and LTP (Figure 4.5) in the dentate gyrus of control offspring. Further, these effects of methimepip in control offspring were similar to effects of PAE on E-S coupling (Figure 4.2) and LTP (Figure 4.5). Conversely, neither PAE nor methimepip treatment had observable effects on raw measurements of granule cell responsiveness to electrical stimulation or on GABAergic feedback inhibition of granule cell responses. Finally, in contrast to our predictions, methimepip did not exacerbate histamine H_3-mediated inhibition of glutamate release (Figure 4.2) or PAE-induced LTP deficit (Figure 4.5).

In the present study, prenatal alcohol exposure failed to alter raw measures of granule cell responsiveness to electrical stimulation, namely the fEPSP slope and PS amplitude (Figure 4.1). In agreement with these observations, previous studies showed that moderate exposure to alcohol during development does not produce alterations in fEPSP and PS input/output curves (Sutherland et al., 1997; Titterness and Christie, 2012; Varaschin et al., 2010). Although no effects of prenatal alcohol exposure were observed in raw measures of fEPSP slope or PS amplitude, there was a remarkable effect when the

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fEPSP slope was plotted against its associated PS amplitude (Figure 4.2). Prenatal alcohol exposure significantly decreased the slope of E-S coupling in the dentate gyrus (Figure 4.2B & 4.2C), suggesting that moderate ethanol consumption during pregnancy reduces the synaptic efficacy of medial perforant path input to dentate granule cells. To our knowledge, this is the first report on prenatal alcohol-induced impairment in E-S coupling. Coupling of fEPSP to PS can be modulated through different mechanisms. For example, blockade of NMDA receptors with dizocilpine (MK-801) reduces E-S coupling (Ferguson and Stone, 2010), and reduced GABAergic inhibition increases E-S coupling (Staff and Spruston, 2003), both effects attributed to post-synaptic mechanisms. Conversely, local dentate gyrus infusion of ghrelin, a drug that facilitates glutamate release, increases E-S coupling by affecting both pre- and post-synaptic loci (Chen et al., 2011).

While measurements of E-S coupling alone cannot distinguish whether the PAE-affected mechanisms are located pre- or post-synaptically, or both, we also investigated whether or not PAE would affect the probability of glutamate release from perforant path nerve terminals. We achieved this by measuring the paired pulse ratio at stimuli intensities that failed to produce a PS. An increase in the fEPSP paired-pulse ratio at low stimulus intensities usually reflects a reduction in the probability of neurotransmitter release (Zucker and Regehr, 2002). However, PAE itself did not alter the paired pulse ratio when compared to control offspring (Figure 4.3), suggesting that changes in E-S coupling are unlikely to derive from reduced glutamate release. Alternatively, a reduction in E-S coupling could also result from increased GABA release (Daoudal et al., 2002). However, as shown in Figure 4.4, PAE did not alter the paired pulse ratio of population

spikes elicited by high stimulus intensity. Hence, given that changes in E-S coupling cannot be explained by changes in glutamate or GABA release, it is likely that the mechanisms are located post-synaptically in the granule cells. Other mechanisms, such as altered NMDA NR1/NR2A subunit ratio (Samudio-Ruiz et al., 2010) or NMDA receptor-dependent ERK1/2 activation (Samudio-Ruiz et al., 2009) are known to be altered in PAE offspring and may be important for a PAE-induced decrease in E-S coupling. A more detailed investigation of these putative mechanisms is one subject for future investigation.

Independent of the putative mechanism(s) of this prenatal alcohol effect, a reduction in granule cell E-S coupling is associated with LTP reduction in the dentate gyrus. For example, Sprague-Dawley rats exhibit lower E-S coupling and LTP when compared to Long Evans rats (Bowden et al., 2011). Increased E-S coupling facilitates LTP (Malik and Chattarji, 2012), while decreasing it results in impaired LTP (Breakwell et al., 1996). Therefore, it is not surprising that the observed reduction of E-S coupling by moderate exposure to alcohol during development was associated with decreased LTP. This teratogenic effect of alcohol has been consistently shown by our laboratory (Sutherland et al., 1997; Varaschin et al., 2010) and others (Titterness and Christie, 2012).

As predicted, methimepip produced some effects in saccharin control offspring similar to prenatal alcohol exposure. Methimepip injection in control rat offspring reduced E-S coupling to similar levels observed in PAE offspring (Figure 4.2A & 4.2C). However, this effect was accompanied by an increase in the fEPSP paired pulse ratio (Figure 4.3A), suggesting that methimepip reduced the probability of glutamate release in control rats. In agreement with this observation, several studies indicate a role for histamine H_3

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receptors in modulating glutamate release in the medial frontal cortex (Welty and Shoblock, 2009), globus pallidus (Osorio-Espinoza et al., 2011b), thalamus (Garduno-Torres et al., 2007), striatum (Molina-Hernandez et al., 2001) and dentate gyrus (Brown and Haas, 1999) of rats. Since no effects were observed in the PS paired-pulse ratio at stimulus intensities sufficient to elicit a large PS (Figure 4.4), it is not likely that the reductions in E-S coupling (Figure 4.2) can be attributed to elevated GABA release.

Given the effect of methimepip on E-S coupling, it was not surprising that methimepip also produced a deficit in LTP in control rats (Figure 4.5B). While it is tempting to speculate that this effect is due to a direct histamine H_3 receptor-mediated decrease of glutamate release in the dentate gyrus, other alternative interpretations warrant consideration. Systemic activation of histamine H_3 receptors by methimepip decreases histamine release in the rat brain (Kitbunnadaj et al., 2005). Endogenous histamine plays an important role in synaptic plasticity by activating postsynaptic H_1 and H_2 receptors. Agonists of these receptors facilitate LTP, while their blockade or genetic ablation impairs LTP (Chang et al., 1997; Dai et al., 2007; Luo and Leung, 2010). Endogenous histamine may also facilitate LTP through a direct action on NMDA receptors (Brown et al., 1995). Likewise, activation of histamine H_3 receptors also inhibits acetylcholine release from cholinergic nerve terminals (Bacciottini et al., 2002). This neurotransmitter is well-established as a procognitive agent (reviewed by Blandina et al., 2004), and dentate gyrus LTP is facilitated by activation α_7 subunit-containing nicotinic cholinergic receptor agonists (Ondrejcak et al., 2012) and suppressed by $\alpha_4\beta_2$ nicotinic cholinergic receptor antagonists (Wang et al., 2006) or by the non-selective muscarinic receptor antagonist atropine (Luo et al., 2008). Therefore, the hypothesis that methimepip reduced

LTP in the dentate gyrus through a combined reduction of glutamate, histamine and acetylcholine release, cannot be ruled out at the present time.

Contrary to our predictions, methimepip did not did not exacerbate PAE-induced deficits in LTP (Figure 4.5B) and even partially restored E-S coupling compared to saline-injected PAE rats (Figures 4.2B and 4.2C). These observations suggest that methimepip and prenatal alcohol exposure may affect baseline and activity-dependent changes in synaptic plasticity via different and possibly independent mechanisms. These effects cannot be attributed to alterations in the probability of glutamate release (Figure 4.3B) or GABAergic inhibition (Figure 4.4B). One possible interpretation for diminished responses to methimepip by PAE rats is that histamine H_3 receptor-mediated inhibition of excitatory transmission at the perforant path - DG synapse is maximally activated in PAE rats, such that the added impact of an H_3 receptor agonist cannot be discerned, at least using the experimental approaches employed in the present study. Putative mechanisms for such an effect could include elevated levels of histamine release, or elevated H_3 receptor-effector coupling or increased constitutive activity by H_3 receptors (Morisset et al., 2000) in PAE rats. The ability of the selective H_3 receptor inverse agonist ABT-239 to reverse PAE-induced LTP deficits (Varaschin et al., 2010) is consistent with this notion. However, the observation that the probability of glutamate release in PAE rats was not different than controls (Figure 4.3) is inconsistent with this interpretation. Another basis for the differential effect of methimepip in control and PAE rats could be the methimepip dose employed in these studies. The 1 mg/kg dose of methimepip was optimal for inhibiting glutamate release in control rats (data not shown). Depending on the nature of the dose-response curve, it is possible that either a larger (or smaller) dose

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of methimepip would have significantly reduced glutamate release probability in PAE rats. This interpretation is supported by a recent demonstration that the facilitation of glutamate release in control rats by the histamine H_3 inverse agonist ABT-239 follows a U-shaped dose-response curve (Varaschin et al., submitted). Unfortunately, more detailed dose response curves using *in vivo* electrophysiological recordings is not a practical approach. Thus, the underlying questions of heightened H_3 receptor responsiveness or differential drug effects will be better investigated using combined neurochemical measures and *in-vitro* electrophysiological recordings in dentate gyrus slices, where the influence of endogenous histamine and neuronal inputs extrinsic to the dentate gyrus are minimized.

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5. Impact of prenatal ethanol exposure on histamine H_3 receptor-mediated modulation of glutamate release and synaptic plasticity in dentate gyrus *in-vitro*.

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5.1 Abstract

Moderate alcohol consumption during gestation results in synaptic plasticity and learning deficits in ethanol-exposed offspring. We have recently found that these deficits are reversed by the procognitive agent ABT-239, an inverse receptor agonist of histamine H_3 receptors. Here, we investigated the hypothesis that moderate ethanol exposure during pregnancy results in a heightened H_3 receptor-mediated inhibition of glutamate release and a deficit in long-term potentiation (LTP) in dentate gyrus slices. Long-Evans rat dams voluntarily consumed either a 0% or 5% ethanol solution for four hours each day throughout gestation. The mean daily ethanol consumption of 2.61 ± 0.15 g/kg/day did

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not affect maternal weight gain, litter size or birth weight. The density of specific denate gyrus binding of [³H]-A349821, an antagonist of histamine H_3 receptors, was not altered by prenatal ethanol exposure. However, histamine H_3 receptor-effector coupling, measured as methimepip-stimulated [³⁵S]-GTPγS binding, was increased in fetal ethanol-exposed offspring. Similarly, methimepip inhibited EPSP responses to a more significant extent in prenatal ethanol-exposed rats than in control. Prenatal ethanol exposure did not affect glutamate release in dentate gyrus slices, based on the paired-pulse ratio method. However, methimepip significantly reduced glutamate release in prenatal ethanol-exposed rats compared to controls. Prenatal ethanol exposure also significantly reduced LTP. This effect was mimicked by methimepip treatment of control rat slices. However, methimepip treatment did not exacerbate the LTP deficit observed in fetal ethanol rats. These results suggest that prenatal ethanol exposure causes long-lasting alterations in H_3 receptor function and the histaminergic regulation of baseline measures of glutamate neurotransmission. While methimepip reduced LTP in controls, the extent to which a heightened H_3 receptor-mediated inhibition of synaptic plasticity contributes to the LTP deficits observed in fetal ethanol-exposed rats is unclear at present.

5.2 Introduction

Alcohol drinking during pregnancy results in long-lasting cognitive impairments. Depending on the level of alcohol exposure, these effects range from severe to subtle and are encompassed by the term Fetal Alcohol Spectrum Disorder (FASD). FASD is a disease estimated to affect approximately 2-5% of children in school age in the United States (May et al., 2009). In animal models, low to moderate ethanol consumption during gestation results in impaired hippocampal-sensitive learning (Brady et al., 2012; Savage et al., 2002; Savage et al., 2010) and long term potentiation (Sutherland et al., 1997; Titterness and Christie, 2012; Varaschin et al., 2010). While multiple mechanisms and neurotransmitter systems are affected by exposure to moderate levels of alcohol during development (reviewed by Valenzuela et al., 2012) it is noteworthy that these changes are usually accompanied by alterations in pre-synaptic markers of activity (Barr et al., 2005; Galindo et al., 2004). Moreover, the dentate gyrus region of the hippocampus, an area of the brain involved in complex memory tasks such as pattern separation (Leutgeb et al., 2007), is particularly sensitive to prenatal alcohol damage (Miki et al., 2008; Perrone-Bizzozero et al., 1998; Samudio-Ruiz et al., 2009; Sutherland et al., 1997).

Histamine neurons project from the tuberomammillary nucleus in the posterior hypothalamus and diffusely innervate most areas of the brain, including those important for cognition such as the prefrontal cortex and the hippocampus (Watanabe et al., 1984). Histamine is released during the waking-phase of the sleep/wake cycle (Mochizuki et al., 1992; Prast et al., 1992) and is involved with increased attention (Horner et al., 2007), memory retention (Frisch et al., 1999) and synaptic plasticity (Brown et al., 1995; Chang et al., 1997). While some of the procognitive effects of histamine may be related to its

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direct facilitation of postsynaptic excitability via activation of histamine H_1 and H_2 receptors (Benetti et al., 2012; Brown et al., 1995; Chang et al., 1997; Dai et al., 2007; Xu et al., 2009), its direct actions on H_3 receptors are mostly detrimental to cognitive processes (Blandina et al., 1996; Flood et al., 1998). This seemingly contradictory effect may be due, in part, to the peculiar characteristic of H_3 receptors in locating predominantly in axonal terminals (Pillot et al., 2002). When in the active state, these receptors couple to $G_{i/o}$ proteins and negatively modulate the release of a number of neurotransmitters, including histamine (Arrang et al., 1985), norepinephrine (Alves-Rodrigues et al., 1998; Di Carlo et al., 2000), acetylcholine (Prast et al., 1994), serotonin (Threlfell et al., 2004) and glutamate (Brown and Haas, 1999; Garduno-Torres et al., 2007; Osorio-Espinoza et al., 2011a).

In recent years, a number of histamine H_3 receptor inverse agonists, drugs capable of decreasing the degree of constitutive activity of these receptors, have been proposed as novel procognitive agents in models of cognition impairment (Brioni et al., 2011; Esbenshade et al., 2008). Although there have been recent advances on treating prenatal ethanol-induced cognitive impairment in animal models (Medina, 2011), to date, there are no rationally designed, clinically proven pharmacotherapeutic intervention to mitigate cognitive deficit in patients with FASD. In principle, these patients could benefit from procognitive drugs such as selective histamine H_3 inverse agonists. We recently demonstrated that the H_3 receptor inverse agonist ABT-239 reversed prenatal ethanol exposure-induce learning deficits (Savage et al., 2010) and LTP deficits (Varaschin et al., 2010). Curiously, ABT-239 did not enhance either learning or synaptic plasticity in control offspring. This differential effect led us to hypothesize that histamine H_3 receptor

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function is increased in prenatal ethanol-exposed offspring, resulting in increased inhibition of glutamate release and impaired synaptic plasticity. We tested this hypothesis using a combination of radiohistochemical and electrophysiological approaches.

5.3 Material and Methods

5.3.1 Material

[^{3}H]-A349821 was generously donated by Abbott Laboratories (Abbott Park, IL, USA). All other reagents were acquired from Sigma-Aldrich Corp (St. Louis, MO, USA) unless indicated otherwise in parenthetical text.

5.3.2 Voluntary drinking paradigm

Long-Evans rats (Harlan Industries, Indianapolis, IN, USA) were group-housed (2-3 rats per cage) in plastic cages at a temperature of 22 °C on a reverse 12-hour dark / 12-hour light schedule (lights on from 2100 to 0900 hours) and provided Harlan Teklad rodent chow and tap water *ad libitum*. All procedures involving the use of live rats were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.

Prenatal alcohol exposure and breeding procedures were described previously (Savage et al., 2010). Briefly, four-month-old Long-Evans rat breeders were single-housed in plastic cages with Harlan Teklad rodent chow and tap water *ad libitum*. After acclimation to the animal facility, all female rats were provided 0.066% saccharin in tap water for four hours each day from 1000 to 1400 hours. The saccharin water contained 0% ethanol on the first and second day, 2.5% ethanol (v/v) on the third and fourth day

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and 5% ethanol on the fifth day and thereafter. Daily four-hour consumption of ethanol was monitored for at least two weeks and then the mean daily ethanol consumption determined for each female. Females whose mean daily ethanol consumption was greater than one standard deviation from the group mean were removed from the study. The remainder of the females were assigned to either a saccharin control or 5% ethanol drinking group and matched such that the mean pre-pregnancy ethanol consumption by each group was similar. Subsequently, females were placed with proven male breeders until pregnant, as indicated by the presence of a vaginal plug. Female rats did not consume ethanol during the breeding procedure. Beginning on Gestational Day 1, rat dams were provided saccharin water containing either 0% or 5% ethanol for four hours a day, from 1000 to 1400 hours. The volume of saccharin water provided to the control group was matched to the mean volume of saccharin water consumed by the ethanol group. Daily four-hour ethanol consumption was recorded for each dam. At birth, litters were culled to ten pups each. Offspring were weaned at 28 days of age and group-housed, two or three males per cage, until used either in the radiolohistochemical studies or *in vitro* electrophysiology studies.

5.3.3 Histamine H_3 receptor density and H_3 receptor-effector coupling

5.3.3.1   Histological sectioning.

Adult male offspring aged 8 to 12 weeks were sacrificed by rapid decapitation. Whole brains were dissected, frozen in isopentane chilled in a dry ice/methanol bath and stored in airtight containers at -80 °C until sectioning. Twelve-μm-thick microtome cryostat sections were collected in the sagittal plane corresponding to Lateral 1.40 mm in the Paxinos & Watson stereotaxic atlas of rat brain (Paxinos and Watson, 1998). The

sections were thaw-mounted onto pre-cleaned Superfrost-Plus$^®$ microscope slides (ColePalmer, Court Vernon Hills, IL, USA) and stored at -80 °C in airtight containers until assay.

5.3.3.2   [^{3}H]-A349821 binding assay.

Tissue sections were incubated in buffer (150 mM Tris-HCl, 5 mM EDTA, 0.1% BSA, pH 7.4 at 25 °C) with 10 nM [^{3}H]-A349821 (Abbott Laboratories, specific activity = 54.0 Ci/mmole) for 60 minutes at 25 °C in the absence (total binding) and presence of 10 μM thioperamide (non-specific binding). After incubation, sections were rinsed twice for 30 seconds each in ice-cold incubation buffer, dipped in ice-cold distilled water, dried and placed in a vacuum desiccator overnight. Sections were then exposed to Kodak Biomax MR film for four months and then the film developed in Kodak D-19 (1:1) and fixed. Microdensitometric measurement of [^{3}H]-A349821 binding was performed using Media Cybernetics Image Pro Plus$^®$ (Silver Spring, MD) on an Olympus BH-2 microscope. An optical density standard curve, expressed in picoCuries / 10^5 μm^2, was established based on autoradiograms of tritium standards. Total and non-specific [^{3}H]-A349821 binding were measured in the superior blade of the dorsal dentate gyrus *stratum moleculare* (total magnification of 3.125 X) the same region where electrophysiological measures were made in dentate gyrus slices. Specific [^{3}H]-A349821 binding, expressed as fmoles bound / 10^5 μm^2, was defined as the difference between total [^{3}H]-A349821 binding and non-specific [^{3}H]-A349821 binding.

