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A Comparison of the Effects of Different Doses of GABAB Receptor Ligands on Spatial Learning and Memory and Memory Flexibility

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A COMPARISON OF THE EFFECTS OF DIFFERENT DOSES OF $GABA_B$ RECEPTOR LIGANDS ON SPATIAL LEARNING AND MEMORY AND MEMORY FLEXIBILITY

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ABSTRACT

A Comparison of the Effects of Different Doses of $GABA_B$ Receptor Ligands on Spatial Learning and Memory and Memory Flexibility

by

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The principal inhibitory neurotransmitter in the brain, gamma amino-butyric acid (GABA), mediates several types of learning and memory. Of the two main receptor subtypes for GABA, the *in vivo* role of $GABA_B$ receptor in learning and memory is less well characterized and the current data often conflict. Based on the current literature, it is unclear, for instance, whether enhancing GABAergic activity via the $GABA_B$ receptor could be beneficial for or detrimental to learning and memory. Hippocampally-dependent learning and memory tasks are of particular interest due to their clinical relevance to patients with schizophrenia or Alzheimer's disease, who exhibit impaired performance in hippocampally-dependent spatial tasks. Further, these clinical populations exhibit alterations to GABAergic and $GABA_B$ receptor markers throughout the brain, including the hippocampus. Before conclusions can be drawn regarding the effect these changes have on these clinical populations, it is crucial that the role of the $GABA_B$ receptor in learning and memory in an unaltered system is understood first. We examined the effect of altered $GABA_B$ receptor activity using several doses of a $GABA_B$ receptor agonist (baclofen) and a $GABA_B$ receptor antagonist (phaclofen) on performance in a hippocampally-dependent learning and memory task, the Morris water maze. Further, we

examined the impact of these ligands on memory flexibility by utilizing reversal training in the Morris water maze. In our first experiment, Sprague-Dawley rats received a dose of baclofen that significantly impaired performance in the Morris water maze, whereas the animals receiving phaclofen exhibited significantly improved performance. Additionally, the phaclofen-treated group demonstrated increased learning flexibility when the rules of the task were changed during reversal training. The goal of the second experiment was to determine whether a lower dose of baclofen would decrease the deficit observed, or whether a higher dose of phaclofen could enhance the enhancement observed. The lower dose of baclofen failed to produce a behavioral deficit, and the higher dose of phaclofen impaired task performance. Interestingly, while the lower dose of baclofen did not affect time to find the hidden platform, it did produce a subtle enhancement of performance during reversal training. Finally, we examined protein levels to determine whether any alterations were related to task performance. Animals treated with a low dose of baclofen or phaclofen and exhibited improved performance during the reversal training also demonstrated a reduction in the glutamate receptor subunit AMPA GluR4 and the phosphorylated serine 892 on the $GABA_{B2}$ receptor subunit. In addition to indicating a role for the $GABA_B$ receptor in memory flexibility and spatial learning and memory, these results suggest a finite range of $GABA_B$ receptor activity that is capable of improving learning.

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CHAPTER 1

INTRODUCTION

Learning and memory are important functions for the survival of any organism; for instance, animals must be able to learn to utilize and recall information from their environment in order to avoid predators or to collect food. Impaired spatial learning and memory and an inability to update previously learned information are prominent symptoms of several diseases and disorders. In order to understand the mechanisms underlying the altered learning and memory in these illnesses, it is important to understand how these processes normally occur. This insight may then allow for the development of treatments directed at improving the deficits in learning and memory. Further, determining the mechanisms involved in normal learning and memory may help researchers understand why some psychological diseases and disorders exhibit certain alterations to learning and memory.

Of particular interest is spatial learning, which depends upon a functional hippocampus, and which is impaired in schizophrenia and Alzheimer's disease (AD). This type of learning is easily investigated in rodents, who must learn to navigate their environment in order to forage for food. Further, this type of learning is readily quantifiable simply based on how much time a rodent spends searching in a specific location for a given reinforcement.

Damage to or malfunction of the hippocampus can greatly affect performance on tasks that require spatial navigation. Further, the cellular circuitry within the hippocampus must be tightly regulated in order to function properly. This regulation appears to be due to the cooperative actions of the excitatory neurotransmitter glutamate

and the inhibitory neurotransmitter gamma amino-butyric acid (GABA). Interestingly, studies demonstrate that patients with schizophrenia or AD exhibit alterations to metabotropic $GABA_B$ receptors within the hippocampus. Possibly, altered activity of these receptors may contribute to the symptoms or etiology of the learning and memory deficits in these disorders. A better understanding of the effect of altered $GABA_B$ inhibitory tone is necessary in order to understand the impact the $GABA_B$ receptor changes have on the clinical populations.

Research Questions

In order to examine the effect of altered $GABA_B$ receptor activity in spatial learning and memory, we administered the $GABA_B$ agonist baclofen or the antagonist phaclofen to rats prior to running them in the Morris water maze task. We also examined behavior in an open field to determine any motoric effects of the ligands. We had a total of two experiments, each run in a separate cohort.

In Cohort 1, animals received a high dose of baclofen (2.0 mg/kg) or a low dose of phaclofen (0.9 mg/kg) via subcutaneous (SC) administration 30 minutes prior to testing in the Morris water maze. In order to determine whether the behavioral effects of baclofen could be reduced and those of phaclofen increased, we tested different doses of the ligands in Cohort 2. In Cohort 2, the dose of baclofen was reduced to 1.0 mg/kg, and the phaclofen dose was increased to 1.25 mg/kg. $GABA_B$ ligands were administered each day before an animal underwent behavioral testing.

CHAPTER 2

REVIEW OF RELATED LITERATURE

GABA Receptors

The primary inhibitory neurotransmitter in the central nervous system (CNS) gamma-aminobutyric acid (GABA) was discovered in 1950 (Roberts, 1956). GABA must be synthesized in neurons via the decarboxylation of glutamate because it cannot cross the blood brain barrier (Olsen, 2002; Roberts, 1956; Watanabe, Maemura, Kanbara, Tamayama, & Hayasaki, 2002). The ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors are the two main receptors for GABA (Enna, 2007; Olsen, 2002).

$GABA_A$ receptors are proteins that span the cellular membrane four times and have several distinct subunits that associate heterogeneously into pentamers (Enna, 2007; Olsen, 2002). The receptors can be made up of any of the subunits, but the major subtypes include α, β, and γ, with the bulk of the receptors being made up of at least one α and one β subunit (Enna, 2007; Olsen, 2002). The composition of the subunits of the $GABA_A$ receptor dictates which ligands can bind to that particular receptor, in addition to determining where the receptors will be located (Möhler, 2009; Olsen, 2002).

Functionally, the $GABA_A$ receptors are ligand-gated ionotropic chloride channels (Enna, 2007; Olsen, 2002). The $GABA_A$ receptors are responsible for the fast-acting inhibitory currents within the CNS (Hevers & Lüddens, 1998; Watanabe et al., 2002). As soon as a ligand (e.g. GABA) binds, the channel opens immediately; however, while the mechanism of action of this receptor is instantaneous, the effects of these channels are very short-lived because as soon as the ligand is dislodged, the channel immediately closes (Watanabe et al., 2002). $GABA_A$ receptors are postsynaptic (Enna, 2007;

Watanabe et al., 2002), and are found throughout the entire CNS (Bowery, Hudson, & Price, 1987; Chu, Albin, Young, & Penney, 1990; Enna, 2007; Hevers & Lüddens, 1998; Olsen & Tobin, 1990). Although there are generally more $GABA_A$ receptors than $GABA_B$, $GABA_B$ receptors typically have a stronger affinity for GABA than $GABA_A$ (Bowery et al., 1987; Chu et al., 1990; Isaacson, Solís, & Nicoll, 1993).

$GABA_B$ receptors are ligand-gated metabotropic G-protein coupled receptors (GPCRs) that are obligate heterodimers. A functional receptor is comprised of two receptor subunits, $GABA_{B1}$ and $GABA_{B2}$ (Bowery et al., 2002; Couve, Moss, & Pangalos, 2000; Enna, 2007; Kohl & Paulsen, 2010). The $GABA_{B1}$ subunit has several isoforms (Farb et al., 2007; Jiang et al., 2012), but the two most common are $GABA_{B1a}$ and $GABA_{B1b}$ (Bowery et al., 2002; Couve et al., 2000; Enna, 2007; Kohlmeier & Kristiansen, 2010). The main difference between the two $GABA_{B1}$ subtypes appears to be related to the receptor's synaptic location; $GABA_{B1a/2}$ receptors generally inhibit presynaptically, whereas $GABA_{B1b/2}$ receptors appear to primarily inhibit postsynaptically (Kohl & Paulsen, 2010; Ladera et al., 2008; Pérez-Garci, Gassmann, Bettler, & Larkum, 2006; Vigot et al., 2006). The make up of the receptor is also influenced by neural region (Foster, Kitchen, Bettler, & Chen, 2013; Vigot et al., 2006), and whether the presynaptic neuron is GABAergic or glutamatergic (Waldmeier, Kaupmann, & Urwyler, 2008).

Each subunit demonstrates a unique function that leaves the receptors essentially non-functional unless it forms a heterodimer with the other subunit type; that is, both a $GABA_{B2}$ subunit and either a $GABA_{B1a}$ or a $GABA_{B1b}$ subunit must couple together to form a working receptor (Enna, 1997; Jones et al., 1998; Kaupmann et al., 1998; Pinard,

Seddik, & Bettler, 2010; Villemure et al., 2005; White et al., 1998). The $GABA_{B1}$ subunits contain the ligand binding sites, whereas the $GABA_{B2}$ subunit couples the receptor complex to G-proteins, as well as brings the heterodimer complex to the cell surface from the endoplasmic reticulum (Bowery et al., 2002; Galvez et al., 2001; Kohl & Paulsen, 2010; Pinard et al., 2010; Robbins et al., 2001). If two $GABA_{B2}$ subunits or any combination of two $GABA_{B1}$ subunits bind together, the resultant $GABA_B$ receptor is dysfunctional. In $GABA_{B1}$-knockout mice, the typical G-protein-linked current is absent; $GABA_{B2}$-knockout mice demonstrate "atypical $GABA_{B1}$-mediated responses," suggesting that $GABA_{B1}$ is capable of coupling to other G-proteins in the absence of $GABA_{B2}$ (Pinard et al., 2010). The $GABA_B$ receptor does not appear to behave like traditional GPCRs when chronically activated or inhibited (Benke, Zemoura, & Maier, 2012). For instance, chronic activation of the $GABA_B$ receptor does not induce the typical down-regulation of the receptor (Fairfax et al., 2004). Interestingly, however, $GABA_B$ receptors may be more sensitive to glutamatergic signaling. Recent data suggest that $GABA_B$ receptors rapidly undergo endocytosis in response to the activation of glutamatergic receptors present in the same dendritic spine (Guetg et al., 2010; Terunuma et al., 2010b). Several serine residues on the intracellular, C-terminus tails of both the $GABA_{B1}$ and $GABA_{B2}$ subunits are the targets of phosphorylation that influence receptor stability (Benke et al., 2012; Calver et al., 2001; Couve, Moss, & Pangalos, 2007; Couve et al., 2002; Fairfax et al., 2004; Gassmann & Bettler, 2012; Guetg et al., 2010; Kuramoto et al., 2007; Terunuma et al., 2010b; Terunuma, Pangalos, & Moss, 2010a). For example, glutamatergic signaling triggers the phosphorylation of both the $GABA_B$ receptor subunits to promote endocytosis. A $GABA_{B1}$ target, serine 867, is phosphorylated via

CaMKII (a calcium-dependent kinase) in response to activated glutamatergic receptors (Terunuma et al., 2010b); increased intracellular calcium concentration via glutamate and calcium channels induces the phosphorylation of serine 783 of the $GABA_{B2}$ subunit via AMPK, which promotes recycling of the $GABA_B$ receptor (Benke et al., 2012). However, once endocytosed, if the serine 783 residue is dephosphorylated through the actions of protein phosphatase 2A (PP2A), the receptor undergoes degradation instead of recycling (Benke et al., 2012).

The $GABA_B$ receptor is slower acting because the effects of cascade sequences activated by the α or β and γ subunits of the coupled G-protein are not immediately evident (Brown & Sihra, 2008). However, the effects of metabotropic receptors are long lasting compared to ionotropic receptors. When a ligand is dislodged from a receptor's binding site, the intracellular signal cascade may still be amplified and propagate, and will persist until it is inactivated within the cell (Brown & Sihra, 2008). Thus, while the metabotropic $GABA_B$ receptors are responsible for a slow inhibitory current, the magnitude of effect is enhanced and longer lasting due to intracellular signal cascades (Bettler, Kaupmann, Mosbacher, & Gassmann, 2004; Couve et al., 2000).

$GABA_B$ receptors are found both pre- and postsynaptically, though there are different mechanisms of action depending on location (Enna, 2007; Kohl & Paulsen, 2010; Misgeld, Bijak, & Jarolimek, 1995; Watanabe et al., 2002). Presynaptic $GABA_B$ receptors may act in a feedback loop as autoreceptors mediating the presynaptic release of GABA (Davies, Starkey, Pozza, & Collingridge, 1991; Kohl & Paulsen, 2010; Misgeld et al., 1995; Zarrindast, Bakhsha, Rostami, & Shafaghi, 2002), or as heteroreceptors mediating the presynaptic release of other neurotransmitters (Bowery,

2010; Kohl & Paulsen, 2010; Sakaba & Neher, 2003; Tiao & Bettler, 2007) such as glutamate (Sakaba & Neher, 2003) or acetylcholine (Morton, Manuel, Bulters, Cobb, & Davies, 2001). These receptors appear to need strong stimulation and large amounts of GABA in the synapse in order to be activated, suggesting some may be located extrasynaptically (Ladera et al., 2008; Misgeld et al., 1995; Pinard et al., 2010). Presynaptic $GABA_B$ receptors inhibit voltage-gated calcium conductance via the β and γ subunits of $G_{i/o}$ G-proteins, the consequence of which is decreased release of vesicular neurotransmitter (Bettler et al., 2004; Couve et al., 2000; Enna, 2007; Padgett & Slesinger, 2010). Depending on which neurotransmitter is prevented from being released, this effect can have an excitatory effect or an inhibitory effect on the subsequent postsynaptic neuron. For instance, if an autoreceptor inhibits the release of GABA onto a postsynaptic neuron, that postsynaptic neuron will have a greater likelihood of depolarizing. This effect is termed disinhibition because the postsynaptic cell is being released from the inhibiting effects of GABA. Conversely, if a heteroreceptor inhibits the release of glutamate, the postsynaptic cell is likely to experience less depolarization due to the lack of excitatory input.

Postsynaptically, a $GABA_B$ receptor can activate inwardly-rectifying potassium channels via the dissociated β and γ subunits of the G-protein complex (Brown & Sihra, 2008; Dascal, 1997; Kohl & Paulsen, 2010; Lewohl et al., 1999; Mark & Herlitze, 2000; Reuveny, 2013), which allows for potassium to efflux out of the cell, leading to hyperpolarization (Bettler et al., 2004; Pinard et al., 2010; Reuveny, 2013). $GABA_B$ receptors also act by inhibiting adenylyl cyclase (Bettler et al., 2004; Enna, 2007; Padgett & Slesinger, 2010). The α subunit dissociates from a G-protein complex and inhibits

adenylyl cyclase, which normally initiates a number of other intracellular cascades, including those that affect short- and long-term memory (Birnbaumer, 2007; Kinney, Starotsa, & Crawley, 2003; Padgett & Slesinger, 2010; Vianna et al., 2000). This mechanism of action may be present at both pre- and postsynaptic neurons, as it has been suggested that the cyclic adenosine monophosphate (cAMP) cascade (which is activated by adenylyl cyclase) may play a role in synaptic signaling via neurotransmitter release, and neuronal excitation (Padgett & Slesinger, 2010; Ulrich & Bettler, 2007).

These studies demonstrate the molecular mechanisms of $GABA_B$ receptors. The overall result of these processes produces a slow, long lasting hyperpolarization of the postsynaptic cell. However, the functional relevance of these receptors to complex behaviors, such as learning and memory, has not been completely determined. Based on the variety and complexity of potential effects of the activation of $GABA_B$ receptors, it is clear why the role of the $GABA_B$ receptor in learning and memory may be convoluted and difficult to unravel.

