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# The Putative Cannabinoid Receptor GPR55 Modulates Synaptic Plasticity in the Hippocampus

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The Putative Cannabinoid Receptor GPR55 Modulates  
Synaptic Plasticity in the Hippocampus

Corinne Marie Badgley

A thesis submitted to the faculty of  
Brigham Young University  
in partial fulfillment of the requirements for the degree of  
Master of Science in Neuroscience

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## ABSTRACT

### The Putative Cannabinoid Receptor GPR55 Modulates Synaptic Plasticity in the Hippocampus

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Endocannabinoids (eCBs) are small molecules that are capable of modulating synaptic plasticity of both excitatory and inhibitory synapses in the brain. While eCBs bind to transient receptor potential vanilloid 1 (TRPV1) and cannabinoid receptor 1 (CB1) in the central nervous system, we recently identified a form of non-CB1, non-TRPV1 mediated long term depression activated by the eCB anandamide at CA1 hippocampal stratum radiatum interneurons. GPR55, an orphan G-protein receptor, has been identified in the hippocampus and is capable of activation by eCBs, making it a good candidate for mediating this non-CB1, non-TRPV1 form of synaptic plasticity. Here we performed whole-cell patch clamp recordings from CA1 stratum radiatum interneurons in rat brain slices to investigate the effect of GPR55 agonist O-1602 on excitatory synapses. We also performed field recordings from CA1 pyramidal cells in rats and GPR55 knockout mice and littermate controls to investigate the effect of GPR55 agonists O-1602 and lysophosphatidylinositol (LPI) on both basal output and electrically induced long-term depression and long-term potentiation in the hippocampus. Application of O-1602 in rats depressed long-term potentiation in CA1 pyramidal cells, and depressed excitatory glutamatergic transmission onto some interneurons. O-1602 had no effect on long-term depression of CA1 pyramidal cells. GPR55<sup>+/+</sup> mice showed an increase in long-term potentiation in the presence of LPI compared to GPR55<sup>-/-</sup> littermates. GPR55<sup>-/-</sup> mice had no change in long-term potentiation when exposed to O-1602, though there was an increase in post-tetanic potentiation with O-1602. In order to examine whether GPR55 has a role in formation of spatial memory, GPR55<sup>-/-</sup> mice were compared to littermate controls during a Morris water maze behavioral task, with a reversal task after 7 days of training. GPR55<sup>-/-</sup> mice did not perform in a different manner on either the training task or the reversal, though there may be a trend of difference in training worth investigating further. This study illustrates a novel pathway for synaptic plasticity modulation through GPR55 in the hippocampus, and may therefore provide valuable insight into both the effects of synthetic and endogenous cannabinoids on the brain and the processes underlying learning and memory.

Keywords: hippocampus, plasticity, GPR55, LPI, O-1602, long term potentiation

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## INTRODUCTION

The hippocampus is required for learning and memory in humans and other mammals and has vast interconnections with the limbic system, cortical association areas and the prefrontal cortex. It has been implicated in many processes, including consolidation of memory of recent events (Scoville and Milner, 2000), declarative memory (Squire, 1992), and encoding spatial and contextual information (Burgess et al., 2002). All of these events are thought to occur by the inherent synaptic plasticity observed between hippocampal neurons. The mechanisms behind these short-term changes in synaptic plasticity are thought to be related to long-term potentiation (LTP) (Bliss and Collingridge, 1993), and long-term depression (LTD) (Dudek and Bear, 1992), which increase and decrease synaptic strength respectively.

### *LTP and LTD in the Hippocampus:*

LTP depends on strengthening synapses and may be involved in encoding memories through increasing the number of active 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoic acid receptors (AMPA) postsynaptically, increasing the size of the synapse, or increasing the amount of presynaptic neurotransmitter release (Malenka and Bear, 2004). LTD in the hippocampus occurs by decreasing postsynaptic receptor density or decreased presynaptic release of neurotransmitter, and may be responsible for clearing old memory traces (Nicholls et al., 2008, Malleret et al., 2010). LTD is of key importance in synaptic plasticity because it allows the continuous alteration of synapses, allowing animals to alter behavior as new information is presented (Nicholls et al., 2008). The processes underlying synaptic plasticity in the hippocampus occur by activation of different presynaptic and postsynaptic receptor types.



Initial research on synaptic plasticity focused on N-methyl-D-aspartate receptor (NMDAR) mediated synaptic plasticity, but recently increased attention has been given to LTP and LTD gated or altered by endocannabinoid (eCB) receptors, as it became apparent that processes other than NMDAR-initiated mechanisms are capable of modulating synaptic plasticity.

#### *Cannabinoids and Plasticity:*

Cannabinoid receptors are sensitive to the exogenous psychoactive component of the marijuana plant *Cannabis sativa*,  $\Delta^9$ -tetrahydrocannabinol (THC). Recently, eCBs or endogenous cannabinoids have been implicated as the natural activators of these receptors. eCBs have been found to work as retrograde messengers within the hippocampus. This form of eCB-mediated synaptic plasticity has been observed in the hippocampus at both excitatory (Gerdeman and Lovinger, 2003) and inhibitory synapses. For example, eCBs have been found to depress inhibitory GABAergic cells presynaptically via cannabinoid receptor 1 (CB1) in a process termed “depolarization-induced suppression of inhibition” (DSI) (Kreitzer and Regehr, 2002). DSI can occur in response to activation from Schaffer collaterals of group I metabotropic glutamate receptor 5 (mGluR5) on CA1 pyramidal cells, which initiate production of eCBs that activate CB1 receptors of nearby cholecystokinin (CCK)-containing interneurons thereby inhibiting the release of GABA (Katona et al., 1999, Hoffman and Lupica, 2000). Another form of postsynaptic mGluR/eCB-dependent LTD was demonstrated to link excitatory neurotransmission and postsynaptic mGluRs with eCB mediated heterosynaptic depression of GABA release from cannabinoid-sensitive interneurons in the stratum radiatum of the hippocampus (Chevalyere and Castillo, 2003), which results in enhanced CA1 pyramidal cell

LTP (Chevaleyre and Castillo, 2004). This research indicates that eCBs are involved in modulating synaptic plasticity in the hippocampus by DSI, and suggest their potential contribution to the processes of learning and memory.

In addition, other somewhat contrasting research has indicated that eCB activation of CB1 can also initiate depression of CA1 LTP directly. Recently it has been demonstrated that both cannabinoid agonists and antagonists can depress LTP in acute slices, as well as impair spatial learning in *in vivo* rat experiments (Abush and Akirav, 2010). While these experiments show that eCBs have an effect on behavior, and especially for hippocampal spatial learning and memory, it has been difficult to draw direct relationships between specific ligand-binding and expected effects. This may be because the eCB system has diverse targets, with different eCB receptors initiating separate signal cascades within the hippocampus. Prior evidence shows that CBs can inhibit CA1 LTP, an effect that can be blocked by CB1 antagonist, however varying results have been gained with different agonist concentrations and stimulation methods (Davies et al., 2002), which also may imply that eCBs are effective to different targets at different concentrations. Thus far it can be concluded that eCBs modulate synaptic plasticity, but their mechanisms of actions and their downstream effects are less clear.

It has also been proposed that eCBs may activate a non-CB1 receptor to mediate acute depression at the CA3-CA1 excitatory synapse (Hajos and Freund, 2002, Rouach and Nicoll, 2003, Nemeth et al., 2008) and recently eCBs have been shown to induce LTD at interneurons in the stratum radiatum by an unknown non-CB1, non-transient receptor potential vanilloid 1 (TRPV1) receptor (Edwards et al., 2010). These findings indicate that eCB signaling is capable of affecting hippocampal output by unknown pathways or receptors. A candidate receptor initiating the eCB-mediated depression of CA1 interneurons has yet to be proposed.

### *G Protein-Coupled Receptor 55:*

A possible non-CB1 cannabinoid receptor candidate involved in hippocampal plasticity is G-protein receptor 55 (GPR55), an orphan G-protein coupled receptor with wide distribution throughout the brain that can be activated by THC, lysophosphatidylinositol (LPI), and most eCBs including anandamide, 2-arachidonylglycerol (2-AG), as well as CB1 antagonists AM251 and SR141716A (Sharir and Abood, 2010). It has been proposed as a novel cannabinoid receptor capable of functioning in the peripheral nervous system separate from CB1, and has been found to initiate a cascade that increases intracellular calcium in neurons of the dorsal root ganglion (Ryberg et al., 2007, Lauckner et al., 2008). GPR55 has been found in the rat hippocampus by in situ hybridization (Sawzdargo et al., 1999), and GPR55 mRNA has been reported in the mouse hippocampus (Ryberg et al., 2007) as well as in tissues outside the central nervous system. Its function in the hippocampus however, has not been examined. Its potential as an endocannabinoid target, as well as an initiator of internal calcium release makes GPR55 an intriguing contender as a non-CB1/non-TRPV1 modulator of synaptic plasticity. GPR55 has also been found in several other tissues, and has been proposed as a promoter of cancer cell proliferation (Andradas et al., 2011), a regulator of osteoclast number and function (Whyte et al., 2009), and as a regulator of inflammatory and neuropathic pain (Staton et al., 2008). The downstream effects and signaling mechanisms have been reported to vary with both ligand binding and tissue specificity, which may allow this receptor to mediate various processes within the body (Henstridge et al., 2010), including altering signaling in the hippocampus.

