Published in final edited form as:

Alcohol Clin Exp Res. 2014 July ; 38(7): 1902–1911. doi:10.1111/acer.12430.

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

Rafael K. Varaschin, Ph.D.¹, Martina J. Rosenberg, Ph.D.¹, Derek A. Hamilton, Ph.D.^{1,2}, and Daniel D. Savage, Ph.D.^{1,2,3}

¹Department of Neurosciences, University of New Mexico, Albuquerque, New Mexico, 87131, USA

²Department of Psychology, University of New Mexico, Albuquerque, New Mexico, 87131, USA

³Department of Pediatrics, University of New Mexico, Albuquerque, New Mexico, 87131, USA

Abstract

We previously reported that prenatal alcohol-induced deficits in dentate gyrus (DG) long-term potentiation (LTP) 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 prenatal alcohol-exposed (PAE) offspring. As the modulation of glutamate release is one important facet 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 PAE rats. Long-Evans rat dams voluntarily consumed either a 0% or 5% ethanol solution four hours daily throughout gestation. Male 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 in PAE offspring. GABAergic feedback inhibition of granule cell responsiveness was not affected by either PAE or methimepip. PAE reduced LTP in the 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 is due to differential drug dose responsiveness will require further investigation. Further, more detailed studies of H₃ receptor-mediated responses *in vitro* may provide clearer insights into the role of the H₃ receptor regulation of excitatory transmission at the perforant path - DG synapse in PAE rats.

Keywords

Fetal alcohol spectrum disorder; Histamine H₃ receptor; Dentate gyrus; Paired-pulse ratio; Long-term potentiation

INTRODUCTION

Prenatal exposure to relatively moderate levels of alcohol causes impairments in several learning tasks sensitive to functional damage in the dentate gyrus, particularly when employing more challenging versions of these tasks (Sutherland et al., 2000; Weeber et al., 2001; Savage et al., 2002; Savage et al., 2010). We, and others, have also observed that prenatal alcohol exposure (PAE) causes long-lasting deficits in dentate granule cell long-term potentiation (LTP) in adult male offspring (Sutherland et al., 1997; Varaschin et al., 2010; Titterness and Christie, 2012) as well as reduced activity-dependent elevations of evoked [³H]-D-aspartate release, a marker of glutamate release (Savage et al., 1998), suggesting a presynaptic mechanism as one component of a LTP deficit in PAE rats. Neurochemical studies of dentate gyrus have shown that metabotropic glutamate receptor subtype 5 (mGluR₅)-mediated phosphorylation of presynaptically-localized growth associated protein 43, as well as a mGluR₅ receptor-mediated potentiation of evoked [³H]-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.

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 include histamine H₃ receptor antagonists or inverse agonists (Fox et al., 2005; Galici et al., 2009; Medhurst et al., 2007). Histamine H₃ 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 H₃ receptors have procognitive actions in a variety of animal models (Brioni et al., 2011; Esbenshade et al., 2008). Conversely, histamine H₃ receptor agonists such as imetit or (R)-alpha-methylhistamine generally impair synaptic plasticity and cognitive processes (Blandina et al., 1996).

Our laboratory previously reported that the histamine H₃ 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 causes a

heightened H₃ 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. In the present study, we tested this hypothesis by examining the effects of the selective H₃ receptor agonist methimepip (Kitbunnadaj et al., 2005) 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.

METHODS

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

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) 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 24 days of age and group-housed, two males per cage, until used in the *in vivo* electrophysiology studies.

In-vivo Electrophysiology

On a given experiment day, one male adult rat offspring, 105 to 140 days of age and weighing 370-500 g received an intraperitoneal injection of 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 Na₂HPO₄, 2 mM NaH₂PO₄; pH = 7.4.) or phosphate-buffered saline alone approximately 2 to 3 hours before the start of electrophysiological recordings. The dose and timing of injection was based on the report that methimipip inhibition of histamine release from rat hypothalamus peaks approximately 150-180 min after i.p. injection. Kitbunnadaj et al., (2005). Methimipip was selected for use given its more than three orders of magnitude greater affinity for H₃ receptors compared to H₁, H₂ and H₄ receptors, as well as its ability to more readily cross the blood brain barrier than other commonly used H₃ receptor agonists (Kitbunnadaj et al., 2005).