$GABA_B$ in the Hippocampus

The hippocampus is split into several subregions through which information flows nearly unidirectionally (Andersen, 2007). Incoming stimuli from the entorhinal cortex (EC) enter the hippocampus via the perforant pathway that feeds into the dentate gyrus (DG). From the DG, information is routed through to Cornu Ammonis (CA) area 3 (CA3) via mossy fiber connections. CA3 then projects to CA1 through the Schaffer collaterals; finally, CA1 projects back to the EC. $GABA_B$ receptors are located throughout the entire CNS, including the hippocampus (Bowery et al., 1987; Chu et al., 1990), and the DG and

CA1 demonstrate higher concentrations of $GABA_B$ receptors than CA3 (Sloviter, Ali-Akbarian, Elliott, Bowery, & Bowery, 1999).

Proper functioning of the hippocampus is required for spatial learning and memory (Barak et al., 2013; Lee, Hunsaker, & Kesner, 2005; Tsien, Huerta, & Tonegawa, 1996). Further, participating in a spatial learning and memory task enhances synaptic plasticity in the hippocampus (Kenney & Manahan-Vaughan, 2013), which may relate to the formation of spatial-related memories (Eyre, Richter-Levin, Avital, & Stewart, 2003). The DG undergoes neurogenesis (Kempermann, Kuhn, & Gage, 1997), a process regulated by $GABA_B$ receptors (Felice, O'Leary, Pizzo, & Cryan, 2012; Giachino et al., 2014). Neurogenesis is correlated with spatial learning and memory (Clelland et al., 2009; Jessberger et al., 2009; Nilsson, Perfilieva, Johansson, Orwar, & Eriksson, 1999), which can also be affected by $GABA_B$ receptors (Arolfo, Zanudio, & Ramirez, 1998).

As described in more detail in the sections below, long-term potentiation (LTP) and synchronous, oscillatory neural firing are well characterized in the hippocampus. These neural correlates to learning and memory, as well as learning and memory behavior, can be affected by altered $GABA_B$ receptor function. Further, several clinical populations that demonstrate spatial learning and memory deficits also exhibit altered $GABA_B$ markers.

$GABA_B$ in Learning and Memory

Oscillatory, synchronous activity is theorized to promote synaptic plasticity such as long-term potentiation (LTP), which in turn is the leading model for the *in vivo* mechanics of learning and memory formation (Buzsaki, 1989; Malenka & Bear, 2004). *In*

vitro (Larson, Wong, & Lynch, 1986) and *in vivo* (Greenstein, Pavlides, & Winson, 1988; Pavlides, Greenstein, Grudman, & Winson, 1988; Stepan et al., 2012) theta frequency stimulation induces LTP within the hippocampus. This effect appears to require stimulation during the peak of, but not at the trough of, the theta oscillation (Hölscher, Anwyl, & Rowan, 1997; Hyman, Wyble, Goyal, Rossi, & Hasselmo, 2003; Orr, Rao, Houston, McNaughton, & Barnes, 2001; Pavlides et al., 1988).

GABA plays an important role in regulating oscillations that influence learning and memory. Synchronized inhibitory postsynaptic potentials (IPSPs) generated by GABA moderate gamma (30-100 Hz) (Mann & Mody, 2010; Traub, 2003; Whittington, Traub, & Jefferys, 1995) and theta (3-12 Hz) (Gong et al., 2009; Xiao et al., 2012) activity in the hippocampus. These frequencies are related to the formation of memories both in humans (Jutras & Buffalo, 2010; Rutishauser, Ross, Mamelak, & Schuman, 2010; Sederberg et al., 2007) and rodents (Axmacher, Mormann, Fernández, Elger, & Fell, 2006; Tort, Komorowski, Manns, Kopell, & Eichenbaum, 2009). For instance, $GABA_B$ antagonism facilitates learning and memory driven by theta rhythms *in vivo* (Staubli, Scafidi, & Chun, 1999). Further, *in vivo,* theta rhythms correlate with better task performance (Olvera-Cortes, Cervantes, & Gonzalez-Burgos, 2002), and blocking theta rhythms impairs learning and memory (Winson, 1978).

The $GABA_B$ receptor also helps regulate and modulate oscillatory activity (Kohl & Paulsen, 2010). For instance, the cooperation of $GABA_A$ and $GABA_B$ receptors increases the synchronization of theta activity in the rat occipital lobe (Xiao et al., 2012). Within the EC, $GABA_A$ receptors control the duration of oscillatory activity, and $GABA_B$ receptors terminate this synchronous activity (Mann, Kohl, & Paulsen, 2009). $GABA_B$

receptors are also capable of altering serotonergic-generated hippocampal theta rhythms via disinhibition of serotonergic neurons within the median raphe nuclei (Li, Varga, Sik, & Kocsis, 2005).

Activation of hippocampal $GABA_B$ receptors is capable of abolishing gamma activity, whereas the blockade of these receptors decreases the number of repetitive stimuli needed to produce gamma activity (Brown, Davies, & Randall, 2007), thus requiring less input to entrain the synchronous activity. *In vivo* recordings of mobile, awake rats indicate that intracerebroventricular (ICV) or intrahippocampal infusions of a $GABA_B$ antagonist induces theta and increases gamma (respectively) rhythms in CA1 (Leung & Shen, 2007). Although the blockade of $GABA_B$ receptors allows for easier entrainment of synchronous activity, the activation of these receptors is also necessary to regulate the persistence of synchronous activity. Together these data suggest that an appropriate amount of activation of $GABA_B$ receptors is crucial for synchronous neural activity associated with learning and memory formation.

$GABA_B$ receptor activity can also induce plasticity between neurons via paired-pulse stimulation, in addition to LTP. These effects, however, appear to be region specific. For instance, studies clearly indicate that $GABA_B$ receptor antagonism in the CA1 enhances synaptic plasticity and LTP (Leung, Peloquin, & Canning, 2008; Morrisett, Mott, Lewis, Swartzwelder, & Wilson, 1991; Olpe et al., 1993; Staubli et al., 1999). Overexpression of the $GABA_{B1a}$ (Wu et al., 2007) and the $GABA_{B1b}$ (Stewart et al., 2009) receptor subunits reduce LTP in the CA1 region of the hippocampus of transgenic mice. While $GABA_{B1b}^{-/-}$ mice with intact presynaptic $GABA_B$ receptors demonstrate the ability to induce LTP in the CA1, $GABA_{B1a}^{-/-}$ mice are unable to induce

LTP, though they exhibit paired-pulse plasticity (Vigot et al., 2006). Taken together, these data demonstrate that blocking $GABA_B$ receptor function within the CA1 enables plasticity, LTP, and the entrainment of synchronous activity associated with learning. Conversely, activating $GABA_B$ receptors impairs these measures of synaptic plasticity.

In addition to LTP of excitatory currents described above, the CA1 has been shown to undergo calcium- and N-methyl-D-aspartate (NMDA)-dependent LTP of $GABA_B$-mediated slow inhibitory postsynaptic currents (sIPSCs); sIPSCs are required to induce LTP between cortico-CA1 synapses (Remondes & Schuman, 2003). LTP of sIPSCs has been suggested to produce a finite window for postsynaptic detection of excitatory inputs to perhaps help modulate rhythmic activities (Huang et al., 2005). For example, rapid excitatory stimulation induces an sIPSC, which then minimizes the impact of any late-arriving excitatory inputs. Similarly, high frequency stimulation of the lateral septal nucleus prevents LTP, an effect that is in part mediated by the activation of $GABA_B$ receptors, and which has been suggested as a regulatory mechanism to prevent an overflow of information from CA3 into other regions (Hasuo & Akasu, 2001). Thus, $GABA_B$-mediated sIPSCs appear to help transmit only pertinent information to and within the hippocampus, which suggests that $GABA_B$ receptors moderate plasticity related to learning and memory.

In contrast to the CA1, *in vitro* activation of $GABA_B$ receptors is necessary for the development of LTP in the DG (Burgard & Sarvey, 1991; Mott & Lewis, 1992; Mott, Lewis, Ferrari, Wilson, & Swartzwelder, 1990; Mott, Xie, Wilson, Swartzwelder, & Lewis, 1993). These effects are due to disinhibition – the activated $GABA_B$ receptor is inhibiting other inhibitory processes, producing a net excitatory effect. Further,

stimulating these cells at frequencies resembling endogenous theta oscillations induces maximal LTP (Mott et al., 1990). Recordings from anesthetized animals demonstrate that a systemically administered $GABA_B$ agonist can produce paired-pulse disinhibition of EPSPs in the DG, whereas systemic administration of a $GABA_B$ antagonist prevents paired-pulse disinhibition (Brucato et al., 1996; Brucato, Mott, Lewis, & Swartzwelder, 1995). Similarly, systemic administration of a $GABA_B$ antagonist decreases the amount of theta-burst stimulation-induced LTP recorded in the DG (Brucato et al., 1996). These facilitatory effects are likely produced only by presynaptic receptors, however, because the activation of postsynaptic $GABA_B$ receptors in the DG produces increased inhibitory current into the neuron (Tao, Higgs, Spain, & Ransom, 2013). Blocking the activation of $GABA_B$ receptors using antagonists is usually effective at reversing the facilitatory effect of $GABA_B$ agonists (Brown et al., 2007; Mott & Lewis, 1992), and antagonists, alone, typically produce no effect (Brown et al., 2007) or impair (Brucato, Morrisett, Wilson, & Swartzwelder, 1992; Mott & Lewis, 1991) LTP and plasticity. The DG, therefore, requires the activation of $GABA_B$ receptors to produce plastic activity

Behaviorally, alteration of GABAergic tone by $GABA_B$ ligands modifies how well an animal learns a task. A general pattern of impaired learning and memory is found with $GABA_B$ agonists (Castellano, Cabib, & Puglisi-Allegra, 1996; Heaney et al., 2012; McNamara & Skelton, 1996; Myhrer, 2003; Stuchlik & Vales, 2009) and enhanced learning and memory after administering $GABA_B$ antagonists (Castellano et al., 1996; Getova & Bowery, 1998). However, the results from $GABA_B$ investigations are not well replicated. In a review, four studies attempting to demonstrate the effects of baclofen (a $GABA_B$ agonist) on the same passive avoidance task found that baclofen either improves,

impairs, or does not alter performance (Myhrer, 2003). Since these four studies utilized the same task in the same manner and all administered baclofen systemically, the differing results could be do to the dosages or strain of animal used, or even to what extent baclofen reached the different regions of the hippocampus.

Additionally, very few studies utilize $GABA_B$ antagonists administered alone in learning and memory tasks, and there are inconsistent results among those studies that have been conducted. For instance, a $GABA_B$ antagonist administered after a passive avoidance task enhanced memory, as measured by increased step-through latencies (Mondadori, Möbius, & Borkowski, 1996). However, another study utilized the same task and found no effect on behavior at low doses of an administered $GABA_B$ antagonist, whereas high doses actually decreased performance (Zarrindast et al., 2002). Differences between these two studies include route of administration (systemic versus intracranial), gender of the animals, and type of animals used (mice versus rats).

Our previous research indicates that altering $GABA_B$ receptor activity affects cued and contextual fear conditioning (Heaney et al., 2012). In this paradigm, a tone is paired with a footshock within a specific environment. The animal is tested for the strength of the association between the tone and footshock (cued association), as well as between the original environment and the footshock (contextual association). If the associations were adequately made, an animal will freeze to the presentation of the tone when presented in a novel environment, demonstrating that the animal recognizes the tone as a predictor of the footshock. Additionally, the animal should freeze when it is placed back into the original training environment, indicating that it remembers the environment where the footshocks occurred. Extinction occurs after repeated presentation of the tone without the

footshock, or being in the original environment without receiving more footshocks; the animal should slowly demonstrate less fear, as indicated by decreased time spent freezing. Impaired performance in this task is marked by a reduction of freezing behavior to either the tone or the original environment before extinction, or increased freezing behavior after extinction.

Systemic pretreatment of baclofen did not affect the acquisition of the associations (Heaney et al., 2012). However, baclofen pretreatment did impair the ability to extinguish or alter the memory of both the cued and contextual associations. Further, administration of baclofen after the initial acquisition only impaired extinction of the contextual association. Acquisition and extinction of contextual fear is guided by the hippocampus (Corcoran & Maren, 2001; Corcoran, Desmond, Frey, & Maren, 2005; Phillips & LeDoux, 1992), as well as by GABA signaling (Makkar, Zhang, & Cranney, 2010). These data suggest that $GABA_B$ receptors may have a role in memory flexibility and updating previously acquired memories that depend on the hippocampus.

Another type of learning and memory that requires the hippocampus is spatial learning and memory (Barak et al., 2013; Lee et al., 2005; Tsien et al., 1996). One of the more prominently used tasks to measure spatial learning and memory in rodents is the Morris water maze (MWM). The MWM was developed by Richard Morris in the 1980s as a way to examine rodent spatial learning and memory (Morris, 1981). This task requires animals to utilize extra-maze cues to locate a hidden platform submerged under opaque water. Rodents are good, yet reluctant, swimmers and are motivated to escape the water as quickly as possible. The MWM task is usually conducted over several days using a circular tank that has been virtually divided into quadrants. Time taken to find the

hidden platform (latency) and performance on a probe trial are the main measures of task comprehension. The faster an animal finds the hidden platform, the more accurately it has learned the spatial location of the hidden platform. Probe trials are usually conducted 24 hours after the last training session. During the probe trial, the hidden platform is removed from the tank and the animals are allowed a free swim period; the amount of time spent in the quadrant of the platform's previous location is measured. The more time spent in the quadrant where the platform used to be located as compared to the other quadrants, the better the animal has learned the task. Further, learning flexibility can be tested in this paradigm by simply changing the location of the hidden platform after the task has been learned. This phase of the MWM is called reversal training and performance can be measured by latency and a probe trial, as done in the initial training phase. Impaired performance is marked by increased latencies compared to controls to find the hidden platform, as well as by equal time spent in all quadrants during the probe trial.

Systemic injections (McNamara & Skelton, 1996; Nakagawa & Takashima, 1997; Nakagawa, Ishibashi, Yoshii, & Tagashira, 1995) and intra-cerebral infusions (Arolfo et al., 1998; Deng et al., 2009) of baclofen, a $GABA_B$ agonist, consistently impair performance in this task. The effect of $GABA_B$ antagonists on performance in this task is more complicated. Depending on the strain of animal used, the same antagonist can produce different effects. For instance, the $GABA_B$ antagonist CGP 36742 has no effect on performance of BALB/c and CF1 mice (Sunyer et al., 2007), whereas C57BL/6J and OF1 mice perform better than controls (John, Sunyer, Höger, Pollak, & Lubec, 2009; Sunyer et al., 2007; Sunyer, Shim, An, Höger, & Lubec, 2009b), and CD1 and DBA/2

mice demonstrate impaired task performance (Sunyer et al., 2007). One of the differences between these strains is the amount of NMDA NR1 receptor subunit and $GABA_{B2}$ receptor subunit proteins in the hippocampus (Sunyer, An, Kang, Höger, & Lubec, 2009a). Compared to C57BL/6J mice, naïve DBA/2 mice demonstrate decreased levels of NR1 and increased levels of $GABA_{B2}$, which could explain the behavioral differences. However, also compared to C57BL/6J mice, OF1, CD1, and CF1 mice also demonstrate increased $GABA_{B2}$ protein levels in the hippocampus. Thus, there must be other differences between these strains of mice that lead to the behavioral differences due to the $GABA_B$ antagonist.

When administered systemically to female rats, CGP 46381, a $GABA_B$ antagonist, increases latency to find the hidden platform (Brucato et al., 1996). The protocol utilized in this study, however, may influence the results. For instance, the rats were only trained for one day, and then immediately given a probe trial after the last training trial. During the training trials, the animals were released from the same quadrant each time, making the task less dependent on the extra-maze spatial cues. Furthermore, the animals were not impaired on the probe trial, indicating they spent more time in the quadrant where the platform had been located as compared to the other quadrants. What is more, the ligand utilized in this study is not commonly used *in vivo* and its effects cannot be compared to other studies. However, CGP 46381 is rather potent compared to other antagonists (Olpe et al., 1993); it is, therefore, possible that this antagonist is binding to both autoreceptors and postsynaptic receptors to produce the memory impairment.

Although these data demonstrate the variation in results typically seen within the $GABA_B$ literature, they also solidify the idea that proper hippocampal $GABA_B$ receptor function is necessary for spatial learning and memory. However, these data do not provide consistent results regarding *how* altering $GABA_B$ receptors affects behavior. Additionally, none of these studies examine memory flexibility via reversal training. Impaired memory flexibility is often associated with the clinical populations that also exhibit impaired spatial learning and memory. Therefore, these deficits may be linked and can be examined together given that memory flexibility and spatial learning and memory are readily measured using the MWM. Determining the role of $GABA_B$ receptors in this type of learning may help lead to better treatment options for these clinical populations.