5.3.3.3   Methimepip-stimulated $[^{35}S]$-GTPγS binding assay

Tissue sections were pre-incubated for 10 minutes in incubation buffer (50 mM Tris-HCl, 100 mM NaCl, 5 mM MgCl$_2$, 0.2 mM EGTA, 2 mM GDP and 100 nM DPCPX; pH 7.4 at 25 °C), and then incubated with 100 pM $[^{35}S]$-GTPγS (Perkin Elmer, specific activity = 1250 Ci/mmole) for 90 minutes at 25 °C in the absence (total binding) and presence of 10 μM unlabelled GTPγS (non-specific binding). Basal $[^{35}S]$-GTPγS binding was defined as the difference between total and non-specific binding. In addition, sections were incubated with fifteen different concentrations of methimepip ranging from 0.1 nM to 5 μM to obtain measures of agonist-stimulated $[^{35}S]$-GTPγS binding. After incubation, sections were rinsed twice for 15 seconds each in ice-cold incubation buffer, dipped in ice-cold distilled water, dried and placed in a vacuum desiccator overnight. Sections were then exposed to Kodak Biomax MR film for four days and then the film developed in Kodak D-19 (1:1) and fixed. Microdensitometric measurements of $[^{35}S]$-GTPγS binding were performed similar to those described above, except that the optical density standard curve was established based on autoradiograms of ^{14}carbon standards. Total, agonist-stimulated and non-specific $[^{35}S]$-GTPγS binding were measured in the superior blade of the dorsal dentate gyrus *stratum moleculare* at 3.125 X total magnification. H$_3$ receptor agonist-stimulated $[^{35}S]$-GTPγS binding was defined as the difference between binding in the absence and presence of methimepip. Percent increase in agonist-stimulated $[^{35}S]$-GTPγS binding was defined as methimepip-stimulated $[^{35}S]$-GTPγS binding divided by basal $[^{35}S]$-GTPγS binding x 100.

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5.3.4 Electrophysiology Studies

Eight-week-old male rats were anesthetized with ketamine (250 mg/kg i.p.) and quickly decapitated for brain removal. Coronal slices containing the dorsal dentate gyrus were obtained as described previously (Mameli et al., 2005). Briefly, whole brain coronal slices (400 μm thick) were obtained with a vibratome immerged in ice-cold cutting solution containing 3 mM KCl, 1.25 mM NaH_2PO_4, 6 mM $MgSO_4$, 26 mM $NaHCO_3$, 0.2 mM $CaCl_2$, 10 mM glucose, 220 mM sucrose, and 0.43 mM ketamine. Slices were then allowed to recover for 40 min at 32 °C in artificial cerebral-spinal fluid (ACSF) containing 126 mM NaCl, 2 mM KCl, 1.25 mM NaH_2PO_4, 1 mM $MgSO_4$, 26 mM $NaHCO_3$, 2 mM $CaCl_2$, 10 mM glucose and oxygenated with 95% O_2 / 5% CO_2. After recovery, slices were maintained in oxygenated ACSF at room temperature until recording. Upon transfer to recording chambers, slices were perfused at a rate of 2 mL/min with 32 °C oxygenated ACSF containing different treatment conditions. Next, a platinum unipolar electrode was placed in the medial portion of the superior blade of the dentate gyrus *stratum moleculare*, and a recording glass electrode (resistance ~3-5 Ω) filled with ACSF placed in the same layer, about 200 μm lateral to the stimulating electrode. Electrically-evoked excitatory post-synaptic field potentials (EPSP) were recorded using a Digitizer model 1440 and AxoPatch 200 amplifier (Molecular Devices, Sunnyvale, CA) with low-pass filter set at 2 kHz. Input/output curves were generated in every recording to determine the stimulus intensity sufficient to elicit maximal EPSP amplitude. All subsequent recordings were performed with stimuli intensities sufficient to elicit 40-50% of maximal EPSP response.

To test the inhibitory effects of methimepip on granule cell responsiveness to electrical stimulation, a baseline where single pulses (75 μs duration) were delivered every 30 seconds was collected for 10 min. Next, increasing concentrations of methimepip were added to ACSF in the following order: 0.3 μM for 10 min, 1 μM for 10 min and 3 μM for 10 min. The perfusing solution was then switched back to ACSF only (wash out) and EPSP responses were recorded for another 10 min. The baseline EPSP amplitude for each rat was averaged and all subsequent evoked EPSPs normalized to that value. Data obtained from the last four minutes at each drug condition were used for statistical analysis.

Paired pulse stimulation of the medial perforant path consisted of two stimulating pulses of 75 μs duration and 40 ms apart. Pairs of pulses were given every 30 seconds and recorded for at least 10 min. Picrotoxin (50 μM) was added to the ACSF solution to prevent GABAergic feedback inhibition of the second pulse. Methimepip (zero or 1 μM) was added to ACSF prior to the input/output curve determination. All subsequent recordings were done with stimulus intensity sufficient to elicit 40-50% of maximal EPSP responses. Paired-pulse ratio (PPR) was measured as the ratio of the EPSP amplitude elicited by the second pulse divided by the EPSP amplitude elicited by the first pulse.

For the LTP experiment, a baseline was recorded for 10 min at stimulus parameters similar to above. Then, slices were tetanized with theta-bursts consisting of four 75 μs pulses at 100 Hz followed by a 200 ms interval and another four pulses at 100 Hz (Alfarez et al., 2003). Theta-bursts were given every 30 seconds for five consecutive times. Post-tetanus recordings proceeded with single pulses evoked every 30 seconds for

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a total of 40 min. For each experiment, the post-tetanus evoked EPSP amplitudes were normalized to the averaged baseline amplitude. The average tetanus-induced increase from baseline throughout the 40 min post-tetanus recording was used for statistical analysis.

5.3.5 Data analysis

All data points are represented as the mean $\pm$ standard error of the mean unless otherwise noted. Prenatal ethanol effects on maternal weight gain, litter size, weight, specific [^{3}H]-A349821 and [^{35}S]-GTPγS binding, and the input/output data in absence of picrotoxin were compared using the two-tailed Student's t-test. The effects of methimepip on normalized EPSP responses in each group (prenatal ethanol-exposed or control) were analyzed using two-way repeated measures ANOVA. The effects of prenatal alcohol exposure and methimepip on the input/output curves, paired pulse ratio and LTP were analyzed by two-way ANOVA with the pregnancy treatment (0% ethanol or 5% ethanol) and the presence or absence of methimepip as independent factors. Post-hoc pairwise multiple comparisons were made using the Student-Newman-Keuls method. For all statistical analyses a value of $P < 0.05$ was deemed significant. All graphs and statistical procedures were performed using SigmaPlot$^{®}$ 11 (Systat Software Inc. San Jose, CA, USA).

5.4 Results

5.4.1 Voluntary alcohol drinking measures

Pregnant dams consumed an average of 2.61± 0.15 g/kg/day throughout gestation. In our previous study, a similar level of alcohol consumption (2.72 $\pm$ 0.13 g/kg/day) resulted in

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blood alcohol concentration of 84 ± 5.5 mg/dL (Savage et al., 2010). No significant effects of prenatal alcohol exposure were observed on maternal weight gain, litter size and pup birth weight (not shown).

5.4.2 Histamine H_3 receptor density and receptor-effector coupling

Figure 5.1A illustrates the distribution of total [^{3}H]-A349821 binding to histamine H_3 receptors in a sagittal section of hippocampal formation from a control rat. In accordance with prior reports (Cumming et al., 1994; Pillot et al., 2002), H_3 receptors are heterogeneously distributed across the hippocampal formation and cortex with relatively higher densities occurring in the dentate gyrus *stratum moleculare*, the *stratum oriens* of hippocampal CA_1 and subiculum and in the middle and outer layers of the parietal cortex. Methimepip-stimulated [^{35}S]-GTPγS binding displayed a similar distribution as [^{3}H]-A349821 binding Figure 5.1C). At the maximally effective concentration of 5 μM methimepip, [^{35}S]-GTPγS binding was 82% greater than basal [^{35}S]-GTPγS binding (Figure 5.1D, open circles).

Prenatal ethanol exposure did not affect specific [^{3}H]-A349821 binding in the dentate gyrus *stratum moleculare* (Figure 5.1B) at a near-saturating radioligand incubation concentration, suggesting that there is no change in total H_3 receptor density between prenatal treatment groups. In contrast, methimepip-stimulated [^{35}S]-GTPγS binding was significantly greater in prenatal ethanol-exposed offspring (Figure 5.1D, filled circles) at agonist concentrations of 100 nM and above. At 5 μM methimepip, [^{35}S]-GTPγS binding was 148% above basal [^{35}S]-GTPγS binding, nearly 80% greater than methimepip-stimulated [^{35}S]-GTPγS binding in control offspring.
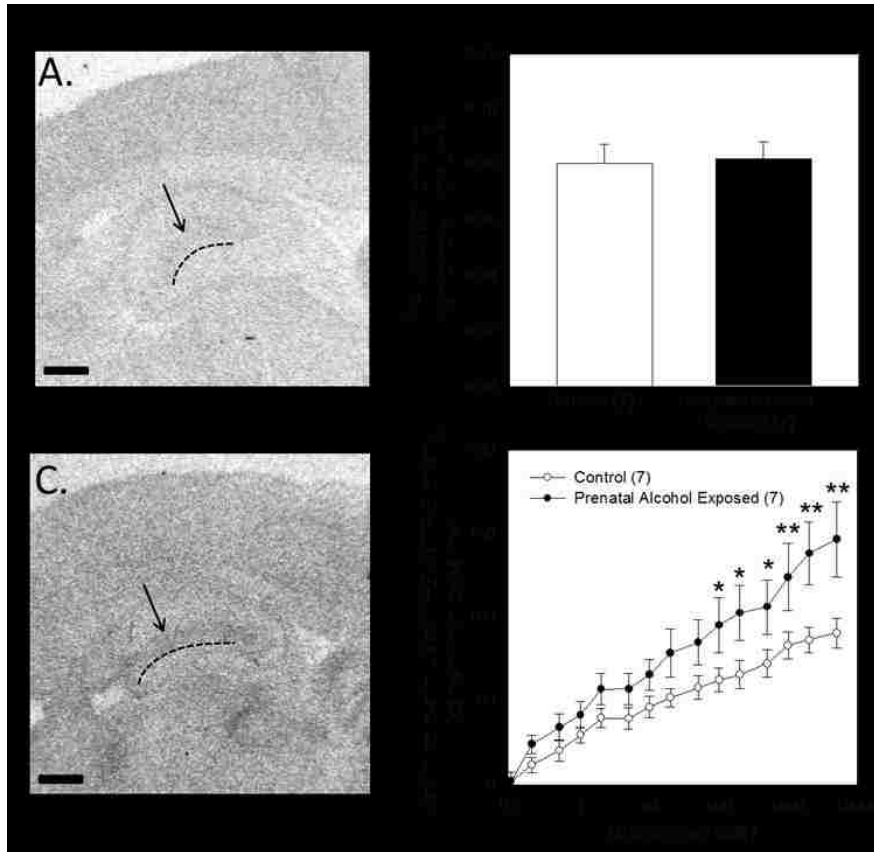
Figure 5.1 Impact of prenatal ethanol exposure on histamine H$_3$ receptor density and H$_3$ receptor effector coupling in the dorsal dentate gyrus of the rat brain.

5.1A: Representative autoradiogram of total [^{3}H]-A349821 binding in sagittal section. The area drawn for density measurements, the superior blade of the dentate gyrus stratum moleculare, is highlighted on the autoradiogram. Black bar denotes a distance of 1 millimeter. 5.1B: Specific [^{3}H]-A349821 binding in the superior blade of the dorsal dentate gyrus stratum moleculare. Data bars represent the mean ± the standard error of the mean binding, expressed as femtomoles / 105 µm2 in seven rats. No statistically significant difference was detected after Student's t-test (t = -0.173, P = 0.866). 5.1C: Representative autoradiogram of methimepip-stimulated [^{35}S]-GTPγS binding using 1 µM methimepip. 5.1D: Methimepip-stimulated [^{35}S]-GTPγS binding in the superior blade of the dorsal dentate gyrus stratum moleculare. Data points represent the mean ± the standard error of the mean, expressed as percent of basal [^{35}S]-GTPγS binding as function of methimepip concentration.   Two-way ANOVA detected a significant effect of prenatal alcohol exposure (F = 44.43, P < 0.001). Asterisks indicate post-hoc P values < 0.05 when compared to the control group. Double asterisks indicate P values < 0.01.

5.4.3 Electrically evoked EPSP responses

The effects of prenatal ethanol exposure and methimepip on electrically evoked EPSP

responses are depicted in Figure 5.2. Increased electrical stimulation of perforant path

fibers in the medial layer of the dentate gyrus stratum moleculare resulted in increased EPSP responses (Figure 5.2A). Prenatal ethanol exposure did not affect input/output responses of granule cells to electrical stimulation (Figure 5.2B). Perfusing slices with increasing concentrations of methimepip (0.3 – 3 µM) resulted in significant inhibition of the EPSP amplitude in prenatal ethanol-exposed rats, but not in controls (Figure 5.2C). This effect was partially reversed during the wash-out phase, when slices were perfused with ACSF only.
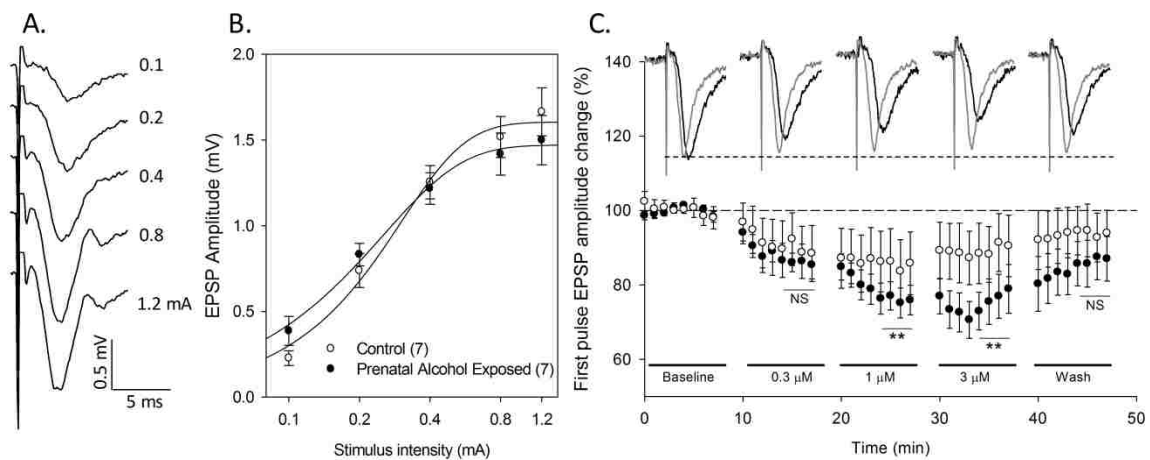
Figure 5.2 Effect of prenatal ethanol exposure on histamine H_3 receptor-mediated inhibition of electrically evoked EPSPs in the dentate gyrus.

5.2A: Representative traces illustrating EPSP evoked by increasing stimulus i intensities from 0.1 – 1.2 mA. 5.2B: Effects of prenatal ethanol exposure on EPSP amplitude evoked by different stimuli intensities. Data points represent the mean ± the standard error of the mean of control (open circles) and prenatal ethanol-exposed offspring (black circles). Sample sizes are denoted in parenthesis. No significant differences were detected between the two pregnancy treatment groups. 5.2C: Effects of H_3 receptor-mediated inhibition of EPSPs in control (open circles) and prenatal alcohol exposed offspring (black circles). Data points represent the percent mean ± the standard error of the mean of EPSP amplitude normalized to baseline values as a function of time. Bars denote the perfusing solution condition during the indicated given intervals of time. Numbers below the bars indicate the micromolar concentration of methimepip in the perfusing solution. A two-way repeated measures ANOVA detected a significant effect of methimepip (F = 7.608, P < 0.001). Post-hoc analysis found significant differences between baseline and different concentrations of methimepip only in the prenatal alcohol exposed group (double asterisks denote where P < 0.01 when compared to baseline values). Inset: Representative EPSP traces of control (gray) and prenatal ethanol-exposed offspring (black) normalized to the baseline (dashed line) at given time intervals. For purposes of illustration, pulses are offset by 2 milliseconds.

5.4.4   Paired pulse ratio

In a separate set of slices, the non-competitive $GABA_A$ receptor antagonist picrotoxin (50 µM) was added to the perfusing ACSF solution, to avoid GABAergic feedback inhibition of the second pulse in paired pulse ratio protocol. Picrotoxin increased EPSP amplitudes in both prenatal treatment groups (Figures 5.3B & 5.3C, compared to Figure 5.2B). Further, the presence of picrotoxin unmasked an increased EPEP responsiveness in prenatal ethanol-exposed rats compared to ACSF-treated control slices at higher current intensities (Figure 5.3C).   The application of 1 µM methimepip did not affect the input/output curve in control rats (Figure 5.3B), but caused a significant reduction in the EPSP responses at higher current intensities in prenatal ethanol-exposed offspring. Typical paired pulse inhibition was observed in both control and prenatal ethanol-exposed slices (Figure 5.3D) and prenatal ethanol exposure did not affect baseline paired pulse ratio measures compared to control offspring (Figure 5.3E).   In contrast, 1 µM methimepip significantly increased the paired pulse ratio in prenatal alcohol exposed offspring, but not in controls (Figure 5.3E).

