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The Role of Age, Sex, and mGluR5 Receptors in Hippocampal Depotentiation in Mice

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Honors Thesis

THE ROLE OF AGE, SEX, AND MGLUR5 RECEPTORS IN HIPPOCAMPAL DEPOTENTIATION IN MICE

by
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ABSTRACT

THE ROLE OF AGE, SEX, AND MGLUR5 RECEPTORS IN HIPPOCAMPAL DEPOTENTIATION IN MICE

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Bachelor of Science

Synaptic plasticity of excitatory hippocampal circuits is one of the primary cellular mechanisms of learning and memory. Synaptic plasticity is also implicated in many disease states such as depression, post-traumatic stress disorder, and Alzheimer disease. Long-term potentiation (LTP) is the plastic strengthening of synapses, while long-term depression (LTD) is the weakening of synapses. Depotentiation is the process of reversing previously established long-term potentiation. Depotentiation has not been studied as thoroughly as long-term potentiation and long-term depression. We examined the role of age, metabotropic glutamate receptor 5 (mGluR5), and sex in the process of CA1 hippocampal depotentiation in young CD-1 mice using field electrophysiology. In juvenile (14-34 days, n=11), adolescent (35-59 days, n=8), and young adult mice (70-100 days, n=5), there were no significant age-related differences in CA1 hippocampal LTP or depotentiation ($p>0.05$). Using MPEP, an antagonist of mGluR5, we showed that hippocampal depotentiation is mGluR5-independent in juvenile mice (control n=11, MPEP n=7, $p>0.05$). This was also the case for adolescent mice (control n=8, MPEP n=7, $p>0.05$). Slices from juvenile female mice (n=6) demonstrated significantly larger

(p=0.030) depotentiation compared to slices from juvenile male mice (n=4) due to increased magnitude of LTP (p=0.032). Our data on adolescent female (n=4) and male (n=4) mice is underpowered to perform statistical analysis. Our results demonstrate the importance of including female subjects in plasticity research.

ACKNOWLEDGEMENTS

I would like to first thank my faculty advisor and mentor, Dr. Edwards for his support and coaching over the last 3 years. I would also like to thank the other members of my thesis committee, Dr. Brown and Dr. Matheson, for their guidance through my thesis process. I am grateful for the work of Bryson Reed and Darien Reed, our lab technicians, who prepared the slices for the experiments. I am also grateful for the work of the other members of the lab who also performed experiments, including those who worked on this project before I joined the lab.

TABLE OF CONTENTS

LIST OF FIGURES

Introduction

Synaptic plasticity is one of the primary cellular mechanisms of encoding memory in the brain. Basic research in synaptic plasticity will be foundational to future understanding of memory processes, as well as pathological conditions involving memory and plasticity. One such disease is Alzheimer's disease, as amyloid-β oligomers can modify synaptic plasticity (Hu et al., 2014; O'Riordan et al., 2018). Abnormal plasticity is also implicated in depression (Jiang et al., 2020), so better understanding of healthy plasticity could assist in the development of depression treatments. Normalizing maladaptive plasticity is an effective way to treat post-traumatic stress disorder as well (Niu et al., 2022). Because of the many applications of synaptic plasticity, a clear understanding of its basic mechanisms will be greatly beneficial to future research and medicine.

Long-Term Potentiation and Long-Term Depression

The first identified form of synaptic plasticity was *N*-methyl-D-aspartate (NMDA) receptor-dependent long-term potentiation (LTP) (Brown et al., 2022). This increase in synaptic strength occurs when additional α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors are added to the membrane of the post-synaptic cell, due to the entrance of calcium into the cell through NMDA receptors (Brown et al., 2022). NMDA receptors are normally blocked by magnesium, but when a dendrite is depolarized by the activity of already-present AMPA receptors letting in sodium, the magnesium is forced out, allowing calcium to enter (Brown et al., 2022; Huganir & Nicoll, 2013). Calcium acts as a second messenger to activate cascades that eventually lead to the insertion of AMPA receptors into the synapse (Malenka & Bear,

2004). Increasing intracellular calcium levels via NMDA receptors can also lead to long-term depression (LTD), or weakening of the synapse (Evans & Blackwell, 2015; Stanton, 1996). Metabotropic glutamate receptors (mGluRs) can also be involved in inducing LTD, sometimes initiating a signaling pathway that leads to endocytosis of AMPA receptors (Gladding et al., 2009; Goh & Manahan-Vaughan, 2013; Jong et al., 2023; Neyman & Manahan-Vaughan, 2008; Nosyreva & Huber, 2005; Popkirov & Manahan-Vaughan, 2011; Snyder et al., 2001). Specifically, mGluRs activate mitogen activated protein kinase (MAPK), and phosphoinositide-3-kinase (PI3K). The activity of these kinases leads to the production of striatal-enriched tyrosine phosphatase (STEP), which removes phosphates from AMPA receptors, leading to their endocytosis (Gladding et al, 2009).

Depotentiation

A third form of synaptic plasticity, depotentiation, involves weakening previously potentiated synapses (Gladding et al., 2009). Depotentiation is not as well studied as LTP and LTD, and it is unclear to what extent depotentiation and LTD involve distinct mechanisms, as both processes involve the weakening of synaptic transmission following low-frequency stimuli (Kamal et al., 1998; Latif-Hernadez et al., 2016; Wagner & Alger, 1996). The ambiguity may be due to the relative difficulty of inducing LTD and depotentiation compared to LTP (Froc et al., 2000; Wagner & Alger, 1996). However, a 5 Hz stimulus can be used to evoke depotentiation, a frequency that does not affect naïve synapses (Kamal et al., 1998; Latif-Hernadez et al., 2016). Additionally, CGP 35348 (an antagonist of $GABA_B$ receptors) prevents the induction of LTD but not depotentiation (Wagner & Alger, 1995). Finally, there may be developmental differences in the

inducibility of these processes such that depotentiation can be induced in all ages but LTD is not found in adults, although the literature is conflicting (Kamal et al., 1998; Kemp et al., 2000; Norris et al., 1996; Wagner & Alger, 1995). Some studies find that LTD can be induced only in older animas (Norris et al., 1996) or that LTD can be induced in all ages (Kamal et al., 1998), but they agree that depotentiation can be induced regardless of age (Kamal et al., 1998; Norris et al., 1996; Wagner & Alger, 1995). Because of these differences, depotentiation is considered to be distinct in some ways from LTD, and further characterization of depotentiation and LTD will enhance understanding of their fundamental and mechanistic differences.