### *Behavioral Effects of Cannabinoids:*

The effects of cannabinoids such as THC on behavior are well documented, and include alterations in memory and cognitive ability (Chaperon and Thiebot, 1999). Altered behaviors induced by cannabinoids are manifestations of synaptic plasticity in the brain (Lupica et al., 2004). eCBs anandamide and 2-AG are present in the hippocampus in high concentrations, and their effects are not fully explained by activation of CB1 alone (Di Marzo et al., 2000). If GPR55 is an eCB target capable of modulating synaptic plasticity shown through electrophysiology, it may also play a role in altering behavior. In order to investigate the role of GPR55 in hippocampal function through behavior, the Morris water maze (Brandeis et al., 1989) has been employed. The goal of this behavioral component to our research is to examine whether there is a link between alterations in electrically induced synaptic plasticity via GPR55 and learning and memory in mice. The involvement of GPR55 was examined by comparing the performance (quantified in latency times) on the maze of GPR55<sup>-/-</sup> mice to GPR55<sup>+/+</sup> mice.

The widespread expression of GPR55 makes discovering its function and mechanism of action within the hippocampus pressing research, as it may modulate learning and memory systems in mammals in addition to having applications in other fields of research. Here, we find evidence supporting GPR55 as a potential eCB target capable of modulating hippocampal synaptic plasticity and neurotransmission. Our research indicates depression of transmission at some, but not all CA3-CA1 interneurons. This study also indicates that GPR55 modulates synaptic plasticity by depressing CA1 LTP, supporting prior research showing that eCBs may initiate different effects on hippocampal function. Further investigation will need to be done in

order to comment definitively on whether GPR55<sup>-/-</sup> mice have different capabilities in spatial memory compared to GPR55<sup>+/+</sup> mice.

## MATERIALS AND METHODS

### *Preparation of Brain Slices:*

Male Sprague-Dawley rats aged P15-27 (Charles River) and male GPR55 knockout and wild type mice P16-32 (University of North Carolina via the Mutant Mouse Regional Resource Center) were used in these studies. Animals were housed in approved conditions with a 12 h light-dark cycle. The experiments were conducted in accordance with the Brigham Young University Institutional Animal Care and Use Committee standards and National Institute of Health guidelines to minimize pain and suffering of animals. All animals were deeply anesthetized with isoflurane using a vapomatic chamber and decapitated, following which their brains were removed rapidly and placed in ice-cold, oxygenated medium containing (in mM): NaCl, 119; NaHCO<sub>3</sub>, 26; KCl, 2.5; NaH<sub>2</sub>PO<sub>4</sub>, 1.0; CaCl<sub>2</sub>, 2.5; MgSO<sub>4</sub>, 0.6; glucose, 11; saturated with 95% O<sub>2</sub>, 5% CO<sub>2</sub> (pH 7.4). The posterior aspect of the brain was glued with cyanoacrylate adhesive to the cutting stage of a vibratome after which 400  $\mu$ m coronal slices were cut and then transferred to a holding chamber containing oxygenated artificial cerebrospinal fluid (ACSF) at room temperature. This cutting technique maintains the integrity of the hippocampal circuitry.

### *Slice Electrophysiology:*

Following an interval of at least 1 h, slices were transferred to a submerged recording chamber and perfused with oxygenated ACSF medium of the same composition as that in the holding chamber at a temperature between 28-32°C. Slices were continuously perfused with ACSF at a flow rate of 2-3 ml/min. A bipolar stainless steel stimulating electrode was placed in the stratum radiatum, at least 400-700  $\mu$ m from the recording electrode to stimulate CA3

glutamatergic afferents of the Schaffer Collateral pathway at 10-50  $\mu$ A for 100  $\mu$ sec at 0.1Hz sampling rate. Recordings were performed in current clamp mode to measure excitatory postsynaptic potentials using an Axopatch 200B amplifier. Data was filtered at 4 kHz and acquired with an axon 1440A digitizer (Molecular Devices) and inputted onto a Dell personal computer (OptiPlex 745) with pClamp10.2 clampex software (Molecular Devices). Stimulation intensity was adjusted to elicit an EPSP of 0.5 mV at the beginning of each experiment. Borosilicate glass patch pipettes (2-3 M $\Omega$ ) were filled with 1 M NaCl for field recording electrodes. EPSPs were evoked and monitored for at least 60 minutes.

Whole-cell recordings were performed in voltage clamp at -65 mV using a multiclamp 700B amplifier (Molecular Devices) and EPSCs are evoked by two pulses separated by 50msec. Borosilicate glass patch pipettes (2-4 M $\Omega$ ) were filled with Cs<sup>+</sup>-gluconate based internal solutions containing (in mM): NaCl, 2; MgCl<sub>2</sub>, 5; HEPES, 20; ATP, 2; GTP, 0.3 and EGTA, 0.6. AMPAR-mediated currents were measured while blocking GABA<sub>A</sub> receptors with bicuculline or picrotoxin (10 $\mu$ M). Stable baseline recordings of AMPAR-mediated currents were obtained at the frequency of 0.1 Hz.

#### *Morris Water Maze:*

The water maze consisted of a large, circular pool (1.67 m in diameter, .4 m in height). A clear platform (7.6 cm in diameter) was placed inside, and the tank was filled with water (22°C) until the top of the platform was submerged by 1 cm. A sufficient amount of powdered skim milk (Augason Farms) was added to make the water opaque and conceal the platform. Four sheets of paper with black and white geometric designs served as visual cues and were attached to four areas of camera scaffolding around the pool. Swim paths were recorded by digital

overhead camera (HeroHD) for each subject, and escape latencies (the time between being placed in the water and finding the platform) were recorded using a stopwatch.

Pre-training acclimation sessions consisting of free swim in the pool for 5 min without the platform were performed the day before acquisition trials began. Acquisition trials occurred over seven consecutive days of four trials per day. The position of the platform remained fixed for all trials for all mice. Four points along the periphery of the pool served as trial start points designated North, South, East, and West, where the mice would be placed facing the wall of the pool. Each starting point was used once per session, with order determined arbitrarily by the tester. Once the platform had been located, the mouse was allowed to stay for 30 s before removal. If a subject failed to locate the platform in less than 120 s, it was manually guided. After the initial seven acquisition sessions, mice were subjected to a reversal test in which the platform was moved to the opposite side of the pool. All other task parameters remained the same.

#### *Analysis:*

For field recordings, the maximal initial value of EPSPs slopes was calculated using pClamp10.2 clampfit software (Molecular Devices). EPSPs measured every 10 s were averaged in 1 minute intervals. Values were normalized to control slope values 5 minutes immediately prior to high frequency stimulus, and subjected to t-tests (paired, two-tailed,  $p < 0.05$ ). An increase in EPSP slope that persisted for longer than 60 min indicated that LTP had been induced. EPSP normalized slope values were compared for significance (unpaired, two tailed t-test) 20-25 min post high frequency stimulus between rat or mouse brain slices treated or not treated with O1602. Only one experiment was performed per slice and the reported n is the



number of slices not the number of animals. Microsoft Excel and Origin (North Hampton, MA) were used to organize, average, graph and to perform statistical analysis on the data.

For whole cell recordings, methods were similar to field recordings, but the EPSC amplitude was determined by measuring against a 10 ms baseline prior to evoking EPSC. The average pre-theta burst AMPAR current from a period of 5 minutes was then compared to a 5-minute period 15-20 minutes post-theta burst to determine statistical significance, using a t test ( $p < 0.05$ ). Theta burst was used to mimic more natural hippocampal activation patterns. Two bursts were given 20 seconds apart. The cell input resistance was monitored throughout the experiment and if changed by more than 10%, the cell was discarded. Interneurons were distinguished from pyramidal cells visually, electrophysiologically and by their higher input resistance.

For statistics of the Morris water maze task, a life regression was conducted to assess the effects of genotype and sessions/trials. Life regression analysis was used in order to account for trials where mice did not find the platform in under 120 sec. These trials are counted as a time above 120 sec, and confidence intervals are used to arrive at predicted median escape latency. An interaction of gender was also tested since both male and female subjects were used.

#### *Materials:*

All salts were purchased from Sigma-Aldrich, Mallinkrodt-Baker or Fisher Scientific. Bicuculline was purchased from Ascent Scientific. Picrotoxin and Lysophosphatidylinositol were purchased from Sigma-Aldrich. O-1602 was purchased from Tocris, Abcam and Ascent Scientific.

## RESULTS

GPR55 is a strong candidate for a novel form of synaptic plasticity that does not occur through the expected CB1 mediated pathway because of its ability to bind eCBs and its potential to activate different signal cascades (Sharir and Abood, 2010). In addition, a function for GPR55 in the hippocampus has not yet been postulated, though its binding has been linked to several different signaling pathways in other regions indicating that it could play a role in modulating synaptic plasticity by a novel mechanism. Therefore, we first wanted to confirm GPR55 receptor expression in the hippocampus. Using quantitative real-time PCR (RT-PCR) we identified the expression of GPR55 in rat hippocampal homogenate (Fig. 1). The GPR55 cDNA from hippocampal homogenates was amplified in a dose dependent manner, demonstrating the presence of GPR55 in increasing cycle number as expected. After amplification, GPR55 cDNA was run out on a 4% agarose gel to indicate the appropriate amplicon size (Fig. 1 inset). From these data we can corroborate that GPR55 is present in rat hippocampus.