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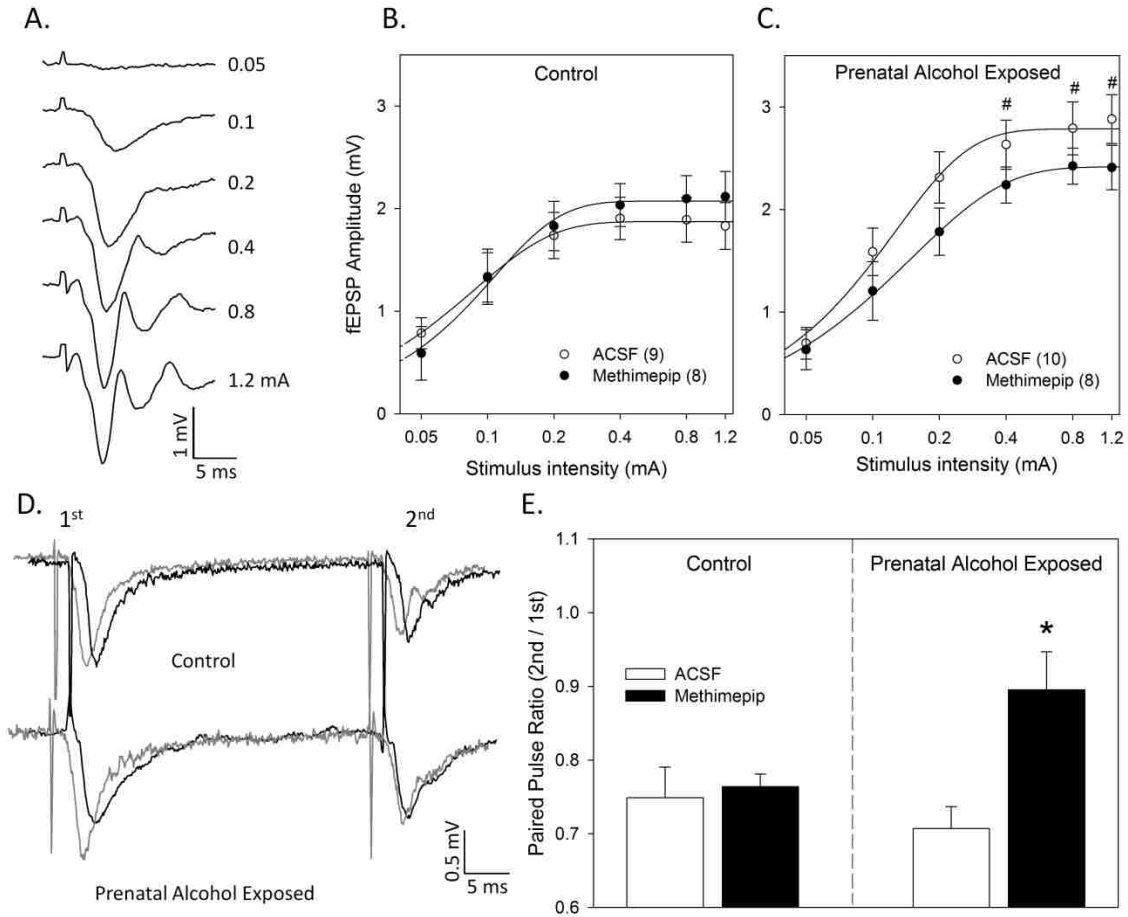
Figure 5.3 Effects of prenatal ethanol exposure on histamine H₃ receptor modulation of electrically-evoked EPSP responses and paired pulse ratio in presence of 50 μM picrotoxin

5.3A: Representative traces illustrating EPSPs evoked by increasing stimulus intensities from 0.05 – 1.2 mA. 5.3B: Effects of methimepip on EPSP responses to different stimulus intensities in control offspring. Data points indicate the mean ± the standard error of the mean of EPSP amplitude in slices perfused with ACSF (open circles) or ACSF containing 1 μM methimepip (black circles). Sample sizes are denoted in parenthesis. 5.3C: Effects of methimepip on EPSP responses to different stimulus intensities in prenatal ethanol-exposed offspring.   Data points indicate the mean ± the standard error of the mean of slices perfused with ACSF (open circles) or 1 μM methimepip (black circles). Pound signs indicate a statistically significant difference between prenatal ethanol-exposed and control offspring in panel B (significant effect of pregnancy treatment at 0.4, 0.8 and 1.2 mA; $F > 4.737$, $P < 0.037$ after two-way ANOVA). Sample sizes are also indicated in parenthesis. 5.3D: Representative EPSP traces evoked by pairs of pulses given at a fixed interpulse interval of 40 milliseconds. Upper traces show paired pulses in ACSF (gray) and methimepip (black) in a control rat. Lower traces illustrate similar conditions in a prenatal ethanol-exposed rat. For purpose of illustration, pulses are offset by 2 milliseconds. 5.3E: Effects of prenatal ethanol exposure on H₃ receptor modulation of the paired pulse ratio. Bars indicate the mean ± the standard error of the mean of the paired pulse ratio obtained by dividing the EPSP amplitude of the second pulse by that of the first pulse in control (left side bars) and prenatal ethanol-exposed offspring (right side bars). Methimepip (filled bars) caused a significant increase in the paired pulse ratio in prenatal alcohol exposed offspring ($P < 0.05$ after two-way ANOVA, denoted by an asterisk), but not in controls.

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5.4.5 Long term potentiation

The effects of prenatal alcohol exposure and methimepip on LTP induced by tetanization with five consecutive theta-bursts are illustrated in Figure 5.4. Tetanization of ACSF-treated control slices resulted in a sustained increase of evoked EPSP amplitude (Figure 5.4A, open circles; Figure 5.4C, left-hand side open bar). Prenatal ethanol-exposed rats exhibited a dramatic reduction in the magnitude and duration of LTP when compared to controls (Figure 5.4B, open circles; Figure 5.4C, right-hand side open bar). Methimepip (1 μM) reduced the magnitude and duration of LTP in control slices (Figure 5.4A, black circles; Figure 5.4C, right-hand side black bar), mimicking the effect of prenatal alcohol exposure. However, methimepip had no significant effect on the LTP deficit observed in ACSF-treated prenatal ethanol-exposed slices (Figure 5.4B, black circles; Figure 5.4C, left-hand side black bar).

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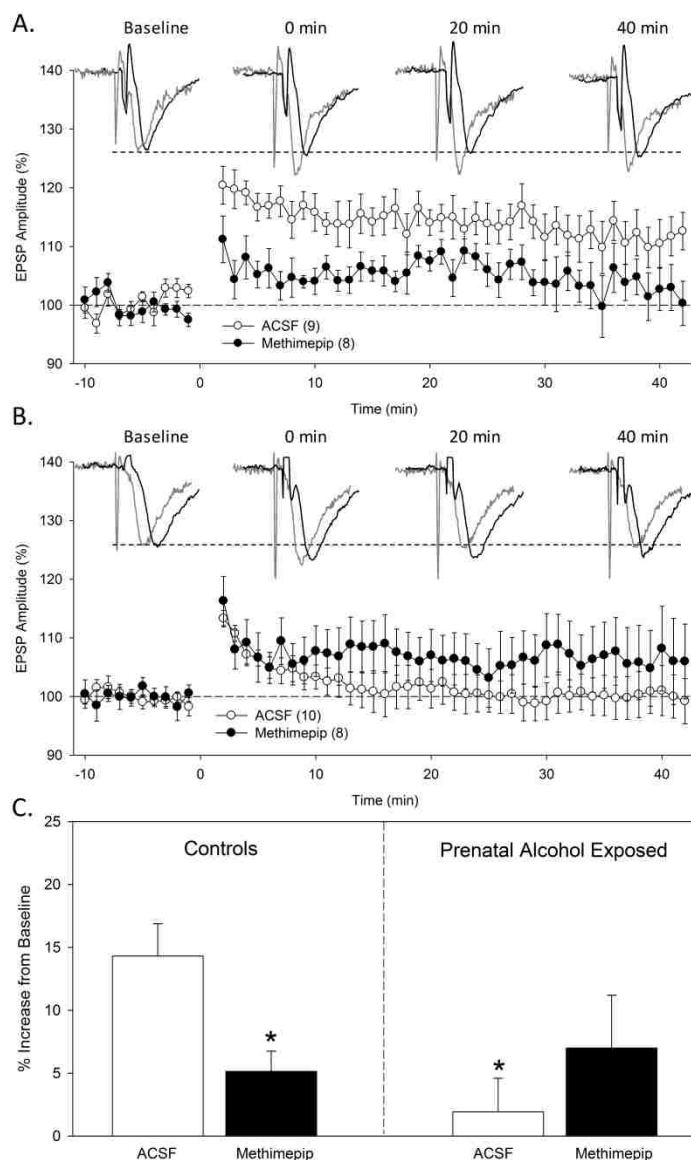
Figure 5.4 Effects of methimepip and prenatal ethanol exposure on LTP in the dentate gyrus.

5.4A: LTP in control offspring. Data points represent the mean ± the standard error of the mean of EPSP amplitude normalized to baseline values in ACSF (open circles) or methimepip (filled cirlces). Sample sizes are indicated in parenthesis. Inset: Representative traces obtained at given time intervals in ACSF (gray) and methimepip-perfused slices (black). Dashed line indicates the baseline. For purposes of illustration, pulses are offset by 2 milliseconds. 5.4B: LTP in prenatal ethanol-exposed offspring. Data points and inset are represented as in 5.4A. 5.4C: Summary of LTP data. Bars represent the mean ± the standard error of the mean increase from baseline during the 40 min post-tetanus recording in control (left-hand side) or prenatal ethanol-exposed offspring (right-hand side) perfused with ACSF (open bars) or methimepip. Two-way ANOVA detected a significant effect of prenatal ethanol exposure and methimepip (F = 6.046, P = 0.020). Asterisks indicate significant differences compared to the ACSF perfused control offspring (P < 0.05 on post-hoc analysis).

5.5 Discussion

The salient findings of this study are that rats prenatally exposed to moderate levels of alcohol have increased histamine H_3 receptor-effector coupling in dentate gyrus (Figure 5.1D) which may contribute to increased H_3 receptor-mediated inhibition of EPSP responses (Figure 5.3C) and glutamate release (Figure 5.3E). Moreover, prenatal ethanol exposure also resulted in striking reduction of the magnitude and duration of LTP in dentate gyrus slices (Figure 5.4B & 5.4C), an effect mimicked by methimepip treatment of control slices (Figure 5.4A & 5.4C). However, in contrast to our prediction, methimepip did not exacerbate the LTP deficit observed in prenatal ethanol-exposed offspring (Figure 5.4B & 5.4C).

The mechanistic basis for elevated H_3 receptor-effector coupling in prenatal ethanol-exposed rats (Figure 5.1D) is currently unknown. Specific [^{3}H]-A349821 binding data measured at a near-saturating concentration of the radioligand (Figure 5.1B) suggests that the total H_3 receptor density is similar between prenatal treatment groups. One possible explanation for heightened H_3 receptor-effector coupling could be a prenatal ethanol-induced alteration in the differential expression of H_3 receptor subtypes within the dentate gyrus. At least three functional isoforms of histamine H_3 receptors are expressed in rats (Morisset et al., 2001). These isoforms, which originate from alternative splicing of the H_3 receptor gene, differ primarily in the length of the amino acid sequence within the third cytoplasmic loop of this transmembrane receptor protein. The longer isoform, identified in rat as rH_{3A}, exhibits higher constitutive activity and lower H_3 receptor agonist affinity compared to the truncated rH_{3B} and rH_{3C} isoforms, which exhibit lower constitutive activity and higher agonist affinity (Bongers et al., 2007; Drutel et al., 2001).

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It is not unreasonable to speculate that prenatal ethanol exposure may have shifted the predominant H_3 isoform in dentate gyrus, reducing the total population of the constitutively active rH_{3A} isoform with a similar increase in the constitutively inactive but more agonist-sensitive rH_{3B} or rH_{3C} isoforms. The differential effect of the H_3 receptor inverse ABT-239 on dentate gyrus LTP *in vivo* (Varaschin *et al*., 2010) is consistent with this notion. Currently, there are no subtype-selective H_3 receptor drugs or antibodies to directly examine this question and the available H_3 receptor antagonist radioligands bind to theses isoforms with similar affinity. One more indirect approach to address the question of altered H_3 receptor agonist affinity would be to conduct an agonist competition of [^{3}H]-A349821 binding study (see Witte et al., 2006) to discern whether prenatal ethanol exposure altered the number or affinity of a "high agonist affinity" population compared to a "lower agonist affinity" population of specific [^{3}H]-A349821 binding sites.

Another possible outcome is that prenatal ethanol exposure did not affect H_3 receptor isoform expression or H_3 receptor affinity for binding agonists, but rather increased the intrinsic activity of the receptor-effector coupling response. One mechanism for this could be an increase in the quantity of $G_{i/o}$ protein levels in the dentate gyrus. However, the observation that basal [^{35}S]-GTPγS binding was similar between prenatal treatment groups (data not shown) does not support this idea. Another explanation could relate to the involvement of intracellular mechanisms that regulate G-protein coupling of metabotropic receptors to their effector mechanisms. While nothing is known about these regulatory processes with respect to H_3 receptors, there is evidence that various protein kinases and other factors regulate the coupling of another $G_{i/o}$ protein-coupled

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receptor, namely the mGluR7 receptor (Sorensen et al., 2002) that also regulates the release of glutamate (Pelkey et al., 2005; Zhang et al., 2008). This is an intriguing prospect relative to the differential receptor-effector coupling response in prenatal ethanol-exposed rats given that some of these regulatory mechanisms target the third cytoplasmic loop of the $mGluR_7$ receptor.

Whatever the mechanistic basis for elevated H_3 receptor-effector coupling in prenatal ethanol-exposed rats, this alteration provides one explanation for methimepip's greater inhibitory effect on EPSPs responses (Figures 5.2C and 5.3C) and glutamate release (Figure 5.3E) in prenatal ethanol-exposed rats. The possible mechanisms by which histamine H_3 receptors inhibit glutamate release may be inferred based on evidence from studies of other presynaptic $G_{i/o}$-protein coupled receptors (GPCRs) (reviewed by Betke et al., 2012). Upon activation, these G-proteins dissociate into α and βγ subunits. The activated $G_{i/o}$ α–subunit inhibits adenylyl-cyclase activity, leading to reduced cAMP levels and, consequentially, reduced protein kinase A (PKA) activity. Presynaptic voltage-gated calcium channels are a substrate for PKA and phosphorylation of these channels increases conductance and facilitates calcium entry into the nerve terminal after an action potential. Since synchronous vesicle fusion to the cell membrane is a calcium-dependent phenomenon, limited availability of free calcium due to low-conductance channels could contribute to reduced neurotransmitter release. Additionally, the $G_{i/o}$ βγ subunits directly interact with voltage gated calcium channels at axon terminals, as well as with soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, respectively inhibiting calcium influx and the exocytotic machinery, further contributing to decreased neurotransmitter release. In a previous study, histamine

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inhibited dentate gyrus granule cell excitability and glutamate release probably by a direct blockade of voltage gated calcium channels by βγ-subunits of $G_{i/o}$ protein (Brown and Haas, 1999; Brown and Reymann, 1996). Here, it remains to be investigated whether or not prenatal alcohol exposure could result in greater interaction between $G_{i/o}$ βγ subunits and voltage gated calcium channels as a potential mechanism for the observed differentiated glutamate release.

One unexpected result in this study was the observation that methimepip did not affect baseline measures of synaptic responses or glutamate release even though the concentration of methimepip used in the slice studies was similar to the maximally effective concentration for producing H_3 receptor-effector coupling (Figure 5.1D). One possibility is that higher concentrations of agonist are required to elicit responses in slice studies than in histological sections. In this case, the concentration of methimepip may have been too low to elicit physiological responses in control slices. In previous studies, dentate gyrus histamine H_3-mediated inhibition of glutamate release by histamine (7 μM) and by the H_3 receptor agonist R-alpha-methylhistamine (10 μM) was clearly demonstrated (Brown and Reymann, 1996). Therefore, it is possible that inhibition of glutamate release would be observed here had the concentrations of methimepip been higher.

Another unanticipated result was the observation that blockade of $GABA_A$ receptors by picrotoxin unmasked an increased responsiveness of granule cells in the dentate gyrus of prenatal alcohol exposed offspring (Figures 5.3A & 5.3B). This increased excitability cannot be explained by an increase in baseline probability of glutamate release, since there were no significant differences in between the paired pulse ratio of control and

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prenatal alcohol exposed offspring in the ACSF condition (Figure 5.3D). Therefore, it is possible that this heightened excitability in is linked to postsynaptic mechanisms independent of histamine H_3 regulation of glutamate release.

As observed previously in *in vivo* electrophysiological studies (Sutherland et al., 1997; Varaschin et al., 2010), prenatal alcohol exposure resulted in striking reduction of LTP in the dentate gyrus. This result further reinforces the notion that synaptic plasticity in the dentate gyrus is particularly sensitive to low levels of alcohol exposure during development. Further, whereas baseline physiological responses were unaffected in control slices at the methimepip concentrations employed here (Figures 5.2B, 5.3B and 5.3E), methimepip caused an LTP deficit in control slices remarkably similar to that observed in ACSF-treated prenatal ethanol-exposed slices (Figure 5.4). This observation suggests that more complex activity-dependent changes in synaptic plasticity may be more sensitive to H_3 receptor-mediated inhibition than less complex baseline measures of synaptic activity.

In contrast to our predictions however, methimepip did not further decrease LTP in prenatal alcohol exposed offspring (Figure 5.4B). This observation may be attributed to the fact that, under the experimental conditions employed in this study, prenatal alcohol exposure reduced LTP to a level where further inhibition of synaptic plasticity was not possible. Therefore, the question of whether or not heightened histamine H_3 mediated inhibition of glutamate release is a causal factor, or one of several contributing mechanisms in prenatal alcohol-induced LTP remains open to further detailed investigation.

6. Discussion

6.1 Summary and significance of results

The salient findings in this study are that a model of limited access, moderate voluntary drinking during pregnancy resulted in altered histamine H_3 modulation of glutamatergic neurotransmission in the dentate gyrus of rat offspring. These changes were observed in absence of gross teratogenic effects, reinforcing the notion that this voluntary drinking paradigm models more subtle aspects of FASD. Further, the prenatal alcohol-induced LTP deficit was amenable by systemic injection of the inverse agonist of H_3 receptors ABT-239, suggesting drugs in this pharmacological category as potential pharmacotherapeutic tools on the treatment of FASD-associated cognitive deficit.