Clinical Relevance

Impaired memory flexibility and spatial learning and memory deficits are hallmarks of both schizophrenia and AD (Addington & Addington, 1999; Albert, 1996; Cherrier, Mendez, & Perryman, 2001; deIpolyi, Rankin, Mucke, Miller, & Gorno-Tempini, 2007; Hanlon et al., 2006; Spieker, Astur, West, Griego, & Rowland, 2012). While these deficits likely stem from a number of pathways, these populations demonstrate changes to $GABA_B$ markers. These alterations could be a result of the particular disorder, or changes to $GABA_B$ receptors and $GABA_B$ receptor function could lead to the development of these disorders. Therefore, determining *how* altered $GABA_B$

receptor function affects learning and memory could provide valuable information regarding the affected $GABA_B$ mechanisms within these disorders.

Expression of $GABA_B$ receptors is reduced in the pyramidal cells of the DG, CA subregions, EC, and within the inferior temporal cortex of postmortem brain tissue from patients with schizophrenia (Kantrowitz, Citrome, & Javitt, 2009; Mizukami et al., 2000). Further, the prefrontal cortex exhibits a decrease in levels of $GABA_{B1a}$ receptor subunits as compared to controls (Ishikawa, Mizukami, Iwakiri, & Asada, 2005), and examination of the cerebellum reveals decreased amounts of $GABA_{B1}$ and $GABA_{B2}$ (Fatemi, Folsom, & Thuras, 2011).

Further, patients with schizophrenia demonstrate other GABAergic alterations. For instance, patients with schizophrenia have decreased parvalbumin and GAD67 expression in GABAergic interneurons (Cherlyn et al., 2010; Gonzalez-Burgos, Hashimoto, & Lewis, 2010; Guidotti et al., 2005; Torrey et al., 2005; Zhao et al., 2007), as well as deficits in frontal cortex chandelier neurons that give rise to oscillatory gamma activity (Kantrowitz et al., 2009). Further changes are apparent in other GABAergic markers like $GABA_A$ receptors (Cherlyn et al., 2010; Deng & Huang, 2006; Gonzalez-Burgos et al., 2010; Zhao et al., 2007), GABA concentration (Öngür, Prescot, McCarthy, Cohen, & Renshaw, 2010), reuptake sites (Wassef, Baker, & Kochan, 2003), and GABA currents (Benes, 2010). Together, these results indicate widespread changes to the $GABA_B$ receptor and GABAergic functioning within this population.

Liu and colleagues (2009) examined the effect of transcranial magnetic stimulation (TMS) of the primary motor cortex on cortical inhibition in controls, medicated, and unmedicated schizophrenia patients. The long-interval cortical silent

period (CSP) is an indication of $GABA_B$ function (Premoli et al., 2014). Medicated patients exhibited an enhanced long-interval CSP as compared to the unmedicated patients. Further, negative symptoms were inversely correlated with the $GABA_B$-mediated transmission, indicating that $GABA_B$ dysfunction could underlie the pathophysiology of these symptoms. Additional differences were evident in the $GABA_A$-mediated short interval CSP, which were weakly correlated with positive symptoms. The authors suggest that the inhibitory currents provided by the GABA receptors could regulate different symptoms of schizophrenia.

Another study examined the effect TMS of the primary motor cortex on CSP between patients at-risk for schizophrenia, first-episode schizophrenia patients and controls (Hasan, Wobrock, et al., 2012b). This study found reductions in the $GABA_A$-mediated CSP in the at-risk and first-episode patients. Compared to both controls and at-risk patients, first-episode patients exhibited an increase in the $GABA_B$-mediated CSP. Further, a similar study found prolonged CSP and impaired LTD-like plasticity in medicated patients compared to controls (Hasan, Nitsche, et al., 2012a). Antipsychotic naïve patients also exhibit deficits in the $GABA_A$-mediated CSP, which were negatively correlated with social cognition (Mehta, Thirthalli, Basavaraju, & Gangadhar, 2014). Further, evidence suggests that presynaptic $GABA_B$ receptors may inhibit $GABA_A$-mediated CSPs, whereas postsynaptic $GABA_B$ receptors may inhibit the TMS-induced motor-evoked potentials (Chu, Gunraj, & Chen, 2008). These data suggest that early disease symptomology may be related to $GABA_A$-mediated or presynaptic $GABA_B$-mediated dysfunction, whereas disease progression may be more affected by altered postsynaptic $GABA_B$ functioning.

While the above experiments examined the CSP only within the primary motor cortex, disturbances to cortical function are also found within the frontal cortex. For instance, patients with schizophrenia exhibit altered gamma activity compared to controls when performing working memory tasks (Barr et al., 2010; Basar-Eroglu et al., 2007; Chen, Stanford, Mao, & Abi-Dargham, 2014; Cho, Konecky, & Carter, 2006). Gamma activity within the frontal cortex (Chen et al., 2014) and working memory (Rogasch, Daskalakis, & Fitzgerald, 2014) are related to GABA activity, and deficits may be ameliorated with GABAergic drugs (Lewis et al., 2008).

Animal models of schizophrenia have demonstrated that $GABA_B$ receptor activity can alter dopamine hyperactivity (Balla et al., 2009; Javitt, Hashim, & Sershen, 2005) and altered glutamatergic activity (Roenker, Gudelsky, Ahlbrand, Horn, & Richtand, 2012), which are both suspected to contribute to symptoms of schizophrenia. Additionally, patients with schizophrenia often exhibit impaired sensorimotor gating. These deficits are recused in animal models by $GABA_B$ receptor activation (Arai et al., 2008; Bortolato, Frau, Aru, Orrù, & Gessa, 2004; Bortolato et al., 2007; Fejgin et al., 2009; Frau et al., 2014), and enhanced with $GABA_B$ receptor blockade (Ma & Leung, 2011). An experimental drug that promotes neurite growth was found to rescue sensorimotor gating deficits; the authors suggest this effect was due to reversing the loss of GABAergic neurons typically seen in the animal model used (Uehara et al., 2012). Further, baclofen improves behavioral deficits suggested to arise from disrupted excitatory-inhibitory signaling induced by altered glutamatergic activity, and restored the disrupted excitatory-inhibitory signaling (Gandal et al., 2012). These data strongly implicate the $GABA_B$ receptor as a potential target in the treatment of schizophrenia.

$GABA_B$ markers are also altered in AD. In a postmortem examination, hippocampal tissue from brains of AD patients was characterized by increases of $GABA_{B1}$ proteins in the CA4 and CA3/2 subfields, which were associated with the progression of neurofibrillary tangle pathology (Iwakiri et al., 2005). Subregions of the DG and CA1 (Chu, Penney, & Young, 1987b) and the superior frontal gyrus (Chu, Penney, & Young, 1987a) had fewer $GABA_B$ receptors; and specifically within the CA1, $GABA_{B1}$ receptors were decreased compared to control tissue (Iwakiri et al., 2005).

A non-coding RNA discovered to produce an alternative splicing of the $GABA_B$ receptor is upregulated in the frontal and temporal cortices of patients with AD (Massone et al., 2011). This alternative splicing is triggered by inflammatory stimuli and results in altered $GABA_B$-mediated signaling, increases amyloid-β (Aβ) secretion, and increases the $A\beta_{42/40}$ ratio. In a study utilizing TMS, AD patients demonstrated an increase in the $GABA_B$-mediated CSP, which was inversely related to scores on a measure of neurological ability (Khedr, Ahmed, Darwish, & Ali, 2011). These studies suggest that $GABA_B$ signaling is altered in AD.

AD affects other GABAergic markers, as well. GABA is decreased in the temporal lobe, parietal lobe, occipital lobe, and cerebellum (Bai et al., 2014; Seidl, Cairns, Singewald, Kaehler, & Lubec, 2001). Decreased amounts of $GABA_A$ receptors and GABAergic neurons are observed in the hippocampus (Chu et al., 1987b; Inaguma, Shinohara, Inagaki, & Kato, 1992). The enzymes responsible for synthesizing and breaking down GABA are decreased within the cerebellum and hippocampus (Burbaeva et al., 2014; Schwab, Yu, Wong, McGeer, & McGeer, 2013). Recent evidence also implicates altered glial function in AD. Postmortem tissue exhibits increased

concentrations of GABA within astrocytes in the hippocampus and temporal cortex (Jo et al., 2014; Wu, Guo, Gearing, & Chen, 2014). Additionally, hippocampal astrocytes also express increased amounts of GAD67 and the astrocytic GABA transporter (Wu et al., 2014).

Aβ produces changes to inhibitory signaling that is similar to the changes observed when $GABA_B$-mediated potassium conductance (via GIRK channels) is altered (Nava-Mesa, Jiménez-Díaz, Yajeya, & Navarro-Lopez, 2013). Additionally, intrahippocampal injections of Aβ decreases the number of neurons expressing GABAergic markers (Villette et al., 2012). Transgenic mice expressing Aβ exhibit impaired neurogenesis and excitatory-inhibitory imbalance in the hippocampus, which is normalized when GABAergic signaling is blocked (Sun et al., 2009). In transgenic mice expressing apolipoprotein E4 (apoE4) and tau, administration of a $GABA_A$ receptor agonist rescues learning and memory deficits (Andrews-Zwilling et al., 2010). When tau expression is removed from this transgenic strain, GABAergic neuron loss and learning and memory deficits are reversed. However, the administration of a $GABA_A$ antagonist eliminates the beneficial effects of the lack of tau. These data suggest that traditional markers of AD may interact with and affect GABAergic signaling.

Similar to the postmortem findings, animal models of AD also display changes to glial function. For instance, hippocampal reactive astrocytes exhibit high GABA content (Jo et al., 2014; Wu et al., 2014), which contributes to increased tonic GABA inhibition, impaired LTP, and learning and memory deficits. Decreasing GABA rescues these deficits.

Patients with Down syndrome (DS) exhibit AD-like neurodegeneration. While postmortem investigations do not necessarily indicate the same changes to GABAergic markers as AD patients (Seidl et al., 2001), animal models do suggest altered GABAergic signaling contributes to the disorder. Transgenic animals exhibit several upregulated GABAergic markers within the hippocampus (Hernández-González et al., 2014), and learning and memory deficits are mediated by the DG (Smith, Kesner, & Korenberg, 2013). Normalizing GABA release rescues hippocampal-dependent learning and memory deficits (Begenisic et al., 2013). Animal models exhibit increased $GABA_B$-mediated GIRK current and GIRK expression within the hippocampus (Best, Cramer, Chakrabarti, Haydar, & Galdzicki, 2012; Best, Siarey, & Galdzicki, 2007; Kleschevnikov, Belichenko, Gall, et al., 2012b), which are suggested to mediate cognitive deficits. When administered a $GABA_B$ receptor antagonist, transgenic animals display normal learning and memory behavior in several domains (Kleschevnikov, Belichenko, Faizi, et al., 2012a).

Interestingly, one of the more extensively studied $GABA_B$ antagonists, CGP 36742, had progressed to Phase II trials to treat AD (Davies, Castaner, & Castaner, 2005; Froestl et al., 2004). Compared to placebo, it improved working memory and attention. However, it has since failed to progress to Phase III testing (Sabbagh, 2009). These data indicate that some of the memory impairments seen in AD patients could be the result of GABA dysfunction, which may be rectified with GABAergic therapeutics.

Together these data indicate that altered $GABA_B$ receptor function is common to patients with schizophrenia and AD. New treatments could focus on these receptors, and the data from the experiments outlined below help identify potential targets within the

$GABA_B$ receptor signaling cascade. These experiments were designed to investigate the impact of altered $GABA_B$ receptor activity on memory flexibility and spatial learning and memory, behavioral paradigms that are affected in clinical populations.

Hypotheses

We were interested in determining whether altered $GABA_B$ receptor activity would affect both memory flexibility and spatial learning and memory. Based on previous research and data from our lab (Heaney et al., 2012), we predicted that the $GABA_B$ agonist baclofen would impair performance in the MWM and be detrimental to memory flexibility. Additionally, we predicted that phaclofen would improve performance and memory flexibility. Further, we predicted that we would find protein level changes within the hippocampus that relate to the behavioral outcomes. Specifically, we examined proteins related to GABAergic and glutamatergic signaling, as well as $GABA_B$ receptor subunits, targets within the $GABA_B$ signaling cascade, and markers of synaptic plasticity. Increases to markers that enhance GABA signaling should be related to impaired performance and increased glutamatergic markers should relate to improved performance. Additionally, we predicted to find increases in markers related to synaptic plasticity in groups that demonstrate improved behavioral performance.

CHAPTER 3

MATERIALS AND METHODS

Subjects

Sixty male Sprague-Dawley rats (Taconic Laboratories, Cambridge City, Indiana) weighing approximately 250-300 g were used. Rats were housed in a temperature and humidity controlled facility (22 ± 1° C), and food and water was provided *ad libitum*. Animals were housed in pairs and kept on a 12:12 light/dark cycle, lights on at 7:00 AM. All procedures were approved by the Institutional Animal Care and Use Committee and were carried out in accordance with NIH guidelines for the care and use of animals.

Drug Treatments

R(+)-Baclofen hydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% physiological saline vehicle at a concentration of 1 mg/mL or 2 mg/mL. Phaclofen (Sigma-Aldrich) was dissolved in vehicle at a concentration of 0.9 mg/mL or 1.25 mg/mL. Compounds were administered 1 mL/kg body weight via subcutaneous (SC) injections each day 30 min before behavioral testing throughout the experiment. Animals were randomly assigned to one of three treatment groups (saline, baclofen, or phaclofen; n=10) in one of two experiments (for a total of n=30 per cohort). In Cohort 1, the baclofen group received 2 mg/kg, and the phaclofen group received 0.9 mg/kg. Based on the behavioral outcomes in Cohort 1, we tested a lower concentration of baclofen (1.0 mg/mL) and a higher concentration of phaclofen (1.25 mg/mL). Due to technical errors during the experiment, data were lost for two of the 1.25 mg/kg phaclofen-treated animals, so they were removed from the analyses.

Morris Water Maze

The MWM task was conducted in a plastic, circular tank (1.8 m diameter and 91 cm tall; San Diego Instruments, San Diego, CA). The tank was filled to a depth of 48 cm with water made opaque using non-toxic tempera paint and temperature held at 25° C ± 2°. A clear, Plexiglas platform was submerged 2.5 cm below the surface of the water. Platform location was randomized between two starting quadrants such that half of the animals from each treatment group started in each location. For each animal, the location of the hidden platform was held constant over successive training days except where noted. Data collected included swim speed, path length, latency to locate the platform, amount of time spent in each quadrant, and amount of time spent swimming around the perimeter of the tank (thigmotaxis).

Open Field

All animals were tested in an open field apparatus consisting of white Plexiglas walls 60 cm x 61 cm x 46 cm. Data collected for each trial included average speed, percent time spent around the perimeter of the maze, and path length.

Behavioral Testing

All procedures were consistent with previous investigations (Kinney et al., 2009; Sabbagh, Heaney, Bolton, Murtishaw, & Kinney, 2012a; Sabbagh et al., 2012b). Across consecutive days, animals were trained to locate a hidden escape platform utilizing distal spatial cues located around the testing room. Each day, animals received one session consisting of four trials; if a subject failed to locate the hidden escape platform within 60

s, an experimenter guided the animal to the platform. The animals were given 20 s on the platform to orient to the distal spatial cues. Animals were given 30 s under a heat lamp in between trials. Training continued until the control group reached a criterion level of average group performance of less than 15 s to locate the hidden platform. Once the criterion was met, a probe trial was conducted 24 h later to examine whether the animals learned the spatial location of the platform or were utilizing a different escape strategy. During the single 60 s probe trial, the escape platform was removed and the animals were allowed to swim freely.

Twenty-four hours following the probe trial, reversal platform training began. The hidden platform was placed into the center of the quadrant 180° from the original training location. Subjects were trained for two days to learn the new location of the hidden platform. Twenty-four hours after the second reversal training day, animals underwent a second probe trial in the same manner as previously described. Immediately after the reversal probe was completed, animals began visible platform training and, thus, only received three trials on this day. The second day of visible training consisted of four trials. The hidden platform was replaced with a visible platform that protrudes from the surface of the water. The visible platform was moved to a new quadrant for each trial and never started in either of the two target quadrants used for hidden or reversal training. Visible platform training was performed to ensure that all subjects were capable of performing the task with similar motoric and visual abilities.

Once MWM training was complete, animals were tested in the open field. Animals were placed into the center of the apparatus and given a single five-minute session. Animals were allowed to move around freely.