We were specifically focused on the CA3-CA1 interneuron synapse as one location to examine eCB modulation because we recently identified a non-CB1/TRPV1 receptor-mediated depression at this synapse in response to anandamide, indicating that an additional eCB receptor is present there (Edwards et al., 2010). In order to examine GPR55 expression at the CA3-CA1 interneuron synapse, whole cell patch clamp recordings were performed on CA1 interneurons with GPR55 agonist O-1602 (5 $\mu$ M). O-1602 caused a slight non-significant ( $p>0.05$ ) depression at 5 $\mu$ M (Fig. 2A), however some individual cells were significantly depressed (Fig. 2B). As a control we examined CA1 pyramidal cells by application of O-1602 (3 $\mu$ M), which did not depress fEPSPs in CA1 pyramidal cells in any experiments (Fig. 2C).

Next, in the process of investigating the role of GPR55 in synaptic plasticity, we wanted to investigate whether LTP was altered when induced using theta-burst protocol in the presence of GPR55 agonist. This was done while recording field excitatory postsynaptic potentials (fEPSPs) from rat hippocampal CA1 pyramidal cells in the stratum radiatum. We confirmed that O-1602 (1  $\mu$ M) application resulted in significantly ( $p < 0.05$ ) reduced LTP when compared to EtOH vehicle control (Fig. 3A). We next examined the effect of O-1602 on LTD, and whether LTD was altered using 1 Hz stimulation in the presence and absence of O-1602 (1 $\mu$ M). We did not observe a significant ( $p > 0.05$ ) difference between trials run with O-1602 and those run with EtOH vehicle control (Fig. 3B).

While the O-1602 data is suggestive that GPR55 is involved in modulating hippocampal plasticity, a GPR55 antagonist is needed to confirm this finding. However, just as there are no consistently reported agonists of GPR55, there are no uniformly accepted antagonists (Sharir and Abood, 2010). Agents cannabidiol and O-1819 have both been shown to inhibit GPR55 in some but not all cases, and their effects on other receptors make them less than ideal negative controls. Therefore, the most reliable negative control for GPR55 experiments is the use of the transgenic GPR55<sup>-/-</sup> mouse.

While using GPR55 littermate and knock-out mice we also chose to broaden our use of agonists to LPI in order to increase our certainty and specificity of activating GPR55, as LPI is an endogenous ligand of GPR55 whereas O-1602 is a manufactured abnormal cannabinoid agonist. It was important to examine the effects of both agonists on GPR55<sup>-/-</sup> mice and their GPR55<sup>+/+</sup> littermates to isolate the effects of these drugs to GPR55 as opposed to other targets. We repeated theta burst induction of CA1 LTP using LPI as a GPR55 agonist in GPR55<sup>-/-</sup> mice and their GPR55<sup>+/+</sup> littermates. Interestingly and in contrast to O-1602 data, LPI caused a

significantly ( $p < 0.05$ ) enhanced LTP in the GPR55<sup>+/+</sup> mice as compared to GPR55<sup>-/-</sup> mice (Fig. 4A). LPI (2 $\mu$ M) also caused an enhancement of LTP in GPR55<sup>+/+</sup> mice compared to EtOH controls. While this increase was not significant ( $p > 0.05$ ) LPI caused a substantial  $14 \pm 3\%$  in LTP compared to EtOH controls (Fig. 4B). In contrast, LPI did not cause any enhancement of LTP ( $.07 \pm 3\%$ ) compared to EtOH controls (Fig. 4C) in GPR55<sup>-/-</sup> mice.

The contrasting results of our O-1602 data raised the question of whether O-1602 was acting specifically on GPR55 to decrease LTP in the rat model. If a receptor other than GPR55 was mediating the observed reduction in LTP, we would continue to see a reduction of LTP in GPR55<sup>-/-</sup> mice. In order to examine this possibility, we induced LTP with a theta burst protocol in the presence of O-1602 (3  $\mu$ M) or EtOH controls using GPR55<sup>-/-</sup> mice (Fig. 5). There was not a significant difference ( $p > 0.05$ ) in LTP induced with and without O-1602 in GPR55<sup>-/-</sup> mice, though some difference was observed between the post-tetanic potentiation (PTP) with O-1602 treated slices, which have a greater PTP than EtOH controls ( $16 \pm 5\%$ ). Since O-1602 had no other effect in GPR55<sup>-/-</sup> mice, it suggests that reduction of LTP in the rat is due to O-1602 activation of GPR55, a result that may be indicative of physiological or functional differences between GPR55 in rat and mouse models.

There are no reports of behavioral testing for spatial memory of GPR55<sup>-/-</sup> and GPR55<sup>+/+</sup> mice, but no differences have been observed in their phenotype or motor skills. Before data collection, mice were observed during the 5 min pretraining session to detect any phenotypic differences in their initial reactions to being placed in water. At the beginning of each session both genotypes were placed into the water facing the pool wall from one of four start points designated North, South, East, and West, and immediately approached the sides of the pool. Initially all mice swam around the perimeter of the pool and spent progressively less time there

as the pretraining session continued. Two of the GPR55<sup>-/-</sup> (29%) and two of the GPR55<sup>+/+</sup> (29%) mice had more labored swimming, characterized by rapid, jerky movements. These mice improved over subsequent training sessions, but were still less likely to stay on the platform and have smooth movements compared to other mice. No other health or motor deficits were observed. At the time of the 5-min pretraining session mice ranged in age from 1.5 to 2 months. The training session consisted of 7 consecutive days where mice searched for the stationary platform four times from four different start points, and two groups were compared to see if GPR55 played a role in learning spatial navigation. Life regression revealed significant decreases in escape latencies ( $p < 0.05$ ) across training sessions for both WT and KO mice (Fig. 6A, B), indicating that both groups were learning the location of the platform over time. Although no significant differences were detected between genotypes ( $p = 0.13$ ), there was a trend towards GPR55<sup>-/-</sup> mice having increased escape latencies. This behavior indicates a possible impediment in navigational learning for GPR55<sup>-/-</sup> mice, although more experiments will need to be done to determine this. For the probe trial, the platform was moved to the opposite side of the pool, and the two genotypes were compared by trial. Figure 6C depicts the results of the reversal test in which the platform was placed on the opposite side of the pool. Escape latencies were not significantly affected by genotype ( $p > 0.05$ ). This result indicates that there is no difference between the GPR55<sup>-/-</sup> and GPR55<sup>+/+</sup> mice in ability to replace the previously learned platform location.

## DISCUSSION

This study is the first to examine the function of GPR55 in the hippocampus as a mediator of non-CB1/non-TRPV1 eCB-mediated synaptic plasticity. Specifically, our observations confirm that GPR55 is present in the hippocampus, suggest that LPI enhances LTP in mice via GPR55 and that O-1602 depresses LTP in rats through a mechanism that is likely independent of GPR55. eCB mediated modulation of synaptic plasticity in the hippocampus is a burgeoning area of research, and more data must be collected in order to create a clearer image of the role of GPR55 in the hippocampus and standardize methods for investigating its effects in the central nervous system.

In previous research, our lab had identified a form of eCB mediated, non-TRPV1/non-CB1 depression of CA1 interneurons. We chose to target GPR55 as a candidate for this effect because of its ability to bind eCBs such as anandamide, its presence in the hippocampus confirmed in our lab by RT-PCR (Fig. 1), and its downstream effectors that have the potential to alter neuronal plasticity by release of intracellular calcium. While other GPCRs such as GPR119 are also good candidates for eCB mediated changes in neurons, GPR55 is comparatively well-characterized and had been reported active in the nervous system, making it a natural first target. GPR119 has yet to be found active in the nervous system, and is predominately expressed in the gastrointestinal system as a mediator of insulin secretion, though our lab has confirmed its expression in rat hippocampal homogenate. GPR55 had been the target of research as a putative third cannabinoid receptor, capable of mediating physiological changes in cells that cannot be from CB1, CB2, or TRP receptors. No research has been published to date about the function of hippocampal GPR55, and thus there is no precedent for our research.

*LPI increases LTP via GPR55 in the mouse model:*

Agonists and antagonists of GPR55 are not well agreed upon, so to test whether GPR55 is capable of modulating synaptic plasticity, we needed a reliable negative control. We found that the most reliable negative controls available are GPR55<sup>-/-</sup> mice. We decided to use lysophosphatidylinositol (LPI) as an agonist, as it is arguably the most potent activator of GPR55 identified thus far for our transgenic mouse experiments. LPI is found in high concentrations in the brain, and as a lipid breakdown product could be a downstream effector of other agonists to activate GPR55. It has also been shown to activate GPR119, so it is not a GPR55 specific agonist, highlighting the importance of using GPR<sup>-/-</sup> mice as negative controls.