Behavioral Correlates of Synaptic Plasticity

When animals navigate a novel environment, hippocampal LTD occurs (Goh & Manahan-Vaughan, 2013; Popkirov & Manahan-Vaughan, 2011). Pharmacological inhibition of LTD during initial exploration prevents the animals from later recognizing the environment as familiar (Goh & Manahan-Vaughan, 2013 Popkirov & Manahan-Vaughan, 2011). LTD is also required for the development of hippocampal place fields, neural maps of an animal's environment (Ashby et al., 2021). The evidence suggests that LTD is important to ensure memories are distinguishable from one another by removing extraneous synaptic connections (Stacho & Manahan-Vaughan, 2022). If the hippocampal field is previously potentiated, exploration of a novel environment leads to depotentiation (Qi et al., 2013).

Project Importance

The characteristics of LTD change with age (Nosyreva & Huber, 2005; Potier et al., 2022), so it is possible that there are also age-related changes in depotentiation. There

is a debate as to whether mGluR5 is required for depotentiation as Qi et al. (2013) found that depotentiation *in vivo* was mGluR5-dependent, while Latif-Hernadez et al. (2016) found that depotentiation *in vitro* was mGluR5-independent. Both studies found that NMDA receptor activity is required for depotentiation (Latif-Hernandez et al., 2016; Qi et al., 2013). Fujii et al. (2020) found that NMDA receptors and group 1 mGluRs (of which mGluR5 is one (Gladding et al., 2009)) are both required for depotentiation, although they did not specifically test mGluR5. Hu et al. (2005) found that while mGluR1 and mGluR5 both contributed to depotentiation, the contribution of mGluR5 was smaller. Sex differences in synaptic plasticity have also been observed (Talani et al., 2023), although sex impacts in depotentiation have not been tested. There is a lack of research in the roles of age, mGluR5, and sex in depotentiation, which this project will help to resolve.

Additional variables that may influence depotentiation are the phase of LTP (early or late) during which depotentiation is initiated and the hippocampal subregion studied (dorsal or ventral). The nature of different phases of LTP, some of which are more resistant to depotentiation than others (Park et al., 2019), is also debated and needs further testing (Abbas et al., 2015). Early or short-term LTP does not require protein synthesis, while late or long-term LTP is believed to be established by protein synthesis (Baltaci et al., 2019; Malenka & Bear, 2004; Park et al., 2019). While LTP is more easily induced (Kouvaros & Papatheodoropoulos, 2016) or has larger magnitude (Dubovyk & Manahan-Vaughan, 2018) in the dorsal hippocampus relative to the ventral hippocampus, LTD is equal in magnitude in the dorsal and ventral hippocampus (Dubovyk & Manahan-Vaughan, 2018; Maggio & Segal, 2009) or greater in the ventral hippocampus (Izaki et

al., 2001). While these variables are important and should be researched in the future, this project will focus on age, mGluR5, and sex to establish broad principles of depotentiation. These broad principles will assist in further research of more specific details.

Results

To test the role of age in CA1 hippocampal depotentiation in mice, we performed field electrophysiology recordings in male and female juvenile (14-34 days), adolescent (35-59 days), and young adult (70-100 days) mice. 15 out of our 54 experiments did not demonstrate significant depotentiation as measured by analysis of variance (ANOVA, $p>0.05$). Experiments that did not demonstrate significant depotentiation were excluded from the following analysis to improve accuracy.

Age-Related Changes in Plasticity in Control Mice

We analyzed the magnitude of depotentiation normalized to baseline recordings and found no statistically significant age-related changes in LTP or depotentiation (Figures 1). We then analyzed the magnitude of depotentiation normalized to LTP recordings and found no statistically significant age-related changes in depotentiation (Figure 2).

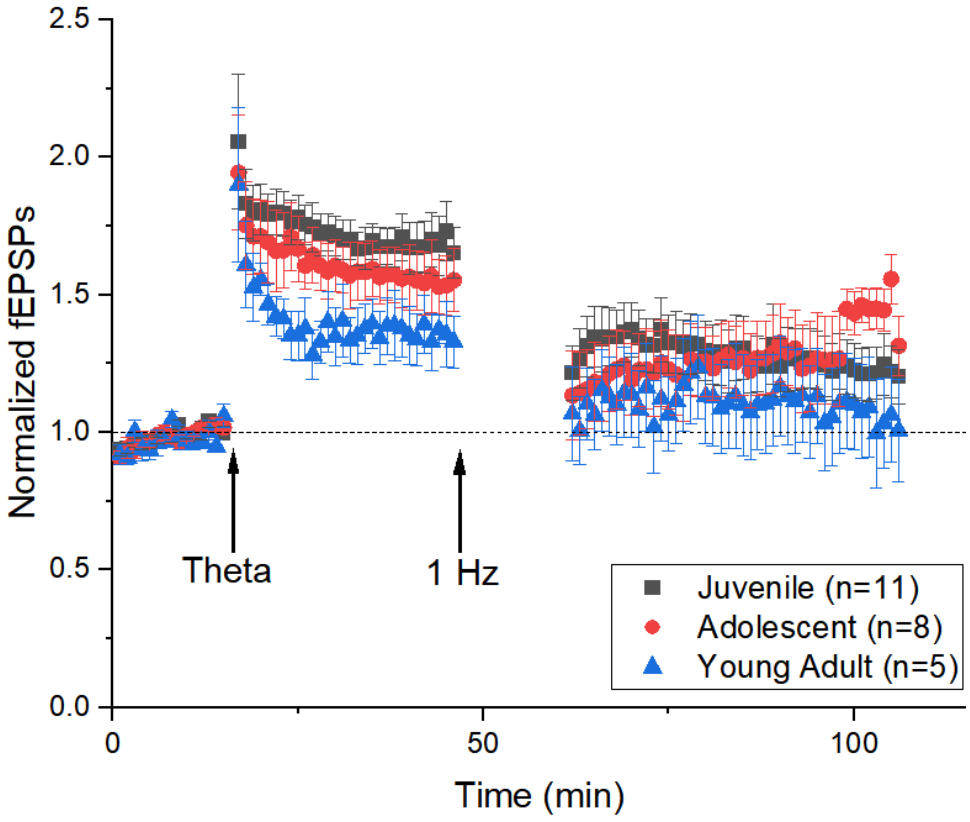

Figure 1: Hippocampal depotentiation in the CA1 is age-independent. During the last five minutes of post-theta burst recording, slices from juvenile mice (n=11) demonstrated LTP of 169% ± 10% of baseline, slices from adolescent mice (n=8) demonstrated LTP of 155% ± 11%, and slices from young adults (n=5) demonstrated LTP of 136% ± 9%. During 1-5 minutes after 1 Hz stimulation, slices from juvenile mice demonstrated depotentiation at 130% ± 11% of baseline, slices from adolescent mice demonstrated depotentiation at 116% ± 16%, and slices from young adults demonstrated depotentiation at 108% ± 13%. During 21-25 minutes after 1 Hz stimulation, slices from juvenile mice demonstrated depotentiation at 128% ± 11% of baseline, slices from adolescent mice demonstrated depotentiation at 126% ± 14%, and slices from young adults demonstrated depotentiation at 110% ± 17%. During 41-45 minutes after 1 Hz stimulation, slices from

juvenile mice demonstrated depotentiation at 122% ± 10% of baseline, slices from adolescent mice demonstrated depotentiation at 146% ± 11%, and slices from young adults demonstrated depotentiation at 104% ± 17%. None of these differences are statistically significant using an analysis of variance (ANOVA) ($p>0.05$).