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), band pass-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 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 4 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 measures, 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 μ A 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.

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.

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 methimipip) 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[®], Version 11.0 (Systat Software Inc., San Jose, CA).

RESULTS

Prenatal Ethanol Exposure Paradigm

Results from voluntary drinking during pregnancy are summarized in Table 1. Pregnant dams stably consumed 2.36 ± 0.13 g/kg/day during the daily four hours period when ethanol was available. In a previous study, maternal ethanol consumption resulted in serum ethanol 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, or offspring weight at time of experimentation (Table 1).

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 1A). In both PAE and control offspring, the maximal fEPSP slope was approximately 8 V/s at a 600 μ A stimulus intensity (Figures 1B & 1C). Population spikes were first observed at a mean stimulus intensity of 120 ± 9 μ A, with maximal amplitude of 15 ± 1 mV at 600 μ A stimulus intensity (Figures 1D & 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.

The effects of PAE and methimepip on E-S coupling are illustrated in Figure 1F & 1G. 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 1F & 1G). The slope of this correlation, known as E-S coupling, has been suggested as an index of synaptic efficacy and granule cell excitability (Richter-Levin et al., 1991). Methimepip injection in control offspring reduced the E-S coupling slope compared to saline (Figure 1F). The E-S coupling slope in saline-treated PAE rats was reduced compared to saline-treated control offspring and was similar to the E-S coupling slope in methimepip-treated controls (Figures 1F & 1G). In contrast to the control group, methimepip treatment of PAE offspring reduce decrease E-S coupling slope further compared to saline-injected PAE offspring (Figure 1G).

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 stimulus intensities that fail to elicit a PS (Figure 2, 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 that could confound 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 2A-B, open circles, $P > 0.05$). Methimepip injection in control offspring resulted in a 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 2A; $F_{(3,20)} = 5.72$ 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 2B). In general, however, there was a trend similar to the effect observed in the methimepip-injected control group.

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 3). Neither PAE nor methimepip altered these parameters under these experimental conditions, suggesting that GABA mediated inhibition of dentate granules cells is not affected using this experimental paradigm.

Long-term Potentiation

The effects of PAE and methimepip on LTP are depicted in Figure 4. 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 4A, open circles). In the control group, the average fEPSP slope potentiation from baseline during the 60 minute recording period was of $23.9 \pm 2.4\%$ (Figure 4C, left-hand side open bar). As shown previously (Varaschin et al., 2010), PAE significantly reduced the magnitude and duration of LTP (Figure 4B, open circles). The PAE-induced LTP deficit was such that the average potentiation in this group was only $16.0 \pm 2.6\%$ above baseline (Figure 4C, right-hand side open bar; $F_{(3,20)} = 5.06$, $P < 0.05$). In addition, methimepip injection in control offspring also impaired LTP (Figure 4A, black circles). The average tetanus-induced potentiation in methimepip-injected controls was of $13.7 \pm 2.6\%$, a level similar to that observed in saline-injected PAE offspring (Figure 4C, left-hand side black bar, $P < 0.05$). LTP in PAE offspring was not further impaired by methimepip injection (Figure 4B, black circles). Average potentiation in this group was $14.6 \pm 2.6\%$, not significantly different from saline-injected PAE offspring (Figure 4C, right-hand side black bar, $P > 0.05$). In contrast to fEPSP slope potentiation results, PAE did not affect potentiation of population spike amplitude, as reported previously (Varaschin et al., 2010), nor did methimepip treatment reduce population spike amplitude in either prenatal treatment group (data not shown).

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 1F), probability of glutamate release (Figure 2) and LTP (Figure 4) 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 1F & 1G) and LTP (Figure 4). 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 granule cell measures affected by PAE (Figures 1F, 1G & 4).