In GPR<sup>+/+</sup> littermate controls we observed a significant increase in LTP in the presence of LPI compared to LTP in GPR<sup>-/-</sup> mice (Fig. 4A). Increased LTP was not present in GPR<sup>-/-</sup> mice, confirming that GPR55 modulates this increase in LTP, and that its activation is sufficient to increase hippocampal LTP in transgenic mice (Fig. 4C). Since we had previously confirmed GPR55 in rat hippocampus, and had observed a novel form of eCB-mediated depression in rat CA1 interneurons, we wanted to examine whether GPR55 agonist would produce the same GPR55 mediated increase in LTP observed in GPR55<sup>+/+</sup> mice in a rat model.

*O-1602 initiates synaptic plasticity of CA1 interneurons and CA1 pyramidal cells in rat:*

As stated before, the agonists of GPR55 are hotly debated, and those that are generally agreed upon are not exclusive for GPR55. We set out to see whether these agonists could potentially mediate the depression at CA1 interneurons that we had originally observed in the presence of R-methanandamide in rats, and potentially be the effector of the increased CA1 LTP we had observed in transgenic mice. We decided to use O-1602, an abnormal cannabinoid that

has been reported as a potent agonist of GPR55 in some reports, though others have found it to be inactive in different models.

We first wanted to see if O-1602 modulated CA1 pyramidal cell synaptic plasticity induced by theta burst protocol to induce LTP as we had observed previously in GPR55<sup>+/+</sup> mice. We found that in the presence of O-1602, LTP in acute rat brain slices was reduced (Fig. 3A). These results may corroborate with the fact that hippocampal eCB receptors can cause reduction in CA1 LTP (Davies et al., 2002, Abush and Akirav, 2010). This decrease in LTP is opposite of what we had observed in the transgenic mice, indicating that O-1602 could be acting through other receptors to modulate synaptic plasticity. Although we observed a decrease in LTP induced in the presence of this putative GPR55 agonist, no effect was observed when LTD was induced in the presence of O-1602 (Fig. 3B). This result indicates that the receptors binding O-1602 do not modulate LTD induced by 1 Hz stimulation. This would suggest that the cascade initiated by GPR55 activation, or at least receptors that bind O-1602 do not affect the processes underlying LTD. It may be that CA1 LTP is necessary to observe a change mediated by targets of O-1602. A decrease in LTP could be attributed to a direct effect on CA1 pyramidal cells possibly modulating intracellular cascades, or an increase in GABA inhibition from interneurons, therefore we examined whole cell recordings from CA1 interneurons to see if O-1602 application caused a change in current.

Whole-cell patch clamp recordings of rat CA1 interneurons showed depression in response to O-1602 in some cases though not in others (Fig. 2A, B). This could be due to the heterogeneity of CA1 interneurons. It would be beneficial to pull cells for quantitative RT-PCR that have been recorded from in order to classify whether O-1602 causes depression in specific interneuron subtypes. If GPR55 is in fact active at some CA1 interneuron subtypes it may reduce



output by decreasing presynaptic CA3 glutamate release as suggested by our paired pulse ratio data.

In order to test whether O-1602 is acting specifically through GPR55, we used GPR55<sup>-/-</sup> mice as an indicator of GPR55 activity. There was no difference in LTP of GPR55<sup>-/-</sup> acute mouse slices that were treated with O-1602 or EtOH control ACSF, however, there does appear to be a difference in the post-tetanic potentiation (PTP), or the plasticity induced directly after the theta burst protocol (Fig. 5). In the presence of O-1602, there is an increased PTP, which could be interpreted to mean that O-1602 does activate a receptor other than GPR55 that is involved in PTP, but not the long-term changes of LTP. It is possible that more than one receptor works in concert in response to O-1602 to cause disinhibition. There is also the possibility that rats and mice express these receptors in different levels with different effectors, and that their activation initiates different outcomes. Past research has revealed significant differences in the behavioral, pharmacological, and molecular structures of mice hippocampus compared to rat hippocampus (McNamara et al., 1996). It is important to consider such differences when attempting to replicate or build upon data across species.

#### *Spatial memory in GPR55<sup>+/+</sup> and GPR55<sup>-/-</sup> mice:*

The hippocampus is known to function in the processes of learning and memory, and more specifically area CA1 functions in storing spatial memory. GPR<sup>+/+</sup> and GPR<sup>-/-</sup> mice have not been found to differ in phenotype or in motor skills thus far, but this is the first study investigating their potential differences in spatial memory. While both GPR<sup>+/+</sup> and GPR<sup>-/-</sup> mice had a significant improvement in escape latency over each session, there were no significant differences between genotypes (Fig. 6A, B). However, it might be argued that there is a

meaningful difference of increased latency times of GPR55<sup>-/-</sup> mice that indicate an increase in sample sizes for both groups may be worthwhile to confirm a significant difference between performance of GPR<sup>+/+</sup> and GPR<sup>-/-</sup> mice. An increased latency time in GPR55<sup>-/-</sup> mice would corroborate our results that GPR55<sup>-/-</sup> mice had reduced LTP compared to their littermate controls, and indicate that GPR55 is normally involved in spatial navigation tasks. An issue with this data set is the large number of trials per session in which mice failed to reach the platform under the 120 sec time limit. This further reduces the amount of data we have reflecting the time it takes each genotype to find the platform, and could potentially be solved by using a smaller pool or larger platform. We controlled for this effect by using a life regression to analyze this data, in order to take into account the trials that can only be accurately described as over 120 sec.

#### *Is GPR55 a cannabinoid receptor?*

As research into GPR55 is relatively new, a complicating issue in observing its effects is the disagreement that exists over what its agonists and antagonists are, and whether they are the same in all cell types, as few results have been replicated. Our results show that GPR55 is capable of modulating synaptic plasticity as shown by our use of transgenic knockout mice. These mice are extremely important to specific research into GPR55 until more specific agonists and antagonists can be developed. O-1602 is also a modulator of synaptic plasticity in the hippocampus, but its specific targets are not well defined (Schicho et al., 2011). O-1602 is an abnormal cannabinoid that binds GPR55 (Johns et al., 2007) and the abnormal cannabidiol (abn-CBD) receptor (McHugh et al., 2010). O-1602 is also a CB1 agonist, and CB1 downstream effects could cause depression, an effect that could be tested by the use of GPR55<sup>-/-</sup>. However,

for this investigation we attempted to control for activation of CB1 by using concentrations of O-1602 well below the 30 $\mu$ M concentration at which CB1 is activated.

We also used LPI as an agonist to assist in clarifying the specificity of these agonists for GPR55. LPI is arguably the most well defined GPR55 agonist that does not activate CB1 (Anavi-Goffer et al., 2012). LPI is a biologically active lysophospholipid that is produced from phosphatidylinositide hydrolysis. It has been found in large quantities in the brain (Oka et al., 2009), and has been linked to inducing ERK  $\frac{1}{2}$  phosphorylation to release intracellular calcium (Oka et al., 2007). In vivo, it has been shown to have neuroprotective properties in a model of transient global ischemia, specifically in CA1 pyramidal cells, even when given 30 min after the ischemic insult (Blondeau et al., 2002). This neuroprotective effect could be due to non-receptor mediated effects such as activating 2-pore domain K<sup>+</sup> channels TREK-1 and TRAAK (Maingret et al., 2000) which would tend to reduce calcium influx through voltage dependent calcium channels and NMDAR. These channels are important for regulation of membrane potential in neurons, and could cause hyperpolarization that could influence our results. However hyperpolarization of neurons would have resulted in a decrease in LTP, rather than the enhanced LTP that we observed with LPI, making it an unlikely influence on our data. Very recently, functional LPI was reported in the hippocampus stimulating [<sup>35</sup>S]GTP $\gamma$ S binding in the rat hippocampus at 10 $\mu$ M (Rojo et al., 2012), confirming that it does activate GPCRs in the hippocampus, but its specific targets are variable. While these studies indicate that it would be a good choice as a GPR55 agonist in the hippocampus, LPI is also linked to other GPCRs, including GPR119.

While GPR55 has been activated or silenced by several synthetic cannabinoids and eCBs, the fact that LPI is the most consistent signaling molecule for GPR55 (Piñeiro and Falasca, 2012)

raises the question of its endogenous signalling molecules, and whether it binds eCBs, lipid products, or both. So while both agonists used in this study are reliable for activating GPR55, neither is perfect in its specificity. More research will need to be done to establish a well-defined protocol for examining GPR55 in the central nervous system, though by using transgenic mice in this project we were able to confirm that GPR55 is capable of modulating plasticity in the hippocampus.

*Significance:*

The hippocampus is responsible for the tasks of learning and encoding memory. These processes are not completely understood, since they are extremely complex and multi-faceted. Synaptic plasticity, or the strengthening or weakening of synaptic connections mediates memory and learning within the hippocampus. We found that GPR55 is capable of modulating hippocampal synaptic plasticity, specifically that its activation is capable of increasing CA1 LTP, a process shown to underlie efficient memory formation. Our findings were vital because of the great interest we have in preventing both the natural decline of memory that occurs with old age, and accelerated memory loss observed in neurodegenerative disease. This research may assist us in better understanding mechanisms of memory formation, thereby opening new channels of research in therapy development for devastating disorders like Alzheimer's and dementia. It is also important to highlight the importance of using GPR55<sup>-/-</sup> mice to better isolate the effect of GPR55, and to consider the other effectors of drugs like O-1602 and LPI in order to better understand synaptic plasticity in the hippocampus.