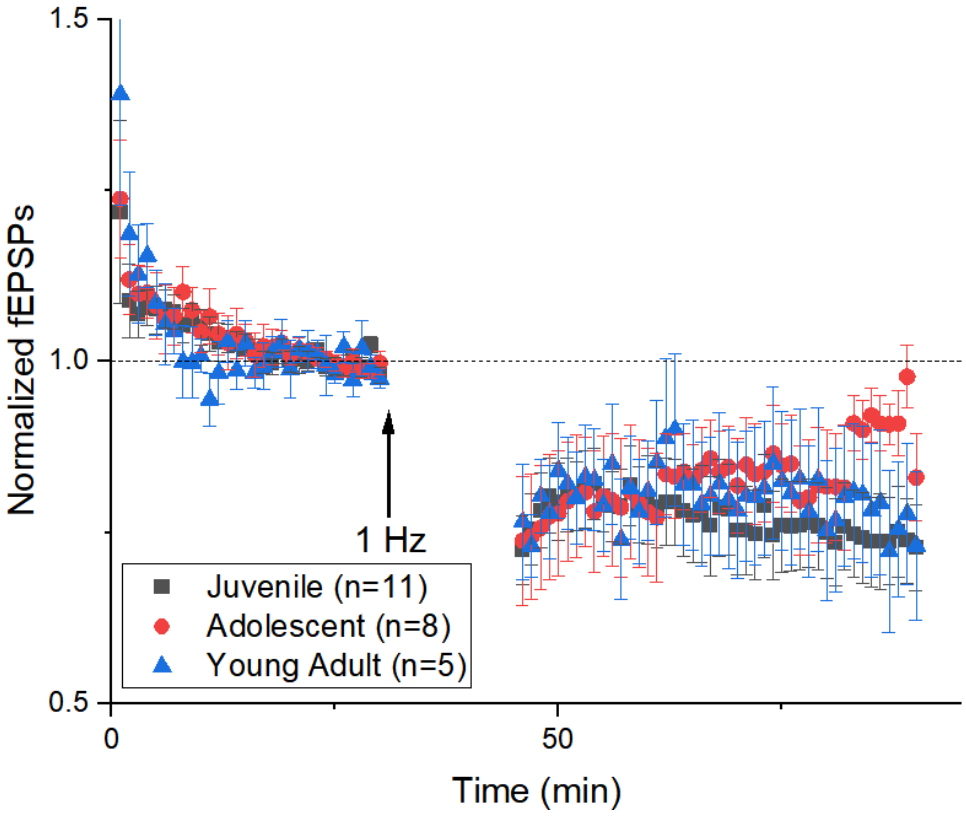

Figure 2: Hippocampal depotentiation in the CA1 is age-independent. Field EPSPs were normalized to LTP rather than to baseline to allow for more accurate comparison of depotentiation. During 1-5 minutes after 1 Hz stimulation, slices from juvenile mice (n=11) demonstrated 23% ± 5% depotentiation, slices from adolescent mice (n=8) demonstrated 24% ± 9% depotentiation, and slices from young adults (n=5) demonstrated 22% ± 6% depotentiation. During 21-25 minutes after 1 Hz stimulation, slices from juvenile mice demonstrated 23% ± 7% depotentiation, slices from adolescent mice

demonstrated 16% ± 6% depotentiation, and slices from young adults demonstrated 20% ± 10% depotentiation. During 41-45 minutes after 1 Hz stimulation, slices from juvenile mice demonstrated 16% ± 8% depotentiation, slices from adolescent mice demonstrated 8% ± 6% depotentiation, and slices from young adults demonstrated 24% ± 10% depotentiation. None of these differences are statistically significant using an analysis of variance (ANOVA, $p>0.05$).

Role of mGluR5 in Depotentiation

We next tested whether CA1 hippocampal depotentiation is mGluR5-dependent by adding 10 μM MPEP to the artificial cerebrospinal fluid (ACSF) 15 minutes before the 1 Hz stimulus. We analyzed the magnitude of depotentiation normalized to baseline recordings and found no statistically significant differences in depotentiation in juveniles between control slices and slices to which MPEP was added, suggesting that CA1 hippocampal depotentiation in juvenile mice is mGluR5-independent (Figure 3). We then analyzed the magnitude of depotentiation normalized to LTP recordings and again found no statistically significant differences in depotentiation (Figure 4). Experiments in adolescent mice also demonstrated no significant differences in LTP or depotentiation between control slices and slices to which MPEP was added, suggesting that CA1 hippocampal depotentiation in adolescent mice is mGluR5-indpendent (Figures 5 and 6).