In the present study, PAE did not alter raw measures of granule cell responsiveness to electrical stimulation, namely the fEPSP slope and PS amplitude (Figure 1B-E). In agreement with these observations, previous studies have shown that moderate exposure to alcohol during prenatal development does not produce alterations in fEPSP and PS input/output curves (Sutherland et al., 1997; Varaschin et al., 2010; Titterness and Christie, 2012). Although no effects of PAE were observed in raw measures of fEPSP slope or PS amplitude,

there was a remarkable effect when the fEPSP slope was plotted against its associated PS amplitude (Figures 1F & 1G). PAE significantly decreased the slope of E-S coupling in the dentate gyrus, suggesting that moderate ethanol consumption during pregnancy reduces the synaptic efficacy of perforant path input to dentate granule cells. To our knowledge, this is the first report on PAE-induced impairment in E-S coupling. It is also noteworthy that this effect was not associated with significant alterations of either the fEPSP slope or the PS amplitude in response to varied electrical stimulus intensities. Therefore, the changes in E-S coupling presented here may be attributed to subtle alterations of fEPSP parameters that were not detected in previous studies (Sutherland et al., 1997; Varaschin et al., 2010).

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 stimulus 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 2), 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 3, PAE did not alter the paired pulse ratio of population spikes elicited by a high stimulus intensity. Thus, 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 examination of these putative mechanisms is one subject for future investigation.

Independent of the putative mechanism(s) of this PAE 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.

As predicted, methimepip produced some effects in saccharin control offspring similar to PAE. Methimepip injection in control rat offspring reduced E-S coupling to similar levels observed in PAE offspring (Figure 1F). This effect was accompanied by an increase in the

fEPSP paired pulse ratio (Figure 2A), suggesting that methimipip reduced the probability of glutamate release in control rats. In agreement with this observation, several studies indicate a role for histamine H₃ receptors in modulating glutamate release in the medial frontal cortex (Welty and Shoblock, 2009), globus pallidus (Osorio-Espinoza et al., 2011), 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 3), it is not likely that the reductions in E-S coupling can be attributed to elevated GABA release.

Given the effect of methimipip on E-S coupling, it was not surprising that methimipip also produced a deficit in LTP in control rats (Figure 4A). This form of synaptic plasticity 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 (reviewed by Malenka and Bear, 2004). This early-LTP is gradually augmented by late-LTP through a mechanism predominantly presynaptic that requires protein synthesis in axon nerve terminals (reviewed by Blundon and Zakharenko, 2008). 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.

While it is tempting to speculate that this effect is due to direct histamine H₃ receptor-mediated decrease of glutamate release in the dentate gyrus, other alternative interpretations warrant consideration. Systemic activation of histamine H₃ receptors by methimipip decreases histamine release in the rat brain (Kitbunnadaj et al., 2005). Endogenous histamine plays an important role in synaptic plasticity by activating postsynaptic H₁ and H₂ 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 H₃ 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 (Ondrejcek 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 methimipip 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, methimipip did not exacerbate PAE-induced deficits in E-S coupling (Figure 1G) or LTP (Figure 4B) compared to saline-injected PAE offspring. These observations suggest that methimipip and PAE 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 2B) or GABAergic inhibition (Figure 3B). One possible interpretation for

diminished responses to methimepip by PAE rats is that histamine H₃ 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 exogenous H₃ 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 H₃ receptor-effector coupling or increased constitutive activity by H₃ receptors (Morisset et al., 2000) in PAE rats. The ability of the selective H₃ 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 2) 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 of methimepip would have significantly reduced glutamate release probability in PAE rats. Unfortunately, more detailed dose response studies using *in vivo* electrophysiological recordings is not a practical approach. Thus, the underlying questions of heightened H₃ 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.

ACKNOWLEDGEMENTS

The authors thank Dr. Kevin O'Hair, Ms. Christie Wilcox, Mr. Nathan Boyer and Ms Denise Cordaro in the UNM Animal Resource Facility for their excellent animal care. The authors would also like to acknowledge Dr. C. Fernando Valenzuela and Dr. Susan Queen for their constructive comments on the preparation of this manuscript.

This work was supported by the National Institute on Alcohol Abuse and Alcoholism grants AA016619, AA017068 and AA019884.

