METABOTROPIC GLUTAMATE RECEPTORS AS TARGETS FOR ANTIEPILEPTIC DRUG THERAPY: A BEHAVIORAL AND ELECTROENCEPHALOGRAPHIC ANALYSIS

BY

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DEDICATION

This endeavor, and indeed my entire existence, is dedicated to my daughter: my best and most beloved work. You are the reason for everything and the center of my Universe, and I simply adore you.
Acknowledgements

I would like to express immense love and appreciation for my husband, without whose continued and unwavering support I would have given up long ago. I could never say it enough, or with enough sincerity, but I am so grateful for you and I love you. Thank you for every single thing you’ve done and continue to do to for our family. I am so very lucky.

I would also like to thank my advisor for his support and guidance. Lastly, thank you to all of my committee members for their help and advisement along this journey. None of this would have been possible without the help of many, and so I heartily thank you all.
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<th>Abbreviation</th>
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<tr>
<td>AED</td>
<td>antiepileptic drug</td>
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<tr>
<td>DBS</td>
<td>deep brain stimulation</td>
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<td>EEG</td>
<td>electroencephalography</td>
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<td>GAERS</td>
<td>genetic absence epilepsy rats from Strasbourg</td>
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<td>GBL</td>
<td>gamma-butyrolactone</td>
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<td>GHB</td>
<td>gamma-hydroxybutyrate</td>
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<td>GPCR</td>
<td>G-protein coupled receptor</td>
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<td>ILAE</td>
<td>International League against Epilepsy</td>
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<tr>
<td>lh/lh</td>
<td>lethargic</td>
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<td>mGluR</td>
<td>metabotropic glutamate receptor</td>
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<tr>
<td>PAM</td>
<td>positive allostetric modulator</td>
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<tr>
<td>PTZ</td>
<td>pentylenetetrazole</td>
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<tr>
<td>SE</td>
<td>status epilepticus</td>
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<tr>
<td>SRS</td>
<td>spontaneous recurrent seizures</td>
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<tr>
<td>SWD</td>
<td>spike wave discharge</td>
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<tr>
<td>TBI</td>
<td>traumatic brain injury</td>
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<tr>
<td>TM</td>
<td>transmembrane</td>
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<tr>
<td>VNS</td>
<td>vagal nerve stimulation</td>
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ABSTRACT

Epilepsy is a relatively common neurological disorder that involves recurrent seizures thought to be caused by an imbalance in the excitatory and inhibitory systems in the brain, in favor of excitation. Unfortunately, some types of epilepsy have drug resistance rates of up to 40%. Classically, targets for antiepileptic drugs have included glutamatergic ion channels. However, interfering with excitatory signalling at these channels can alter normal neural communication and can have negative side effects, including cognitive dysfunction and fatigue, which are among the most common complaints from patients. Given the high rate of drug-resistant patients, and the high rate of deleterious side effects, there is an obvious need to develop novel drugs with novel targets and mechanisms of action. Compounds targeting metabotropic glutamate receptors (mGluRs; specifically mGlur2 and 3) have shown promise in other hyperexcitatory neural disorders, and some have shown efficacy in models of epilepsy as well. The goal of these studies was to demonstrate a conclusive behavioral effect of partial mGluR active compounds in reducing the severity of seizures associated with two models of epilepsy. Both models that were used are models of generalized seizure, although one is characterized by convulsive seizures (pilocarpine model) and the other is characterized by the lack of convulsive seizure, or an “absence” seizure (GBL model). Mice were given mGluR2/3 active drugs either before or after seizure onset and behavioral observations relating to seizure were measured. In some cases, the mice had been implanted with tethered EEG devices to record neural activity that coincided with behavioral observations and measures. It was demonstrated that in generalized models of seizure, mGluR2/3 active agonists had some efficacy at reducing seizure severity. The
effect of modulators at mGluR2 was also investigated. One in particular, CBiPES had a modest but significant effect in reducing the behavioral severity of pilocarpine induced seizures, but not of GBL induced seizures. In conclusion, mGluR2/3 remains a valid target for antiepileptic drug development. The ideal compound would yield higher response rates for patients while boasting a minimal negative side effect profile.
CHAPTER ONE: INTRODUCTION

I. EPILEPSY

A. History of Epilepsy: Demonic Possession or Divine Power?

Epilepsy, a cluster of neurological disorders characterized by spontaneous seizure activity, has been described for thousands of years. Historically, epilepsy has been classified as both good and evil; as either a symptom of demonic or divine possession.