Tissue Collection

Following the open field test, rats were euthanized via CO_2 asphyxiation. Tissue was rapidly dissected out and flash frozen for western blotting analyses. Right hippocampi were used for analyses due to previous research indicating that the right hippocampus is more involved in spatial learning and memory than the left hippocampus (Bohbot et al., 1998; Burgess, Maguire, & O'Keefe, 2002; Shinohara et al., 2010).

SDS-PAGE/Western Blotting

Brain tissue was homogenized in RIPA lysis buffer (Cell Signaling, Danvers, MA) with 1 mM DTT, 1 mM PMSF, 20 μg/mL aprotinin, and 0.1% SDS added. Lysates were centrifuged at 15,000xg for 15 minutes at 4°C; the supernatants were then collected and protein concentrations were determined using the bicinchoninic acid assay (Pierce, Rockford, IL). Samples were loaded at a total of 20 μg into 8% or 10% acrylamide gels (gel percentage was based on target protein size) and separated via SDS-PAGE (Laemmli, 1970).

For membranes imaged using the Typhoon 9410 Variable Mode Imager (GE Healthcare Life Sciences, Piscataway, NJ), proteins were then transferred to a nitrocellulose membrane and blocked in 1x TBS with 5% BSA, 0.05% Tween-20, and 0.02% sodium azide. Membranes were incubated in primary antibody overnight mixed in 1x TBS with 5% BSA and 0.05% Tween-20 (rabbit anti-$GABA_{B1}$, 1:2000, Cell Signaling; rabbit anti-$GABA_{B2}$, 1:1000, Cell Signaling; rabbit anti-phosphoserine 892-$GABA_{B2}$, 1:1000, Novus Biologicals, Littleton, CO; mouse anti-GAD67, 1:2000, Millipore, Billerica, MA; mouse or rabbit anti-β-actin, 1:10,000, ProteinTech, Chicago,

IL). The next day, membranes were incubated in HRP-conjugated secondary antibodies mixed in 5% milk-TBS-Tween-20 (goat anti-mouse or goat anti-rabbit, 1:5000, Vector Laboratories, Burlingame, CA) and then probed with Amersham ECL Plus (GE Healthcare Life Sciences) and imaged via the Typhoon 9410 Imager (GE Healthcare Life Sciences). Band intensity was determined via ImageQuant 5.2 (GE Healthcare Life Sciences).

For membranes imaged using the Odyssey CLx Infrared Imager (Li-Cor Biosciences, Lincoln, NE), proteins were transferred to a nitrocellulose membrane and blocked in 5% non-fat skim milk mixed in 1x PBS with 0.01% sodium azide. Membranes were incubated in primary antibody overnight mixed in the blocking solution with 0.1% Tween-20 added (rabbit anti-AMPA receptor GluR4, 1:1000, Cell Signaling; rabbit anti-GIRK2, 1 μg/mL, Abcam, Cambridge, MA; rabbit anti-kalirin, 1:750 for kalirin-5 and 1:7000 for kalirin-7, 9, and 12, Millipore; mouse or rabbit anti-β-actin, 1:20,000, ProteinTech). The next day, membranes were incubated in IRDye near-infrared secondary antibodies (IRDye 680 goat anti-mouse, 1:10,000, IRDye 800 donkey anti-goat, and IRDye 800 goat anti-rabbit, 1:5000, Li-Cor Biosciences) mixed in the blocking solution plus 0.1% Tween-20 and then imaged via the Odyssey CLx Infrared Imaging System (Li-Cor Biosciences).

All western blots were analyzed by normalizing the densities of the protein of interest to the density of β-actin for each individual sample. A proportion was determined for each normalized value of the treatment group protein bands compared to the averaged normalized values for saline control groups run in the same gel. These proportional values were used for analysis.

Statistical Analyses

All measures of hidden, reversal, and visible platform performance (path length, latency, thigmotaxis, and swim speed) were analyzed using repeated measures analysis of variance (RM-ANVOA) across days. Probe trial time in target quadrant as compared to time in the other quadrants and annulus crossings data were analyzed using within-subjects one-way ANOVA. Measures of open field data (speed, percent time in perimeter, path length) were analyzed via one-way ANOVA. Western blot results were analyzed using one-way ANOVA. Tukey post-hoc comparisons were performed following a significant one-way ANOVA result where applicable. In order to better detect subtle differences, the hidden platform latency across days was also analyzed separately between the 0.9 mg/kg phaclofen group and controls with RM-ANOVA, and all western blot analyses were performed between a single treatment group and the control. Although there were planned comparisons to compare performance between ligand groups, the two control groups were significantly different; therefore, these analyses were not conducted.

CHAPTER 4

RESULTS

Morris Water Maze

Hidden Platform Training and Probe

Performance of the two control groups were analyzed in order to determine whether we could combine groups and compare within ligand groups. The control group for Cohort 1 (2.0 mg/kg baclofen, 0.9 mg/kg phaclofen) had a significantly lower latency across days compared to the Cohort 2 (1.0 mg/kg baclofen, 1.25 mg/kg phaclofen) controls ($F_{1,78}$=4.407, $p<0.05$; see fig. 1). Therefore, we were unable to compare the different concentrations of the ligands together and analyzed each cohort separately.

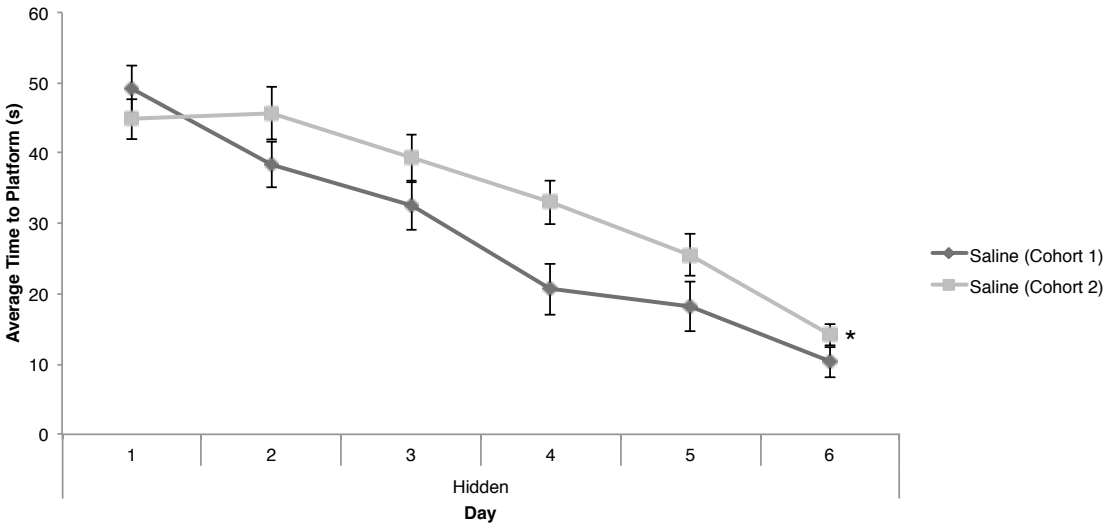

Figure 1. Morris Water Maze Latency of the Saline Controls from Both Cohorts. Compared to the controls from Cohort 1, the average latency (± SEM) for the controls from Cohort 2 was significantly increased across hidden training days. * = $p<0.05$ compared to Cohort 1.

The 2.0 mg/kg baclofen-treated group was significantly impaired in the training phase across days as compared to the control group (see fig. 2a; $F_{2,117}$=79.748, $p<0.001$; Tukey post-hoc saline vs baclofen $p<0.001$). The group that received the 1.0 mg/kg baclofen dose (fig. 2b), however, did not exhibit this deficit and was not significantly different from the control group ($F_{2,109}$=1.704, $p>0.05$). Treatment with 0.9 mg/kg of phaclofen (fig. 2a) produced a learning and memory enhancement during the hidden platform training across days compared to the control group ($F_{1,78}$=5.046, $p<0.05$). While the 1.25 mg/kg phaclofen-treated group did not significantly differ from the controls ($F_{2,109}$=1.704, $p>0.05$), this dose of phaclofen did not produce the enhancement seen with the lower dose (fig. 2b).

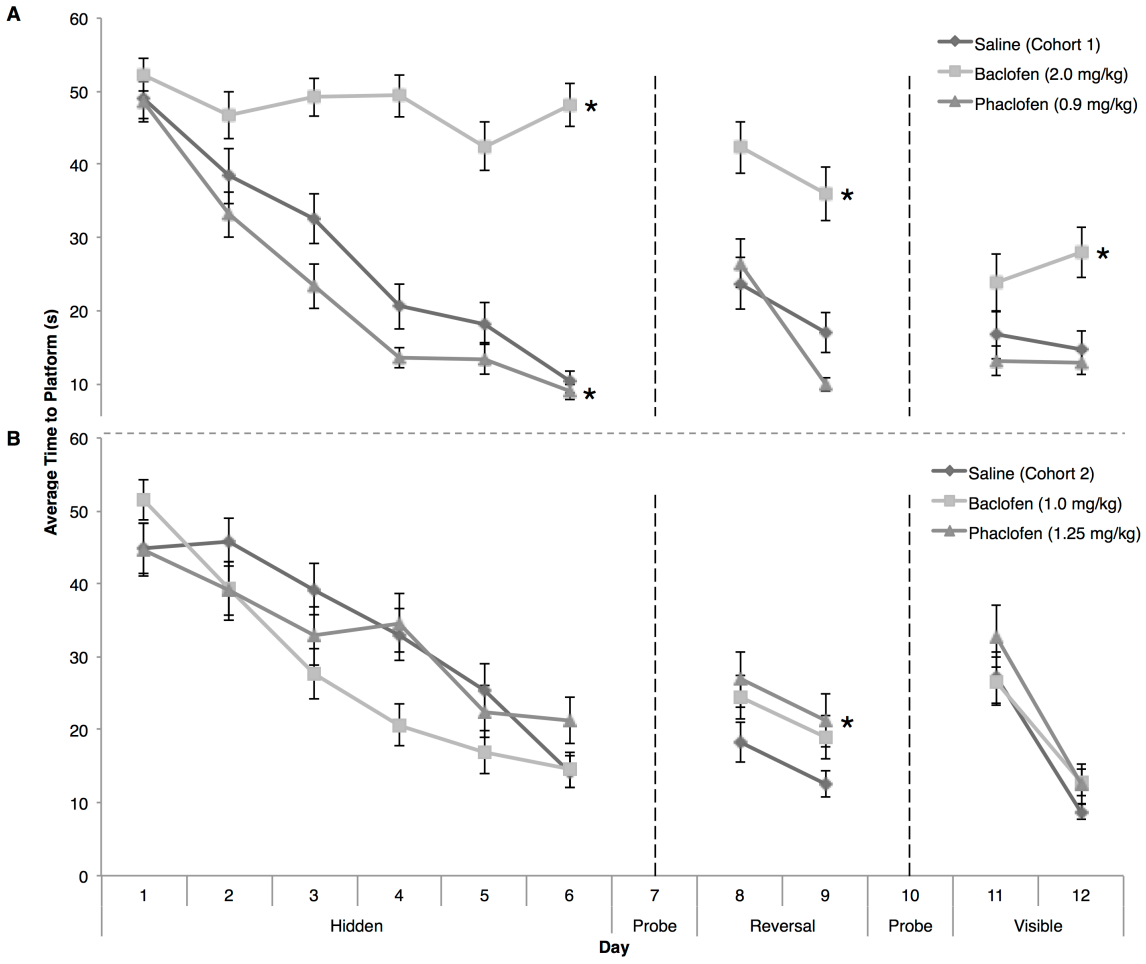

Figure 2. Morris Water Maze Latency of Each Group During Hidden, Reversal, and Visible Training. Average latency (± SEM) was increased by administration of 2.0 mg/kg baclofen, but decreased by 0.9 mg/kg phaclofen (a) during hidden training. Latency remained significantly elevated for reversal and visible training for the 2.0 mg/kg baclofen group. Administration of 1.25 mg/kg phaclofen significantly increased latency during reversal training (b). Treatment of 1.0 mg/kg baclofen did not impair performance. * = $p<0.05$ compared to saline controls.

During the probe trial (see fig. 3a), the 0.9 mg/kg phaclofen-treated ($F_{3,36}=15.477$, $p<0.001$), 1.0 mg/kg baclofen-treated ($F_{3,36}=20.075$, $p<0.001$), and control groups demonstrated a selective search (cohort 1 saline: $F_{3,36}=14.657$, $p<0.001$; cohort 2 saline: $F_{3,36}=16.095$, $p<0.001$; for all: Tukey post-hoc percent time in target quadrant vs percent

time in each non-target quadrant $p<0.01$). The 2.0 mg/kg baclofen-treated ($F_{3,36}=11.189$, $p<0.001$) and 1.25 mg/kg phaclofen-treated ($F_{3,28}=3.804$, $p<0.05$) groups did not exhibit a selective search for the target quadrant (Tukey post-hoc analyses show that percent time in target quadrant is not $p<0.05$ vs all non-target quadrants). However, all groups except the 2.0 mg/kg baclofen-treated group ($F_{3,36}=0.545$, $p>0.05$) demonstrated a significant number of annulus crossings (fig. 3b) over the physical space where the platform was previously located versus analogous locations in other quadrants (cohort 1 saline: $F_{3,36}=9.199$, $p<0.001$; 0.9 mg/kg phaclofen: $F_{3,36}=5.974$, $p<0.01$; cohort 2 saline: $F_{3,36}=15.468$, $p<0.001$; 1.0 mg/kg baclofen: $F_{3,36}=11.917$, $p<0.001$; 1.25 mg/kg phaclofen: $F_{3,28}=6.13$, $p<0.01$; Tukey post-hoc analyses show that number of annulus crossings in target quadrant vs all other quadrants $p<0.05$ for all groups). These data suggest that the only group that was impaired in learning the location of the hidden platform was the 2.0 mg/kg baclofen-treated group.

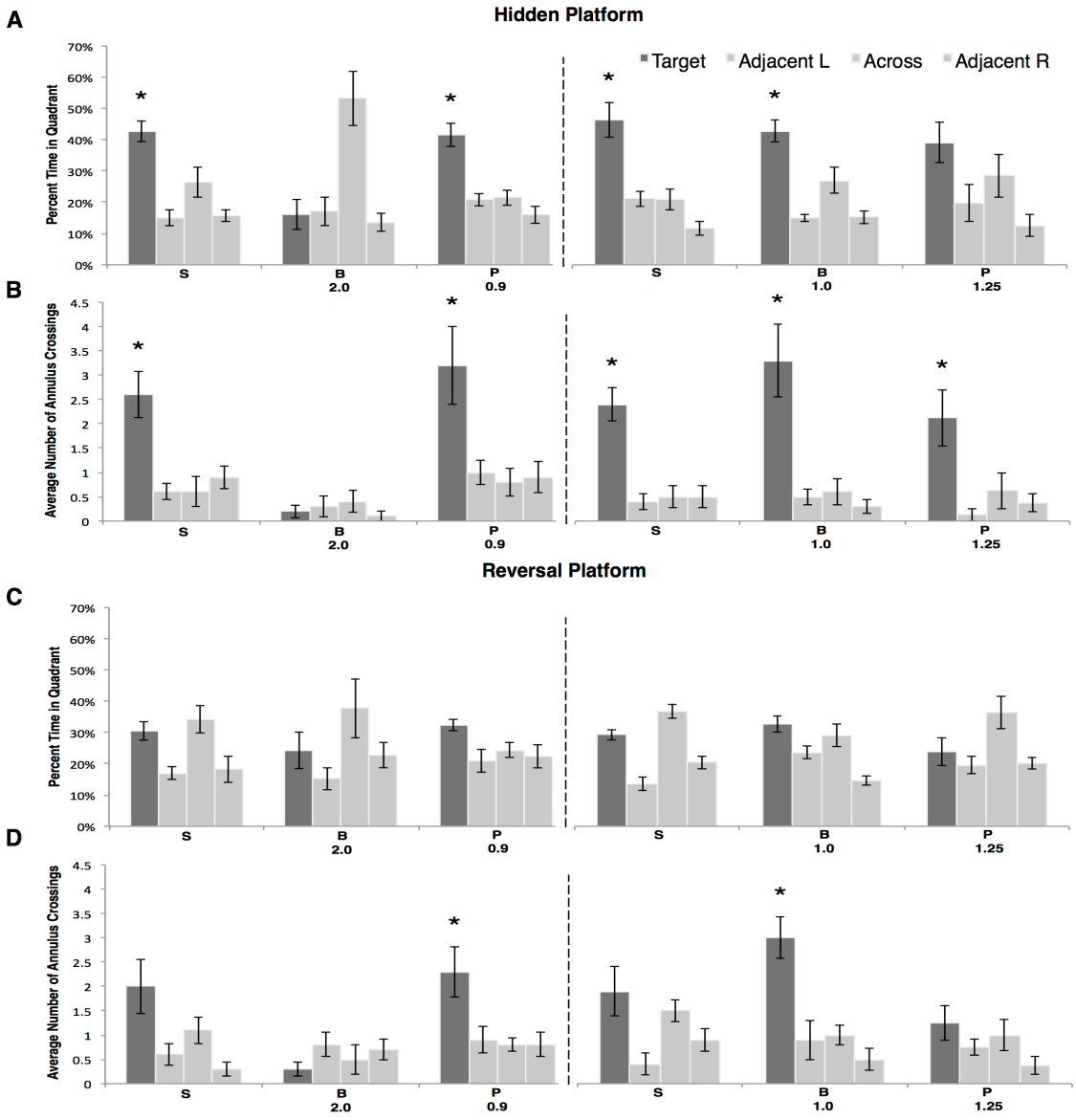

Figure 3. Hidden and Reversal Probe Trial Performance. Average percent time (± SEM) spent in each quadrant (a, c) during the hidden training probe was significant for the control, 0.9 mg/kg phaclofen, and the 1.0 mg/kg baclofen groups; the 2.0 mg/kg baclofen and 1.25 mg/kg phaclofen groups did not exhibit a significant search. The controls, 0.9 mg/kg phaclofen, 1.0 mg/kg baclofen, and 1.25 mg/kg phaclofen groups did display significant average number of crossings (± SEM) over the analogous location of the platform (b, d). Average percent time (± SEM) spent in each quadrant during the reversal training probe (c) was not significant for any group, but average number of crossings (± SEM) over the analogous location of the platform (d) during the reversal training probe was significant for the 0.9 mg/kg phaclofen- and 1.0 mg/kg baclofen-treated groups only. * = $p<0.05$ for percent time or number of crossings for the target quadrant as compared to the non-target quadrants.