REFERENCES

1. Bacciottini L, Passani MB, Giovannelli L, Cangioli I, Mannaioni PF, Schunack W, Blandina P. 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.* 2002; 15(10):1669–80. [PubMed: 12059975]
2. Blandina P, Giorgetti M, Bartolini L, Cecchi M, Timmerman H, Leurs R, Pepeu G, Giovannini MG. Inhibition of cortical acetylcholine release and cognitive performance by histamine H₃ receptor activation in rats. *Br J Pharmacol.* 1996; 119(8):1656–64. [PubMed: 8982515]
3. Blandina P, Efofudebe M, Cenni G, Mannaioni P, Passani MB. Acetylcholine, histamine, and cognition: two sides of the same coin. *Learn Mem.* 2004; 11(1):1–8. [PubMed: 14747511]
4. Blundon JA, Zakharenko SS. Dissecting the components of long-term potentiation. *Neuroscientist.* 2008; 14(6):598–608. [PubMed: 18940785]
5. Bowden JB, Abraham WC, Harris KM. Differential effects of strain, circadian cycle, and stimulation pattern on LTP and concurrent LTD in the dentate gyrus of freely moving rats. *Hippocampus.* 2011
6. Breakwell NA, Rowan MJ, Anwyl R. Metabotropic glutamate receptor dependent EPSP and EPSP-spike potentiation in area CA1 of the submerged rat hippocampal slice. *J Neurophysiol.* 1996; 76(5):3126–35. [PubMed: 8930260]

7. Brioni JD, Esbenshade TA, Garrison TR, Bitner SR, Cowart MD. Discovery of histamine H₃ antagonists for the treatment of cognitive disorders and Alzheimer's disease. *J Pharmacol Exp Ther*. 2011; 336(1):38–46. [PubMed: 20864505]
8. Brown RE, Fedorov NB, Haas HL, Reymann KG. Histaminergic modulation of synaptic plasticity in area CA1 of rat hippocampal slices. *Neuropharmacology*. 1995; 34(2):181–90. [PubMed: 7617144]
9. Brown RE, Haas HL. On the mechanism of histaminergic inhibition of glutamate release in the rat dentate gyrus. *J Physiol*. 1999; 515(3):777–786. [PubMed: 10066904]
10. Chang M, Saito H, Abe K. Cimetidine inhibits the induction of long-term potentiation in the dentate gyrus of rats in vivo. *Jap J Pharmacol*. 1997; 74(3):281–3. [PubMed: 9268089]
11. Chen L, Xing T, Wang M, Miao Y, Tang M, Chen J, Li G, Ruan DY. Local infusion of ghrelin enhanced hippocampal synaptic plasticity and spatial memory through activation of phosphoinositide 3-kinase in the dentate gyrus of adult rats. *Eur J Neurosci*. 2011; 33(2):266–75. [PubMed: 21219473]
12. Dai H, Kaneko K, Kato H, Fujii S, Jing Y, Xu A, Sakurai E, Kato M, Okamura N, Kuramasu A. Selective cognitive dysfunction in mice lacking histamine H₁ and H₂ receptors. *Neuroscience Res*. 2007; 57(2):306–13. others.
13. Daoudal G, Hanada Y, Debanne D. Bidirectional plasticity of excitatory postsynaptic potential (EPSP)-spike coupling in CA1 hippocampal pyramidal neurons. *Proc Natl Acad Sci USA*. 2002; 99(22):14512–7. [PubMed: 12391303]
14. Esbenshade TA, Browman KE, Bitner RS, Strakhova M, Cowart MD, Brioni JD. The histamine H₃ receptor: an attractive target for the treatment of cognitive disorders. *Br J Pharmacol*. 2008; 154(6):1166–81. [PubMed: 18469850]
15. Ferguson AL, Stone TW. Glutamate-induced depression of EPSP-spike coupling in rat hippocampal CA1 neurons and modulation by adenosine receptors. *Eur J Neurosci*. 2010; 31(7):1208–18. [PubMed: 20345917]
16. Fox GB, Esbenshade TA, Pan JB, Radek RJ, Krueger KM, Yao BB, Browman KE, Buckley MJ, Ballard ME, Komater VA. 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*. 2005; 313(1):176–90. others. [PubMed: 15608077]
17. Fujimoto K, Mizuguchi H, Fukui H, Wada H. Presynaptic localization of histamine H₃-receptors in rat brain. *Biochem Biophys Res Comm*. 1991; 177(3):907–12. [PubMed: 1647769]
18. Galici R, Boggs JD, Aluisio L, Fraser IC, Bonaventure P, Lord B, Lovenberg TW. JNJ-10181457, a selective non-imidazole histamine H(3) receptor antagonist, normalizes acetylcholine neurotransmission and has efficacy in translational rat models of cognition. *Neuropharmacology*. 2009; 56(8):1131–7. [PubMed: 19345233]
19. Galindo R, Frausto S, Wolff C, Caldwell KK, Perrone-Bizzozero NI, Savage DD. Prenatal ethanol exposure reduces mGluR₅ receptor number and function in the dentate gyrus of adult offspring. *Alcohol Clin Exp Res*. 2004; 28(10):1587–97. [PubMed: 15597093]
20. Garduno-Torres B, Trevino M, Gutierrez R, Arias-Montano JA. Pre-synaptic histamine H₃ receptors regulate glutamate, but not GABA release in rat thalamus. *Neuropharmacology*. 2007; 52(2):527–35. [PubMed: 17027043]
21. Giannoni P, Medhurst AD, Passani MB, Giovannini MG, Ballini C, Corte LD, Blandina P. 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. *J Pharmacol Exp Ther*. 2010; 332(1):164–72. [PubMed: 19815811]
22. Jedlicka P, Hoon M, Papadopoulos T, Vlachos A, Winkels R, Pouloupoulos A, Betz H, Deller T, Brose N, Varoqueaux F. Increased dentate gyrus excitability in neuroligin-2-deficient mice in vivo. *Cereb Cortex*. 2011; 21(2):357–67. others. [PubMed: 20530218]
23. Kitbunnadaj R, Hashimoto T, Poli E, Zuiderveld OP, Menozzi A, Hidaka R, de Esch IJ, Bakker RA, Menge WM, Yamatodani A. N-substituted piperidinyl alkyl imidazoles: discovery of