6.1.1 Effects of prenatal alcohol exposure

One striking observation in this study was the consistency of the voluntary drinking paradigm and its effects on the offspring. The experiments presented in chapters 3 to 5 spanned over 9 different breeding rounds. Average ethanol daily intake varied from 2.82 ± 0.13 g/kg/day (chapter 3) to 2.40 ± 0.13 and 2.61 ± 0.15 g/kg/day (Chapters 4 and 5 respectively). These levels of ethanol consumption did not induce changes in maternal weight gain during pregnancy, size of the litters and pup birth weight. No changes were observed in maternal care after birth or in pup weight gain to weaning (Staples et al., in preparation) or adult offspring weights (Chapter 3). The average blood alcohol concentration measured in pregnant dams (Chapter 3) was of 84.0 ± 5.5 mg/dL, a level considered moderate for rats (Valenzuela et al., 2012). These data are in contrast to those observed in models that aimed for higher blood alcohol concentrations (on the order of 300 mg/dL or higher), where high pup mortality, arrested growth and anatomical

abnormalities are commonly observed (Bonthius et al., 1988; Bonthius and West, 1988). The moderate blood alcohol concentrations achieved here happened by voluntary drinking, instead of forced gavage, gastric intubation, liquid ethanol-containing or vapor inhalation of alcohol, other models commonly used in the study of prenatal alcohol effects. This is important, because as opposed to these more commonly employed models, the voluntary drinking paradigm minimizes the stress involved with highly invasive alcohol-administration procedures or with the consumption of an unfamiliar diet, as well as the malnutrition associated with consumption of ethanol-derived "empty calories" (Savage et al., 2010). These stress-effects are important confounding factors when studying teratogen effects of alcohol, thus resulting in the addition of an extra control group for administration-procedure effects that may incur increased expenses and unnecessary animal use (Redila et al., 2006; Titterness and Christie, 2012). Moreover, the limited-access component of the paradigm is significant because it emulates important aspects of social drinking observed in humans (for example, having a couple of drinks during "happy-hour") (Savage et al., 2010). Intermittent alcohol exposure during development was previously shown to result in greater effects in the offspring than constant "*ad-libitum*" exposure (Bonthius et al., 1988; Maier and West, 2001). Together, these characteristics make of the voluntary drinking paradigm an attractive model for the study of subtle forms of FASD.

In addition to the lack of gross teratogenic effects, most baseline measures of glutamatergic neurotransmission investigated in this manuscript were not affected by prenatal alcohol exposure. There were no differences in raw measures of granule cell responsiveness to electrical stimulation, either *in-vivo* (Chapters 3 and 4) or *in-vitro*

(Chapter 5), nor in basal probability of glutamate release (Chapters 4 and 5). These findings are consistent with previous studies, where similar levels of prenatal ethanol exposure failed to alter synaptic number, strength and basal release of markers for glutamate (Savage et al., 2002; Savage et al., 1998; Sutherland et al., 1997). Two important observations diverge from this trend: First, prenatal alcohol exposure reduced the population spike amplitude in response to given fEPSP-slopes *in-vivo* (Chapter 4, Figure 4.2). These changes could not be explained by decreased glutamate release from presynaptic nerve terminals or by increased GABAergic inhibition by interneurons in the dentate gyrus, at least with the techniques employed here (Figures 4.3 and 4.4). Since GABA and glutamate release in the dentate gyrus were not affected by prenatal alcohol, it is possible that decreased E-S coupling is related to changes in the postsynaptic neurons. Second, prenatal alcohol increased *in-vitro* EPSP amplitude in response to electrical stimuli when GABA$_A$ receptors were blocked by picrotoxin (Chapter 5, Figure 5.3). These data indicate that, in the absence of GABAergic inhibition, there is a greater excitability of granule cells in the dentate gyrus of prenatal alcohol exposed offspring. This differential increase in EPSP responses, however, was not observed in experiments where GABA$_A$ inhibition was intact (Chapter 5, Figure 5.2). Moreover, an increase in EPSP responses *in-vitro* is difficult to reconcile with a decrease in E-S coupling *in-vivo*. Clearly, these effects are complex and will require further investigation.

Prenatal exposure to moderate levels of alcohol produces more evident effects in activity-dependent measures of hippocampal function (Savage et al., 2002; Savage et al., 1998). In the present study, voluntary, limited access drinking of moderate amounts of alcohol throughout pregnancy reduced LTP in the dentate gyrus of alcohol-exposed offspring, an

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effect consistent across different breeding rounds. These effects are in accordance with those observed in previous studies (Sutherland et al., 1997; Titterness and Christie, 2012). Further, LTP reduction was evident only in a sub-maximal induction protocol (three high-frequency stimuli trains). This initial prenatal alcohol-induced LTP impairment was overcome when repeated tetanizing stimuli were delivered, in a protocol that elicited maximal-LTP (Chapter 3, Figure 3.3). This observation is an important reminder that moderate consumption of alcohol during pregnancy results in subtle effects in the offspring, and that these effects are often elusive (Valenzuela et al., 2012). A clear example may be taken from measures of learning and memory in models of moderate pregnancy drinking, where deficits were only observed in very challenging tasks (Hunt et al., 2009; Savage et al., 2002). It is also noteworthy that prenatal alcohol exposed offspring still possess the same LTP capacity as control offspring, illustrated here by a lack of significant differences in the maximal LTP-induction protocol (Figure 3.3A and 3.3B). This capability may parallel previous observations in behavioral experiments in animal models and in humans, where the impaired ability to learn a specific task is rescued by cognitive intervention or by repeated training on the task (reviewed by Kodituwakku and Kodituwakku, 2011). Together, these observations lend support to the hypothesis that prenatal alcohol-induced cognitive and synaptic plasticity impairments are not irreversible, opening a broad array of possibilities for therapeutic intervention.

The mechanisms through which prenatal alcohol impairs submaximal-LTP are not yet completely understood. It is noteworthy, however, that tetanization of perforant path fibers in prenatal alcohol exposed offspring, whether *in-vivo* or *in-vitro,* resulted in immediate potentiation of granule cell responses to the same extent observed in control

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offspring, the difference being that these responses rapidly decayed back to baseline levels in prenatal alcohol exposed offspring. As speculated in chapter 3's discussion, these mechanisms may relate to impairments in the mechanisms controlling late-LTP or LTP2/3 (Malenka and Bear, 2004; Raymond, 2007). One suggested mechanism relates to reduced metabotropic glutamate receptor signaling in prenatal alcohol exposed offspring (Galindo et al., 2004). While a reduction in metabotropic glutamate signaling itself does not indicate whether these changes are pre- or postsynaptically located, Galindo and collaborators also found that, associated with a decrease in expression of metabotropic receptors type 5 ($mGluR_5$), are decreased phosphorylation of growth associated protein 43 (GAP43) and decreased activity-dependent potentiation of glutamate release, both phenomena thought to take place exclusively in presynaptic nerve terminals. Moreover, sustained signaling from $mGluR_5$ receptors may contribute to increased levels of intracellular calcium, a mechanism required for development of sustained LTP (Raymond, 2007). Therefore, reduced $mGluR_5$ signaling in presynaptic nerve terminals and consequent decreased mobilization of intracellular calcium stores could negatively impact the development of late-LTP or LTP2/3. This view is complemented by recent findings indicating that sustained LTP requires protein synthesis in presynaptic nerve terminals that, in turn, increase the turnover of presynaptic vesicles and neurotransmitter release (Johnstone and Raymond, 2011). In addition to presynaptic protein synthesis, sustainment of LTP may require calcium influx at the soma of presynaptic cells, fast axonal transport of proteins (Barnes et al., 2010), nitric oxide signaling (Johnstone and Raymond, 2011), activation metabotropic glutamate receptor type 1 and downstream ERK-dependent activation of cAMP response element-binding

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protein (CREB) (Ran et al., 2009; Ran et al., 2012). Although previous studies exemplify alterations in some of the above mentioned mechanisms (Samudio-Ruiz et al., 2009), the full extent of the impact of prenatal alcohol exposure on presynaptic mechanisms of LTP is far from being completely understood.

6.1.2 Histamine H_3 receptor modulation of glutamate release and LTP in control offspring

Inhibition of glutamate release mediated by histamine H_3 receptors has been illustrated in numerous studies (Brown and Haas, 1999; Brown and Reymann, 1996; Chang et al., 1998; Garduno-Torres et al., 2007; Molina-Hernandez et al., 2001). As mentioned previously, these receptors reside predominantly in presynaptic nerve terminals (Pillot et al., 2002), where they couple to $G_{i/o}$ proteins and inhibit neurotransmitter release, possibly via blockade of calcium channels (Brown and Haas, 1999). In agreement with these findings, it was shown in Chapter 5 that functional histamine H_3 receptors are expressed by neurons in the dentate gyrus of rats (Figure 5.1). Systemic activation of these receptors by the agonist methimepip *in-vivo* resulted only in a trend to inhibition of population spikes (Chapter 4, Figure 4.1) and no significant effect on fEPSP slope. However, methimepip did significantly reduce E-S coupling (Figure 4.2), similar to that observed in prenatal alcohol exposed offspring. Methimepip-injection in control offspring *in-vivo* significantly inhibited the probability of glutamate release from presynaptic nerve terminals (Figure 4.3), which may, in part, explain the reduced E-S coupling in these animals (Daoudal et al., 2002). These observations were not replicated in the *in-vitro* measures of probability of glutamate release, where concentrations of methimepip up to 3 µM failed to inhibit granule cell responsiveness to electrical

stimulation or the probability of glutamate release from perforant path nerve terminals in control rats (Figures 5.2 and 5.3). These differential observations may be due to the dose/concentrations chosen for this study. The selection of the dose used in Chapter 4 (*in-vivo* experiments) was based on studies by Kitbunnadaj and collaborators (2005) and on our pilot experiments. The concentrations used in the *in-vitro* experiments were based on pilot experiments, and were kept intentionally low to facilitate detection of a heightened function of these receptors in the prenatal alcohol exposed group. Therefore, it is possible that histamine H_3 mediated inhibition of glutamate release could be achieved with increased concentrations of methimepip. A detailed investigation of methimepip modulation of glutamate release in the dentate gyrus of control animals, however, was beyond the scope of this study.

Both *in-vivo* and *in-vitro*, activation of H_3 receptors by methimepip reduced LTP to levels similar to those observed in prenatal alcohol exposed offspring (Figures 4.5 and 5.4). One serendipitous finding was that, at least *in-vitro,* inhibition of LTP did not require decreased probability of glutamate release. In fact, methimepip-induced impairment of LTP was evident while no effect was observed in the probability of glutamate release or in granule cell responses to electrically evoked glutamate release. These differential effects of methimepip *in-vitro* strongly suggest that histamine H_3 modulation of glutamate release and of LTP are controlled by different mechanisms, and that the latter is more sensitive to lower concentrations of the agonist. This is an interesting observation because, until this date, most studies investigating the role of these receptors in learning and memory hypothesize that modulation of cognitive processes by histamine H_3 receptors is directly linked to their ability to modulate neurotransmitter release

(reviewed by Brioni et al., 2011; Esbenshade et al., 2008; Passani and Blandina, 2011). Although negative modulation of neurotransmitter release – in this case glutamate – indeed has an unequivocal and detrimental impact on cognition and its underlying cellular processes, further consideration needs to be paid to other mechanisms downstream of $G_{i/o}$ coupled receptors. As mentioned above, previous studies indicate that expression of sustained LTP requires protein synthesis in presynaptic nerve terminals (i.e.: Barnes et al., 2010; Johnstone and Raymond, 2011), and that gene transcription during LTP is regulated by CREB (Ran et al., 2012), a transcription factor whose activity is directly related to the intracellular levels of cAMP (reviewed by Barco and Marie, 2011). This CREB-dependent protein synthesis is negatively modulated by $GABA_B$ receptors (Helm et al., 2005), another type of presynaptically located $G_{i/o}$ coupled receptor that, when activated, promote a gradual decline of potentiation in synapses tetanized with high frequency stimuli (Huang and Hsu, 2004). Interestingly, this $GABA_B$ receptor-mediated depotentiation is only evident when submaximal LTP is elicited, but not when maximal LTP is elicited (Huang and Hsu, 2004). It has not been established to date whether or not histamine H_3 receptors modulate protein synthesis required in sustained LTP. However, these receptors are clearly capable of modulating intracellular cAMP levels and, consequentially, modulating activity of protein kinase A (reviewed by Haas et al., 2008) and possibly CREB phosphorylation, nuclear translocation and gene transcription (Scott Bitner, 2012). Detailed studies of these mechanisms should be subject of further investigation.

An alternative interpretation for histamine H_3 receptor-mediated inhibition of LTP is related to decreased histamine release from histaminergic nerve terminals in the dentate

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gyrus. Activation of histamine H_1 and H_2 receptors by endogenous histamine is thought to facilitate synaptic plasticity in the hippocampus (Luo and Leung, 2010). Conversely, pharmacological blockade or genetic ablation of these receptors results in impaired LTP (Chang et al., 1997; Dai et al., 2007). Since activation of histamine H_3 receptors results in decreased histamine release (i.e.: Kitbunnadaj et al., 2005), it is possible that the reduced LTP observed after methimepip treatment *in-vivo* or *in-vitro* could be due to reduced tonal activation of H_1 or H_2 receptors by endogenous histamine. Therefore, it would be of interest to study whether or not methimepip-induced LTP deficit in control rats could be rescued by concomitant treatment with agonists of H_1 or H_2 receptors.

Secondary to these observations are the effects of the inverse agonist ABT-239 on LTP and glutamate release in the rat dentate gyrus. Contrary to its predicted effect as a cognitive enhancer, ABT-239 did not facilitate learning and memory retention in behavioral experiments, even in challenging tasks where cognitive enhancement might be readily detected (Savage et al., 2010). These observations are reminiscent of the effects in control offspring *in-vivo* (Chapter 3, Figure 3.4), where ABT-239 failed to enhance LTP. In *in-vitro* experiments, ABT-239 increased granule cell responses to electrical stimulation and facilitated glutamate release from perforant path nerve terminals (Varaschin et al., submitted; Appendix A, Figure A.1) and reversed methimepip-induced LTP deficit (Appendix A, Figure A.2). However, ABT-239 alone did not enhance LTP *in-vitro* (Figure A.3). The differential effects of ABT-239 on probability of glutamate release and LTP further reinforce the notion that histamine H_3 receptors modulate these two phenomena by different mechanisms.

<u>6.1.3 Histamine H_3 receptor-effector coupling, modulation of glutamate release and LTP in prenatal alcohol exposed offspring</u>

Effects of prenatal alcohol exposure on histamine H_3 modulation of glutamate release and LTP in the dentate gyrus were the main focus of this dissertation. Prenatal alcohol exposure increased methimepip-stimulated $[^{35}S]$-GTPγS binding in the dentate gyrus (Chapter 5, Figure 5.1) and in the medial frontal cortex (data not shown) of rats, suggesting a heightened receptor-effector coupling in these regions. This increased response to methimepip could not be explained by a greater number of histamine H_3 receptors, since there were no significant differences in the $[^3H]$-A349821 binding in these brain regions. Whether or not changes in receptor-effector coupling are related to post-translational modifications in the histamine H_3 receptor, thus altering its conformation and facilitating interaction with G-protein; or in altered conformation or function of G-protein itself, is not known. Interestingly, these changes do not appear to be restricted to histamine H_3 receptors. Preliminary data indicates that adenosine A_1 receptors and cannabinoid CB_1 receptors among others also have their receptor-effector coupling proprieties altered by prenatal alcohol exposure (Allen et al., in preparation). Therefore, prenatal alcohol exposure is likely to result in complex interactions between multiple neurotransmitter systems, leading to outcomes that are often not easily predicted.

Specific to histamine H_3 receptors, the increased receptor-effector coupling in prenatal alcohol exposed offspring resulted in heightened H_3-mediated inhibition of glutamate release, at least in the *in-vitro* experiments (Figure 5.3). Heightened receptor-effector coupling could be explained by a shift in the predominant H_3 isoform in the dentate gyrus. As mentioned in the introduction, alternative splicing of the histamine H_3 gene

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yields at least three functional isoforms that are naturally expressed in the rat brain (reviewed by Bakker, 2004). These isoforms differ by a truncation in their third intracellular loop. The truncated isoforms (rH_{3B} and rH_{3C}) exhibit greater affinity for H_3 agonists than the non-truncated isoform (rH_{3A}). On the other hand, the non-truncated isoform possess greater constitutive activity than the truncated isoforms. Since their pharmacological characteristics differ dramatically, a prenatal alcohol induced shift in the predominant isoform (from rH_{3A} to rH_{3B} or rH_{3C}) could explain some of the effects observed in the present study. For example, the effect of an inverse agonist like ABT-239 should be more easily detected in animals that preferentially express the constitutively-active rH_{3A} isoform than in animals that express predominantly isoforms rH_{3B} or rH_{3C}. Indeed, ABT-239 facilitated glutamate release in the dentate gyrus of control offspring (Varaschin et al., submitted), an effect expected from this inverse agonist. Still, the same concentration in prenatal alcohol exposed offspring resulted in no effect (data not shown). Moreover, due to the lower agonist-affinity of the isoform rH_{3A} predominant in the control offspring, methimepip-induced inhibition of glutamate release would be discrete, perhaps undetectable at lower concentrations of the agonist, but should be considerably high in prenatal alcohol exposed offspring. Indeed, methimepip decreased glutamate release in prenatal alcohol exposed offspring in concentrations that failed to produce any significant effect in controls (Figure 5.3). Therefore, given the evidence presented in this dissertation, the hypothesis of a shift in the predominant H_3 isoform is plausible, and an investigation of which isoforms are present in control and prenatal alcohol offspring appears to be a logical next step.

Lastly, there are the effects of histamine H_3 modulation of LTP in the dentate gyrus. Contrary to the original hypothesis, methimepip did not further decrease LTP in prenatal alcohol exposed offspring, neither *in-vivo* nor *in-vitro*. One parsimonious explanation for these observations is that LTP in these animals could be already maximally impaired by prenatal alcohol, and that further agonism of H_3 receptors would not be effective in exacerbating this LTP deficit. Another explanation would be that residual histamine in dentate gyrus slices or *in-vivo* could be already maximally activating the aforementioned hypothetical H_3 mechanisms that modulate presynaptic sustenance of LTP. This hypothesis is supported by the ABT-239 reversal of prenatal alcohol-induced LTP deficit observed *in-vivo* (Chapter 3), but is not supported by the lack of effect of ABT-239 on LTP in the *in-vitro* experiments (data not shown). Besides, these differential effects strengthen the view that H_3-mediated inhibition of glutamate release and H_3-mediated inhibition of LTP are controlled by different putative mechanisms. Although a detailed investigation of these mechanisms was beyond the primary objectives of this study, a clearer understanding of these phenomena could greatly contribute for the understanding of histaminergic regulation of synaptic processes involved with cognition, and the understanding of how prenatal alcohol exposure may alter this regulation.