In antiquity, the treatment of seizures largely depended on what the culture believed was the root cause of the disease. Some societies believed the cause was related to occult phenomena (the result of some witchcraft or magic), or perhaps the end result of excess phlegms or humors in the brain (a more rational and empirical explanation, Gross, 1992; Elferink, 1999; Todman, 2008). Even though the causes may have been mystical, treatments were often based on what the ancients knew about the pathophysiology of the brain. These treatments were rudimentary at best, barbaric at worst. Various herbal tinctures or concoctions would be recommended. There is also substantial evidence that skull trephining was used to treat various neurological problems, including epilepsy (Gross, 1992; Missios, 2007). In fact, the earliest known use of skull craniotomy is thought to have occurred between 8000 and 5000 BC (Sperati, 2007).

As Christianity became popular throughout the Middle Ages, empirical evidence for the causes and treatments of seizures competed with more mystical explanations and answers (Diamantis et al., 2010). Religious zealots and physicians alike were in the business of treating the disease. Treatments may range from some of the more successful remedies of the past, or something more supernatural in an effort to cure the epileptic of what was sure to be a demonic possession. On the other hand, the experiences of some
epileptic persons led theologians to believe seizures were from a more positive and divine source, and perhaps should not be treated so much as studied for religious insights. Epileptics often report sensory experiences, such as visions, sounds, smells, and so forth that directly precede their seizure and were thought to be of a religious nature (Devinsky and Lai, 2008); this “aura” was looked at as an other-worldly message that only bolstered the mystical explanations for epilepsy.

With the passage of time and the advancement of the modern era as we know it, the spiritual and occult-based explanations fell to the more empirical and rational explanations born from the scientific method (Diamantis et al., 2010; Sidiropoulou et al., 2010). Treatments have become more and targeted, seeking to treat only the seizures themselves while sparing normal brain function and producing minimal side effects. Looking into the future, understanding the cause and course of epileptogenesis will yield the perfect treatment; a cure, an antiepileptogenic. For now however, neurologists must rely on treating the symptom of the disease (the seizure) until a cure for the cause of the disease is found.

B. Classifications/Causes of Epilepsies

Today, the epilepsies are classified according to their etiology and the clinical features of seizure that is exhibited. The types of epilepsy are briefly reviewed below.

1. Generalized versus Partial, Simple versus Complex

When a seizure type is defined as “generalized”, this means the neurological correlate of the seizure encompasses the entire brain network and the entire body is involved in the abnormal motor activity (Dreifuss et al., 1989; Benbadis, 2001; Chang and Lowenstein, 2003). Generalized seizures can be further classified according to the
presence or lack of convulsive ictal activity, which is described in more detail in section 2. Often, a very strong genetic component is causal in generalized epilepsy (Gutierrez-Delicado and Serratosa, 2004; Michelucci et al., 2012), and typically the genetic mutation involves one or more ion channelopathies (Chang and Lowenstein, 2003).