## FUTURE DIRECTIONS

As stated previously, GPR55<sup>-/-</sup> mice are necessary to further establish and characterize GPR55 in the hippocampus. It would be of interest to examine whether LPI induces change in basal transmission on CA1 interneurons directly using whole cell patch clamp on transgenic mice. If LPI alters current on GPR55<sup>+/+</sup> mice, and not GPR55<sup>-/-</sup> mice, we will further establish that LPI is activating GPR55 specifically, and obtain more information about the functional location of GPR55 in the hippocampus.

If GPR55 does mediate the depression we observed previously in CA1 interneurons, it could imply that the increased CA1 LTP we observed is a result of disinhibition, the process by which CB1 and mGluR receptors can both mediate eCB induced increases in CA1 LTP by reducing the output of inhibitory interneurons on excitatory CA1 pyramidal cells (Kreitzer and Regehr, 2002). In order to investigate this possibility, these experiments should be repeated in the presence of bicuculline, a GABA<sub>A</sub> antagonist that will isolate the CA3-CA1 excitatory pyramidal cell synapse. If an increase in LTP is still present with bicuculline in the ACSF, we will be able to establish that the observed enhancement of LTP is independent of GABA<sub>A</sub> interneurons, and therefore whether GPR55 works through disinhibition of CA1 pyramidal cells similar to CB1.

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## FIGURE LEGENDS

*Figure 1.* GPR55 mRNA expression in rat hippocampus. A) Hippocampal homogenate demonstrates the presence of GPR55 in 1000 ng (black), 300 ng (red) and 100 ng samples (cyan). Inset: 4% agarose gel to confirm amplicon size, left to right, 50bp ladder (50 bp, 100 bp, and 150 bp shown), GPR55 no template control, and GPR55 (76bp).

*Figure 2.* O-1602 mediates depression of some CA1 interneuron EPSCs, but not CA1 pyramidal cells. A) Application of O-1602 (5  $\mu$ M) induces slight ( $p > 0.05$ ) depression of EPSCs (EPSC amplitudes after 10-15 minutes in O-1602;  $n=8$ , inset: representative EPSC traces and resistance) in whole cell recordings of CA1 interneurons but B) significant depression in only a few interneurons. C) However, no depression was observed in field recordings of CA1 pyramidal cells ( $p > 0.05$ , inset: representative EPSP traces).

*Figure 3.* O-1602 mediates depression of CA1 LTP, but does not alter LTD in the rat. A) O-1602 (1  $\mu$ M) significantly ( $p < 0.05$ ;  $n = 15$ ) decreased theta burst induced LTP as compared to EtOH vehicle control (0.01%;  $n = 14$ ) as measured using field excitatory postsynaptic potentials (fEPSPs). B) O-1602 (1  $\mu$ M) did not alter ( $p > 0.05$ ;  $n=8$ ) LTD induced by 1 Hz as compared to EtOH vehicle control (0.01%;  $n = 10$ ) as measured using fEPSPs. Error bars indicate SEM. Inset: representative EPSP traces, scale bar: 100 pA, 10 ms.

*Figure 4.* GPR55 activation increases CA1 LTP in transgenic mice. A) GPR55<sup>-/-</sup> mice had significantly reduced ( $p < 0.05$ ;  $n=9$ ) theta burst induced LTP as compared to littermate wild-type controls ( $n=15$ ) in the presence of lysophosphatidylinositol (LPI; 2  $\mu$ M). B) LPI (2  $\mu$ M) had a trend to increase ( $p > 0.05$ ;  $n=15$ , from Fig. A) theta burst induced LTP as compared to EtOH vehicle control (0.05%;  $n = 13$ ) in GPR55<sup>+/+</sup> mice as measured using fEPSPs. C) LPI did not alter ( $p > 0.05$ ;  $n = 9$ , from Fig. A) theta burst induced LTP in as compared to EtOH vehicle control (0.05%;  $n = 8$ ) in GPR55<sup>-/-</sup> mice as measured using fEPSPs. Error bars indicate SEM. Inset: representative EPSP traces, scale bar: 100 pA, 10 ms.

*Figure 5.* O-1602 does not alter long-term potentiation in GPR55<sup>-/-</sup> mice. The putative GPR55 agonist O-1602 (3  $\mu$ M) did not alter ( $p > 0.05$ ;  $n = 6$ ) theta burst induced LTP in as compared to EtOH vehicle control (0.05%;  $n = 5$ ) in GPR55<sup>-/-</sup> mice as measured using fEPSPs. Error bars indicate SEM. Inset: representative EPSP traces, scale bar: 100 pA, 10 ms.

*Figure 6.* GPR55<sup>-/-</sup> mice do not have impaired spatial memory compared to littermate GPR55<sup>+/+</sup> controls. A) GPR55<sup>-/-</sup> ( $n=7$ ) and GPR55<sup>+/+</sup> mice ( $n=7$ ) did not ( $p > 0.05$ ) have different predicted median escape latency times (sec) in the acquisition period (sessions 1-7) of the Morris water maze task. Inset represents platform (red) location in pool (blue). B) Close up of sessions 2-7 during acquisition period. C) GPR55<sup>-/-</sup> ( $n=7$ ) and GPR55<sup>+/+</sup> mice ( $n=7$ ) did not ( $p > 0.05$ ) have different predicted median escape latency times (sec) in the reversal period (session 8) of the Morris water maze task. Inset represents platform (red) location in pool (blue) with North (N), South (S), East (E), and West (W) start locations indicated.

# FIGURES

Figure 1.

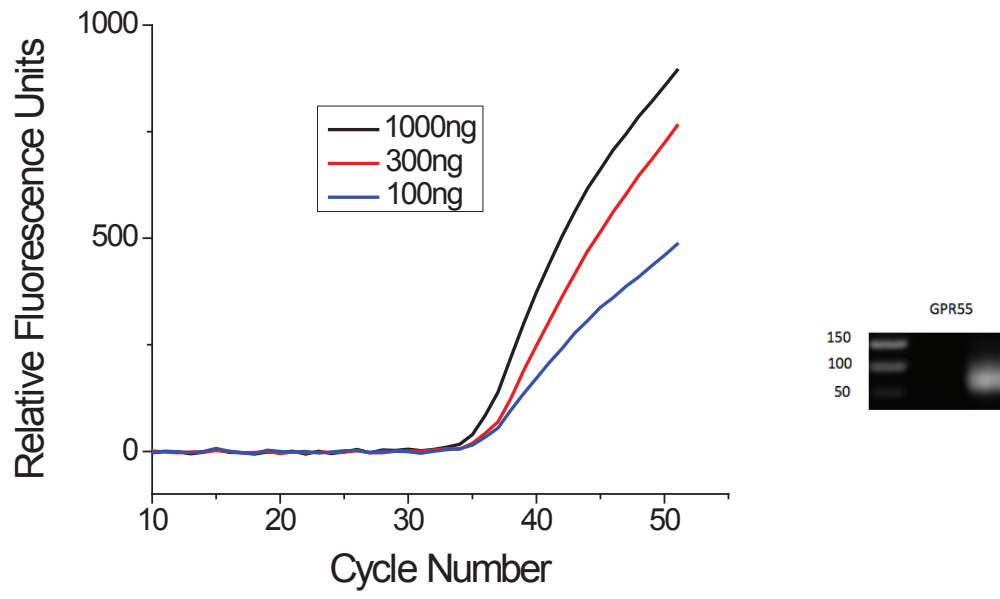
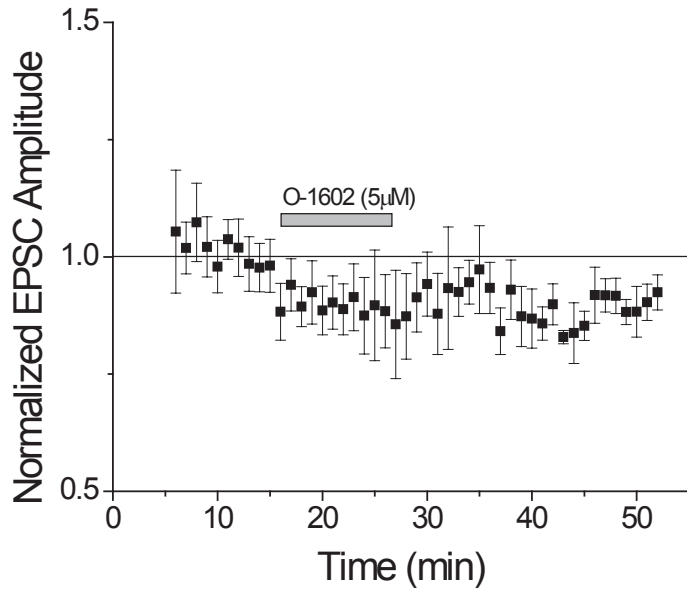
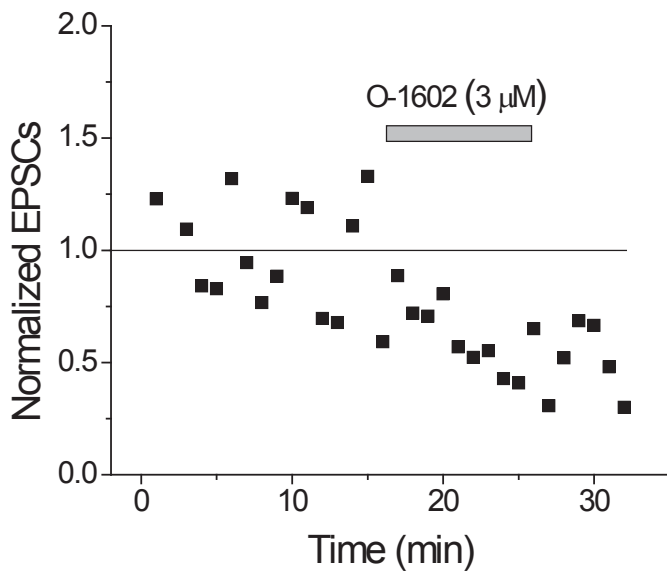


Figure 2.