6.2 Critique and future studies

It would be an overambitious assumption to expect that studies such as the ones presented in this dissertation would uncover definitive answers for complicated phenomena laying in the overlap of broad areas of research, like synaptic plasticity, cognition, development and teratogenic effects of alcohol. Such is the nature of science that scientific enterprises often encounter novel findings, but with these findings also come novel questions and

hypotheses. Likewise, the present study encountered multiple questions that were left unanswered, some of which were explicitly posed throughout this manuscript, while others only implicitly mentioned.

For example, the question of whether or not prenatal alcohol exposure could result in increased histamine H_3 receptor affinity for methimepip was not addressed in the binding experiments presented in Chapter 5. Multiple affinity sites in mixed receptor population, such as H_3 receptors with multiple isoforms, can be investigated by displacement of antagonist binding with increasing concentrations of a given agonist. In this experiment, histological brain sections are incubated with a near-saturating concentration of a radiolabeled antagonist (for example [^{3}H]-A349821 used in Chapter 5). Next, in different incubating baths, increasing concentrations of an unlabeled agonist are added (for example, methimepip). The agonist will then compete with the antagonist for the receptor binding sites, thus decreasing the amount of radiolabeled-antagonist bound to receptors in a concentration-dependent manner. Next, after exposing film to the histological sections in order to generate autoradiograms, it is possible to quantify binding of the radiolabeled antagonist in the brain regions of interest at each concentration of the agonist. Analysis of these data should, then, give an estimate of the affinity for the agonist, number of affinity states in the case of multiple isoforms, and the proportion of each individual isoform in relation to the total population. Therefore, in the hypothesis that prenatal alcohol exposure shifting the balance of the predominant subpopulation of histamine H_3 receptors in favor of the isoforms that possess greater agonist affinity, and given that the total number of receptors would remain unaltered, there would be an observable shift to the left in the competitive-binding curve.

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In the possibility of altered affinity, further investigation would require the elucidation of the predominant isoforms in brain regions of interest in prenatal alcohol exposed offspring. Detailed investigation of the localization of histamine H_3 receptor isoforms in the rat brain is currently limited by the unavailability of specific antibodies or radioligands for these isoforms. Nevertheless, an investigation of the cells that express these isoforms could be applied to circumvent these limitations. This would be achieved by *in-situ* hybridization of RNA probes directed to mRNA of each isoform. Additionally, retrograde labeling of perforant path nerve terminals should indicate if the cells that express specific H_3 isoforms are the same that project into the dentate gyrus.

A second limitation in the present study was that electrophysiological measures of glutamate release and LTP were restricted to the dentate gyrus of the hippocampus. As mentioned in the introduction, characteristics of the dentate gyrus such as its behavioral significance to cognitive process, synaptic configuration, organized laminar structure and vulnerability to teratogenic effects of alcohol offer remarkable advantages in the study of prenatal alcohol effects on synaptic plasticity. However, prenatal alcohol induced changes in H_3 receptor-effector coupling are not unique to the dentate gyrus (Allen et al., in preparation). Other regions where glutamatergic plasticity is of crucial importance to cognitive processing, such as the medial frontal cortex, dorsal- and medial-parietal cortex are also affected. Therefore, a complete understanding of prenatal alcohol-induced alteration of H_3-mediated inhibition of glutamate release and LTP requires an investigation in these areas as well.

Moreover, studies of synaptic plasticity in the dentate gyrus conducted here were limited to LTP induction of perforant path to granule cell synapses. Although LTP is an

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important cellular aspect of memory storage, other neurophysiological events are also involved with information processing and memory formation, such as long-term depression, LTP depontentiation and spike-time dependent plasticity among others (reviewed by Chaillan et al., 2008). Curiously, these other forms of synaptic plasticity have been neglected by researchers in the field or FASD, and the effects of prenatal alcohol in these neurophysiological phenomena are currently unknown (Medina, 2011). In addition, the electrophysiological experiments conducted here utilized simple recordings of electrically evoked field potentials in neuronal populations, either *in-vitro* or under urethane anesthesia. Certainly, interpretation of prenatal alcohol effects would benefit from refinement of these techniques, for example, moving from extracellular recordings *in-vitro* into whole cell patch clamp and quanta analysis (i.e.: Ran et al., 2012), or moving from urethane anesthetized recordings *in-vivo* into awake, freely-moving recordings (i.e.: Doyere et al., 1997). Additionally, the effects observed here should be confirmed using other techniques available.

Studies here were limited to histamine H_3 receptors. Preliminary data indicates that histamine H_2 receptors are also affected by prenatal alcohol exposure (unpublished observations). Since histamine H_1 and H_2 receptors participate in modulation of synaptic plasticity (Chang et al., 1997; Dai et al., 2007), it would be of interest to investigate the effects of agonists and antagonists of these receptors in prenatal alcohol-induced LTP deficit in the dentate gyrus. Together, these experiments would greatly advance the current knowledge of FASD-associated cognitive impairment as well as histaminergic modulation of synaptic plasticity in the brain.

Lastly, histamine H_3 receptors act as heteroreceptors in a number of neurotransmitter systems, and are known to regulate release of not only glutamate, but also acetylcholine, dopamine, serotonin and histamine. These neurotransmitters, in turn, play an important modulatory role of cognitive processes (reviewed by Blandina et al., 2004; Esbenshade et al., 2008; Haas et al., 2008; Passani and Blandina, 1998). Consequently, prenatal alcohol-induced alteration of H_3 receptors could have a potential impact in each of these other neurotransmitters. Here, the effects of alcohol drinking during pregnancy on H_3-mediated inhibition of glutamate release were investigated in some detail. Yet, it is not known whether these effects generalize into other neurotransmitters or if they are specific to glutamate release. Further studies will be necessary to elucidate this question.

6.3 Final remarks

It was hypothesized in this study that *prenatal exposure to moderate levels of ethanol increased histamine H_3 receptor-effector coupling and H_3-mediated inhibition of glutamate release, contributing to a synaptic plasticity deficit in the dentate gyrus of rats.*

This hypothesis was supported by findings of increased agonist-stimulated $[^{35}S]$-GTPγS binding in the dentate gyrus, increased methimepip-inhibition of the probability of glutamate release *in-vitro* and by reversal of prenatal alcohol-induced LTP deficit *in-vivo*. In addition, this hypothesis was indirectly supported by methimepip-induced LTP deficit and by methimepip reduction of E-S coupling *in-vivo*, mimicking in control offspring some of the effects observed in prenatal alcohol exposed offspring. Finally, the hypothesis was neither supported nor refuted by lack of prenatal alcohol-induced changes in the baseline probability of glutamate release *in-vivo* and *in-vitro,* by the lack of further

LTP impairment in methimepip-injected prenatal alcohol exposed rats, and by the lack of LTP enhancement in ABT-239 control offspring.

Together, the results presented here suggest that prenatal alcohol-induced heightening of histamine H_3 receptor-mediated of glutamate release is one contributing factor in LTP impairment caused by moderate exposure to alcohol during development.

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Appendix A: Supplemental data

The effects of ABT-239 on electrically-evoked excitatory post-synaptic field potentials (EPSP) in brain slices containing the dorsal dentate gyrus are depicted in Figure A.1. Methods are similar to those employed in Chapter 5. Electrical stimulation of perforant path fibers in ACSF perfused slices resulted in typical EPSPs responses that increased with stimulus intensity (Figure A.1A). While controls exhibited mean maximal amplitude of 1.83 ± 0.23 mV, ABT-239 significantly increased the amplitude of EPSP responses to stimuli above 0.4 mA, reaching maximal amplitude of 2.66 ± 0.22 mV at 1.2 mA (Figure A.1B). In ACSF perfused slices, pairs of pulses collected at stimuli intensities sufficient to elicit 40-50% of maximal EPSP amplitude and at fixed interpulse-interval of 40 ms resulted in typical paired-pulse inhibition (Figure A.1C). ABT-239 increased the amplitude of the first pulse proportionally greater than that of the second pulse, thus significantly decreasing the paired-pulse ratio (Figure A.1D) (Varaschin et al., submitted).

Effects of ABT-239 on methimepip-induced LTP deficit in control offspring *in-vitro* are illustrated in Figure A.2 and A.3. Methods are similar to those employed in Chapter 5. As shown in Figure 5.4, methimepip reduced LTP in the dentate gyrus of control offspring. Concomitant application of ABT-239 (300 nM) successfully reversed methimepip-induced LTP deficit (Figure A.2, gray squares). Moreover, ABT-239 alone did not affect LTP when compared to ACSF-perfused control slices (Figure A.3).
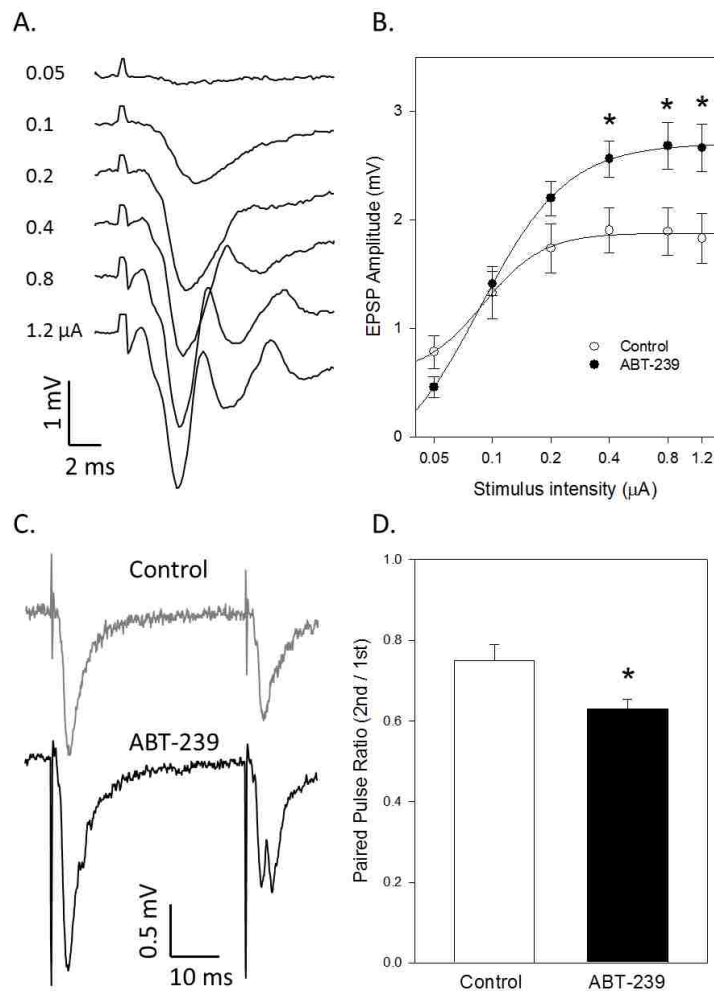
Figure A.1 Effect of ABT-239 on electrically-evoked EPSPs and paired-pulse ratio in dorsal dentate gyrus slices.

A.1A: Traces obtained from a slice perfused with ACSF after stimulation of perforant-path afferent fibers using electrical stimuli ranging from 0.05 - 1.2 mA. A.1B: Averaged EPSP responses for different stimulus intensities are illustrated as the mean ± the standard error of the mean. Open circles indicate control slices perfused with ACSF only (n = 10) and closed circles represent slices perfused with ACSF containing ABT-239 (300 nM, n = 9). Asterisks denote data significantly greater than control (Student's two-tailed t-test; t < -2.4, p < 0.05). A.1C: Traces obtained from electrically-evoked EPSPs by pairs of pulses with stimulus intensities sufficient to elicit 40-50% of maximal amplitude. Upper trace (light gray) illustrates the typical response of a slice perfused with ACSF. The lower trace (black) represents a response from a slice perfused with ACSF containing 300 nM of ABT-239. A.1D: Paired-pulse ratio, expressed as the amplitude of the second evoked EPSP divided by the amplitude of the first EPSP. Bars represent the mean ± standard error of the mean of slices perfused with ACSF only (open, n = 10) and ACSF containing ABT-239 (filled, n = 9). Asterisks denote data significantly less than control (Student's two-tailed t-test; t = 2.41, p = 0.029).
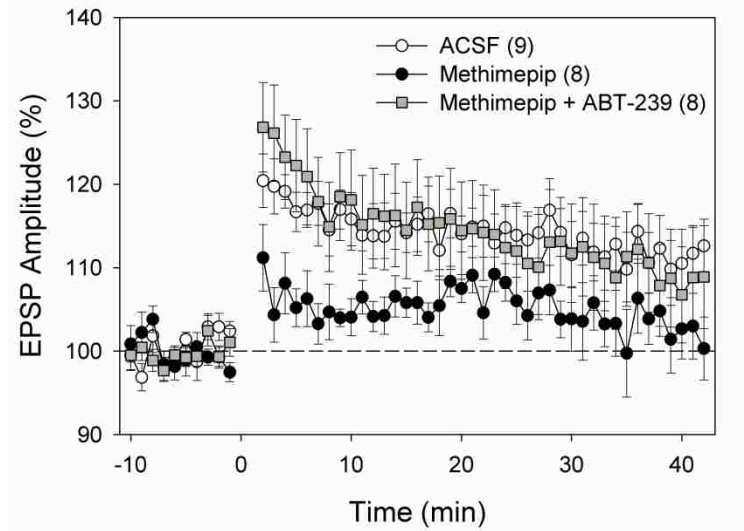
Figure A.2 ABT-239 reverses methimepip-induced LTP deficit in the dentate gyrus of control offspring *in-vitro*

LTP in control offspring. Data points represent the mean ± the standard error of the mean of EPSP amplitude normalized to baseline values in ACSF (open circles) or methimepip (filled cirlces). Concomitant application of methimepip and ABT-239 (gray squares) reversed methimepip-induced LTP deficit. Sample sizes are indicated in parenthesis.

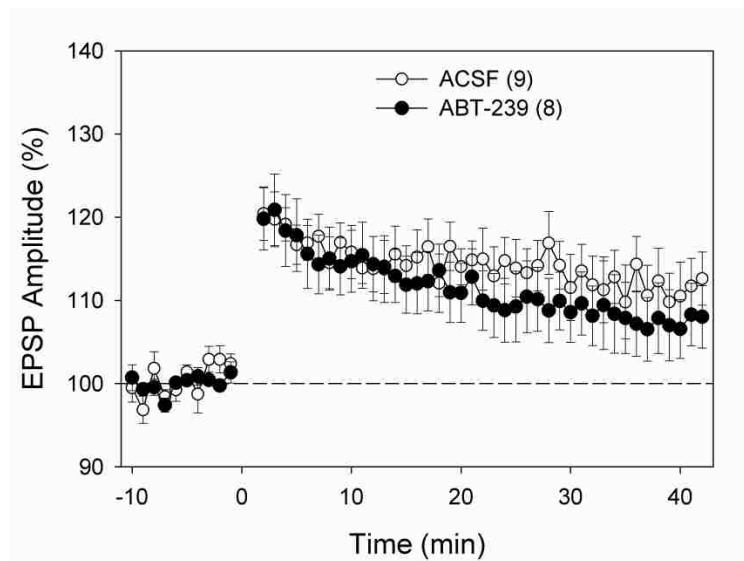**Figure A.3 ABT-239 alone does not potentiate LTP in the dentate gyrus of control offspring *in-vitro***

LTP in control offspring. Data points represent the mean ± the standard error of the mean of EPSP amplitude normalized to baseline values in ACSF (open circles) or ABT-239 (filled cirlces). Sample sizes are indicated in parenthesis.

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List of abbreviations

5HT	5-hidroxy-triptamine (serotonin)
A-349821	(4'-[3-(2R,5R-dimethylpyrrolidin-1-yl)propoxy]biphenyl-4-yl)morpholin-4-ylmethanone
ABT-239	[4-(2-{2-[(2R)-2-methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile]
ACh	Acetylcholine
ACSF	Artificial cerebral-spinal fluid
AMPA	2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid
ANOVA	Analysis of variance
BAC	Blood alcohol concentration
CA1	*Cornu Ammonis* area 1
CA3	*Cornu Ammonis* area 3
c-AMP	Cyclic-adenosine monophosphate
c-GMP	Cyclic-guanosine monophosphate
CREB	cAMP response element-binding
DAG	Diacylglycerol
DG	*Dentate gyrus*
EPSP	Excitatory postsynaptic potential
ERK	Extracellular-signal-regulated kinases
E-S coupling	fEPSP-to-PS coupling
ES_{40}	Effective stimulus intensity to elicit 40% of maximal response
FAS	Fetal alcohol syndrome
FASD	Fetal alcohol spectrum disorders
fEPSP	Field excitatory postsynaptic potential
GABA	γ-Aminobutyric acid

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GAP43	Growth associated protein 43
GPRC	G-protein coupled receptor
GTP	Guanosine-5'-triphosphate
GTPγS	Guanosine-5'-O-[gamma-thio]triphosphate
HDC	Histidine-decarboxylase
HFS	High frequency stimulus
I/O	Input/output
IP3	Inositol tri-phosphate
LTD	Long-term depression
LTP	Long-term potentiation
MAPK	Mitogen-activated protein (MAP) kinases
Methimepip	4-(1H-imidazol-4-ylmethyl)-1-methylpiperidine
mGluR	Metabotropic glutamate receptor
mRNA	Messenger ribonucleic acid
NMDA	N-methyl-D-aspartate
PAE	Prenatal alcohol exposed
PKA	Protein kinase A
PP	Perforant path
PPR	Paired-pulse ratio
PS	Population spike
RNA	Ribonucleic acid
SNARE	Soluble NSF (N-ethylmaleimide-sensitive factor) attachment protein receptor
TMN	Tuberomammillary nucleus
VGCC	Voltage-gated calcium channels

References

Abel EL, Jacobson S, Sherwin BT. 1983. In utero alcohol exposure: functional and structural brain damage. Neurobehavioral toxicology and teratology 5(3):363-6.

Acsady L, Kamondi A, Sik A, Freund T, Buzsaki G. 1998. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 18(9):3386-403.

Acsady L, Pascual M, Rocamora N, Soriano E, Freund TF. 2000. Nerve growth factor but not neurotrophin-3 is synthesized by hippocampal GABAergic neurons that project to the medial septum. Neuroscience 98(1):23-31.