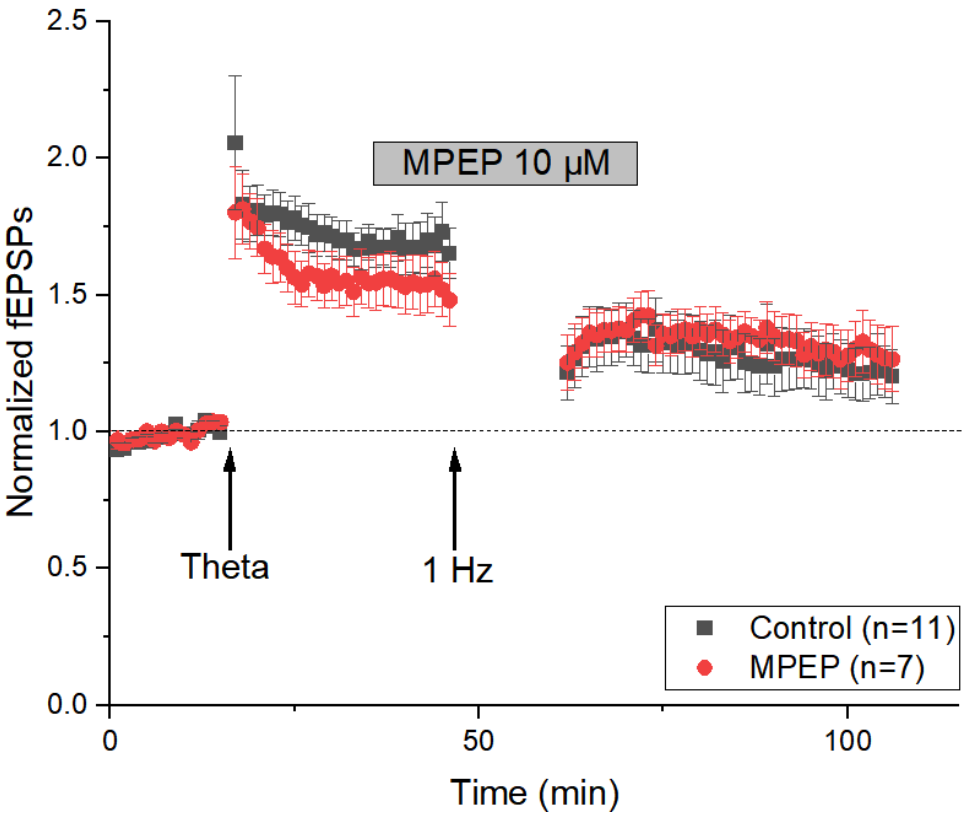

Figure 3: Hippocampal depotentiation in the CA1 in juvenile mice is mGluR5-independent. During the last five minutes of post-theta burst recording, slices with control ACSF (n=11) demonstrated LTP of 169% ± 10% of baseline and slices with MPEP ACSF (n=7) demonstrated LTP of 153% ± 10%. During 1-5 minutes after 1Hz stimulation, slices with control ACSF demonstrated depotentiation at 130% ± 11% of baseline while slices with MPEP ACSF demonstrated depotentiation at 132% ± 9%. During 21-25 minutes after 1 Hz stimulation, slices with control ACSF demonstrated depotentiation at 128% ± 11% of baseline while slices with MPEP ACSF demonstrated depotentiation at 135% ± 8%. During 41-45 minutes after 1 Hz stimulation, slices with control ACSF demonstrated depotentiation at 122% ± 10% of baseline, while slices with MPEP ACSF demonstrated depotentiation at 129% ± 11%. None of these differences are statistically significant using a two-tailed, type three t-test ($p>0.05$).

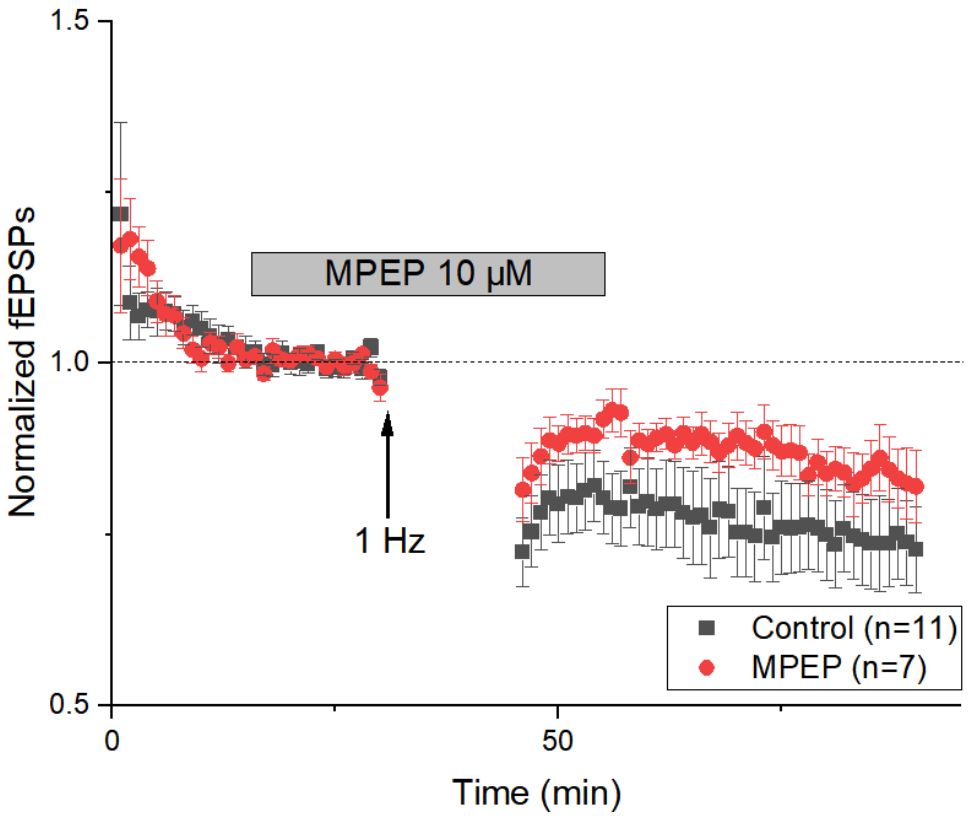

Figure 4: Hippocampal depotentiation in the CA1 in juvenile mice is mGluR5-independent. Field EPSPs were normalized to LTP rather than to baseline to allow for more accurate comparison of depotentiation. During 1-5 minutes after 1 Hz stimulation, slices with control ACSF (n=11) demonstrated 23% ± 5% depotentiation, while slices with MPEP ACSF (n=12) demonstrated 14% ± 4% depotentiation. During 21-25 minutes after 1 Hz stimulation, slices with control ACSF demonstrated 23% ± 7% depotentiation, while slices with MPEP ACSF demonstrated 11% ± 3% depotentiation. During 41-45 minutes after 1 Hz stimulation, slices with control ACSF demonstrated 26% ± 6% depotentiation, while slices with MPEP ACSF demonstrated 16% ± 5% depotentiation. Neither of these differences are statistically significant using a two-tailed, type three t-test ($p>0.05$).