A.



B.



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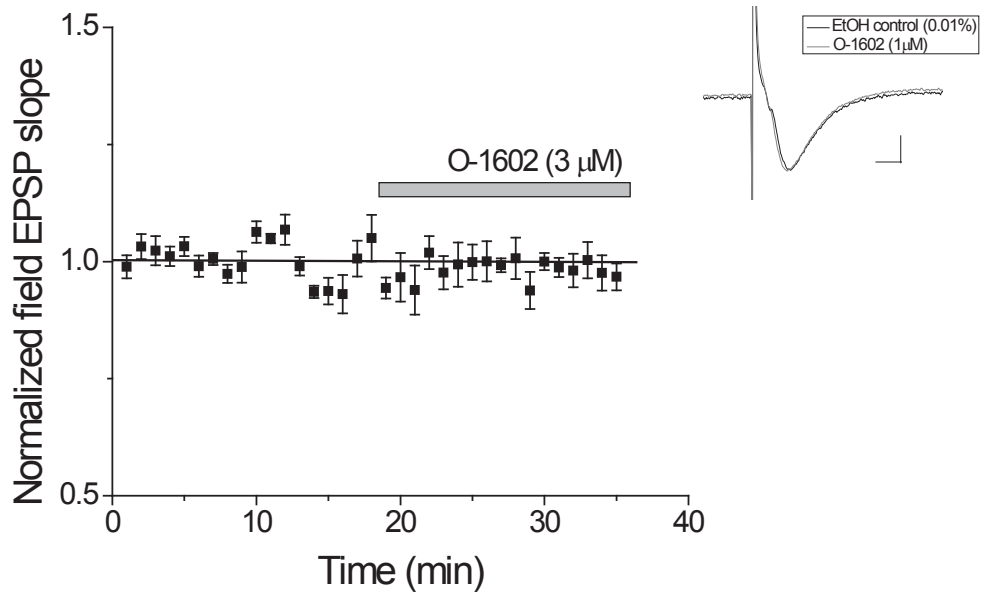
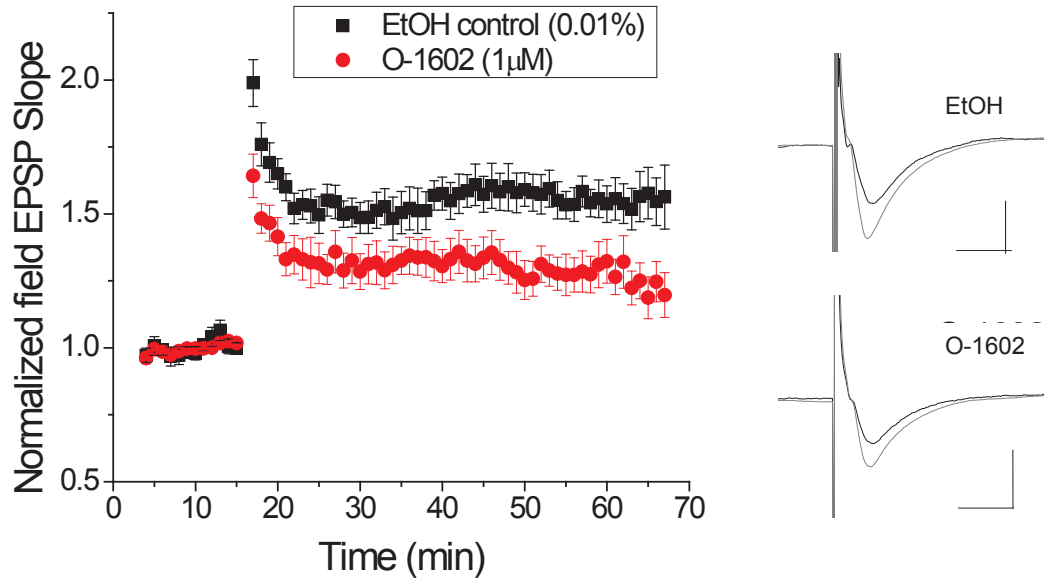


Figure 3.

A.



B.

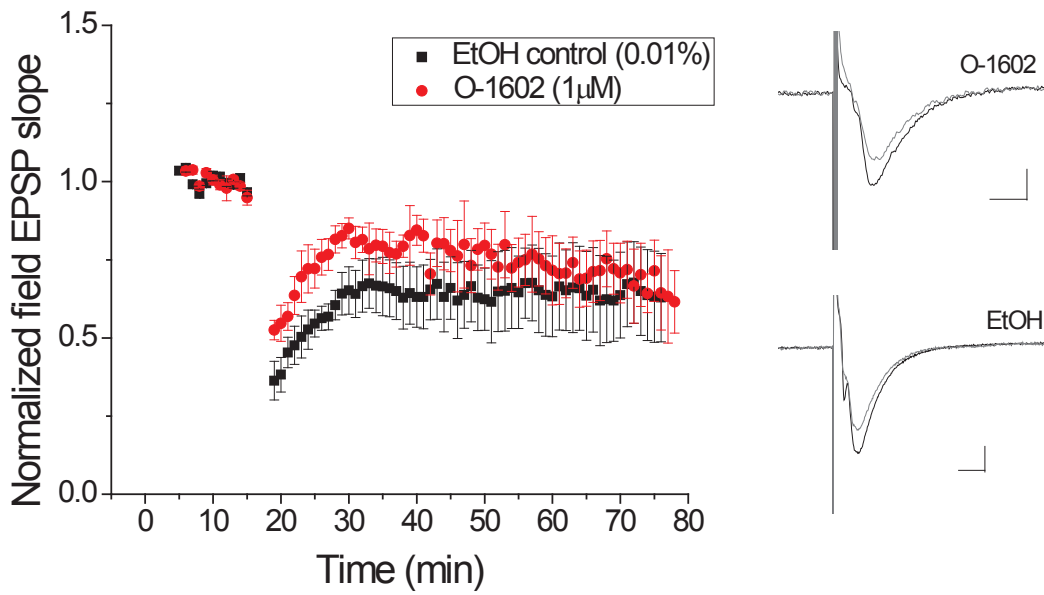
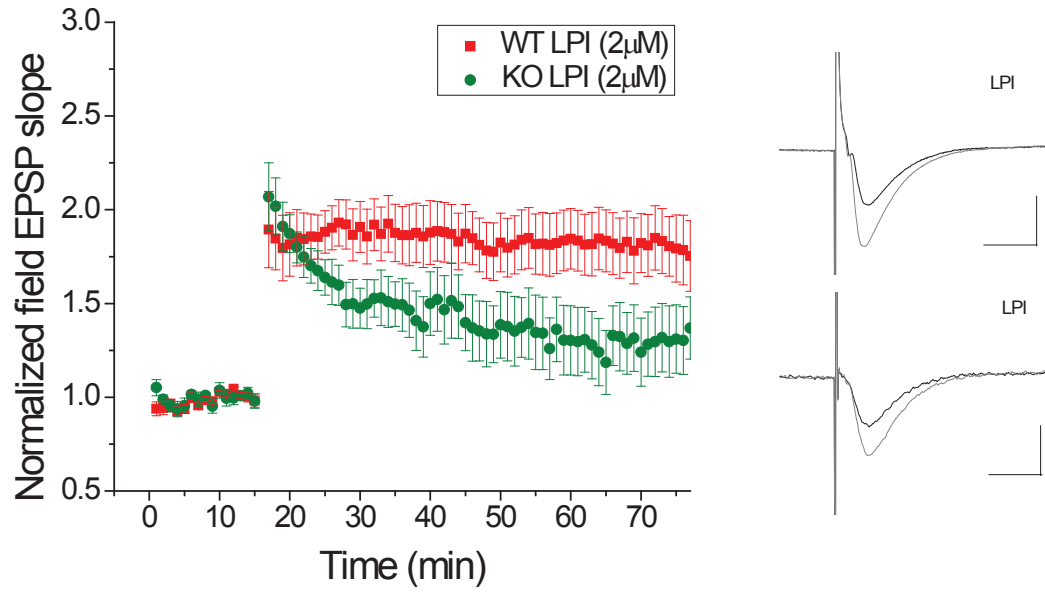
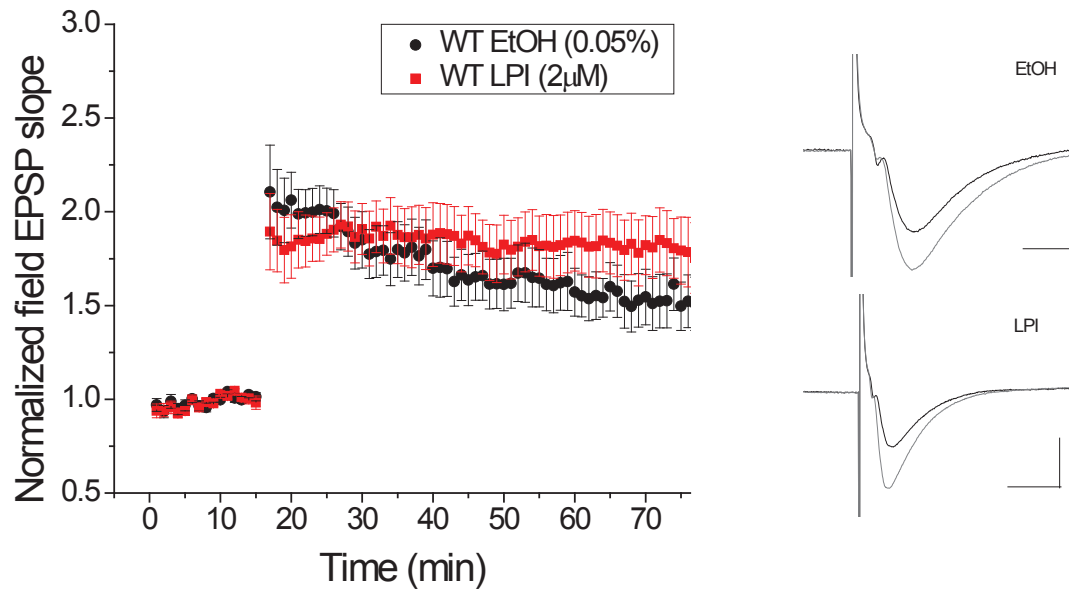