Alfarez DN, Joels M, Krugers HJ. 2003. Chronic unpredictable stress impairs long-term potentiation in rat hippocampal CA1 area and dentate gyrus in vitro. The European journal of neuroscience 17(9):1928-34.

Allan AM, Weeber EJ, Savage DD, Caldwell KK. 1997. Effects of prenatal ethanol exposure on phospholipase C-beta 1 and phospholipase A2 in hippocampus and medial frontal cortex of adult rat offspring. Alcoholism, clinical and experimental research 21(8):1534-41.

Altman J, Bayer SA. 1990. Migration and distribution of two populations of hippocampal granule cell precursors during the perinatal and postnatal periods. The Journal of comparative neurology 301(3):365-81.

Alvarez EO, Banzan AM. 1996. Hippocampal histamine receptors: possible role on the mechanisms of memory in the rat, II. Journal of neural transmission 103(1-2):147-56.

Alvarez EO, Banzan AM. 2001. Functional regional distribution of histamine receptors in the rat hippocampus: modulation of learning of an active avoidance response. Journal of neural transmission 108(11):1249-61.

Alves-Rodrigues A, Timmerman H, Willems E, Lemstra S, Zuiderveld OP, Leurs R. 1998. Pharmacological characterisation of the histamine H3 receptor in the rat hippocampus. Brain research 788(1-2):179-86.

Amaral DG. 1978. A Golgi study of cell types in the hilar region of the hippocampus in the rat. The Journal of comparative neurology 182(4 Pt 2):851-914.

Amaral DG, Kurz J. 1985. An analysis of the origins of the cholinergic and noncholinergic septal projections to the hippocampal formation of the rat. The Journal of comparative neurology 240(1):37-59.

Amaral DG, Scharfman HE, Lavenex P. 2007. The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). Progress in brain research 163:3-22.

Andrews JS. 1996. Possible confounding influence of strain, age and gender on cognitive performance in rats. Brain Res Cogn Brain Res 3(3-4):251-67.

Aragon AS, Kalberg WO, Buckley D, Barela-Scott LM, Tabachnick BG, May PA. 2008. Neuropsychological study of FASD in a sample of American Indian children: processing simple versus complex information. Alcoholism, clinical and experimental research 32(12):2136-48.

Arrang JM, Garbarg M, Schwartz JC. 1983. Auto-inhibition of brain histamine release mediated by a novel class (H3) of histamine receptor. Nature 302(5911):832-7.

Arrang JM, Garbarg M, Schwartz JC. 1985. Autoregulation of histamine release in brain by presynaptic H3-receptors. Neuroscience 15(2):553-62.

Bacciottini L, Passani MB, Giovannelli L, Cangioli I, Mannaioni PF, Schunack W, Blandina P. 2002. Endogenous histamine in the medial septum-diagonal band complex increases the release of acetylcholine from the hippocampus: a dual-probe microdialysis study in the freely moving rat. Eur J Neurosci 15(10):1669-80.

Bakker RA. 2004. Histamine H3-receptor isoforms. Inflamm Res 53(10):509-16.

Barco A, Marie H. 2011. Genetic approaches to investigate the role of CREB in neuronal plasticity and memory. Molecular neurobiology 44(3):330-49.

Bardgett ME, Points M, Kleier J, Blankenship M, Griffith MS. 2010. The H3 antagonist, ciproxifan, alleviates the memory impairment but enhances the motor effects of MK-801 (dizocilpine) in rats. Neuropharmacology 59(6):492-502.

Barnes DE, Walker DW. 1981. Prenatal ethanol exposure permanently reduces the number of pyramidal neurons in rat hippocampus. Brain research 227(3):333-40.

Barnes SJ, Opitz T, Merkens M, Kelly T, von der Brelie C, Krueppel R, Beck H. 2010. Stable mossy fiber long-term potentiation requires calcium influx at the granule cell soma, protein synthesis, and microtubule-dependent axonal transport. The Journal of neuroscience : the official journal of the Society for Neuroscience 30(39):12996-3004.

Barnes WG, Hough LB. 2002. Membrane-bound histamine N-methyltransferase in mouse brain: possible role in the synaptic inactivation of neuronal histamine. Journal of neurochemistry 82(5):1262-71.

Barr AM, Hofmann CE, Phillips AG, Weinberg J, Honer WG. 2005. Prenatal ethanol exposure in rats decreases levels of complexin proteins in the frontal cortex. Alcoholism, clinical and experimental research 29(11):1915-20.

Bauer-Moffett C, Altman J. 1977. The effect of ethanol chronically administered to preweanling rats on cerebellar development: a morphological study. Brain research 119(2):249-68.

Bekkers JM. 1993. Enhancement by histamine of NMDA-mediated synaptic transmission in the hippocampus. Science 261(5117):104-6.

Benetti F, da Silveira CK, da Silva WC, Cammarota M, Izquierdo I. 2012. Histamine reverses a memory deficit induced in rats by early postnatal maternal deprivation. Neurobiology of learning and memory 97(1):54-8.

Betke KM, Wells CA, Hamm HE. 2012. GPCR mediated regulation of synaptic transmission. Progress in neurobiology 96(3):304-21.

Blandina P, Efoudebe M, Cenni G, Mannaioni P, Passani MB. 2004. Acetylcholine, histamine, and cognition: two sides of the same coin. Learning & memory 11(1):1-8.

Blandina P, Giorgetti M, Bartolini L, Cecchi M, Timmerman H, Leurs R, Pepeu G, Giovannini MG. 1996. Inhibition of cortical acetylcholine release and cognitive performance by histamine H3 receptor activation in rats. Br J Pharmacol 119(8):1656-64.

Bliss TV, Gardner-Medwin AR. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. J Physiol 232(2):357-74.

Bliss TV, Lomo T. 1973. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 232(2):331-56.

Blundon JA, Zakharenko SS. 2008. Dissecting the components of long-term potentiation. Neuroscientist 14(6):598-608.

Bongers G, Krueger KM, Miller TR, Baranowski JL, Estvander BR, Witte DG, Strakhova MI, van Meer P, Bakker RA, Cowart MD and others. 2007. An 80-amino acid deletion in the third intracellular loop of a naturally occurring human histamine H3 isoform confers pharmacological differences and constitutive activity. The Journal of pharmacology and experimental therapeutics 323(3):888-98.

Bonthius DJ, Goodlett CR, West JR. 1988. Blood alcohol concentration and severity of microencephaly in neonatal rats depend on the pattern of alcohol administration. Alcohol 5(3):209-14.

Bonthius DJ, West JR. 1988. Blood alcohol concentration and microencephaly: a dose-response study in the neonatal rat. Teratology 37(3):223-31.

Bowden JB, Abraham WC, Harris KM. 2011. Differential effects of strain, circadian cycle, and stimulation pattern on LTP and concurrent LTD in the dentate gyrus of freely moving rats. Hippocampus.

Brady ML, Allan AM, Caldwell KK. 2012. A limited access mouse model of prenatal alcohol exposure that produces long-lasting deficits in hippocampal-dependent learning and memory. Alcoholism, clinical and experimental research 36(3):457-66.

Breakwell NA, Rowan MJ, Anwyl R. 1996. Metabotropic glutamate receptor dependent EPSP and EPSP-spike potentiation in area CA1 of the submerged rat hippocampal slice. Journal of neurophysiology 76(5):3126-35.

Brioni JD, Esbenshade TA, Garrison TR, Bitner SR, Cowart MD. 2011. Discovery of histamine H3 antagonists for the treatment of cognitive disorders and Alzheimer's

disease. The Journal of pharmacology and experimental therapeutics 336(1):38-46.

Brown RE, Fedorov NB, Haas HL, Reymann KG. 1995. Histaminergic modulation of synaptic plasticity in area CA1 of rat hippocampal slices. Neuropharmacology 34(2):181-90.

Brown RE, Haas HL. 1999. On the mechanism of histaminergic inhibition of glutamate release in the rat dentate gyrus. Journal of Physiology-London 515(3):777-786.

Brown RE, Reymann KG. 1995. Metabotropic glutamate receptor agonists reduce paired-pulse depression in the dentate gyrus of the rat in vitro. Neurosci Lett 196(1-2):17-20.

Brown RE, Reymann KG. 1996. Histamine H3 receptor-mediated depression of synaptic transmission in the dentate gyrus of the rat in vitro. J Physiol 496 (Pt 1):175-84.

Carr JL, Agnihotri S, Keightley M. 2010. Sensory processing and adaptive behavior deficits of children across the fetal alcohol spectrum disorder continuum. Alcoholism, clinical and experimental research 34(6):1022-32.

Chaillan FA, Truchet B, Roman FS. 2008. Extracellular recordings of rodents in vivo: their contribution to integrative neuroscience. Journal of integrative neuroscience 7(2):287-313.

Chang M, Saito H, Abe K. 1997. Cimetidine inhibits the induction of long-term potentiation in the dentate gyrus of rats in vivo. Japanese journal of pharmacology 74(3):281-3.

Chang M, Saito H, Abe K. 1998. Histamine H3 receptor-mediated inhibition of excitatory synaptic transmission in the rat dentate gyrus in vivo. Jpn J Pharmacol 77(3):251-5.

Chen L, Xing T, Wang M, Miao Y, Tang M, Chen J, Li G, Ruan DY. 2011. Local infusion of ghrelin enhanced hippocampal synaptic plasticity and spatial memory through activation of phosphoinositide 3-kinase in the dentate gyrus of adult rats. The European journal of neuroscience 33(2):266-75.

Chen Z, Sugimoto Y, Kamei C. 1999. Effects of intracerebroventricular injection of alpha-fluoromethylhistidine on radial maze performance in rats. Pharmacology, biochemistry, and behavior 64(3):513-8.

Chernoff GF. 1977. The fetal alcohol syndrome in mice: an animal model. Teratology 15(3):223-9.

Choi IY, Allan AM, Cunningham LA. 2005. Moderate fetal alcohol exposure impairs the neurogenic response to an enriched environment in adult mice. Alcoholism, clinical and experimental research 29(11):2053-62.

Christie BR, Abraham WC. 1994. Differential regulation of paired-pulse plasticity following LTP in the dentate gyrus. Neuroreport 5(4):385-8.

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Clapham J, Kilpatrick GJ. 1992. Histamine H3 receptors modulate the release of [3H]-acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of H3 receptor subtypes. Br J Pharmacol 107(4):919-23.

Clelland CD, Choi M, Romberg C, Clemenson GD, Jr., Fragniere A, Tyers P, Jessberger S, Saksida LM, Barker RA, Gage FH and others. 2009. A functional role for adult hippocampal neurogenesis in spatial pattern separation. Science 325(5937):210-3.

Coles CD, Kable JA, Taddeo E. 2009. Math performance and behavior problems in children affected by prenatal alcohol exposure: intervention and follow-up. Journal of developmental and behavioral pediatrics : JDBP 30(1):7-15.

Coles CD, Lynch ME, Kable JA, Johnson KC, Goldstein FC. 2010. Verbal and nonverbal memory in adults prenatally exposed to alcohol. Alcoholism, clinical and experimental research 34(5):897-906.

Collingridge GL. 2003. The induction of N-methyl-D-aspartate receptor-dependent long-term potentiation. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 358(1432):635-41.

Costa ET, Savage DD, Valenzuela CF. 2000. A review of the effects of prenatal or early postnatal ethanol exposure on brain ligand-gated ion channels. Alcohol Clin Exp Res 24(5):706-15.

Cumming P, Gjedde A, Vincent S. 1994. Histamine H3 binding sites in rat brain: localization in the nucleus of the solitary tract. Brain research 641(2):198-202.

Dai H, Kaneko K, Kato H, Fujii S, Jing Y, Xu A, Sakurai E, Kato M, Okamura N, Kuramasu A and others. 2007. Selective cognitive dysfunction in mice lacking histamine H1 and H2 receptors. Neuroscience research 57(2):306-13.

Daoudal G, Hanada Y, Debanne D. 2002. Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. Proceedings of the National Academy of Sciences of the United States of America 99(22):14512-7.

de Almeida MA, Izquierdo I. 1986. Memory facilitation by histamine. Archives internationales de pharmacodynamie et de therapie 283(2):193-8.

Di Carlo G, Ghi P, Orsetti M. 2000. Effect of R-(-)-alpha-methylhistamine and thioperamide on in vivo release of norepinephrine in the rat hippocampus. Progress in neuro-psychopharmacology & biological psychiatry 24(2):275-84.

Doyere V, Srebro B, Laroche S. 1997. Heterosynaptic LTD and depotentiation in the medial perforant path of the dentate gyrus in the freely moving rat. Journal of neurophysiology 77(2):571-8.

Drutel G, Peitsaro N, Karlstedt K, Wieland K, Smit MJ, Timmerman H, Panula P, Leurs R. 2001. Identification of rat H3 receptor isoforms with different brain expression and signaling properties. Molecular pharmacology 59(1):1-8.

Ericson H, Blomqvist A, Kohler C. 1989. Brainstem afferents to the tuberomammillary nucleus in the rat brain with special reference to monoaminergic innervation. J Comp Neurol 281(2):169-92.

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Ericson H, Blomqvist A, Kohler C. 1991. Origin of Neuronal Inputs to the Region of the Tuberomammillary Nucleus of the Rat-Brain. Journal of Comparative Neurology 311(1):45-64.

Eriksson KS, Sergeeva O, Brown RE, Haas HL. 2001. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. J Neurosci 21(23):9273-9.

Eriksson KS, Stevens DR, Haas HL. 2000. Opposite modulation of histaminergic neurons by nociceptin and morphine. Neuropharmacology 39(12):2492-8.

Esbenshade TA, Browman KE, Bitner RS, Strakhova M, Cowart MD, Brioni JD. 2008. The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. Br J Pharmacol 154(6):1166-81.

Esbenshade TA, Fox GB, Krueger KM, Miller TR, Kang CH, Denny LI, Witte DG, Yao BB, Pan L, Wetter J and others. 2005. Pharmacological properties of ABT-239 [4-(2-{2-[(2R)-2-Methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile]: I. Potent and selective histamine H3 receptor antagonist with drug-like properties. J Pharmacol Exp Ther 313(1):165-75.

Farr KL, Montano CY, Paxton LL, Savage DD. 1988. Prenatal ethanol exposure decreases hippocampal 3H-vinylidene kainic acid binding in 45-day-old rats. Neurotoxicology and teratology 10(6):563-8.

Faucard R, Armand V, Heron A, Cochois V, Schwartz JC, Arrang JM. 2006. N-methyl-D-aspartate receptor antagonists enhance histamine neuron activity in rodent brain. J Neurochem 98(5):1487-96.

Ferguson AL, Stone TW. 2010. Glutamate-induced depression of EPSP-spike coupling in rat hippocampal CA1 neurons and modulation by adenosine receptors. The European journal of neuroscience 31(7):1208-18.

Filgueiras CC, Krahe TE, Medina AE. 2010. Phosphodiesterase type 1 inhibition improves learning in rats exposed to alcohol during the third trimester equivalent of human gestation. Neuroscience letters 473(3):202-7.

Flood JF, Uezu K, Morley JE. 1998. Effect of histamine H2 and H3 receptor modulation in the septum on post-training memory processing. Psychopharmacology 140(3):279-84.

Foley AG, Prendergast A, Barry C, Scully D, Upton N, Medhurst AD, Regan CM. 2009. H(3) Receptor Antagonism Enhances NCAM PSA-Mediated Plasticity and Improves Memory Consolidation in Odor Discrimination and Delayed Match-to-Position Paradigms. Neuropsychopharmacology.

Fox GB, Esbenshade TA, Pan JB, Radek RJ, Krueger KM, Yao BB, Browman KE, Buckley MJ, Ballard ME, Komater VA and others. 2005. Pharmacological properties of ABT-239 [4-(2-{2-[(2R)-2-Methylpyrrolidinyl]ethyl}-benzofuran-5-yl)benzonitrile]: II. Neurophysiological characterization and broad preclinical efficacy in cognition and schizophrenia of a potent and selective histamine H3 receptor antagonist. J Pharmacol Exp Ther 313(1):176-90.

118

Fox GB, Pan JB, Faghih R, Esbenshade TA, Lewis A, Bitner RS, Black LA, Bennani YL, Decker MW, Hancock AA. 2003. Identification of novel H3 receptor (H3R) antagonists with cognition enhancing properties in rats. Inflamm Res 52 Suppl 1:S31-2.

Frisch C, Hasenohrl RU, Huston JP. 1999. Memory improvement by post-trial injection of lidocaine into the tuberomammillary nucleus, the source of neuronal histamine. Neurobiology of learning and memory 72(2):69-77.

Fujimoto K, Mizuguchi H, Fukui H, Wada H. 1991. Presynaptic localization of histamine H3-receptors in rat brain. Biochem Biophys Res Commun 177(3):907-12.

Gaarskjaer FB. 1981. The hippocampal mossy fiber system of the rat studied with retrograde tracing techniques. Correlation between topographic organization and neurogenetic gradients. The Journal of comparative neurology 203(4):717-35.

Galici R, Boggs JD, Aluisio L, Fraser IC, Bonaventure P, Lord B, Lovenberg TW. 2009. JNJ-10181457, a selective non-imidazole histamine H(3) receptor antagonist, normalizes acetylcholine neurotransmission and has efficacy in translational rat models of cognition. Neuropharmacology 56(8):1131-7.

Galindo R, Frausto S, Wolff C, Caldwell KK, Perrone-Bizzozero NI, Savage DD. 2004. Prenatal ethanol exposure reduces mGluR5 receptor number and function in the dentate gyrus of adult offspring. Alcohol Clin Exp Res 28(10):1587-97.

Garduno-Torres B, Trevino M, Gutierrez R, Arias-Montano JA. 2007. Pre-synaptic histamine H3 receptors regulate glutamate, but not GABA release in rat thalamus. Neuropharmacology 52(2):527-35.

Giannoni P, Medhurst AD, Passani MB, Giovannini MG, Ballini C, Corte LD, Blandina P. 2010. Regional differential effects of the novel histamine H3 receptor antagonist 6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1H-3-benzazepin-7-yl)oxy]-N-methyl-3-pyridine carboxamide hydrochloride (GSK189254) on histamine release in the central nervous system of freely moving rats. The Journal of pharmacology and experimental therapeutics 332(1):164-72.

Gianoulakis C. 1990. Rats exposed prenatally to alcohol exhibit impairment in spatial navigation test. Behavioural brain research 36(3):217-28.