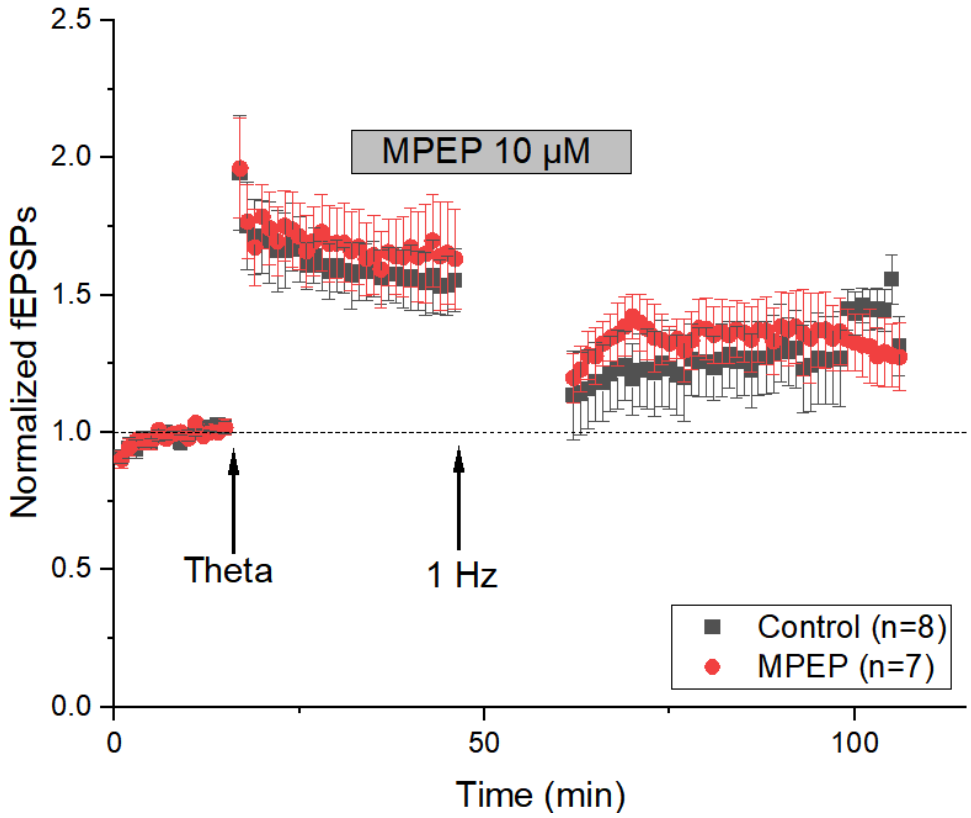

Figure 5: Hippocampal depotentiation in the CA1 in adolescent mice is mGluR5-independent. During the last five minutes of post-theta burst recording, slices with control ACSF (n=8) demonstrated LTP of 155% ± 11% of baseline and slices with MPEP ACSF (n=7) demonstrated LTP of 165% ± 15%. During 1-5 minutes after 1 Hz stimulation, slices with control ACSF demonstrated depotentiation at 116% ± 15% of baseline while slices with MPEP ACSF demonstrated depotentiation at 124% ± 8%. During 21-25 minutes after 1 Hz stimulation, slices with control ACSF demonstrated depotentiation at 126% ± 14% of baseline while slices with MPEP ACSF demonstrated depotentiation at 135% ± 9%. During 41-45 minutes after 1 Hz stimulation, slices with control ACSF demonstrated depotentiation at 146% ± 12% of baseline, while slices with MPEP ACSF demonstrated depotentiation at 129% ± 12%. None of these differences are statistically significant using a two-tailed, type three t-test ($p>0.05$).

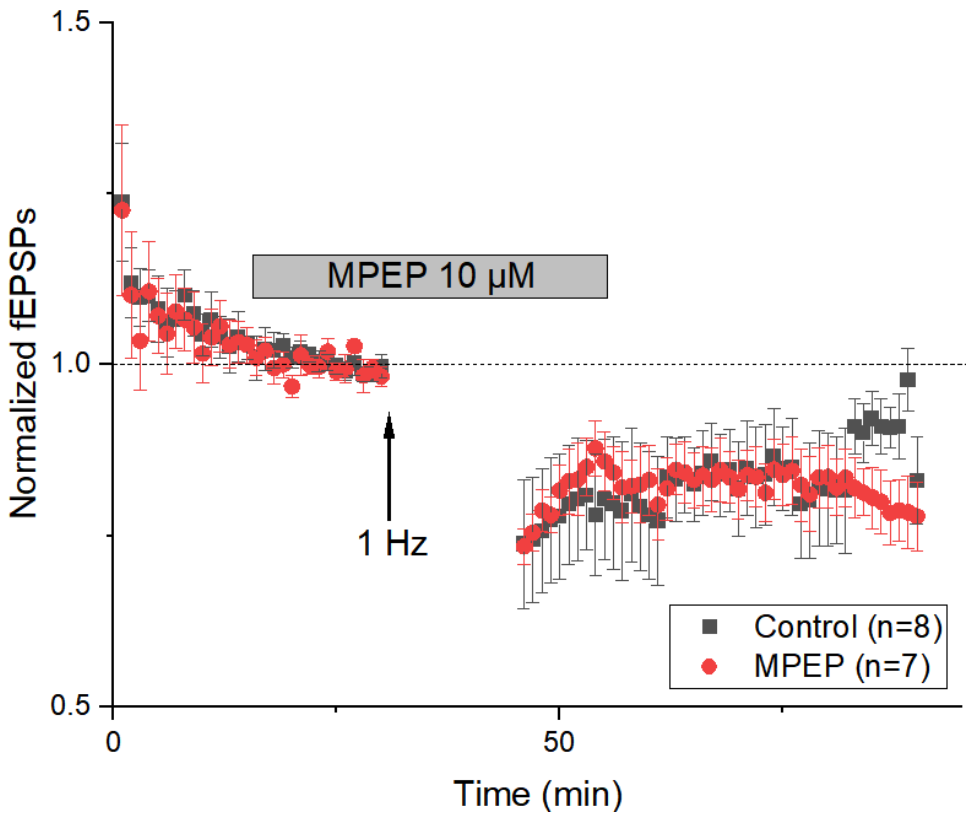

Figure 6: Hippocampal depotentiation in the CA1 in adolescent mice is mGluR5-independent. Field EPSPs were normalized to LTP rather than to baseline to allow for more accurate comparison of depotentiation. During 1-5 minutes after 1 Hz stimulation, slices with control ACSF (n=8) demonstrated 24% ± 9% depotentiation, while slices with MPEP ACSF (n=7) demonstrated 24% ± 3% depotentiation. During 21-25 minutes after 1 Hz stimulation, slices with control ACSF demonstrated 16% ± 6% depotentiation, while slices with MPEP ACSF demonstrated 17% ± 4% depotentiation. During 41-45 minutes after 1 Hz stimulation, slices with control ACSF demonstrated 8% ± 6% depotentiation, while slices with MPEP ACSF demonstrated 21% ± 5% depotentiation. Neither of these differences are statistically significant using a two-tailed, type three t-test ($p>0.05$)

Sex-Related Differences in Plasticity

To determine the role of sex in CA1 hippocampal depotentiation in mice, we separated our previous experiments by sex. We analyzed the magnitude of depotentiation normalized to baseline recordings and found significantly increased LTP in slices from female mice compared to male mice (Figure 7). When we analyzed the magnitude of depotentiation normalized to LTP recordings, we found that slices from female mice demonstrated more depotentiation than slices from male mice, suggesting that CA1 hippocampal depotentiation in juvenile mice is sex-dependent (Figure 8). Our data on adolescent mice is currently underpowered to perform statistical analysis (Figure 9, 10). We did not separate MPEP experiments or experiments in young adults by sex due to a low number of experiments in females in those groups.