Conversely, “partial” seizures typically involve only a specific region or regions of the brain and body, and are now commonly referred to as focal seizures (Dreifuss et al., 1989; Chang and Lowenstein, 2003; Berg and Scheffer, 2011). However, focal seizures often evolve into the generalized type, spreading across the entire brain and recruiting the entire body into the ictal process (Dreifuss et al., 1989; Blumenfeld et al., 2009). This is known as a focal, or partial, seizure with secondary generalization. Focal seizures are the most common type of seizure disorder in adults (Dreifuss et al., 1989; Chang and Lowenstein, 2003); the most common of the focal epilepsies is mesial temporal lobe epilepsy, which affects approximately 2.5 million Americans (Engel, 1996; Sharma et al., 2007). The cause of focal epilepsy is often some restricted lesion, created by an injury, stroke, other head trauma, or hippocampal sclerosis (Dalby and Mody, 2001; Chang and Lowenstein, 2003; Janszky et al., 2003; O’Dell et al., 2012). Partial seizures can be even further classified by the maintenance or lack of consciousness during seizures. People with simple partial seizure types do not exhibit impaired consciousness, while those with complex partial seizure types do (Dreifuss et al., 1989; Norden and Blumenfeld, 2002; Chang and Lowenstein, 2003; Englot and Blumenfeld, 2009).

2. Convulsive versus Non-convulsive
Historically, the terms used to describe a generalized seizure type were “grand mal” (convulsive) and “petit mal” (Sidiropoulou et al., 2010). Today, convulsive seizures are categorized as either tonic, clonic, tonic/clonic, myoclonic or atonic (Benbadis, 2001). These various forms of convulsive seizures are differentiated by type and extent of motor involvement and typical electroencephalographic (EEG) signature: tonic seizures are characterized by general muscle stiffening and low amplitude, fast EEG activity; clonic seizures involve repeating contraction of musculature and initially fast and low amplitude EEG that evolves to slower and high amplitude activity; tonic/clonic seizures are a mixed type of the previous two seizure classifications, and finally myoclonic seizures involve smaller amplitude sporadic jerks and generalized spikes on the EEG (Dreifuss et al., 1989; Benbadis, 2001). Atonic seizures are slightly different and are defined by an abrupt and general loss of muscle tone and generalized spikes on the EEG with interictal flattening (Benbadis, 2001).

Non-convulsive seizures are referred to as absence seizures. During an absence seizure, there is brief loss of consciousness, behavioral arrest and a stereotyped EEG signature with high amplitude, low frequency activity predominating (Crunelli and Leresche, 2002; Chang and Lowenstein, 2003). There may be some very small repetitive motor movements associated with absence activity, usually eye lid flutter or oral automatisms (Holmes et al., 1987). Further, there are typical and atypical forms of absence seizures. Atypical absence seizures are unlike typical absence seizures primarily because they exhibit a different EEG frequency than the 3 Hz activity of a typical absence seizure (Yagi et al., 1980; Benbadis, 2001). Also, typical absence seizures can be elicited by hyperventilation, while atypical absences cannot (Holmes et al., 1987).
3. Childhood Epilepsy

Epilepsy is considerably more common in children than in adults, and several forms of epilepsy that occur in childhood do not continue into adulthood; a peripubescent patient may experience spontaneous remittance from their seizures with the onset of adulthood (Eriksson and Koivikko, 1997). Prognosis for young epileptics is favorable (Ureña-Hornos et al., 2004), with some evidence suggesting 70 to 80% spontaneously remit and do not continue to experience seizures in adulthood (Berg and Shinnar, 1994). By far the most common epilepsy of childhood is absence epilepsy (Crunelli and Lerescue, 2002).

There may be greater concern for the social impact of epilepsy in childhood than in adulthood, and this is certainly an area where more study is needed (Rantanen et al., 2012). It has been demonstrated that some social and psychological functioning can be impaired in children with epilepsy, although the cause of this phenomenon is most likely due to a confluence of issues and is not well understood (Rodenburg et al., 2011). The cognitive impact of epilepsy is also of increased concern in childhood. Some studies have suggested most children with epilepsy possess normal cognitive functioning, but there is also significant evidence that the use of certain AEDs in childhood can have negative consequences on cognitive performance (Aldenkamp, 1997; Bourgeois, 2004; Bootsma et al., 2006; You, 2012). Alternatively, there is also suggestion that environmental and individual factors play a greater role in the cognitive functioning of children with epilepsy than does the use of antiepileptic drugs (AEDs, Mandelbaum and Burack, 1997, 1999).