Girard TA, Xing HC, Ward GR, Wainwright PE. 2000. Early postnatal ethanol exposure has long-term effects on the performance of male rats in a delayed matching-to-place task in the Morris water maze. Alcoholism, clinical and experimental research 24(3):300-6.

Gonzalez-Burgos I, Feria-Velasco A. 2008. Serotonin/dopamine interaction in memory formation. Progress in brain research 172:603-23.

Greenbaum RL, Stevens SA, Nash K, Koren G, Rovet J. 2009. Social cognitive and emotion processing abilities of children with fetal alcohol spectrum disorders: a comparison with attention deficit hyperactivity disorder. Alcoholism, clinical and experimental research 33(10):1656-70.

Grover LM, Teyler TJ. 1990. Two components of long-term potentiation induced by different patterns of afferent activation. Nature 347(6292):477-9.

Guerri C. 1998. Neuroanatomical and neurophysiological mechanisms involved in central nervous system dysfunctions induced by prenatal alcohol exposure. Alcoholism, clinical and experimental research 22(2):304-12.

Haas HL. 1984. Histamine potentiates neuronal excitation by blocking a calcium-dependent potassium conductance. Agents Actions 14(3-4):534-7.

Haas HL, Sergeeva OA, Selbach O. 2008. Histamine in the nervous system. Physiol Rev 88(3):1183-241.

Hajos M, Siok CJ, Hoffmann WE, Li S, Kocsis B. 2008. Modulation of hippocampal theta oscillation by histamine H3 receptors. J Pharmacol Exp Ther 324(1):391-8.

Hamilton DA, Kodituwakku P, Sutherland RJ, Savage DD. 2003. Children with Fetal Alcohol Syndrome are impaired at place learning but not cued-navigation in a virtual Morris water task. Behav Brain Res 143(1):85-94.

Hanson JW, Jones KL, Smith DW. 1976. Fetal alcohol syndrome. Experience with 41 patients. JAMA : the journal of the American Medical Association 235(14):1458-60.

Helm KA, Haberman RP, Dean SL, Hoyt EC, Melcher T, Lund PK, Gallagher M. 2005. GABAB receptor antagonist SGS742 improves spatial memory and reduces protein binding to the cAMP response element (CRE) in the hippocampus. Neuropharmacology 48(7):956-64.

Henderson GI, Schenker S. 1977. The effect of maternal alcohol consumption on the viability and visceral development of the newborn rat. Research communications in chemical pathology and pharmacology 16(1):15-32.

Hirst WD, Stean TO, Rogers DC, Sunter D, Pugh P, Moss SF, Bromidge SM, Riley G, Smith DR, Bartlett S and others. 2006. SB-399885 is a potent, selective 5-HT6 receptor antagonist with cognitive enhancing properties in aged rat water maze and novel object recognition models. European journal of pharmacology 553(1-3):109-19.

Horner WE, Johnson DE, Schmidt AW, Rollema H. 2007. Methylphenidate and atomoxetine increase histamine release in rat prefrontal cortex. European journal of pharmacology 558(1-3):96-7.

Huang CC, Hsu KS. 2004. Local protein synthesis and GABAB receptors regulate the reversibility of long-term potentiation at murine hippocampal mossy fibre-CA3 synapses. The Journal of physiology 561(Pt 1):91-108.

Hunt PS, Jacobson SE, Torok EJ. 2009. Deficits in trace fear conditioning in a rat model of fetal alcohol exposure: dose-response and timing effects. Alcohol 43(6):465-74.

Jacobson JL, Jacobson SW, Sokol RJ, Ager JW, Jr. 1998. Relation of maternal age and pattern of pregnancy drinking to functionally significant cognitive deficit in infancy. Alcohol Clin Exp Res 22(2):345-51.

Jedlicka P, Hoon M, Papadopoulos T, Vlachos A, Winkels R, Poulopoulos A, Betz H, Deller T, Brose N, Varoqueaux F and others. 2011. Increased dentate gyrus excitability in neuroligin-2-deficient mice in vivo. Cerebral cortex 21(2):357-67.

Johnstone VP, Raymond CR. 2011. A protein synthesis and nitric oxide-dependent presynaptic enhancement in persistent forms of long-term potentiation. Learning & memory 18(10):625-33.

Jones KL, Smith DW. 1973. Recognition of the fetal alcohol syndrome in early infancy. Lancet 302(7836):999-1001.

Jones KL, Smith DW, Ulleland CN, Streissguth P. 1973. Pattern of malformation in offspring of chronic alcoholic mothers. Lancet 1(7815):1267-71.

Kamei C, Okumura Y, Tasaka K. 1993. Influence of histamine depletion on learning and memory recollection in rats. Psychopharmacology (Berl) 111(3):376-82.

Kaminski M, Rumeau-Rouquette C, Schwartz D. 1976. [Alcohol consumption among pregnant women and outcome of pregnancy (author's transl)]. Revue d'epidemiologie, medecine sociale et sante publique 24(1):27-40.

Karlstedt K, Senkas A, Ahman M, Panula P. 2001. Regional expression of the histamine H(2) receptor in adult and developing rat brain. Neuroscience 102(1):201-8.

Kelly SJ, Leggett DC, Cronise K. 2009. Sexually dimorphic effects of alcohol exposure during development on the processing of social cues. Alcohol and alcoholism 44(6):555-60.

Kim CK, Kalynchuk LE, Kornecook TJ, Mumby DG, Dadgar NA, Pinel JP, Weinberg J. 1997. Object-recognition and spatial learning and memory in rats prenatally exposed to ethanol. Behavioral neuroscience 111(5):985-95.

Kim WR, Sun W. 2011. Programmed cell death during postnatal development of the rodent nervous system. Development, growth & differentiation 53(2):225-35.

Kitbunnadaj R, Hashimoto T, Poli E, Zuiderveld OP, Menozzi A, Hidaka R, de Esch IJ, Bakker RA, Menge WM, Yamatodani A and others. 2005. N-substituted piperidinyl alkyl imidazoles: discovery of methimepip as a potent and selective histamine H3 receptor agonist. J Med Chem 48(6):2100-7.

Kodituwakku PW. 2009. Neurocognitive profile in children with fetal alcohol spectrum disorders. Dev Disabil Res Rev 15(3):218-24.

Kodituwakku PW, Kodituwakku EL. 2011. From research to practice: an integrative framework for the development of interventions for children with fetal alcohol spectrum disorders. Neuropsychology review 21(2):204-23.

Kohler C. 1985. Intrinsic projections of the retrohippocampal region in the rat brain. I. The subicular complex. The Journal of comparative neurology 236(4):504-22.

Kohler C, Hall H, Ogren SO, Gawell L. 1985. Specific in vitro and in vivo binding of 3H-raclopride. A potent substituted benzamide drug with high affinity for dopamine D-2 receptors in the rat brain. Biochemical pharmacology 34(13):2251-9.

Lebel C, Rasmussen C, Wyper K, Walker L, Andrew G, Yager J, Beaulieu C. 2008. Brain diffusion abnormalities in children with fetal alcohol spectrum disorder. Alcoholism, clinical and experimental research 32(10):1732-40.

Lee I, Solivan F. 2010. Dentate gyrus is necessary for disambiguating similar object-place representations. Learning & memory 17(5):252-8.

Lemoine P, Haronsseau H, Borteryu JP, Menuet JC. 1968. Les enfants de parents alcooliques: anomalies observees a propos de 127 cas. Ouest Med(25):476-482.

Leutgeb JK, Leutgeb S, Moser MB, Moser EI. 2007. Pattern separation in the dentate gyrus and CA3 of the hippocampus. Science 315(5814):961-6.

Liu L, Zhang S, Zhu Y, Fu Q, Gong Y, Ohtsu H, Luo J, Wei E, Chen Z. 2007. Improved learning and memory of contextual fear conditioning and hippocampal CA1 long-term potentiation in histidine decarboxylase knock-out mice. Hippocampus 17(8):634-41.

Loughlin SE, Foote SL, Bloom FE. 1986. Efferent projections of nucleus locus coeruleus: topographic organization of cells of origin demonstrated by three-dimensional reconstruction. Neuroscience 18(2):291-306.

Lubke J, Deller T, Frotscher M. 1997. Septal innervation of mossy cells in the hilus of the rat dentate gyrus: an anterograde tracing and intracellular labeling study. Experimental brain research. Experimentelle Hirnforschung. Experimentation cerebrale 114(3):423-32.

Lundquist F, Fugmann U, Klaning E, Rasmussen H. 1959. The metabolism of acetaldehyde in mammalian tissues: reactions in rat-liver suspensions under anaerobic conditions. Biochem J 72:409-19.

Luo L, Chen WH, Wang M, Zhu DM, She JQ, Ruan DY. 2008. Modulation of long-term potentiation by individual subtypes of muscarinic acetylcholine receptor in the rat dentate gyrus. Hippocampus 18(10):989-95.

Luo T, Leung LS. 2010. Endogenous histamine facilitates long-term potentiation in the hippocampus during walking. The Journal of neuroscience : the official journal of the Society for Neuroscience 30(23):7845-52.

Magloczky Z, Acsady L, Freund TF. 1994. Principal cells are the postsynaptic targets of supramammillary afferents in the hippocampus of the rat. Hippocampus 4(3):322-34.

Maier SE, West JR. 2001. Drinking patterns and alcohol-related birth defects. Alcohol research & health : the journal of the National Institute on Alcohol Abuse and Alcoholism 25(3):168-74.

Malenka RC, Bear MF. 2004. LTP and LTD: an embarrassment of riches. Neuron 44(1):5-21.

Malik R, Chattarji S. 2012. Enhanced intrinsic excitability and EPSP-spike coupling accompany enriched environment-induced facilitation of LTP in hippocampal CA1 pyramidal neurons. Journal of neurophysiology 107(5):1366-78.

Malisza KL, Allman AA, Shiloff D, Jakobson L, Longstaffe S, Chudley AE. 2005. Evaluation of spatial working memory function in children and adults with fetal alcohol spectrum disorders: a functional magnetic resonance imaging study. Pediatric research 58(6):1150-7.

Mameli M, Zamudio PA, Carta M, Valenzuela CF. 2005. Developmentally regulated actions of alcohol on hippocampal glutamatergic transmission. The Journal of neuroscience : the official journal of the Society for Neuroscience 25(35):8027-36.

Manahan-Vaughan D, Reymann KG, Brown RE. 1998. In vivo electrophysiological investigations into the role of histamine in the dentate gyrus of the rat. Neuroscience 84(3):783-90.

Martin D, Savage DD, Swartzwelder HS. 1992. Effects of prenatal ethanol exposure on hippocampal ionotropic-quisqualate and kainate receptors. Alcoholism, clinical and experimental research 16(4):816-21.

May PA, Gossage JP, Kalberg WO, Robinson LK, Buckley D, Manning M, Hoyme HE. 2009. Prevalence and epidemiologic characteristics of FASD from various research methods with an emphasis on recent in-school studies. Developmental disabilities research reviews 15(3):176-92.

Medhurst AD, Atkins AR, Beresford IJ, Brackenborough K, Briggs MA, Calver AR, Cilia J, Cluderay JE, Crook B, Davis JB and others. 2007a. GSK189254, a novel H3 receptor antagonist that binds to histamine H3 receptors in Alzheimer's disease brain and improves cognitive performance in preclinical models. The Journal of pharmacology and experimental therapeutics 321(3):1032-45.

Medhurst AD, Briggs MA, Bruton G, Calver AR, Chessell I, Crook B, Davis JB, Davis RP, Foley AG, Heslop T and others. 2007b. Structurally novel histamine H3 receptor antagonists GSK207040 and GSK334429 improve scopolamine-induced memory impairment and capsaicin-induced secondary allodynia in rats. Biochem Pharmacol 73(8):1182-94.

Medina AE. 2011. Fetal alcohol spectrum disorders and abnormal neuronal plasticity. The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry 17(3):274-87.

Medina AE, Krahe TE, Ramoa AS. 2006. Restoration of neuronal plasticity by a phosphodiesterase type 1 inhibitor in a model of fetal alcohol exposure. J Neurosci 26(3):1057-60.

Miki T, Yokoyama T, Sumitani K, Kusaka T, Warita K, Matsumoto Y, Wang ZY, Wilce PA, Bedi KS, Itoh S and others. 2008. Ethanol neurotoxicity and dentate gyrus development. Congenital anomalies 48(3):110-7.

Miller TR, Baranowski JL, Estvander BR, Witte DG, Carr TL, Manelli AM, Krueger KM, Cowart MD, Brioni JD, Esbenshade TA. 2008. A robust and high-capacity [(35)S]GTPgammaS binding assay for determining antagonist and inverse agonist pharmacological parameters of histamine H(3) receptor ligands. Assay Drug Dev Technol 6(3):339-49.

Mochizuki T, Yamatodani A, Okakura K, Horii A, Inagaki N, Wada H. 1992. Circadian rhythm of histamine release from the hypothalamus of freely moving rats. Physiology & behavior 51(2):391-4.

Molina-Hernandez A, Nunez A, Sierra JJ, Arias-Montano JA. 2001. Histamine H3 receptor activation inhibits glutamate release from rat striatal synaptosomes. Neuropharmacology 41(8):928-34.

Monk BR, Leslie FM, Thomas JD. 2012. The effects of perinatal choline supplementation on hippocampal cholinergic development in rats exposed to alcohol during the brain growth spurt. Hippocampus.

Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-Lacombe J, Stark H, Schunack W, Ganellin CR, Schwartz JC, Arrang JM. 2000. High constitutive activity of native H3 receptors regulates histamine neurons in brain. Nature 408(6814):860-4.

Morisset S, Sasse A, Gbahou F, Heron A, Ligneau X, Tardivel-Lacombe J, Schwartz JC, Arrang JM. 2001. The rat H3 receptor: gene organization and multiple isoforms. Biochemical and biophysical research communications 280(1):75-80.

Mulvihill JJ, Klimas JT, Stokes DC, Risemberg HM. 1976. Fetal alcohol syndrome: seven new cases. American journal of obstetrics and gynecology 125(7):937-41.

Naie K, Manahan-Vaughan D. 2005. Pharmacological antagonism of metabotropic glutamate receptor 1 regulates long-term potentiation and spatial reference memory in the dentate gyrus of freely moving rats via N-methyl-D-aspartate and metabotropic glutamate receptor-dependent mechanisms. Eur J Neurosci 21(2):411-21.

Nardelli A, Lebel C, Rasmussen C, Andrew G, Beaulieu C. 2011. Extensive deep gray matter volume reductions in children and adolescents with fetal alcohol spectrum disorders. Alcoholism, clinical and experimental research 35(8):1404-17.

Niccols A. 2007. Fetal alcohol syndrome and the developing socio-emotional brain. Brain Cogn 65(1):135-42.

Nio E, Kogure K, Yae T, Onodera H. 1991. The effects of maternal ethanol exposure on neurotransmission and second messenger systems: a quantitative autoradiographic study in the rat brain. Brain research. Developmental brain research 62(1):51-60.

O'Leary DM, O'Connor JJ. 1997. Potentiation of synaptic transmission in the rat dentate gyrus in vitro by (S)-3,5-dihydroxyphenylglycine ((S)-DHPG). Neuroscience letters 229(1):29-32.

Oishi Y, Huang ZL, Fredholm BB, Urade Y, Hayaishi O. 2008. Adenosine in the tuberomammillary nucleus inhibits the histaminergic system via A1 receptors and promotes non-rapid eye movement sleep. Proc Natl Acad Sci U S A 105(50):19992-7.

Olianas M, Oliver AP, Neff NH. 1984. Correlation between histamine-induced neuronal excitability and activation of adenylate cyclase in the guinea pig hippocampus. Neuropharmacology 23(9):1071-4.

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Ondrejcak T, Wang Q, Kew JN, Virley DJ, Upton N, Anwyl R, Rowan MJ. 2012. Activation of alpha7 nicotinic acetylcholine receptors persistently enhances hippocampal synaptic transmission and prevents Ass-mediated inhibition of LTP in the rat hippocampus. European journal of pharmacology 677(1-3):63-70.

Osorio-Espinoza A, Alatorre A, Ramos-Jimenez J, Garduno-Torres B, Garcia-Ramirez M, Querejeta E, Arias-Montano JA. 2011a. Pre-synaptic histamine H receptors modulate glutamatergic transmission in rat globus pallidus. Neuroscience 176:20-31.

Osorio-Espinoza A, Alatorre A, Ramos-Jimenez J, Garduno-Torres B, Garcia-Ramirez M, Querejeta E, Arias-Montano JA. 2011b. Pre-synaptic histamine H(3) receptors modulate glutamatergic transmission in rat globus pallidus. Neuroscience 176:20-31.

Palacios JM, Garbarg M, Barbin G, Schwartz JC. 1978. Pharmacological characterization of histamine receptors mediating the stimulation of cyclic AMP accumulation in slices from guinea-pig hippocampus. Molecular pharmacology 14(6):971-82.

Panula P, Yang HY, Costa E. 1984. Histamine-containing neurons in the rat hypothalamus. Proc Natl Acad Sci U S A 81(8):2572-6.

Passani MB, Blandina P. 1998. Cognitive implications for H3 and 5-HT3 receptor modulation of cortical cholinergic function: a parallel story. Methods and findings in experimental and clinical pharmacology 20(8):725-33.

Passani MB, Blandina P. 2011. Histamine receptors in the CNS as targets for therapeutic intervention. Trends in pharmacological sciences 32(4):242-9.

Paxinos G, Watson C. 1998. The rat brain in stereotaxic coordinates. San Diego: Academic Press.

Pei J, Job J, Kully-Martens K, Rasmussen C. 2011. Executive function and memory in children with Fetal Alcohol Spectrum Disorder. Child neuropsychology : a journal on normal and abnormal development in childhood and adolescence 17(3):290-309.

Pelkey KA, Lavezzari G, Racca C, Roche KW, McBain CJ. 2005. mGluR7 is a metaplastic switch controlling bidirectional plasticity of feedforward inhibition. Neuron 46(1):89-102.

Perrone-Bizzozero NI, Isaacson TV, Keidan GM, Eriqat C, Meiri KF, Savage DD, Allan AM. 1998. Prenatal ethanol exposure decreases GAP-43 phosphorylation and protein kinase C activity in the hippocampus of adult rat offspring. J Neurochem 71(5):2104-11.

Pierog S, Chandavasu O, Wexler I. 1977. Withdrawal symptoms in infants with the fetal alcohol syndrome. The Journal of pediatrics 90(4):630-3.