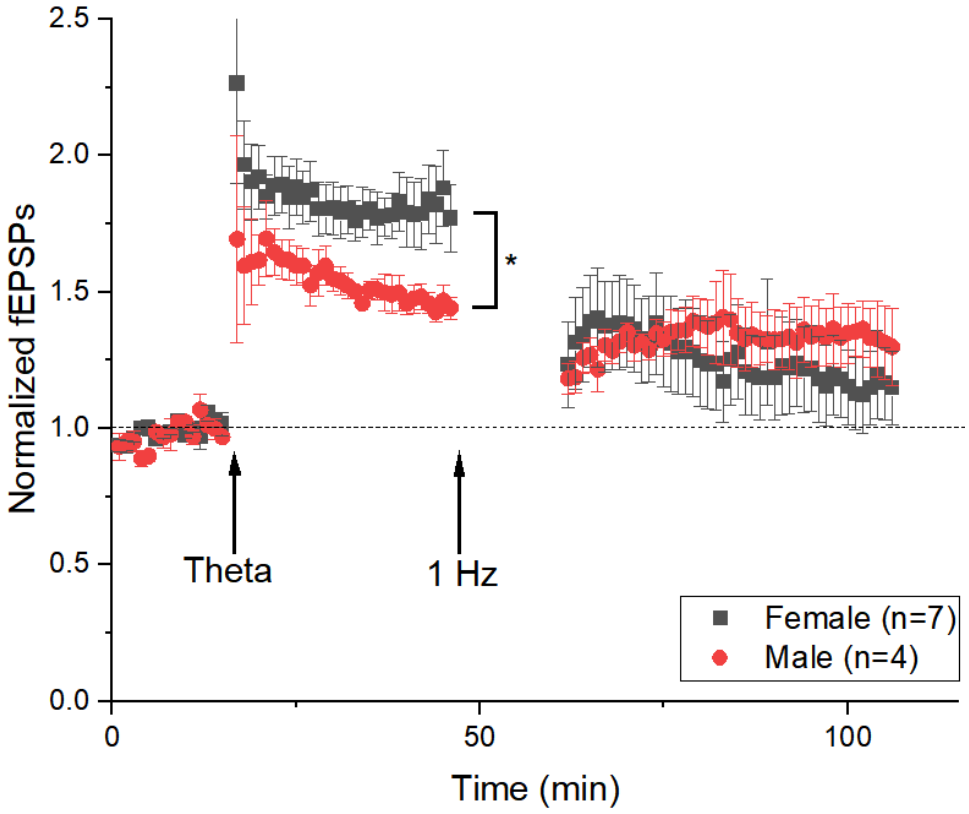

Figure 7: Hippocampal LTP in the CA1 is sex-dependent in juveniles. During the last 5 minutes of post-theta burst recording, slices from female mice (n=7) demonstrated LTP of 182% ± 13% of baseline, while slices from male mice (n=4) demonstrated LTP of 146% ± 4%. During 1-5 minutes after 1 Hz stimulation, slices from female mice demonstrated depotentiation at 134% ± 117% of baseline while slices from male mice demonstrated depotentiation at 122% ± 6%. During 21-25 minutes after 1 Hz stimulation, slices from female mice demonstrated depotentiation at 123% ± 16% of baseline while slices from male mice demonstrated depotentiation at 137% ± 14%. During 41-45 minutes after 1 Hz stimulation, slices from female mice demonstrated depotentiation at 116% ± 14% of baseline, while slices from male mice demonstrated continued potentiation at 133% ± 12%. The difference in LTP magnitude during the last 5 minutes

of post-theta recording is statistically significant using a two-tailed, type three t-test (p=0.032), but there were no significant differences in depotentiation.

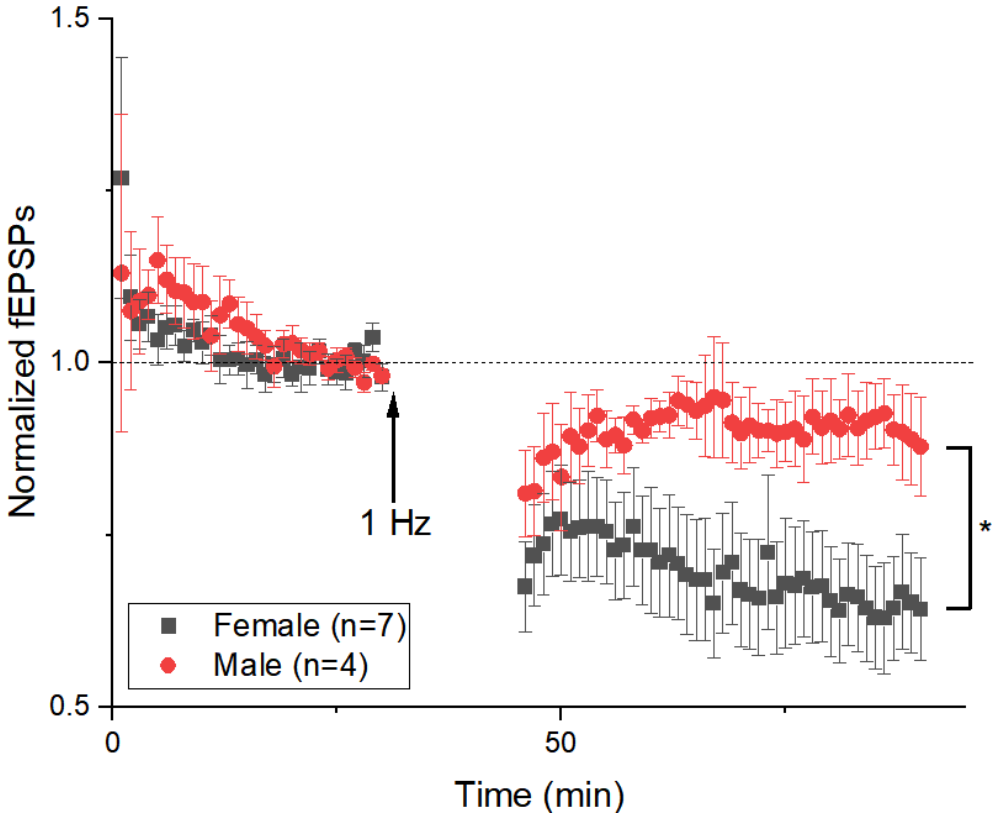

Figure 8: Hippocampal depotentiation in the CA1 is sex-dependent in juveniles. Field EPSPs were normalized to LTP rather than to baseline to allow for more accurate comparison of depotentiation. During 1-5 minutes after 1 Hz stimulation, slices from female mice (n=10) demonstrated 27% ± 7% depotentiation, while slices from male mice (n=7) demonstrated 15% ± 9% depotentiation. During 21-25 minutes after 1 Hz stimulation, slices from female mice demonstrated 32% ± 8% depotentiation, while slices from male mice demonstrated 6% ± 10% depotentiation. During 41-45 minutes after 1 Hz stimulation, slices from female mice demonstrated 35% ± 8% depotentiation, while slices from male mice demonstrated 7% ± 7% additional potentiation. Although the differences

in depotentiation during 1-5 minutes after 1 Hz stimulation and 21-25 minutes after 1 Hz stimulation are not statistically significant using a two-tailed, type three t-test ($p>0.05$), the difference in depotentiation during 41-45 minutes after 1Hz stimulation is statistically significant ($p=0.030$).