- methimepip as a potent and selective histamine H₃ receptor agonist. *J Med Chem.* 2005; 48(6): 2100–7. others. [PubMed: 15771452]
24. Luo L, Chen WH, Wang M, Zhu DM, She JQ, Ruan DY. Modulation of long-term potentiation by individual subtypes of muscarinic acetylcholine receptor in the rat dentate gyrus. *Hippocampus.* 2008; 18(10):989–95. [PubMed: 18548582]
 25. Luo T, Leung LS. Endogenous histamine facilitates long-term potentiation in the hippocampus during walking. *J Neurosci.* 2010; 30(23):7845–52. [PubMed: 20534833]
 26. Malenka RC, Bear MF. LTP and LTD: an embarrassment of riches. *Neuron.* 2004; 44(1):5–21. [PubMed: 15450156]
 27. Malik R, Chattarji S. Enhanced intrinsic excitability and EPSP-spike coupling accompany enriched environment-induced facilitation of LTP in hippocampal CA1 pyramidal neurons. *J Neurophysiol.* 2012; 107(5):1366–78. [PubMed: 22157122]
 28. Medhurst AD, Atkins AR, Beresford IJ, Brackenborough K, Briggs MA, Calver AR, Cilia J, Cluderay JE, Crook B, Davis JB. GSK189254, a novel H₃ receptor antagonist that binds to histamine H₃ receptors in Alzheimer's disease brain and improves cognitive performance in preclinical models. *J Pharmacol Exp Ther.* 2007; 321(3):1032–45. others. [PubMed: 17327487]
 29. Molina-Hernandez A, Nunez A, Sierra JJ, Arias-Montano JA. Histamine H₃ receptor activation inhibits glutamate release from rat striatal synaptosomes. *Neuropharmacology.* 2001; 41(8):928–34. [PubMed: 11747897]
 30. Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-Lacombe J, Stark H, Schunack W, Ganellin CR, Schwartz JC, Arrang JM. High constitutive activity of native H₃ receptors regulates histamine neurons in brain. *Nature.* 2000; 408(6814):860–4. [PubMed: 11130725]
 31. Ondrejcek T, Wang Q, Kew JN, Virley DJ, Upton N, Anwyl R, Rowan MJ. Activation of alpha7 nicotinic acetylcholine receptors persistently enhances hippocampal synaptic transmission and prevents Ass-mediated inhibition of LTP in the rat hippocampus. *Eur J Pharmacol.* 2012; 677(1-3): 63–70. [PubMed: 22200627]
 32. Osorio-Espinoza A, Alatorre A, Ramos-Jimenez J, Garduno-Torres B, Garcia-Ramirez M, Querejeta E, Arias-Montano JA. Pre-synaptic histamine H(3) receptors modulate glutamatergic transmission in rat globus pallidus. *Neuroscience.* 2011; 176:20–31. [PubMed: 21195747]
 33. Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Academic Press; San Diego: 1998.
 34. Richter-Levin G, Segal M, Sara S. An alpha 2 antagonist, idazoxan, enhances EPSP-spike coupling in the rat dentate gyrus. *Brain Res.* 1991; 540(1-2):291–4. [PubMed: 1675916]
 35. Samudio-Ruiz SL, Allan AM, Sheema S, Caldwell KK. Hippocampal N-methyl-D-aspartate receptor subunit expression profiles in a mouse model of prenatal alcohol exposure. *Alcohol Clin Exp Res.* 2010; 34(2):342–53. [PubMed: 19951292]
 36. Samudio-Ruiz SL, Allan AM, Valenzuela CF, Perrone-Bizzozero NI, Caldwell KK. Prenatal ethanol exposure persistently impairs NMDA receptor-dependent activation of extracellular signal-regulated kinase in the mouse dentate gyrus. *J Neurochem.* 2009; 109(5):1311–23. [PubMed: 19317851]
 37. Savage DD, Becher M, de la Torre AJ, Sutherland RJ. Dose-dependent effects of prenatal ethanol exposure on synaptic plasticity and learning in mature offspring. *Alcohol Clin Exp Res.* 2002; 26(11):1752–8. [PubMed: 12436066]
 38. Savage DD, Cruz LL, Duran LM, Paxton LL. Prenatal ethanol exposure diminishes activity-dependent potentiation of amino acid neurotransmitter release in adult rat offspring. *Alcohol Clin Exp Res.* 1998; 22(8):1771–7. [PubMed: 9835293]
 39. Savage DD, Rosenberg MJ, Wolff CR, Akers KG, El-Emawy A, Staples MC, Varaschin RK, Wright CA, Seidel JL, Caldwell KK. Effects of a novel cognition-enhancing agent on fetal ethanol-induced learning deficits. *Alcohol Clin Exp Res.* 2010; 34(10):1793–802. others. [PubMed: 20626729]
 40. Sloviter RS. Feedforward and feedback inhibition of hippocampal principal cell activity evoked by perforant path stimulation: GABA-mediated mechanisms that regulate excitability in vivo. *Hippocampus.* 1991; 1(1):31–40. [PubMed: 1669342]
 41. Staff NP, Spruston N. Intracellular correlate of EPSP-spike potentiation in CA1 pyramidal neurons is controlled by GABAergic modulation. *Hippocampus.* 2003; 13(7):801–5. [PubMed: 14620875]