Pillot C, Heron A, Cochois V, Tardivel-Lacombe J, Ligneau X, Schwartz JC, Arrang JM. 2002. A detailed mapping of the histamine H(3) receptor and its gene transcripts in rat brain. Neuroscience 114(1):173-93.

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Prast H, Dietl H, Philippu A. 1992. Pulsatile release of histamine in the hypothalamus of conscious rats. Journal of the autonomic nervous system 39(2):105-10.

Prast H, Fischer H, Philippu A. 1994. Release of acetylcholine in the ventral striatum is influenced by histamine receptors. Agents and actions 41 Spec No:C85-6.

Ran I, Laplante I, Bourgeois C, Pepin J, Lacaille P, Costa-Mattioli M, Pelletier J, Sonenberg N, Lacaille JC. 2009. Persistent transcription- and translation-dependent long-term potentiation induced by mGluR1 in hippocampal interneurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 29(17):5605-15.

Ran I, Laplante I, Lacaille JC. 2012. CREB-dependent transcriptional control and quantal changes in persistent long-term potentiation in hippocampal interneurons. The Journal of neuroscience : the official journal of the Society for Neuroscience 32(18):6335-50.

Randall CL, Taylor J, Walker DW. 1977. Ethanol-induced malformations in mice. Alcoholism, clinical and experimental research 1(3):219-24.

Randall CL, Taylor WJ. 1979. Prenatal ethanol exposure in mice: teratogenic effects. Teratology 19(3):305-11.

Rasmussen C, Pei J, Manji S, Loomes C, Andrew G. 2009. Memory strategy development in children with foetal alcohol spectrum disorders. Developmental neurorehabilitation 12(4):207-14.

Rasmussen DD, Boldt BM, Bryant CA, Mitton DR, Larsen SA, Wilkinson CW. 2000. Chronic daily ethanol and withdrawal: 1. Long-term changes in the hypothalamo-pituitary-adrenal axis. Alcohol Clin Exp Res 24(12):1836-49.

Raymond CR. 2007. LTP forms 1, 2 and 3: different mechanisms for the "long" in long-term potentiation. Trends Neurosci 30(4):167-75.

Raymond CR, Thompson VL, Tate WP, Abraham WC. 2000. Metabotropic glutamate receptors trigger homosynaptic protein synthesis to prolong long-term potentiation. J Neurosci 20(3):969-76.

Redila VA, Olson AK, Swann SE, Mohades G, Webber AJ, Weinberg J, Christie BR. 2006. Hippocampal cell proliferation is reduced following prenatal ethanol exposure but can be rescued with voluntary exercise. Hippocampus 16(3):305-11.

Reyes E, Wolfe J, Savage DD. 1989. The effects of prenatal alcohol exposure on radial arm maze performance in adult rats. Physiology & behavior 46(1):45-8.

Reymann KG, Frey JU. 2007. The late maintenance of hippocampal LTP: requirements, phases, 'synaptic tagging', 'late-associativity' and implications. Neuropharmacology 52(1):24-40.

Ribak CE, Seress L. 1983. Five types of basket cell in the hippocampal dentate gyrus: a combined Golgi and electron microscopic study. Journal of neurocytology 12(4):577-97.

Ribak CE, Vaughn JE, Saito K. 1978. Immunocytochemical localization of glutamic acid decarboxylase in neuronal somata following colchicine inhibition of axonal transport. Brain research 140(2):315-32.

Richter-Levin G, Segal M, Sara S. 1991. An alpha 2 antagonist, idazoxan, enhances EPSP-spike coupling in the rat dentate gyrus. Brain research 540(1-2):291-4.

Riley EP, McGee CL. 2005. Fetal alcohol spectrum disorders: an overview with emphasis on changes in brain and behavior. Exp Biol Med (Maywood) 230(6):357-65.

Roussinov K, Yonkov D. 1976. Effect of imidazole, papaverine and histamine on learning and memory in albino rats. Acta physiologica et pharmacologica Bulgarica 2(2):49-57.

Ruat M, Traiffort E, Arrang JM, Leurs R, Schwartz JC. 1991. Cloning and tissue expression of a rat histamine H2-receptor gene. Biochem Biophys Res Commun 179(3):1470-8.

Sakai N, Sakurai E, Yanai K, Mirua Y, Watanabe T. 1998. Depletion of brain histamine induced by alpha-fluoromethylhistidine enhances radial maze performance in rats with modulation of brain amino acid levels. Life sciences 62(11):989-94.

Samudio-Ruiz SL, Allan AM, Sheema S, Caldwell KK. 2010. Hippocampal N-methyl-D-aspartate receptor subunit expression profiles in a mouse model of prenatal alcohol exposure. Alcoholism, clinical and experimental research 34(2):342-53.

Samudio-Ruiz SL, Allan AM, Valenzuela CF, Perrone-Bizzozero NI, Caldwell KK. 2009. Prenatal ethanol exposure persistently impairs NMDA receptor-dependent activation of extracellular signal-regulated kinase in the mouse dentate gyrus. J Neurochem 109(5):1311-23.

Savage DD, Becher M, de la Torre AJ, Sutherland RJ. 2002. Dose-dependent effects of prenatal ethanol exposure on synaptic plasticity and learning in mature offspring. Alcoholism, clinical and experimental research 26(11):1752-8.

Savage DD, Cruz LL, Duran LM, Paxton LL. 1998. Prenatal ethanol exposure diminishes activity-dependent potentiation of amino acid neurotransmitter release in adult rat offspring. Alcohol Clin Exp Res 22(8):1771-7.

Savage DD, Montano CY, Otero MA, Paxton LL. 1991. Prenatal ethanol exposure decreases hippocampal NMDA-sensitive [3H]-glutamate binding site density in 45-day-old rats. Alcohol 8(3):193-201.

Savage DD, Montano CY, Paxton LL, Kasarskis EJ. 1989. Prenatal ethanol exposure decreases hippocampal mossy fiber zinc in 45-day-old rats. Alcoholism, clinical and experimental research 13(4):588-93.

Savage DD, Rosenberg MJ, Wolff CR, Akers KG, El-Emawy A, Staples MC, Varaschin RK, Wright CA, Seidel JL, Caldwell KK and others. 2010. Effects of a novel cognition-enhancing agent on fetal ethanol-induced learning deficits. Alcoholism, clinical and experimental research 34(10):1793-802.

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Scharfman HE. 1995. Electrophysiological evidence that dentate hilar mossy cells are excitatory and innervate both granule cells and interneurons. Journal of neurophysiology 74(1):179-94.

Scharfman HE. 2007. The CA3 "backprojection" to the dentate gyrus. Progress in brain research 163:627-37.

Schlicker E, Betz R, Gothert M. 1988. Histamine H3 receptor-mediated inhibition of serotonin release in the rat brain cortex. Naunyn Schmiedebergs Arch Pharmacol 337(5):588-90.

Schlicker E, Fink K, Detzner M, Gothert M. 1993. Histamine inhibits dopamine release in the mouse striatum via presynaptic H3 receptors. J Neural Transm Gen Sect 93(1):1-10.

Schlicker E, Fink K, Hinterthaner M, Gothert M. 1989. Inhibition of noradrenaline release in the rat brain cortex via presynaptic H3 receptors. Naunyn Schmiedebergs Arch Pharmacol 340(6):633-8.

Scott Bitner R. 2012. Cyclic AMP response element-binding protein (CREB) phosphorylation: a mechanistic marker in the development of memory enhancing Alzheimer's disease therapeutics. Biochemical pharmacology 83(6):705-14.

Shen F, Smith JA, Chang R, Bourdet DL, Tsuruda PR, Obedencio GP, Beattie DT. 2011. 5-HT(4) receptor agonist mediated enhancement of cognitive function in vivo and amyloid precursor protein processing in vitro: A pharmacodynamic and pharmacokinetic assessment. Neuropharmacology 61(1-2):69-79.

Sherin JE, Elmquist JK, Torrealba F, Saper CB. 1998. Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. J Neurosci 18(12):4705-21.

Sloviter RS. 1991. Feedforward and feedback inhibition of hippocampal principal cell activity evoked by perforant path stimulation: GABA-mediated mechanisms that regulate excitability in vivo. Hippocampus 1(1):31-40.

Smith DE, Davies DL. 1990. Effect of perinatal administration of ethanol on the CA1 pyramidal cell of the hippocampus and Purkinje cell of the cerebellum: an ultrastructural survey. Journal of neurocytology 19(5):708-17.

Sorensen SD, Macek TA, Cai Z, Saugstad JA, Conn PJ. 2002. Dissociation of protein kinase-mediated regulation of metabotropic glutamate receptor 7 (mGluR7) interactions with calmodulin and regulation of mGluR7 function. Molecular pharmacology 61(6):1303-12.

Soriano E, Frotscher M. 1994. Mossy cells of the rat fascia dentata are glutamate-immunoreactive. Hippocampus 4(1):65-9.

Staff NP, Spruston N. 2003. Intracellular correlate of EPSP-spike potentiation in CA1 pyramidal neurons is controlled by GABAergic modulation. Hippocampus 13(7):801-5.

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Steinbusch HW. 1991. Distribution of histaminergic neurons and fibers in rat brain. Comparison with noradrenergic and serotonergic innervation of the vestibular system. Acta Otolaryngol Suppl 479:12-23.

Stevens DR, Eriksson KS, Brown RE, Haas HL. 2001. The mechanism of spontaneous firing in histamine neurons. Behav Brain Res 124(2):105-12.

Strakhova MI, Nikkel AL, Manelli AM, Hsieh GC, Esbenshade TA, Brioni JD, Bitner RS. 2009. Localization of histamine H4 receptors in the central nervous system of human and rat. Brain research 1250:41-8.

Streissguth AP. 1976. Psychologic handicaps in children with the fetal alcohol syndrome. Annals of the New York Academy of Sciences 273:140-5.

Streissguth AP, Barr HM, Sampson PD. 1990. Moderate prenatal alcohol exposure: effects on child IQ and learning problems at age 7 1/2 years. Alcohol Clin Exp Res 14(5):662-9.

Streissguth AP, Barr HM, Sampson PD, Bookstein FL. 1994. Prenatal alcohol and offspring development: the first fourteen years. Drug Alcohol Depend 36(2):89-99.

Sutherland RJ, McDonald RJ, Savage DD. 1997. Prenatal exposure to moderate levels of ethanol can have long-lasting effects on hippocampal synaptic plasticity in adult offspring. Hippocampus 7(2):232-8.

Sutherland RJ, McDonald RJ, Savage DD. 2000. Prenatal exposure to moderate levels of ethanol can have long-lasting effects on learning and memory of adult rat offspring. Psychobiology(28):532-539.

Swanson LW. 1982. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain research bulletin 9(1-6):321-53.

Takagi H, Morishima Y, Matsuyama T, Hayashi H, Watanabe T, Wada H. 1986. Histaminergic axons in the neostriatum and cerebral cortex of the rat: a correlated light and electron microscopic immunocytochemical study using histidine decarboxylase as a marker. Brain Res 364(1):114-23.

Takahashi K, Lin JS, Sakai K. 2006. Neuronal activity of histaminergic tuberomammillary neurons during wake-sleep states in the mouse. J Neurosci 26(40):10292-8.

Takeda N, Inagaki S, Taguchi Y, Tohyama M, Watanabe T, Wada H. 1984. Origins of histamine-containing fibers in the cerebral cortex of rats studied by immunohistochemistry with histidine decarboxylase as a marker and transection. Brain Res 323(1):55-63.

Taube JS, Schwartzkroin PA. 1988. Mechanisms of long-term potentiation: EPSP/spike dissociation, intradendritic recordings, and glutamate sensitivity. J Neurosci 8(5):1632-44.

Testa M, Quigley BM, Eiden RD. 2003. The effects of prenatal alcohol exposure on infant mental development: a meta-analytical review. Alcohol and alcoholism 38(4):295-304.

Thomas JD, Idrus NM, Monk BR, Dominguez HD. 2010. Prenatal choline supplementation mitigates behavioral alterations associated with prenatal alcohol exposure in rats. Birth defects research. Part A, Clinical and molecular teratology 88(10):827-37.

Threlfell S, Cragg SJ, Kallo I, Turi GF, Coen CW, Greenfield SA. 2004. Histamine H3 receptors inhibit serotonin release in substantia nigra pars reticulata. The Journal of neuroscience : the official journal of the Society for Neuroscience 24(40):8704-10.

Titterness AK, Christie BR. 2012. Prenatal ethanol exposure enhances NMDAR-dependent long-term potentiation in the adolescent female dentate gyrus. Hippocampus 22(1):69-81.

Tully K, Bolshakov VY. 2010. Emotional enhancement of memory: how norepinephrine enables synaptic plasticity. Molecular brain 3:15.

Uecker A, Nadel L. 1998. Spatial but not object memory impairments in children with fetal alcohol syndrome. American journal of mental retardation : AJMR 103(1):12-8.

Vaglenova J, Pandiella N, Wijayawardhane N, Vaithianathan T, Birru S, Breese C, Suppiramaniam V, Randal C. 2008. Aniracetam reversed learning and memory deficits following prenatal ethanol exposure by modulating functions of synaptic AMPA receptors. Neuropsychopharmacology 33(5):1071-83.

Valenzuela CF, Morton RA, Diaz MR, Topper L. 2012. Does moderate drinking harm the fetal brain? Insights from animal models. Trends in neurosciences 35(5):284-92.

Varaschin RK, Akers KG, Rosenberg MJ, Hamilton DA, Savage DD. 2010. Effects of the cognition-enhancing agent ABT-239 on fetal ethanol-induced deficits in dentate gyrus synaptic plasticity. The Journal of pharmacology and experimental therapeutics 334(1):191-8.

Vertes RP, Fortin WJ, Crane AM. 1999. Projections of the median raphe nucleus in the rat. The Journal of comparative neurology 407(4):555-82.

Wang L, Gantz I, DelValle J. 1996. Histamine H2 receptor activates adenylate cyclase and PLC via separate GTP-dependent pathways. The American journal of physiology 271(4 Pt 1):G613-20.

Wang Y, Sherwood JL, Lodge D. 2006. The alpha4beta2 nicotinic acetylcholine receptor agonist TC-2559 impairs long-term potentiation in the dentate gyrus in vivo. Neuroscience letters 406(3):183-8.

Watanabe T, Taguchi Y, Shiosaka S, Tanaka J, Kubota H, Terano Y, Tohyama M, Wada H. 1984. Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. Brain research 295(1):13-25.

Weeber EJ, Savage DD, Sutherland RJ, Caldwell KK. 2001a. Fear conditioning-induced alterations of phospholipase C-beta1a protein level and enzyme activity in rat hippocampal formation and medial frontal cortex. Neurobiol Learn Mem 76(2):151-82.

Weeber EJ, Savage DD, Sutherland RJ, Caldwell KK. 2001b. Fear conditioning-induced alterations of phospholipase C-beta1a protein level and enzyme activity in rat hippocampal formation and medial frontal cortex. Neurobiology of learning and memory 76(2):151-82.

Welty N, Shoblock JR. 2009. The effects of thioperamide on extracellular levels of glutamate and GABA in the rat prefrontal cortex. Psychopharmacology (Berl) 207(3):433-8.

West JR, Pierce DR. 1984. The effect of in utero ethanol exposure on hippocampal mossy fibers: an HRP study. Brain research 317(2):275-9.

Willoughby KA, Sheard ED, Nash K, Rovet J. 2008. Effects of prenatal alcohol exposure on hippocampal volume, verbal learning, and verbal and spatial recall in late childhood. Journal of the International Neuropsychological Society : JINS 14(6):1022-33.

Witte DG, Yao BB, Miller TR, Carr TL, Cassar S, Sharma R, Faghih R, Surber BW, Esbenshade TA, Hancock AA and others. 2006. Detection of multiple H3 receptor affinity states utilizing [3H]A-349821, a novel, selective, non-imidazole histamine H3 receptor inverse agonist radioligand. Br J Pharmacol 148(5):657-70.

Wouterlood FG, Sauren YM, Steinbusch HW. 1986. Histaminergic neurons in the rat brain: correlative immunocytochemistry, Golgi impregnation, and electron microscopy. J Comp Neurol 252(2):227-44.

Wozniak JR, Muetzel RL, Mueller BA, McGee CL, Freerks MA, Ward EE, Nelson ML, Chang PN, Lim KO. 2009. Microstructural corpus callosum anomalies in children with prenatal alcohol exposure: an extension of previous diffusion tensor imaging findings. Alcoholism, clinical and experimental research 33(10):1825-35.

Wu GY, Han XH, Zhuang QX, Zhang J, Yung WH, Chan YS, Zhu JN, Wang JJ. 2012. Excitatory effect of histamine on rat spinal motoneurons by activation of both H(1) and H(2) receptors in vitro. Journal of neuroscience research 90(1):132-42.

Wyper KR, Rasmussen CR. 2011. Language impairments in children with fetal alcohol spectrum disorders. Journal of population therapeutics and clinical pharmacology = Journal de la therapeutique des populations et de la pharamcologie clinique 18(2):e364-76.

Xu LS, Fan YY, He P, Zhang WP, Hu WW, Chen Z. 2009. Ameliorative effects of histamine on spatial memory deficits induced by scopolamine infusion into bilateral dorsal or ventral hippocampus as evaluated by the radial arm maze task. Clinical and experimental pharmacology & physiology 36(8):816-21.

Yum DS, Cho JH, Choi IS, Nakamura M, Lee JJ, Lee MG, Choi BJ, Choi JK, Jang IS. 2008. Adenosine A1 receptors inhibit GABAergic transmission in rat tuberomammillary nucleus neurons. J Neurochem 106(1):361-71.

Zhang CS, Bertaso F, Eulenburg V, Lerner-Natoli M, Herin GA, Bauer L, Bockaert J, Fagni L, Betz H, Scheschonka A. 2008. Knock-in mice lacking the PDZ-ligand motif of mGluR7a show impaired PKC-dependent autoinhibition of glutamate release, spatial working memory deficits, and increased susceptibility to pentylenetetrazol. The Journal of neuroscience : the official journal of the Society for Neuroscience 28(34):8604-14.

Zhou D, Lebel C, Lepage C, Rasmussen C, Evans A, Wyper K, Pei J, Andrew G, Massey A, Massey D and others. 2011. Developmental cortical thinning in fetal alcohol spectrum disorders. NeuroImage 58(1):16-25.

Zucker RS, Regehr WG. 2002. Short-term synaptic plasticity. Annual review of physiology 64:355-405.