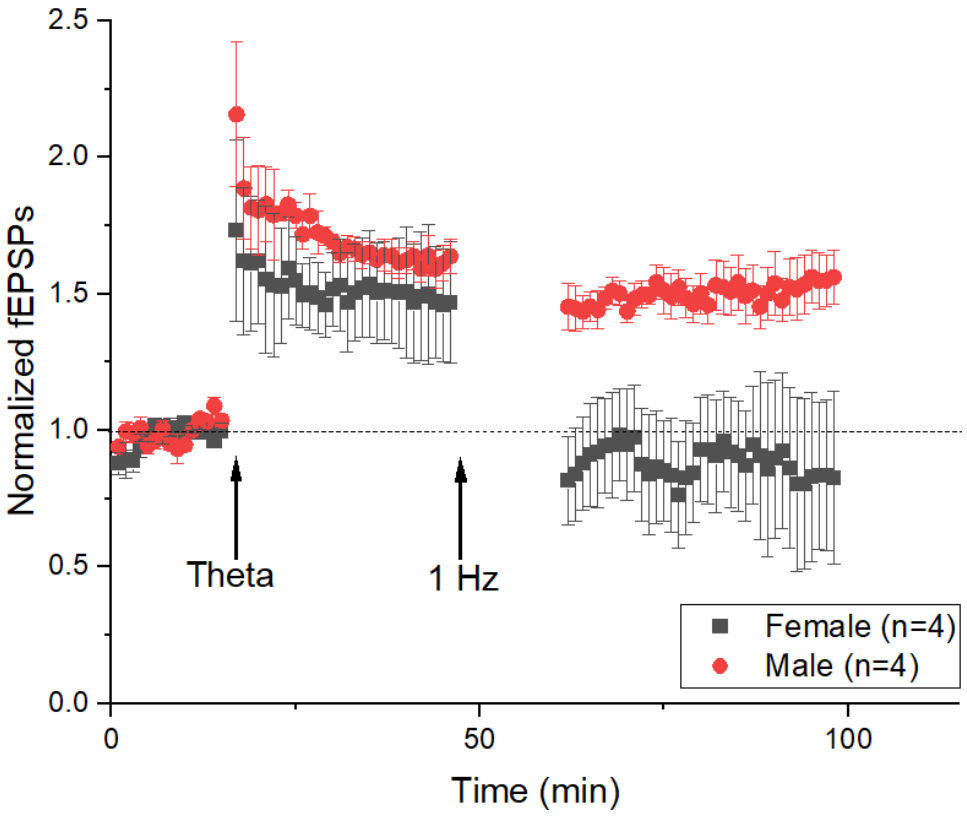

Figure 9: Hippocampal depotentiation in the CA1 in adolescents. During the last 5 minutes of post-theta burst recording, slices from female mice (n=4) demonstrated LTP of 148% ± 23% of baseline, while slices from male mice (n=4) demonstrated LTP of 161% ± 7%. During 1-5 minutes after 1 Hz stimulation, slices from female mice demonstrated depotentiation at 87% ± 19% of baseline while slices from male mice demonstrated depotentiation at 145% ± 9%. During 21-25 minutes after 1 Hz stimulation, slices from female mice demonstrated depotentiation at 91% ± 17% of baseline while

slices from male mice showed continued potentiation at 152% ± 3%. The data is underpowered to perform statistical analysis.

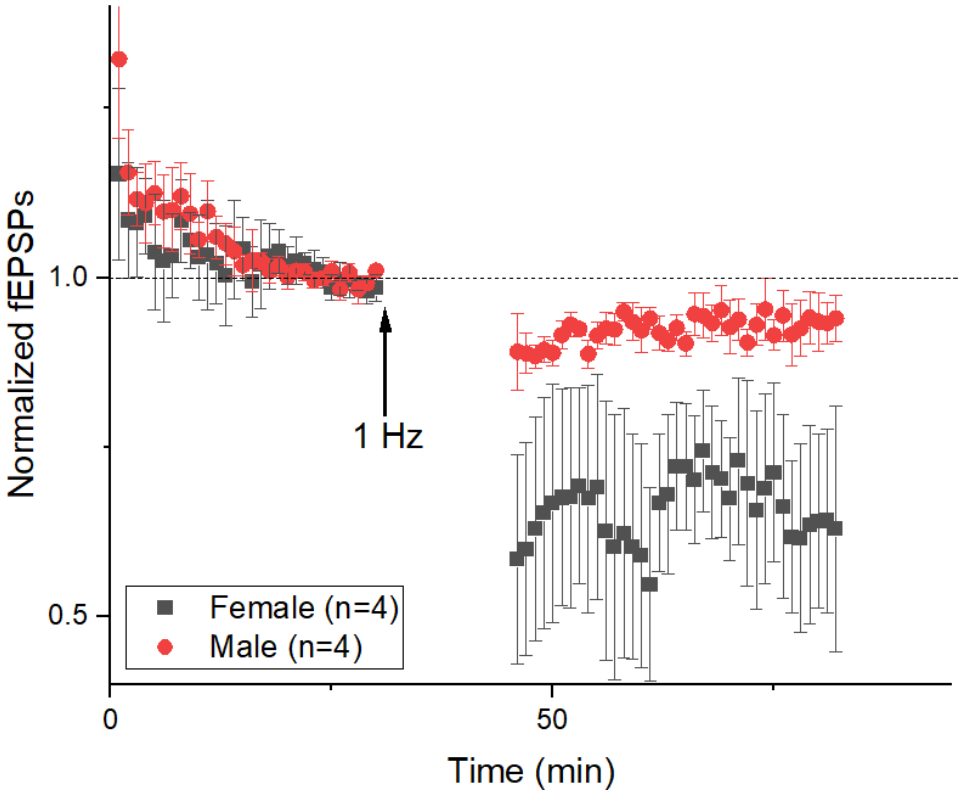

Figure 10: Hippocampal depotentiation in the CA1 in adolescents. Field EPSPs were normalized to LTP rather than to baseline to allow for more accurate comparison of depotentiation. During 1-5 minutes after 1 Hz stimulation, slices from female mice (n=4) demonstrated 37% ± 16% depotentiation, while slices from male mice (n=6) demonstrated 11% ± 2% depotentiation. During 21-25 minutes after 1 Hz stimulation, slices from female mice demonstrated 29% ± 11% depotentiation, while slices from male mice demonstrated 6% ± 4% additional potentiation. The data is underpowered to perform statistical analysis.

Discussion

We found that hippocampal depotentiation in the CA1 in mice is age-independent, mGluR5-independent, but sex-dependent in juveniles. This highlights the importance of using both male and female animals in plasticity research.