Reversal Platform Training and Probe

Across the two days of reversal training, the 2.0 mg/kg baclofen-treated ($F_{2,117}$=20.244, $p<0.001$; Tukey post-hoc analysis saline vs 2.0mg/kg baclofen $p<0.001$) and the 1.25 mg/kg phaclofen-treated ($F_{2,109}$=3.226, $p<0.05$; Tukey post-hoc analysis saline vs 1.25 mg/kg phaclofen $p<0.05$) required significantly more time to find the platform as compared to the control group (see fig. 2). We further examined reference memory between groups by analyzing latency to find the hidden platform during first trial of the second day of reversal training (see fig. 4). As compared to controls, the 0.9 mg/kg phaclofen-treated group ($F_{2,27}$=17.471, $p<0.001$, Tukey post-hoc analysis saline vs 0.9 mg/kg phaclofen $p<0.01$) appeared to learn the new spatial location of the platform during the reversal training more effectively. This effect was not seen in the 1.25 mg/kg phaclofen-treated group ($F_{2,25}$=1.015, $p>0.05$). Further, the 2.0 mg/kg baclofen-treated group was significantly impaired compared to controls ($F_{2,27}$=17.471, $p<0.001$, Tukey post-hoc analysis saline vs 2.0 mg/kg baclofen $p<0.05$), but the 1.0 mg/kg baclofen-treated group was not ($F_{2,25}$=1.015, $p>0.05$). These data suggest that treatment with 0.9 mg/kg phaclofen significantly improved memory flexibility for the new platform location, whereas 2.0 mg/kg baclofen significantly impaired performance similar to acquisition.

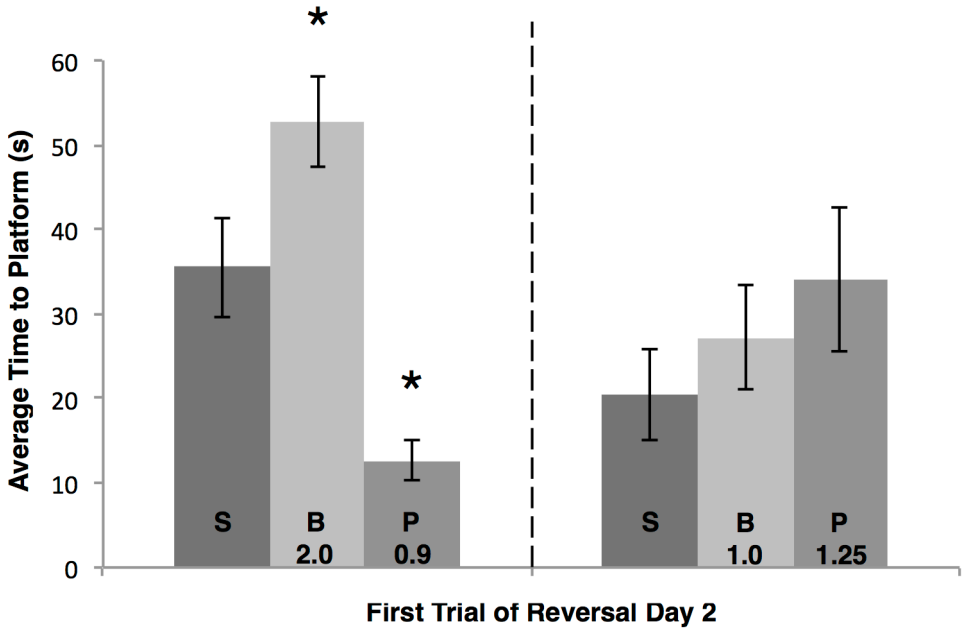

Figure 4. Additional Reversal Training Performance Analysis. Average latency (± SEM) to find the platform during the first trial of the second day of reversal training was significantly increased by 2.0 mg/kg baclofen treatment but decreased by 0.9 mg/kg phaclofen treatment. * = $p<0.05$ compared to saline controls.

During the reversal probe trial (see fig. 3c), none of the groups demonstrated a selective search based on percent time spent in the target quadrant compared to the non-target quadrants (cohort 1 saline: $F_{3,36}=6.019$, $p<0.01$; 2.0 mg/kg baclofen: $F_{3,36}=2.309$, $p>0.05$; 0.9 mg/kg phaclofen: $F_{3,36}=2.852$, $p>0.05$; cohort 2 saline: $F_{3,36}=24.551$, $p<0.001$; 1.0 mg/kg baclofen $F_{3,36}=9.232$, $p<0.001$; 1.25 mg/kg phaclofen: $F_{3,28}=4.152$, $p<0.05$; for all groups with significant analyses Tukey post-hoc analysis percent time in target quadrant vs percent time in non-target quadrants $p>0.05$). This result is not surprising since the groups were only given two days to learn the new location of the platform. However, when annulus crossings were analyzed (see fig. 3d), the 0.9 mg/kg phaclofen-treated and 1.0 mg/kg baclofen-treated groups demonstrated a selective

preference for the new target location (phaclofen: $F_{3,36}$=5.089, p<0.01; baclofen: $F_{3,36}$=11.482, p<0.001; for both groups Tukey post-hoc analysis number of crossings in target quadrant vs number of crossings in each non-target quadrant p<0.05). The controls (cohort 1 saline: $F_{3,36}$=4.812, p<0.05; cohort 2 saline: $F_{3,36}$=4.275, p<0.05; Tukey post-hoc analysis number of crossings in target quadrant vs number of crossings in non-target quadrants, p>0.05;), 2.0 mg/kg baclofen-treated ($F_{3,36}$=0.872, p>0.05) and 1.25 mg/kg phaclofen-treated ($F_{3,28}$=1.849, p>0.05) groups did not demonstrate this specificity for the new platform location.

Visible Platform Training

Across the two days of visible training, the 2.0 mg/kg baclofen-treated group required more time to find the visible platform as compared to the control group (see fig. 2; $F_{2,87}$=7.891, p<0.001; Tukey post-hoc analysis saline vs 2.0 mg/kg baclofen p<0.01). This effect was not seen for the 0.9 mg/kg phaclofen-treated group ($F_{2,87}$=7.891, p<0.001; Tukey post-hoc analysis saline vs 0.9 mg/kg phaclofen p>0.05), the 1.0 mg/kg baclofen-treated ($F_{2,81}$=1.424, p>0.05), or 1.25 mg/kg phaclofen-treated groups ($F_{2,81}$=1.424, p>0.05). While significantly higher compared to controls, the average latency for 2.0 mg/kg baclofen-treated group to find the platform decreases across the various stages of this experiment (see fig. 2). Further, performance on the first day of visible training for the high dose of baclofen did not significantly differ between groups ($F_{2,87}$=2.868, p>0.05); the difference in latency as measured across days for visible training stems from the performance difference on the second day of visible training. If treatment with baclofen were impairing this group's ability to physically solve the task, we would expect

performance to be stagnant across the entire experiment. Therefore, we do not believe the 2.0 mg/kg baclofen treatment affected this group's ability to detect the platform.

Swim Speed, Thigmotaxis, and Open Field

Open field testing was conducted to determine if drug treatment produced locomotor deficits or produced an anxiety-like phenotype. While the 2.0 mg/kg baclofen dose did result in significantly decreased swim speeds during reversal ($F_{2,117}$=8.37, $p<0.001$) and visible training ($F_{2,117}$=8.37, $p<0.001$; for both phases Tukey post-hoc analysis saline vs 2.0 mg/kg baclofen $p<0.01$; see fig. 5), there were no significant differences in speed during the hidden platform training ($F_{5,585}$=28.185, $p<0.001$; Tukey post-hoc saline vs 2.0 mg/kg baclofen $p>0.05$) or during the open field task ($F_{2,27}$=1.106, $p>0.05$; see fig. 6a). No significant speed differences in the open field task were detected for the 0.9 mg/kg phaclofen-treated group ($F_{2,27}$=1.106, $p>0.05$), the 1.0 mg/kg baclofen-treated group ($F_{2,25}$=3.246, $p>0.05$), or 1.25 mg/kg phaclofen-treated groups ($F_{2,25}$=3.246, $p>0.05$).

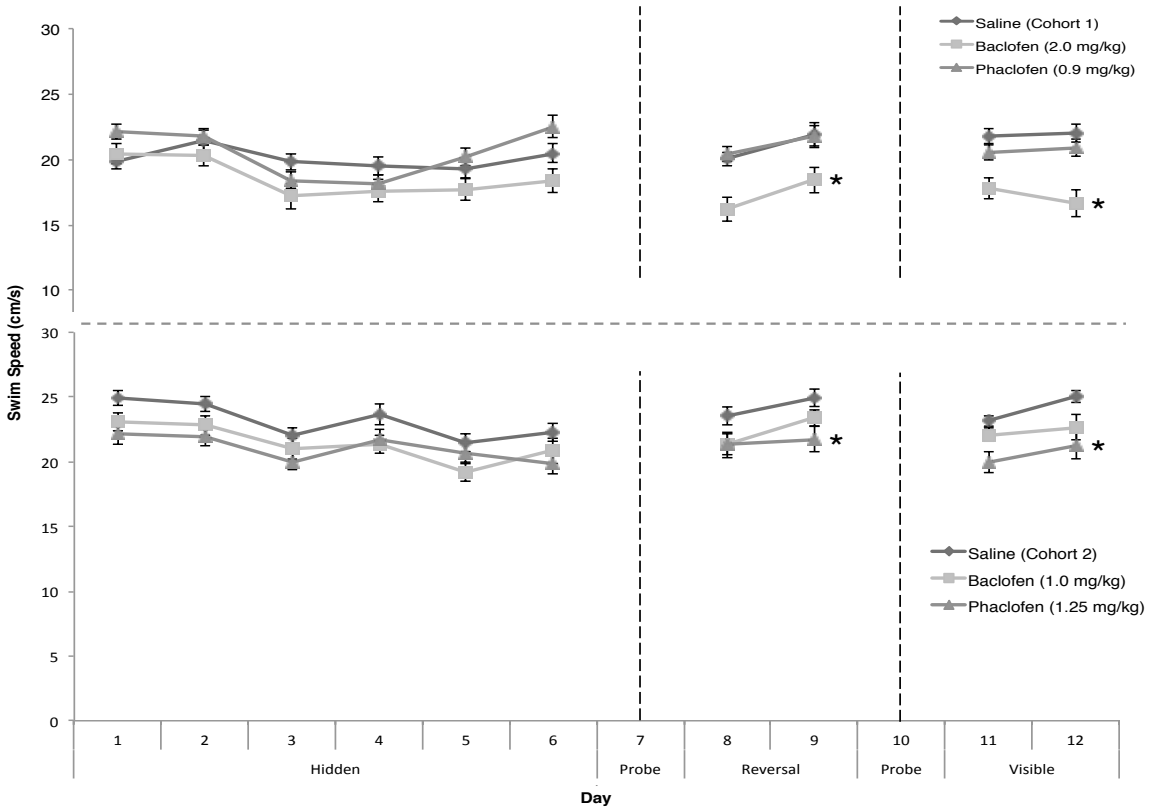

Figure 5. Swim Speed Performance. Treatment with 2.0 mg/kg baclofen and 1.25 mg/kg phaclofen significantly decreased average (± SEM) swim speed (cm/s) during reversal and visible training.

The 2.0 mg/kg baclofen-treated group exhibited significantly elevated thigmotaxis (see fig. 7) during hidden platform training ($F_{2,117}$=6.797, p<0.001), reversal training ($F_{2,117}$=36.942, p<0.001), and visible training ($F_{2,117}$=36.942, p<0.001; for all phases Tukey post-hoc saline vs 2.0 mg/kg baclofen p<0.001). In order to evaluate if elevated thigmotaxis was associated with an anxiety phenotype, we examined anxiety-like behavior in an open field task. No significant differences appeared for the amount of time either cohort (2.0 mg/kg baclofen/0.9 mg/kg phaclofen: $F_{2,27}$=0.678, p>0.05; 1.0 mg/kg baclofen/1.25 mg/kg phaclofen: $F_{2,25}$=0.186, p>0.05) spent along the perimeter during the open field task compared to controls (see fig. 6b). Further, no significant differences

appeared for path length for either cohort (2.0 mg/kg baclofen/0.9 mg/kg phaclofen: $F_{2,27}$=1.099, p>0.05; 1.0 mg/kg baclofen/1.25 mg/kg phaclofen: $F_{2,25}$=3.248, p>0.05; see fig. 6c).

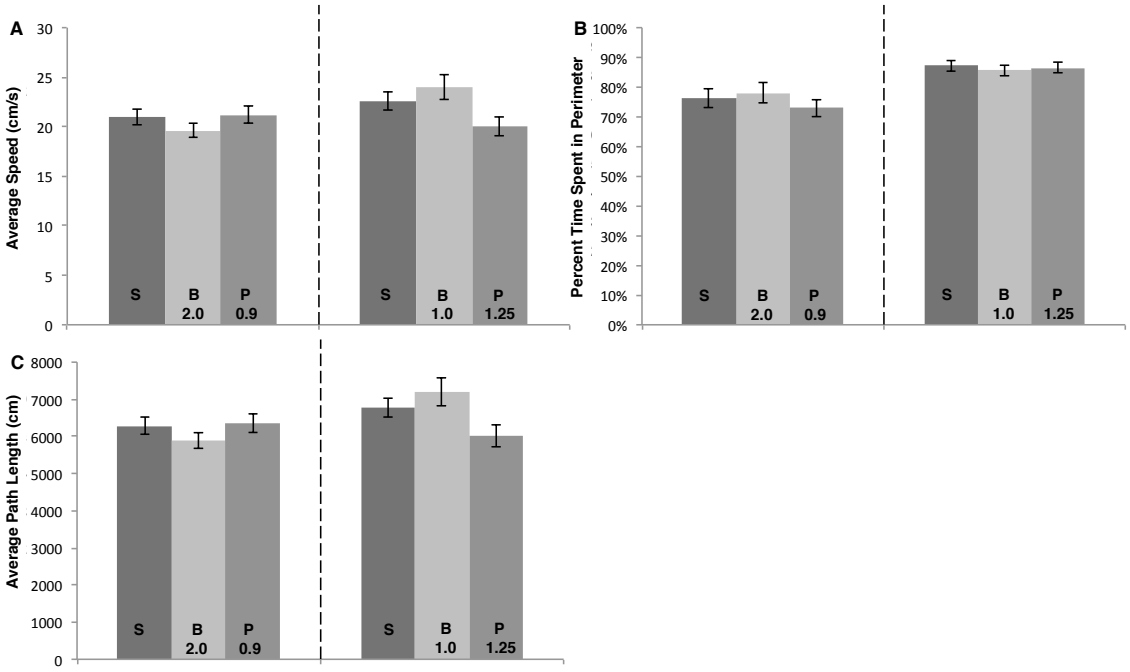

Figure 6. Open Field Performance. Ligand treatment did not affect average speed (± SEM cm/s; a), percent time in perimeter (± SEM; b), or average path length (± SEM cm; c).