Figure 4.

A.



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C.

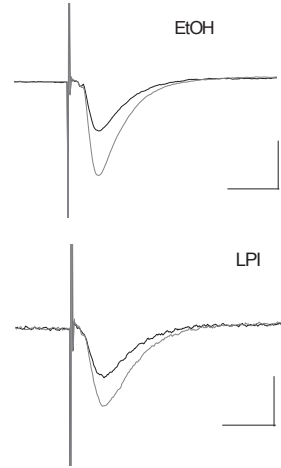
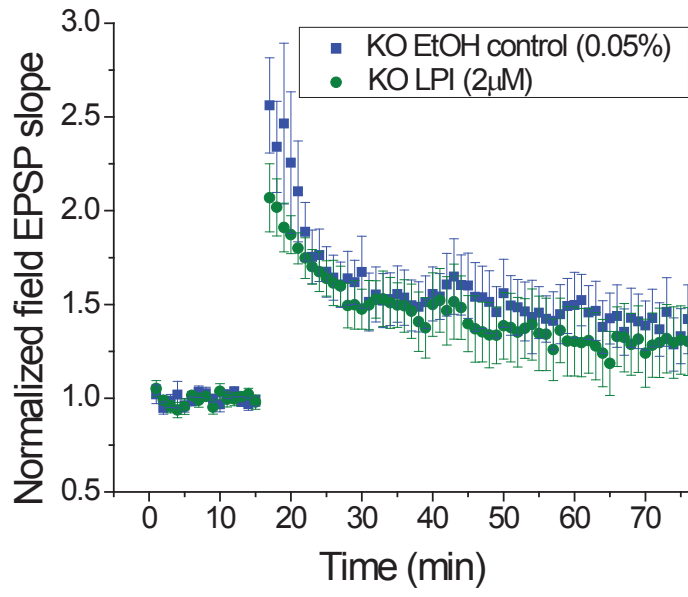


Figure 5.

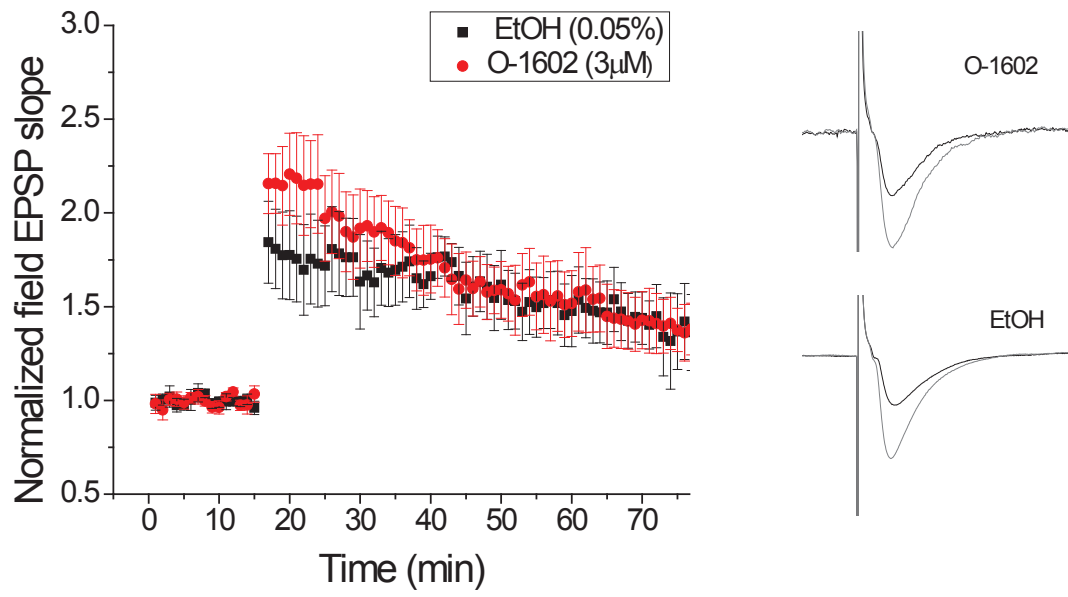
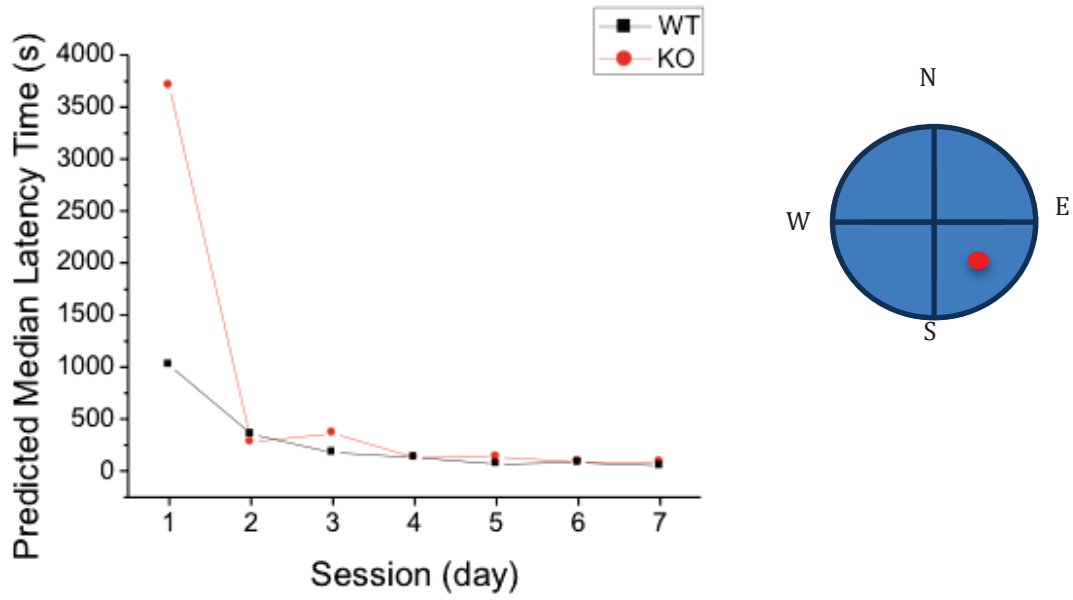


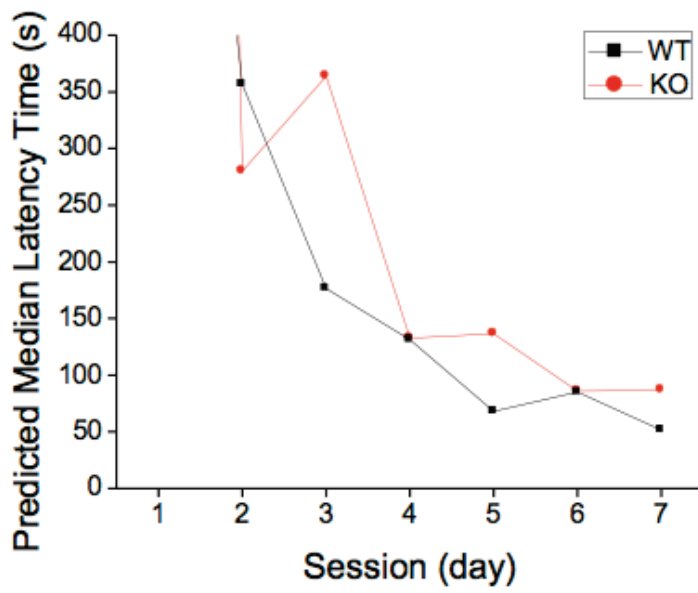


Figure 6.