42. Sutherland RJ, McDonald RJ, Savage DD. Prenatal exposure to moderate levels of ethanol can have long-lasting effects on hippocampal synaptic plasticity in adult offspring. *Hippocampus*. 1997; 7(2):232–8. [PubMed: 9136052]
43. Sutherland RJ, McDonald RJ, Savage DD. Prenatal exposure to moderate levels of ethanol can have long-lasting effects on learning and memory of adult rat offspring. *Psychobiology*. 2000; (28):532–539.
44. Titterness AK, Christie BR. Prenatal ethanol exposure enhances NMDAR-dependent long-term potentiation in the adolescent female dentate gyrus. *Hippocampus*. 2012; 22(1):69–81. [PubMed: 21080406]
45. Varaschin RK, Akers KG, Rosenberg MJ, Hamilton DA, Savage DD. Effects of the cognition-enhancing agent ABT-239 on fetal ethanol-induced deficits in dentate gyrus synaptic plasticity. *J Pharmacology Exp Ther*. 2010; 334(1):191–8.
46. Wang Y, Sherwood JL, Lodge D. The alpha4beta2 nicotinic acetylcholine receptor agonist TC-2559 impairs long-term potentiation in the dentate gyrus in vivo. *Neurosci Lett*. 2006; 406(3): 183–8. [PubMed: 16935422]
47. Weeber EJ, Savage DD, Sutherland RJ, Caldwell KK. Fear conditioning-induced alterations of phospholipase C-beta1a protein level and enzyme activity in rat hippocampal formation and medial frontal cortex. *Neurobiol Learn Mem*. 2001; 76(2):151–82. [PubMed: 11502147]
48. Welty N, Shoblock JR. The effects of thioperamide on extracellular levels of glutamate and GABA in the rat prefrontal cortex. *Psychopharmacology (Berl)*. 2009; 207(3):433–8. [PubMed: 19795107]
49. Zucker RS, Regehr WG. Short-term synaptic plasticity. *Ann Rev Physiol*. 2002; 64:355–405. [PubMed: 11826273]