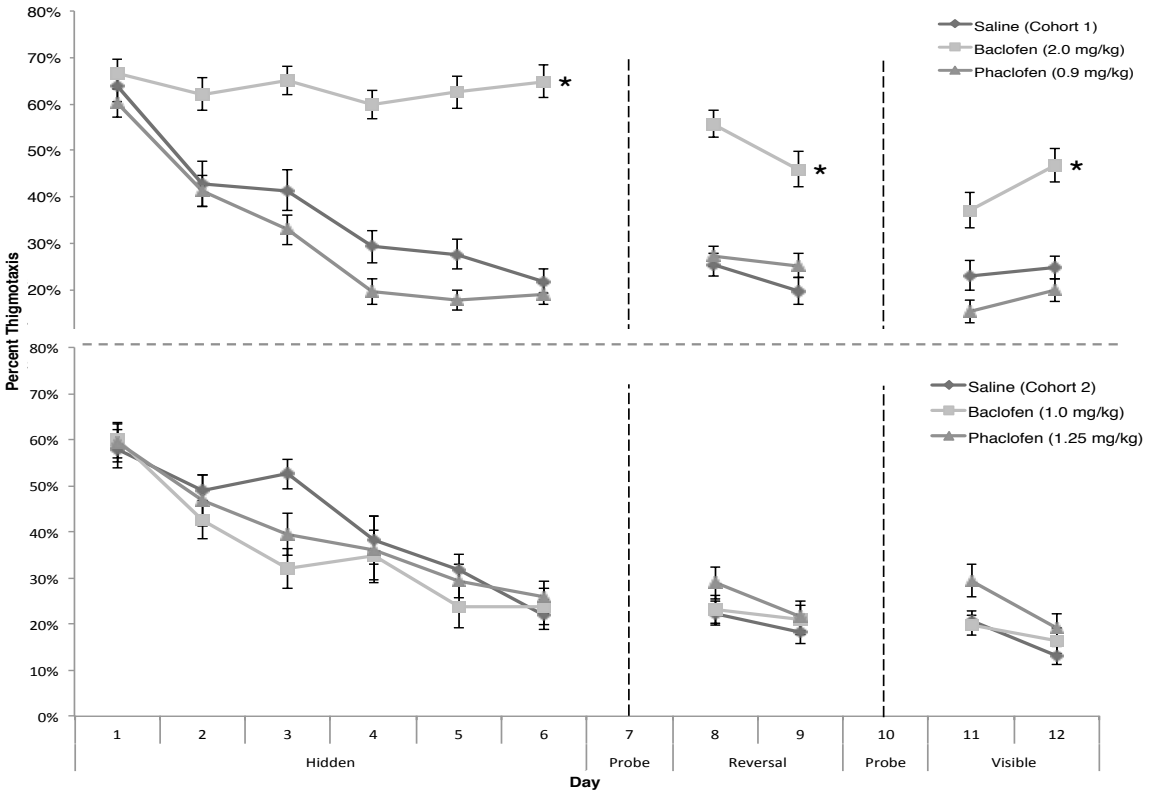

Figure 7. Thigmotaxis Performance. Percent thigmotaxis (± SEM) was significantly elevated in the 2.0 mg/kg baclofen-treated group. * = $p<0.05$ compared to saline controls.

SDS-PAGE/Western Blotting

We examined total protein levels from the right hippocampus for several GABAergic markers including $GABA_B$ receptor subunits, phosphorylated serine 892 $GABA_{B2}$ (pSer892), and GAD67. We also analyzed total protein levels for several kalirin isoforms, a marker of synapse formation (Ma, Kiraly, Gaier, Wang, Kim, Levine, et al., 2008a; Ma, Wang, Ferraro, Mains, & Eipper, 2008b; Penzes & Jones, 2008). Effects on potassium channel expression were examined via GIRK2, an inwardly-rectifying potassium channel, which $GABA_B$ receptors activate via G-proteins (Cramer, Best, Stoffel, Siarey, & Galdzicki, 2010; Fowler, Aryal, Suen, & Slesinger, 2007; Lüscher, Jan,

Stoffel, Malenka, & Nicoll, 1997). Additionally, we investigated total expression of AMPA GluR4 to examine glutamatergic activity on hippocampal interneurons (Leranth, Szeidemann, Hsu, & Buzsaki, 1996).

Both the 0.9 mg/kg phaclofen-treated ($F_{1,18}$=4.852, $p<0.05$) and the 1.0 mg/kg baclofen-treated ($F_{1,18}$=4.654, $p<0.05$) groups exhibited a significant reduction in the total expression of the AMPA subunit GluR4 as compared to the saline controls (see fig. 8a). No differences in GluR4 were found between the 2.0 mg/kg baclofen-treated ($F_{1,18}$=1.845, $p>0.05$) or 1.25 mg/kg phaclofen-treated ($F_{1,18}$=0.114, $p>0.05$) groups compared to controls.

The 0.9 mg/kg phaclofen-treated group also exhibited a significant increase in GIRK2 compared to controls (see fig. 8b; $F_{1,18}$=5.253, $p<0.05$). GIRK2 expression in the 2.0 mg/kg baclofen-treated group ($F_{1,18}$=1.076, $p>0.05$), 1.0 mg/kg baclofen-treated group ($F_{1,18}$=1.018, $p>0.05$), or the 1.25 mg/kg phaclofen-treated group ($F_{1,18}$=0.79, $p>0.05$) did not significantly differ from the control groups.

A significant reduction of pSer892 (fig. 8c) was observed in the 0.9 mg/kg phaclofen-treated ($F_{1,18}$=5.775, $p<0.05$) and 1.0 mg/kg baclofen-treated ($F_{1,18}$=4.64, $p<0.05$) groups compared to controls. No differences were found for the 2.0 mg/kg baclofen-treated ($F_{1,18}$=0.097, $p>0.05$) and 1.25 mg/kg phaclofen-treated ($F_{1,18}$=0.153, $p>0.05$) groups.

A significant reduction was found for the kalirin-7 isoform in the 2.0 mg/kg baclofen-treated group compared to controls (see fig. 8d; $F_{1,18}$=5.451, $p<0.05$); no differences were found for kalirin-5, kalirin-9, or kalirin-12 (kalirin-5: $F_{1,18}$=1.257, $p>0.05$; kalirin-9: $F_{1,18}$=1.787, $p>0.05$; kalirin-12: $F_{1,18}$=0.209, $p>0.05$). Interestingly,

while the 1.0 mg/kg baclofen-treated group did not exhibit the same behavioral deficits, the kalirin-5 ($F_{1,18}$=7.501, $p<0.05$), kalirin-7 ($F_{1,18}$=9.844, $p<0.01$), and kalirin-9 ($F_{1,18}$=8.308, $p<0.05$) isoforms were significantly decreased compared to controls; kalirin-12 was not changed ($F_{1,18}$=0.017, $p>0.05$). No differences were found between the 0.9 mg/kg phaclofen-treated (kalirin-5: $F_{1,18}$=0, $p>0.05$; kalirin-7: $F_{1,18}$=0.868, $p>0.05$; kalirin-9: $F_{1,18}$=0.503, $p>0.05$; kalirin-12: $F_{1,18}$=0.537, $p>0.05$) or 1.25 mg/kg phaclofen-treated (kalirin-5: $F_{1,18}$=0.003, $p>0.05$; kalirin-7: $F_{1,18}$=0.019, $p>0.05$; kalirin-9: $F_{1,18}$=0.382, $p>0.05$; kalirin-12: $F_{1,18}$=0.002, $p>0.05$) groups and the controls for any of the kalirin isoforms.

A significant increase in total GAD67 levels were detected for the 2.0 mg/kg baclofen-treated group as compared to controls (see fig. 8e; $F_{1,18}$=4.585, $p<0.05$). No differences were found between the 0.9 mg/kg phaclofen-treated ($F_{1,18}$=0.137, $p>0.05$), 1.0 mg/kg baclofen-treated ($F_{1,18}$=0.157, $p>0.05$) or 1.25 mg/kg phaclofen-treated ($F_{1,18}$=1.125, $p>0.05$) groups compared to controls.

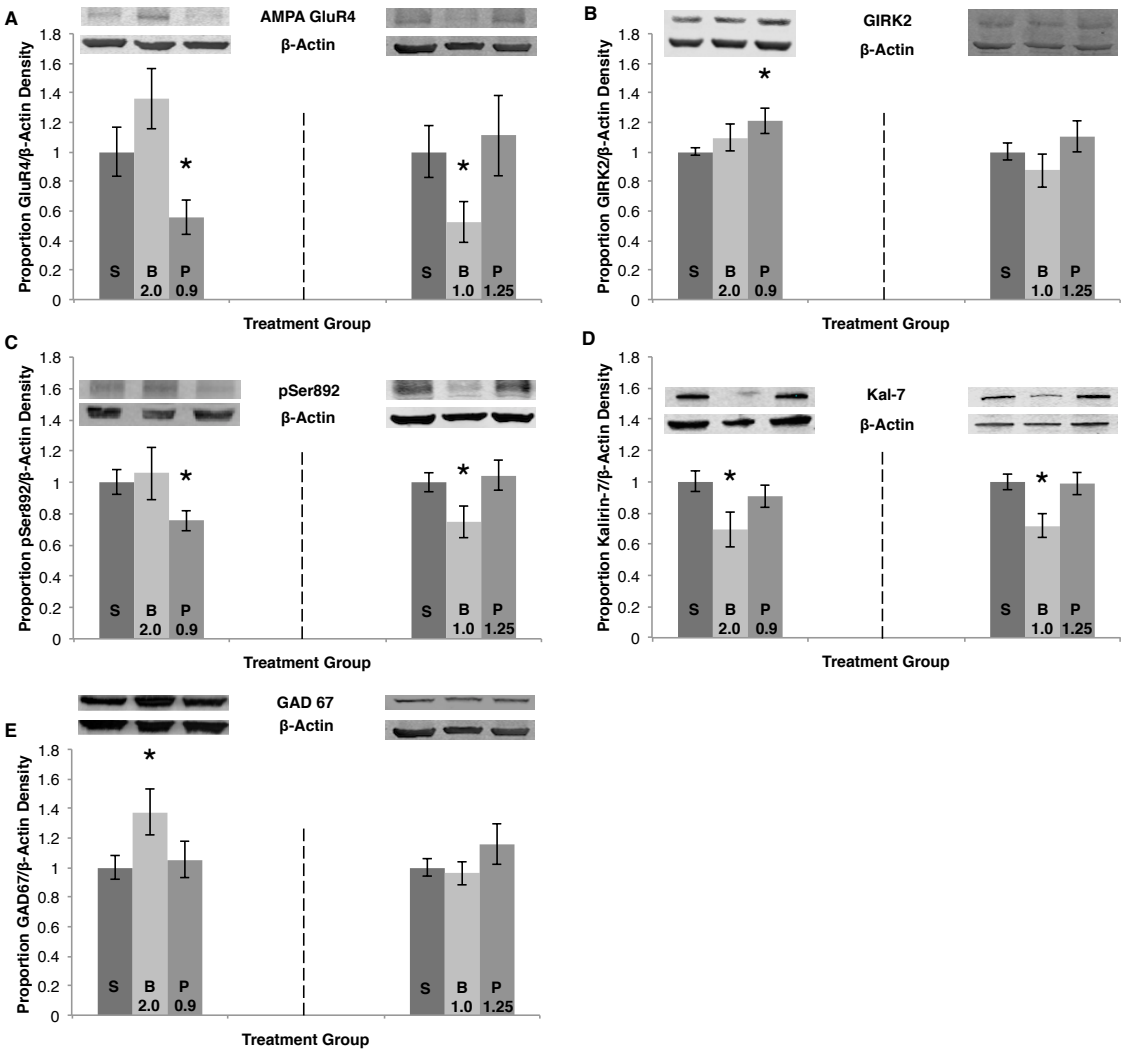

Figure 8. Western Blot Data and Representative Images. Treatment with 0.9 mg/kg phaclofen and 1.0 mg/kg baclofen significantly decreased the average proportion (± SEM) of AMPA GluR4 (a) and pSer892 (c). The average proportion (± SEM) of GIRK2 (b) was significantly increased by 0.9 mg/kg phaclofen treatment. Baclofen treatment (both 2.0 and 1.0 mg/kg) decreased the average proportion (± SEM) of kalirin-7 (d); 2.0 mg/kg baclofen significantly increased the average proportion (± SEM) of GAD67 (e). * = $p<0.05$ compared to saline controls.

No significant differences were detected between groups for the $GABA_B$ receptor subunits $GABA_{B1a}$ (saline vs 2.0 mg/kg baclofen, $F_{1,18}=2.212$; saline vs 0.9 mg/kg phaclofen, $F_{1,18}=0.708$; saline vs 1.0 mg/kg baclofen, $F_{1,18}=3.027$; saline vs 1.25 mg/kg

phaclofen, $F_{1,18}$=0) or $GABA_{B1b}$ (saline vs 2.0 mg/kg baclofen, $F_{1,18}$=2.53; saline vs 0.9 mg/kg phaclofen, $F_{1,18}$=0.176; saline vs 1.0 mg/kg baclofen, $F_{1,18}$=1.545; saline vs 1.25 mg/kg phaclofen, $F_{1,18}$=0.091) for any group ($p>0.05$ for all groups; see fig. 9). The 1.0 mg/kg baclofen treatment, however, did produce a reduction in $GABA_{B2}$ ($F_{1,18}$=9.113, $p<0.05$), but none of the other treatments (saline vs 2.0 mg/kg baclofen, $F_{1,18}$=2.112; saline vs 0.9 mg/kg phaclofen, $F_{1,18}$=0.013; saline vs 1.25 mg/kg phaclofen, $F_{1,18}$=1.475) affected total amount of $GABA_{B2}$ ($p>0.05$ for all groups; see fig. 9).

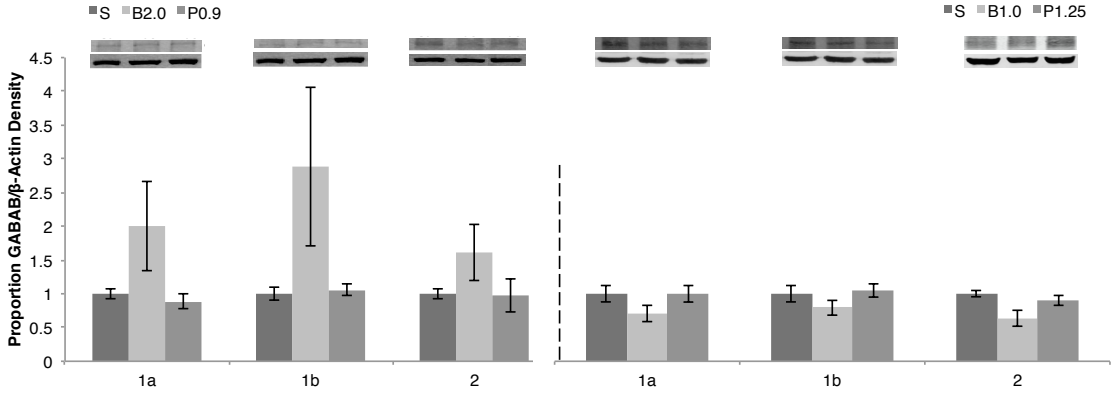

Figure 9. Non-significant Western Blot Data and Representative Images. Ligand treatment did not significantly affect the average proportion (± SEM) of $GABA_{B1}$ or $GABA_{B2}$ subunits.

CHAPTER 5

SUMMARY, DISCUSSION, AND FUTURE DIRECTIONS

In the above studies, we investigated the effect of different doses of baclofen (1.0 mg/kg or 2.0 mg/kg SC) and phaclofen (0.9 mg/kg or 1.25 mg/kg SC), administered each day throughout the experiment, on performance of rats run in the MWM. Our study provides novel data indicating that the lower dose of phaclofen enhanced the acquisition of spatial memory, as exhibited by significantly decreased latency to find the hidden platform across days. The 0.9 mg/kg SC dose of phaclofen also enhanced performance in learning the new location of the hidden platform on the second day of reversal training. Furthermore, the 0.9 mg/kg phaclofen-treated animals demonstrated a preference for the new platform location as indicated by a significant number of annulus crossings for the new platform location during the reversal platform probe. These data indicate a subtle enhancement of learning in the initial acquisition of a spatial task, as well as when the conditions shift. Administration of a higher dose of phaclofen did not improve upon or even mimic the beneficial effect of the lower dose. Our data indicate that the 1.25 mg/kg dose impaired performance in locating the new platform location in the reversal phase while it did not alter the acquisition of the task compared to controls.