The finding that age has no significant effect on depotentiation is consistent with previous research suggesting that depotentiation is inducible in all age groups (Kamal et al., 1998; Norris et al., 1996; Wagner & Alger, 1995). Kamal et al. (1998) found that the magnitude of saturated depotentiation in 2 week old animals was significantly greater than in 36 week old animals. However, our research only uses animals aged 2 to 14 weeks, and we did not employ repeated low frequency stimulation to achieve saturated depotentiation, which Kamal et al. (1998) did. Our success in inducing depotentiation in animals older than 5 weeks may support the distinction between LTD and depotentiation on the basis of age, as Kemp et al. (2000) and Wagner & Alger (1995) failed to induce LTD using a 1 Hz stimulus in animals older than 6 weeks. However, others have observed LTD in animals as old as 6 to 24 months (Kamal et al., 1998; Norris et al., 1996), so further research is needed to clarify age-related differences in LTD and depotentiation. In any case, it is clear that the magnitude of depotentiation is not moderated by age.

Existing literature is conflicting on the role of mGluR5 in depotentiation. Zho et al. (2002) found that 10 μM MPEP application blocked DHPG-induced depotentiation. DHPG is a general agonist of group 1 mGluRs (mGluR1 and mGluR5), but MPEP is specific to mGluR5 (Zho et al., 2002). If DHPG-induced depotentiation by activating only mGluR1, depotentiation should not have been eliminated by MPEP, suggesting that

mGluR5 is essential to DHPG-induced depotentiation. Hu et al. (2005) confirmed the role of mGluR5 in depotentiation using a seizure model. MPEP was less effective at preventing LTP reversal than mGluR1 antagonists, but still significantly reduced depotentiation compared to controls (Hu et al., 2005). Qi et al. (2013) found that MPEP injection blocked depotentiation when animals were exploring a novel environment. However, our finding that low-frequency induced depotentiation is mGluR5-independent are supported by Latif-Hernandez et al. (2016), who also used low frequency stimulation (5 Hz) to induce depotentiation in acute slices and showed that 40 μM MPEP did not prevent depotentiation. Huang et al. (2001) also found that depotentiation induced by 2 Hz stimulation was mGluR-independent, using the general mGluR group I antagonist AIDA (500 μM).

It is possible that DHPG-induced, seizure-induced, and novelty-induced depotentiation use different mechanisms than depotentiation induced by low frequency stimulation. It is also possible that 1 Hz-induced depotentiation (which we used), 2 Hz-induced depotentiation (Huang et al., 2001), and 5 Hz-induced depotentiation (Latif-Hernandez et al., 2016) are mediated by different mechanisms. Experiments using low frequency electrical stimulation consistently suggest that depotentiation is mGluR5-indpendent, while experiments using other methods of depotentiation suggest that it is mGluR5-dependent. Further comparative studies are needed to clarify these differences.

A potential limitation of our research is the application of MPEP both before, during, and after the 1 Hz stimulus. Latif-Hernandez et al. (2016) used a similar protocol that involved drug application throughout and surrounding the stimulus window, but Neyman and Manahan-Vaughan (2008) found that the effects of mGluR5 antagonism

before low frequency stimulation on LTD were different than the effects of mGluR5 antagonism after low frequency stimulation.

Another potential limitation of our research is that the post theta recording for some experiments is longer than 30 minutes. One study found that depotentiation is more difficult to induce at greater intervals from the time of potentiation (Zho et al., 2002), so it is possible that experiments with different post theta recording times should not be combined. However, Zho et al. (2002) found no significant difference in depotentiation induced 10 minutes and 30 minutes after potentiation; the only significant differences were between 3 minutes and greater intervals. Additionally, other studies focused on larger time intervals have not found significant differences in depotentiation based on length of time potentiated (Hu et al., 2005; Park et al., 2019). These studies suggest that it is viable to include experiments with slight variation in time potentiated.

The finding that slices from females depotentiated more readily than slices from males is unique, largely due to the infrequent use of females in research and the lack of research on depotentiation. One study that measured LTD in male and female rats did not find a significant difference due to sex (Golitabari et al., 2022).

Conclusion

Using electrophysiology in hippocampal slices from young mice, we determined that depotentiation in the CA1 region is influenced by sex, but is not significantly influenced by age, and does not depend on the activity of mGluR5.

Future Directions

We plan to perform more MPEP experiments using female mice to determine if there are sex differences in the role of mGluR5 in hippocampal depotentiation. We also

plan to perform more control experiments using adolescent and young adult female mice to determine if the sex differences in depotentiation seen in juveniles are also present in adolescents and young adults. We also plan to perform experiments using the NMDA receptor antagonist D-AP5 to determine the role of NMDA receptors in hippocampal depotentiation in mice.

Methods

All mice were housed and handled as approved by Brigham Young University's Institutional Animal Care and Use Committee and the directions of the National Institute of Heath relative to experiments involving animals.

Slices were prepared using the standard procedure for Dr. Edwards' lab (Miller et al., 2019). Mice were anesthetized using isoflurane and euthanized using a rodent guillotine. The brain was removed and sliced coronally to 400 μm using a vibratome in holding solution at 0 °C. Slices were transferred to ACSF at room temperature where they were oxygenated for at least 60 minutes. ACSF concentrations were as follows: 2.5 mM $CaCl_2$, 2.5 mM KCl, 1 mM NaH_2PO_4, 1.3 mM $MgSO_4$, 119 mM NaCl, 26 mM $NaHCO_3$, 11 mM glucose. The final osmolarity of the ACSF was 296 mOsm.

Slices were placed individually in recording chambers filled with oxygenated ACSF, which was replaced at a rate of one to two mL/minute and held at an average temperature of 27 – 32 °C. A bipolar stainless steel stimulating electrode and a borosilicate glass recording electrode filled with 1 M NaCl were placed 500-600 μm apart in the stratum radiatum of the CA1 region of the hippocampus. Excitatory postsynaptic potentials (EPSPs) were evoked using 0.1 Hz stimulus of 100-200 μA. After establishing a 15 minute baseline, LTP was induced using a theta burst: two sets of 40 stimuli given at

5 Hz, at a current 50% greater than was used to record the baseline. After the theta burst, the current was reduced to the baseline level and EPSPs were recorded for 30 minutes. Depotentiation was then induced using a 1 Hz stimulus for 15 minutes, at the same current used for baseline and post-theta burst recording. EPSPs were recorded for 45 minutes following the 1 Hz stimulation.

Drug Application

For experiments involving MPEP, 0.24 mg of MPEP (purchased from Cayman) was dissolved in 100 μL of dimethyl sulfoxide and was added to 100 mL of oxygenated ACSF. This 10 μM MPEP solution was perfused into the well holding the slice for 15 minutes prior to the 1 Hz stimulus, as well as for the entire 1 Hz stimulus.

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