FIGURE 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). Effects of saline (open circles) or methimepip (black circles) on PS

amplitude responses to a given fEPSP slope (E-S coupling) in control offspring (1F) or PAE offspring (1G). Lines indicate the average linear regression for saline (dashed) and methimepip-injected (solid) offspring. Data points represent the mean $\pm$ the S.E.M. Sample sizes are denoted in parenthesis.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

FIGURE 2.

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 (2A) and PAE offspring (2B) at given interpulse intervals. Inset: Representative traces of the rise phase of fEPSP responses evoked by the first (solid line) and second (dotted line) electrical pulse in saline-injected (gray) or methimepip-injected offspring (black) at given interpulse intervals. Data points represent the

mean $\pm$ the S.E.M. Sample sizes are denoted in parenthesis. Asterisks denote data significantly greater than the saline-injected control group ($P < 0.05$).

FIGURE 3.

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 (3A) and PAE offspring (3B) at given interpulse intervals. 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 $\pm$ the S.E.M. Sample

sizes are denoted in parenthesis. No statistically significant differences were detected in either treatment group.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

FIGURE 4.

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 (4A) or PAE offspring (4B). Inset: Representative traces of fEPSP responses of saline (gray) or methimepip injected (black) before and after tetanus. 4C: Average post-tetanus increase from baseline levels in saline (white bars) or methimepip injected (black bars) control (left) and PAE offspring (right). Data bars represent the mean $\pm$

the S.E.M. Sample sizes are denoted in parenthesis. Asterisks denote data significantly decreased compared to the saline-injected control group ($P < 0.05$).

Table 1

Daily maternal ethanol consumption and impact on weight gain and offspring outcomes. Data are expressed as the mean $\pm$ the S.E.M. and sample sizes are denoted in parenthesis.

Prenatal Group	Daily Ethanol Consumption^a	Maternal Weight Gain During Pregnancy^b	Pup Birth Weight^c	Litter Size^d	Weight at experiment^e
Control	N/A	110 $\pm$ 6 (18)	8.9 $\pm$ 0.4 (25)	10.8 $\pm$ 0.5 (18)	512 $\pm$ 12 (25)
PAE	2.4 $\pm$ 0.1 (14)	98 $\pm$ 7 (14)	8.0 $\pm$ 0.3 (24)	11.0 $\pm$ 0.6 (14)	506 $\pm$ 11 (24)

^aGrams ethanol /kg body weight /day, averaged throughout pregnancy.

^bNet wet gain between Gestational Days 0 and 20, in grams.

^cAverage offspring weight, in grams.

^dNumber of live births per litter.

^eOffspring body weight, in grams