Interestingly, while the higher dose of baclofen produced a significant performance deficit throughout the experiment, the lower baclofen dose did not negatively impact performance. Further, the low dose of baclofen did not significantly alter behavior during reversal training compared to controls, and may have facilitated a preference for the new platform location as indicated by the significant number of annulus crossing in the new platform location during the reversal probe. Treatment with

2.0 mg/kg baclofen did produce significant differences for swim speed during the reversal and visible sessions; because there were no swim speed differences during the initial hidden platform training, these results are inconsistent with a gross motor impairment that could be impairing MWM performance. Evaluation of performance in the open field task also did not indicate a motoric deficit due to baclofen. Therefore, it is unlikely that motoric impairment contributed to the deficits observed. Similarly, the elevated thigmotaxis in the MWM appears to reflect an altered search strategy by the 2.0 mg/kg baclofen group, rather than an anxiety phenotype, since no differences in anxiety-like behavior were observed in the open field test. Overall, the higher doses of both the agonist and antagonist produced some impairment in spatial learning. However, lower doses of those same compounds exhibited positive behavioral impacts and appeared to facilitate learning. These data suggest that there is an optimal, finite range of activation within which the $GABA_B$ receptor must operate for proper functioning or to enhance learning. That is, too little or too much activation may be as deleterious as too little or too much antagonism to spatial learning and memory. This possible finite range may account for some of the differences observed in previous investigations.

In order to identify potential mechanisms responsible for the behavioral data, we also examined alterations to total protein targets due to ligand administration. Interestingly, both the administration of 0.9 mg/kg phaclofen and 1.0 mg/kg baclofen throughout the MWM task produced a significant decrease in the total hippocampal expression of the AMPA receptor subunit GluR4. Though only the 0.9 mg/kg phaclofen-treated group exhibited a significant enhancement in reference memory during reversal training, both groups demonstrated a significant preference for the new platform location

during the reversal platform probe based on the annulus crossings. These data suggest a possible beneficial alteration in the interplay between the GABAergic and glutamatergic systems. Sagata et al. (2010) found that GluR4 knockouts behaved normally, and even exhibited a subtle, yet significant, spatial learning and memory enhancement. The authors also concluded that the GluR4 knockouts demonstrated "weaker perseveration tendency and higher behavioural flexibility" compared to controls (Sagata et al. 2010), which is consistent with the data obtained in the present study. The decreased expression of GluR4 in the present study may therefore be related to the enhanced spatial learning and memory and learning flexibility.

Further, both of these treatment groups that exhibited enhanced flexibility also exhibited a decrease in pSer892. Phosphorylation of serine 892 on the $GABA_{B2}$ receptor is implicated in stabilizing $GABA_B$ receptors at the surface of the cell membrane (Couve et al., 2002; Fairfax et al., 2004). Recent evidence suggests that pSer892 decreases desensitization of the $GABA_B$ receptor (Benke et al., 2012) by preventing the uncoupling of the $GABA_B$ receptor from G protein-coupled inwardly rectifying potassium channels (GIRK), a process guided by K^+ channel tetramerization domain 12 (KCTD12; Adelfinger et al., 2014; Turecek et al., 2014). KCTD12 may also aid in the signaling precision of $GABA_B$ receptors by preventing receptor internalization and promoting desensitization (Ivankova et al., 2013; Schwenk et al., 2010). Therefore, it is possible that the decrease in pSer892 may encourage receptor desensitization, decreased GIRK signaling, and enhanced signaling precision via the mechanisms of KCTD12 (see fig. 10).

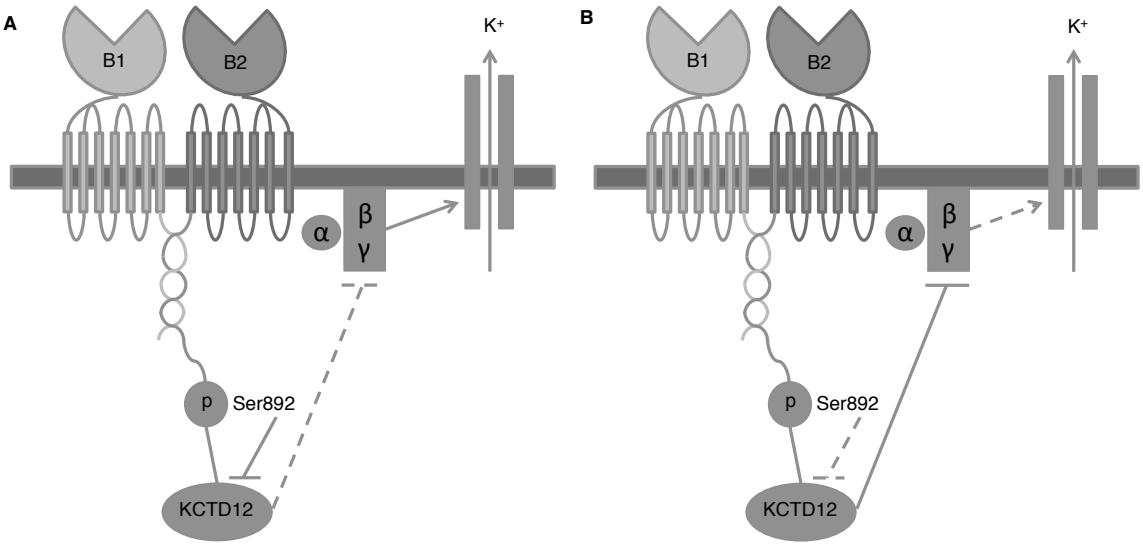

Figure 10. Proposed Effect of Ligands on pSer892. Phosphorylation at Serine 892 on the $GABA_{B2}$ receptor subunit inhibits KCTD12 (a). With less pSer892, KCTD12 is able to prevent potassium channel activation by inhibiting the beta-gamma G-protein subunits (b). Dashed lines represent less activity.

Interestingly, the 0.9 mg/kg phaclofen-treated group also showed a significant increase in GIRK2 expression. This potassium channel is activated by $G_{i/o}$ G-proteins (Cui, Ho, Kim, & Cho, 2010; Jelacic, Kennedy, Wickman, & Clapham, 2000; Mark & Herlitze, 2000), and over-expression is typically associated with impaired learning and memory (Best et al., 2012; Harashima, Jacobowitz, Stoffel, et al., 2006a; Harashima, Jacobowitz, Witta, et al., 2006b). Possibly, the up-regulation of the GIRK subunit identified in this experiment is in response to the loss of the $G_{i/o}$ signal due to the antagonist treatment. *In vitro* studies demonstrate that $GABA_B$ receptors do not respond to chronic agonist activation by down regulating overactive receptors, the typical response for other G-protein coupled receptors (Fairfax et al., 2004). It may be likely, therefore, that targets downstream of the G-protein signal cascade up- or down-regulate based on changes to $GABA_B$ receptor activity. Additionally, given the decrease in

pSer892, which could enhance the uncoupling mechanism of KCTD12 described above, it is possible that the increase in this GIRK subunit may be an attempt to regain function of an overly-silenced channel.

Treatment with 1.0 mg/kg baclofen or 0.9 mg/kg phaclofen may enhance synaptic plasticity as indicated by the decreases in AMPA GluR4 and pSer892, as well as the increase in GIRK2 for the phaclofen-treated group. The regulation of AMPA at a synapse is a well known marker of synaptic plasticity (Kessels & Malinow, 2009). Because pSer892 is implicated in receptor stability, decreased expression could demonstrate changes related to the strengthening or weakening of plastic synapses (Gerrow & Triller, 2010; Mao et al., 2009). Further evidence that the 0.9 mg/kg phaclofen treatment may be facilitating synaptic plasticity stems from the changes observed to GIRK expression. GIRK has been demonstrated to be necessary for the depotentiation of long-term potentiation (Chung et al., 2009a), and increased NMDA receptor activity upregulates GIRK channels (Chung, Qian, Ehlers, Jan, & Jan, 2009b). Therefore, the altered expression of AMPA GluR4, pSer892, and GIRK2 could indicate increased synaptic plasticity due to the administration of low doses of $GABA_B$ receptor ligands.

The above data may also indicate alterations to presynaptic $GABA_B$ receptors. If the low dose of phaclofen blocks both pre- and postsynaptic receptors, it is possible phaclofen antagonism is creating a synergistic effect to enhance learning and memory. For instance, phaclofen may antagonize the activity of presynaptic heteroreceptors on glutamatergic neurons, leading to the prevention of $GABA_B$ receptor-mediated reduction in glutamate release. This blockade would result in more glutamate reaching the postsynaptic neuron (Vigot et al., 2006; Waldmeier et al., 2008). Phaclofen blockade of

postsynaptic $GABA_B$ receptors decreases the hyperpolarizing effects of endogenous GABA receptor activation, thus enabling the postsynaptic neuron to depolarize more easily. Other studies demonstrate that the antagonism of $GABA_B$ receptors could enhance NMDA-mediated spatial learning and memory (Davis, Butcher, & Morris, 1992; McNamara & Skelton, 1993; Shapiro, 2001; Vorhees & Williams, 2006). Furthermore, $GABA_B$-induced potassium conductance appears to have a negative relationship with NMDA receptor conductance (Sanders, Berends, Major, Goldman, & Lisman, 2013), such that $GABA_B$ receptors possibly actively suppress NMDA receptor activity. It is, therefore, possible that phaclofen may enhance learning and memory through both pre- and postsynaptic mechanisms.

Evidence suggests that low concentrations of baclofen preferentially bind to presynaptic receptors (Pinard et al., 2010; Yoon & Rothman, 1991). Therefore, it is possible that the low dose of baclofen used in this study could be selectively activating autoreceptors and preventing the presynaptic release of GABA. Recent evidence also demonstrates a direct connection between $GABA_B$ signaling and both excitatory (e.g. LTP, AMPA receptor number) and inhibitory (e.g. GIRK activation) mechanisms (Terunuma et al., 2014). Future studies could examine this relationship between $GABA_B$ and NMDA receptor activity on spatial learning and memory, perhaps through the dual-administration of GABAergic and glutamatergic ligands.

Treatment with 2.0 mg/kg baclofen produced a significant increase in hippocampal GAD67. The increase in GAD67 protein levels suggests an increased production of intracellular GABA, and it has been suggested that intracellular GABA concentrations can influence the release of vesicular GABA (Overstreet & Westbrook,

2001). Combined with the actions of baclofen (decreased presynaptic neurotransmitter release and increased postsynaptic inhibition) and the non-significant trend of increased $GABA_B$ receptor subunits, these data suggest a state of over-inhibition of hippocampal neurons. Intracellular GABA can also be slowly released in an action potential-independent manner (Schoffelmeer, Wardeh, & Vanderschuren, 2001), which may increase the tonic levels of extracellular GABA. This increased extracellular GABA may then start to bind to GABA receptors; because some of the $GABA_B$ receptors are likely occupied by baclofen, it is plausible that the released GABA would then bind to $GABA_A$ receptors – again, producing more postsynaptic inhibition. It is well established that enhancing GABA tone via $GABA_A$ receptors impairs learning and memory (Brioni, Nagahara, & McGaugh, 1989; Kim et al., 2012; Torkaman-Boutorabi et al., 2013; Zarrindast et al., 2002). Therefore, it is plausible that the administration of 2.0 mg/kg baclofen could produce learning and memory deficits through the activation of both $GABA_A$ and $GABA_B$ receptors.

Interestingly, the 1.0 mg/kg dose of baclofen produced a significant decrease of kalirin-5, kalirin-7, and kalirin-9, but did not cause an increase in GAD67 or produce a behavioral deficit. It is possible that the behavioral deficits seen in the 2.0 mg/kg baclofen group are a product of an interaction between the decrease in kalirin-7, specifically (described below), and the increased GAD67. Possibly, the lack of a behavioral deficit and subtle enhancement in the 1.0 mg/kg baclofen group could be due to an interaction of the decreased kalirin isoforms, pSer892, and GluR4. Or the changes to pSer892 and GluR4 alone could indicate changes to synaptic plasticity (as described above), perhaps via a presynaptic, kalirin-independent mechanism.

The administration of 2.0 mg/kg baclofen produced a significant decrease in kalirin-7, a key component to the formation of synapses, as well as a marker of excitatory postsynaptic density (Ma et al., 2008b). If we examine these results under the theoretical framework of long-term potentiation, which suggests that increased activity of neurons strengthens neuronal connections and synapses, in conjunction with the increased GAD67 data, 2.0 mg/kg baclofen treatment may be promoting the removal of over-inhibited synapses from the synaptic network. The decrease in kalirin-7 suggests a decrease in synaptogenesis, a reduction of dendritic spines, or decreased ability for synapse rearrangement (Ma et al., 2008b), which coincides with the idea of synaptic pruning. Reducing neuronal connections or the decreased ability for synapses to reorganize, particularly in the hippocampus, could explain the spatial memory impairment in the 2.0 mg/kg baclofen-treated group. If a lack of synaptic strengthening and the pruning of those synaptic connections were to occur, a substantial learning deficit would result, as was demonstrated in this study. The implications of these data may shed light on mechanisms responsible for the spatial learning and memory decreases associated with certain psychiatric and neurological disorders.

Alternatively, the impaired performance of the 2.0 mg/kg baclofen group could be due to a failure to adapt new strategies to escape the MWM. Given that performance on the last day of hidden training was so similar to the first day, the lack of improvement could be a result of this group using an escape strategy not predicated on locating the platform. Previous studies indicate that reduced synaptic plasticity is related to poor search strategy use (Garthe, Behr, & Kempermann, 2009; Gil-Mohapel et al., 2013; Santin et al., 2009). Therefore, administration of 2.0 mg/kg baclofen could negatively

impact synaptic plasticity, which in turn negatively affects the use of spatially-driven search strategies.

Impaired memory flexibility and spatial learning and memory are key features of both Alzheimer's disease and schizophrenia (Addington & Addington, 1999; Albert, 1996; Cherrier et al., 2001; deIpolyi et al., 2007; Hanlon et al., 2006; Spieker et al., 2012). Further, these diseases also exhibit altered GABAergic markers (Bai et al., 2014; Burbaeva et al., 2014; Schwab et al., 2013; Seidl et al., 2001), $GABA_B$ markers (Chu et al., 1987a; Massone et al., 2011; Young, 1987), and kalirin-7 (Mandela & Ma, 2012; Murray et al., 2012; Youn et al., 2007). Together with the data from this study, further examination of the impact of altered GABAergic and $GABA_B$ receptor signaling as it relates to spatial learning and memory in these diseases is warranted.

A limitation to the current studies is our inability to determine whether the subunits of GABA receptors were part of functional or membrane-expressed receptors. While we attempted to address this issue by examining pSer892, which is implicated in membrane stability of the $GABA_B$ receptor, we cannot demonstrate that these B2 subunits were actually expressed at the surface of the membrane. Furthermore, many of our of conclusions are drawn from studies examining changes demonstrated *in vitro* that have not yet been confirmed to take place *in vivo*. Another limitation stems from the route of injection utilized in these studies. While the data indicate behavioral and protein changes related to ligand administration, we can not determine the ligand concentration once it reaches the central nervous system and, therefore, the exact impact these ligands have on certain neural regions.

Possibly, $GABA_B$ receptor activation is modulated by stress. While the MWM is not as stressful as other tasks (like fear conditioning), the task does produce some initial stress as the animals learn how to escape. Future experiments could examine the effect of $GABA_B$ receptor ligands on other, non-stress inducing behavioral tasks, such as the radial arm maze. Further, investigations of how $GABA_B$ ligands alter the function of other markers implicated in receptor stability, and the impact those changes have on memory flexibility and spatial learning and memory, would help expand our knowledge of $GABA_B$ mechanisms of action. Additionally, the examination of the effect of altered presynaptic versus postsynaptic $GABA_B$ receptor activity would be another interesting future direction. The data from the above studies indicate an impact of altered $GABA_B$ receptor activity on glutamatergic targets, and examining this link could also help identify potential therapeutic targets. As reviewed above, *in vitro* investigations indicate that $GABA_B$ ligands produce different effects within the DG and CA1. Future experiments could examine the behavioral effect of $GABA_B$ ligands infused into these discrete brain regions to determine whether these *in vitro* data translate *in vivo*.