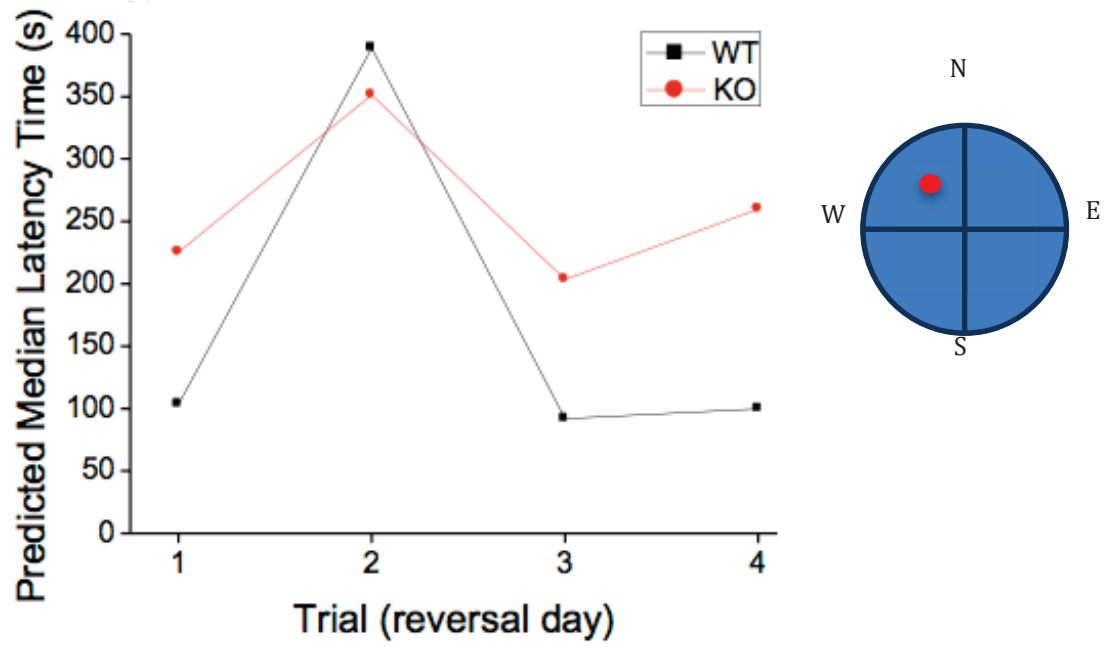
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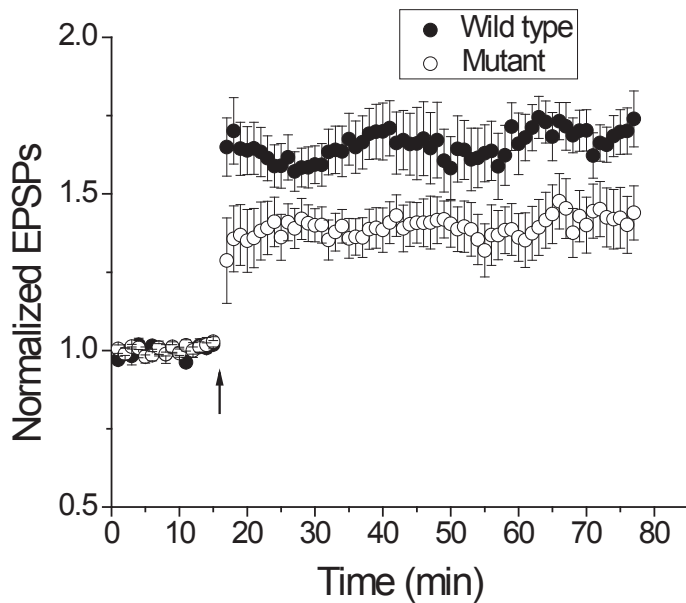


C.



## APPENDIX

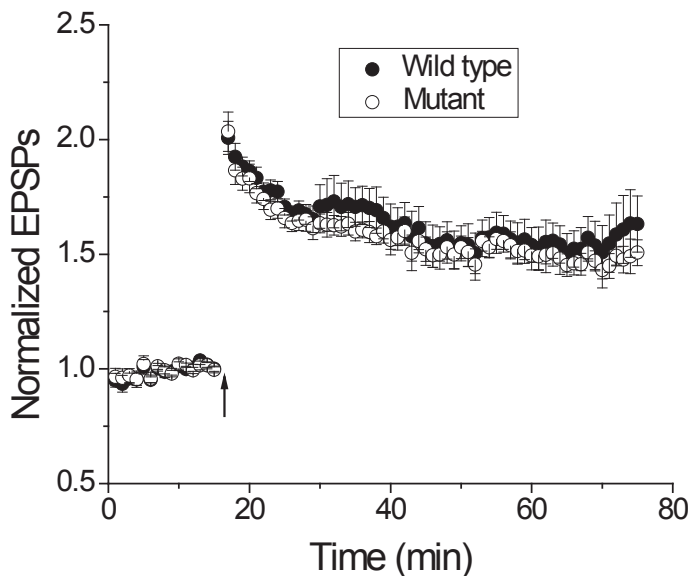
A.



In addition to my thesis project, I collaborated with Dr. Laura Bridgewater by collecting electrophysiological data from hippocampal slices of nuclear bone morphogenetic protein 2 (nBmp2) transgenic mice.

Field recordings from hippocampal CA1 pyramidal cells of nBmp2 transgenic mice. Theta burst induced LTP was examined in nBmp2<sup>-/-</sup> mice vs. nBmp2<sup>+/+</sup> mice to examine the potential for nBmp2 as a modulator of synaptic plasticity. A) We found that nBmp2<sup>-/-</sup> mice ( $n=12$ ) aged 2.5-3.5 months had significant ( $p<0.05$ ) reduction differences in LTP compared to nBmp2<sup>+/+</sup> mice ( $n=15$ ). B) However, 3-4 week old nBmp2<sup>-/-</sup> mice ( $n=8$ ) exhibited no difference ( $p>0.05$ ) compared to nBmp2<sup>+/+</sup> mice ( $n=5$ ), indicating a potential developmental change in expression or function for hippocampal nBMP2 in mice.

B.



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**EDUCATION**

**Brigham Young University** Provo, UT  
MS, Neuroscience. Phi Kappa Phi. 2012  
Thesis: The putative cannabinoid receptor GPR55 modulates synaptic plasticity in the hippocampus.

**University of California, San Diego** La Jolla, CA  
BS, Physiology and Neuroscience 2008

**AWARDS**

**Outstanding Presentation Abstract** 2012  
Intermountain Graduate Research Symposium

**Graduate Mentoring Award** 2011-2012  
Brigham Young University

**Active Member of Phi Kappa Phi Honors Society** 2011-2012  
Brigham Young University Chapter

**Physiology and Developmental Biology Department Tuition Award** 2010-2012  
Brigham Young University

**Teacher's Assistantship (4)** 2010-2012  
Brigham Young University  
Department of Physiology and Developmental Biology, Brigham Young University

**Research Assistantship** 2011  
Department of Physiology and Developmental Biology, Brigham Young University

**Life Sciences Department Award** 2011  
Brigham Young University

**RESEARCH EXPERIENCE**

**Brigham Young University** Provo, UT  
Advisor: Dr. Jeffrey Edwards 2010-Present  
The effect of GPR55 activation on synaptic plasticity in the hippocampus.  
-Bred and managed colony of GPR55 transgenic mice.

- Dissected out whole rodent brains and prepared acute slices using vibratome.
- Examined output of hippocampal CA1 pyramidal cells using field electrophysiology on acute rat/mouse brain slices.
- Investigated effect of GPR55 agonists on CA1 interneurons using whole-cell patch clamp techniques.
- Developed and carried out Morris water maze for transgenic mice for behavioral testing.
- Analyzed data using OriginLab, ClampFit and Excel software.

Collaborated with Dr. Laura Bridgewater collecting field electrophysiological recordings from nBmp2 transgenic mice.

## TEACHING EXPERIENCE

### **Brigham Young University**

Teaching Assistant, Introduction to Neuroscience  
Teaching Assistant, Neuroanatomy

Provo, UT  
Fall 2010, Fall 2011  
Winter 2011, Winter 2012

### **University of California, San Diego**

Teaching Assistant, Modern Topics in Biology

La Jolla, CA  
Spring 2008

## PUBLICATIONS

Cordner D, Mayo J, Ventura J, **Badgley C**, Wallmann A, Andreasen B, Barrow J, Edwards JG, Capecchi M, Bridgewater LC. Mice Bearing a Targeted Mutation of nBmp2 Display Decreased Memory Capabilities. **In Preparation.**

## PRESENTATIONS

**Badgley, C.M.** (2012) The putative cannabinoid receptor GPR55 modulates synaptic plasticity in the hippocampus. Intermountain Graduate Research Symposium Abstracts. Oral presentation delivered at the Intermountain Graduate Research Symposium, Logan, UT, April 2012.

## REFERENCES

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