The intended goal of the current experiment was to determine whether altered $GABA_B$ receptor activity would affect both memory flexibility and spatial learning and memory. Overall, our data suggest that $GABA_B$ receptor activity due to low-levels of ligand administration, regardless of agonist or antagonist, enhances spatial learning and memory. Conversely, $GABA_B$ receptor activity due to high-levels of ligand administration impairs spatial learning and memory. Additionally, we identified behavioral and molecular changes similar to what is seen in Alzheimer's disease and schizophrenia. Together, these data provide insight to the *in vivo* mechanisms of $GABA_B$

receptor activity, as well as potential mechanisms associated with $GABA_B$ receptor activity, memory flexibility, and spatial learning and memory.

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Curriculum Vitae

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Education and Training

Spring 2012-Spring 2015	University of Nevada, Las Vegas Las Vegas, NV 89154	Ph.D.	Experimental Psychology Behavioral Neuroscience

Dissertation: A Comparison of the Effects of Different Doses of $GABA_B$ Receptor Ligands on Spatial Learning and Memory and Memory Flexibility

Advisor: Dr. Jefferson W. Kinney

Fall 2009-Spring 2012	University of Nevada, Las Vegas Las Vegas, NV 89154	M.A.	Experimental Psychology Behavioral Neuroscience

Thesis: An Investigation of the Role of $GABA_B$ Ligands on Cued and Contextual Fear Conditioning **Advisor:** Dr. Jefferson W. Kinney

Fall 2003-Spring 2007	Boston University Boston, MA 02215	B.A.	Psychology, Biology minor

Teaching Experience

Undergraduate Courses

Introductory Psychology (Fall 2012-Fall 2013)

Physiological Psychology (Fall 2013-Spring 2014)

Research Interests

My research interests include investigating the role of the inhibitory neurotransmitter receptor $GABA_B$ in learning and memory. Specifically, I am interested in how alterations to this system contributes to neurological and psychiatric disorders including schizophrenia, autism, and Alzheimer's disease. I am further interested in the dynamic relationship between GABA and the excitatory neurotransmitter glutamate in the process of learning and memory, and in the function of the hippocampus.

Research Skills and Techniques

- Experience with the appropriate care, use, and husbandry of animal subjects, including rodents and ground squirrels;
- Expertise in several behavioral tasks, including the Morris water maze, radial arm maze, Barnes maze, cued and contextual fear conditioning, operant conditioning procedures, open field, novel object recognition, acoustic startle and prepulse

inhibition, tail flick nociception task, and general screening for basic sensory function and reflexes;
- Proficient at aseptic surgical techniques, stereotaxic surgical procedures, and wound closure techniques;
- Skilled in neural tissue collection (including transcardial perfusion and dissection of specific structures), sectioning, and immunohistochemistry techniques;
- Trained in preparation and maintenance of cell cultures;
- Experienced in biochemical assays including end point and qRT-PCR; DNA and RNA extraction; Bradford and bicinchonic assays; western blotting; and ELISA;
- Experience in programming software associated with behavioral testing, including Smart and Startle;
- Supervised and trained numerous undergraduate research assistants on the above listed laboratory techniques.

Honors and Awards

Fall 2014	**APF/COGDOP Graduate Research Scholarship** External competitive scholarship to support submitted research proposal.
Spring 2014; Fall 2014	**Edward Lovinger Psychology Scholarship** Department-wide competitive scholarship.
Fall 2010; Fall 2011; Fall 2012; Fall 2013; Fall 2014	**University of Nevada, Las Vegas Graduate and Professional Student Association Travel Grant** University-wide competitive grant to help cover travel expenses to professional conferences. Awarded to attend the annual Society for Neuroscience meeting.
Spring 2011; Spring 2013; Spring 2014	**University of Nevada, Las Vegas Graduate and Professional Student Association Research Forum First Place Presentation Award** University-wide graduate and professional research forum to share research with peers and faculty.
Summer 2013	**Dean's Graduate Student Stipend Award** College of Liberal Arts competitive summer stipend awarded to five students in recognition of their academic and research productivity. Three students from each department within the college may be nominated each year.
Spring 2013	**College of Liberal Arts Nominee for Graduate College Outstanding Thesis Award** University-wide competitive accolade awarded to the single most outstanding thesis completed the previous academic year. One student is nominated from each college.

Fall 2012	**Sierra Nevada Chapter of the Society for Neuroscience Travel Award** Travel award granted to members of the Sierra Nevada Chapter of the Society for Neuroscience to attend the Chapter's 2012 annual research symposium, held at the University of Nevada, Reno.
Fall 2012	**Patricia Sastaunik Scholarship** University-wide competitive scholarship.
Summer 2012	**Graduate College Summer Session Scholarship** University-wide competitive scholarship to enable graduate students to engage in research over the summer.
Spring 2005; Fall 2006	**Boston University Dean's List**

Publications

Bolton MM, **Heaney CF**, Murtishaw AS, Sabbagh JJ, Magcalas CM, Kinney JW (2015). Postnatal alterations in $GABA_B$ receptor tone produce sensorimotor gating deficits and protein level differences in adulthood. *International Journal of Developmental Neuroscience* 2015(41):17-27.

Sabbagh JJ, Murtishaw AS, Bolton MM, **Heaney CF**, Langhardt M, Kinney JW (2013). Chronic ketamine produces altered distribution of parvalbumin-positive cells in the hippocampus of adult rats. *Neuroscience Letters* 550:69-74.

Heaney CF, Bolton MM, Murtishaw AS, Sabbagh JJ, Magcalas CM, Kinney JW (2012). Baclofen administration alters fear extinction and GABAergic protein levels. *Neurobiology of Learning and Memory* 98(2012):261-271.

Bolton MM, **Heaney CF**, Sabbagh JJ, Murtishaw AS, Magcalas CM, Kinney JW (2012). Deficits in emotional learning and memory in an animal model of schizophrenia. *Behavioural Brain Research* 233(1):35-44.

Sabbagh JJ, **Heaney CF**, Bolton MM, Murtishaw AS, Kinney JW (2012). Examination of ketamine-induced deficits in sensorimotor gating and spatial learning. *Physiology & Behavior* 107(2012):355-363.

Sabbagh JJ, **Heaney CF**, Bolton MM, Murtishaw AS, Ure JA, Kinney JW (2012). Administration of donepezil does not rescue galanin-induced spatial learning deficits. *International Journal of Neuroscience* 122(12):742-747.

Di Pietro NC, Mashhoon Y, **Heaney C**, Yager LM, Kantak KM (2008). Role of dopamine D1 receptors in the prefrontal dorsal agranular insular cortex in mediating cocaine self-administration in rats. *Psychopharmacology* 200:81-91.

Manuscripts Submitted or in Preparation

Heaney CF, Bolton MM, Murtishaw AS, Langhardt MA, Kinney JW. Dose response effect of $GABA_B$ ligands on spatial learning and memory. Submitted March 2015 to *Neurobiology of Learning and Memory*.

Heaney CF, Kinney JW. $GABA_B$ in Learning and Memory. In preparation.

Hensleigh EM, Murtishaw AS, Treat MD, **Heaney CF**, Bolton MM, Sabbagh JJ, Kinney JW, van Breukelen F. The effect of torpor on spatial memory in ground squirrels (*Spermophilus lateralis*) throughout a hibernation season. In preparation to submit to *Learning & Behavior*.

Murtishaw AS, **Heaney CF**, Bolton MM, Kinney JW. An acute inflammatory response improves learning and memory deficits and reduces pathological markers in a diabetes animal model of Alzheimer's disease. In preparation to submit to *Brain, Behavior, and Immunity*.

Presentations and Posters

Heaney CF, Bolton MM, Murtishaw AS, Langhardt MA, Kinney JW. GABAB ligand dose-dependent changes in spatial learning and hippocampal GABAergic and plasticity proteins. Presented at the American Chemical Society Southern Nevada Local Section Annual Poster Exhibition and Competition. Las Vegas, NV, November 2014.

Heaney CF, Bolton MM, Murtishaw AS, Langhardt MA, Kinney JW. GABAB ligand dose-dependent changes in spatial learning and hippocampal GABAergic and plasticity proteins. Presented at 24th Neuropharmacology Conference 2014: GABAergic Signaling in Health and Disease. Washington, DC, November 2014.

Heaney CF, Bolton MM, Murtishaw AS, Langhardt MA, Kinney JW. Evaluation of multiple doses of GABA(B) ligands on learning and memory. Program No. 784.07. 2014 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2014. Online.

Bolton MM, **Heaney CF**, Murtishaw AS, Langhardt MA, Kinney JW. Interactions of behavioral training and ketamine administration on changes in parvalbumin positive neurons. Program No. 265.05. 2014 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2014. Online.

Langhardt MA, Murtishaw AS, **Heaney CF**, Bolton MM, Belmonte KCD, Hagins PM, Kinney JW. Facilitation of GABAB receptor function modulates chronic inflammatory effects. Program No. 790.21. 2014 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2014. Online.

Murtishaw AS, **Heaney CF**, Bolton MM, Belmonte KD, Hagins PM, Langhardt MA, Kinney JW. Chronic LPS-induced inflammation and insulin signaling disruption in a diabetic model of Alzheimer's Disease. Program No. 790.19. 2014 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2014. Online.

Heaney CF (2014, March). The effects of baclofen and phaclofen on performance in the Morris water maze. Talk presented at the annual UNLV GPSA Research Forum, Las Vegas, NV.

Heaney CF, Bolton MM, Murtishaw AS, Kinney JW. The effects of baclofen and phaclofen on performance in the Morris water maze. Program No. 516.12. 2013 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2013. Online.

Bolton MM, **Heaney CF**, Murtishaw AS, Kinney JW. Developmental alteration of GABAB receptor function results in behavioral deficits in adulthood. Program No. 721.23. 2013 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2013. Online.

Langhardt MA, Bolton MM, **Heaney CF**, Murtishaw AS, Nagl, SL, Kinney JW. Evaluation of ketamine-induced changes in spatial working memory and GABAergic systems. Program No. 364.04. 2013 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2013. Online.

Murtishaw AS, **Heaney CF**, Bolton MM, Langhardt MA, Belmonte KCD, Kinney JW. An acute LPS-induced inflammatory response in a diabetic model of Alzheimer's disease. Program No. 238.11. 2013 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2013. Online.

Heaney CF (2013, March). Alterations to Inhibitory Signaling During Development Produce Deficits in Adulthood. Talk presented at the annual UNLV GPSA Research Forum, Las Vegas, NV.

Heaney CF, Bolton MM, Murtishaw AS, Sabbagh JJ, Magcalas CM, Kinney JW (2012, November). Changes in $GABA_B$ receptor tone in development produces behavioral deficits in adulthood. Poster presented at the Sierra Nevada Chapter of the Society for Neuroscience annual research symposium, Reno, NV.

Heaney CF, Bolton MM, Murtishaw AS, Sabbagh JJ, Magcalas CM, Kinney JW. Changes in $GABA_B$ receptor tone in development produces behavioral deficits in adulthood. Program No. 39.05. 2012 Neuroscience Meeting Planner. New Orleans, LA: Society for Neuroscience, 2012. Online.

Bolton MM, **Heaney CF**, Sabbagh JJ, Murtishaw AS, Magcalas CM, Kinney JW. Comparison of postnatal ketamine dosage on behavioral deficits in adulthood. Program No. 771.27. 2012 Neuroscience Meeting Planner. New Orleans, LA: Society for Neuroscience, 2012. Online.

Murtishaw AS, Sabbagh JJ, **Heaney CF**, Bolton MM, Magcalas CM, Langhardt MA, Kinney JW. Ketamine-induced behavioral impairments and alterations in hippocampal GABAergic neuron distribution. Program No. 497.16. 2012 Neuroscience Meeting Planner. New Orleans, LA: Society for Neuroscience, 2012. Online.

Sabbagh JJ, Murtishaw AS, **Heaney CF**, Bolton MM, Magcalas CM, Kinney JW. Chronic calcium dysregulation produces cognitive deficits and biochemical changes relevant to Alzheimer's disease. Program No. 544.16. 2012 Neuroscience Meeting Planner. New Orleans, LA: Society for Neuroscience, 2012. Online.

Heaney CF, Bolton MM, Murtishaw AS, Magcalas CM, Sabbagh JJ, Kinney JW (2012, March). An investigation of $GABA_B$ ligands on cued and contextual fear conditioning. Talk presented at the annual UNLV Psychology Department Research Fair, Las Vegas, NV.

Heaney CF (2012, March). Role of altered $GABA_B$ function on cued and contextual fear conditioning and extinction. Talk presented at the annual UNLV GPSA Research Forum, Las Vegas, NV.

Heaney CF, Bolton MM, Murtishaw AS, Sabbagh JJ, Magcalas CM, Kinney JW. An investigation of the effects of alterations in $GABA_B$ receptor function on learning and memory. Program No. 613.13. 2011 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2011. Online.

Bolton MM, **Heaney CF**, Sabbagh JJ, Murtishaw AS, Kinney JW. Comparison of an adult and developmental model of schizophrenia. Program No. 790.08. 2011 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2011. Online.

Sabbagh JJ, Bolton MM, **Heaney CF**, Murtishaw AS, Kinney JW. Deficits in emotional learning and memory in an animal model of schizophrenia. Program No. 790.11. 2011 Neuroscience Meeting Planner. Washington, DC: Society for Neuroscience, 2011. Online.

Heaney CF (2011, March). The effects of $GABA_B$ ligands on learning and memory. Talk presented at the annual UNLV GPSA Research Forum, Las Vegas, NV.

Heaney CF, Sabbagh JJ, Bolton MM, Murtishaw AM, Santa Ana IK, Kinney JW. An investigation of alterations in GABAergic tone in an animal model of schizophrenia. Program No. 62.11. 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Sabbagh JJ, **Heaney CF**, Bolton MM, Ure JA, Kinney JW. Donepezil and galanin interactions in learning and memory and a model of cholinergic loss. Program No. 747.15. 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online.

Sabbagh JJ, **Heaney CF**, Bolton MM, Ambrose T, Kinney JW. Efficacy of acute versus chronic administration of an NMDA receptor antagonist to induce an animal model of schizophrenia. Program No. 443.6. 2009 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2009. Online.

Grants Contributed To

Years inclusive 2014-2017	"A comparison of altered presynaptic versus postsynaptic GABA(B) receptor function on simple and complex behaviors" submitted 10/2013 to the National Institutes of Health (NIH), Neurobiology of Learning and Memory Study Section (LAM). PI – Jefferson Kinney.

Service and Other Work

Fall 2013-present	**Las Vegas Brain Bee** Event organizer and volunteer. Along with other members of the Nevada Brain Bee Association, I organized the first annual Las Vegas Brain Bee, a high school neuroscience competition designed to increase neuroscience awareness and to encourage high school students to pursue careers in neuroscience.
Fall 2011-Spring 2012; Fall 2013-Spring 2014	**Experimental Student Committee** Cohort Representative. Duties included communicating the needs of my cohort to the Experimental Student Committee, as well as soliciting feedback from my cohort regarding any departmental issues.
Summer 2013; Summer 2014	**UNLV Summer Bridge Math Tutor** The Summer Bridge Program is designed to help incoming freshmen and current students place into a 100-level math course at UNLV. I helped to identify and provide one-on-one support for high-risk math anxious students, as well as tutor other students as they studied to take the UNLV Math Placement Exam.
Spring 2013-present	**Outreach Undergraduate Mentoring Program** Mentor. Working with OUMP, I mentor undergraduates from under-represented minority groups who are interested in pursuing a graduate degree in psychology.
Spring 2012-present	**Brain Awareness Week** Event organizer and volunteer. Together with other UNLV students, I visit local elementary and middle schools to encourage students to become interested in neuroscience. We help students to become aware of the functions of the brain, the importance of protecting their brains, as well as some of the diseases that can affect the brain.

Professional Memberships

2013-present	**American Association for the Advancement of Science** Student member
2013-present	**Association for Women in Science** Junior member
2013-present	**International Behavioral Neuroscience Society** Student member

2012-2013 **Society for the Teaching of Psychology**
Student member

2011-present **Sierra Nevada Chapter of the Society for Neuroscience**
Student member

2009-present **Society for Neuroscience**
Student member

Other Memberships

2013-present **Nevada Brain Bee Association**
Board member and charter member. The goal of this association is to organize the Las Vegas Brain Bee. Duties include fundraising, visiting high schools to generate enthusiasm for and participation in the event, and general organization of the event.

2011-present **Graduate Neuroscience Association**
Charter member. This association was formed to enable graduate students interested in neuroscience to keep abreast of the current research being done in the field.

2009-2013 **Neuroscience Journal Club**
Charter member. As a graduate member, I helped to guide the discussion of neuroscience journal articles, as well as help teach neuroscience principles to the undergraduate members.

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