4. Epileptogenesis
The process by which a normal brain acquires epilepsy is extraordinarily complex. Processes or events that make certain individuals experience seizures may not have the same epileptogenic effect on others. In 1989, the International League Against Epilepsy (ILAE) defined the methods of epileptogenesis that are reviewed below, and while some adjustments have been recently suggested to update the ILAE report, (Dreifuss et al., 1989; Berg et al., 2010; Berg and Scheffer, 2011) there is significant debate over the new updates and altered definitions of some of the causes of epilepsy. Further, the older, more well defined terms are much more commonly used in epilepsy clinics and in everyday practice today (Okuma, 2004; Panayiotopoulos, 2012). For these reasons the original terms used to define the causes of epilepsy in the 1989 report will be examined.

a. Symptomatic

Symptomatic epilepsy refers to epilepsy with a known cause (Dreifuss et al., 1989). Symptomatic epilepsy may be caused by such things as traumatic brain injury (Chen et al., 2009), focal lesions (Engel, 2001), brain tumors (Rudà et al., 2010), neurological malformations (Guerrini, 2005), or even high fever (Dubé et al., 2009). While each of these negative events may result in a seizure, the occurrence of one seizure does not necessarily mean a diagnosis of epilepsy (Dreifuss et al., 1981; Fisher and Leppik, 2008; Hesdorffer et al., 2009). Patients can show sensitivity to any of the above mentioned causes and demonstrate altered neuropathology to such an extent that seizures occur spontaneously and sporadically across a patient’s life (Mauri-Llerda et al., 2000; Beleza, 2012).
Two genetic syndromes are also known to be the cause of symptomatic epilepsy; those are Lennox-Gastaut Syndrome (Dulac and N’Guyen, 1993; Crumrine, 2002) and West Syndrome (Trevathan, 2002). Lennox-Gastaut Syndrome is a progressive syndrome that includes multiple seizure types and other neurological issues including cognitive dysfunction of various degrees (Dulac and N’Guyen, 1993; Oguni et al., 1996). West syndrome is also known by the descriptor of the specific seizure type involved, infantile spasms (Trevathan, 2002), and can evolve over time into other generalized forms of epilepsy (Lombroso, 1983).

b. Idiopathic and Cryptogenic

Idiopathic refers to epilepsy of an unknown cause, although a genetic component is often strongly suggested (Dreifuss et al., 1989). Childhood and juvenile absence epilepsy are examples of idiopathic epilepsies (Holmes et al., 1987; Porter, 1993; Benbadis, 2001). Idiopathic epilepsies are often generalized across the brain and not limited to a focal area (Benbadis and Lüders, 1996); although some focal idiopathic epilepsies, such as benign rolandic epilepsy (Gregory and Wong, 1984) do exist.

Epilepsy of a truly unknown cause, in which no genetic mutation is discovered and diagnostic imaging is normal, is known as cryptogenic (Dreifuss et al., 1989). West syndrome and Lennox-Gastaut syndrome are both examples of cryptogenic and symptomatic epilepsies (Crumrine, 2002; Trevathan, 2002). It is understood that the seizures are caused by the syndromes, but what is causing the syndromes is not known. It may be rare when the cause of epilepsy cannot be determined, but it is safe to assume that what is considered cryptogenic today may well be explained in the future (Shorvon, 2011).
5. Related Seizure Disorder - Alcohol Withdrawal

There are some neurological conditions that can lead to seizures, but are not any form of epilepsy. One such factor is the withdrawal from alcohol. Chronic alcohol consumption can lead to an imbalance of excitatory and inhibitory mechanisms in the brain (McIntosh and Chick, 2004). In alcoholics, this balance is tipped towards chronic inhibition; sudden cessation of drinking can lead to hyperexcitation in neural circuitry and seizures may result (Rogawski, 2005; Hughes, 2009). Multiple withdrawals from alcohol result in a kindling-like effect, such that with each successive cycle, withdrawal symptoms get progressively worse, or “kindle” together to produce progressively more severe symptoms including seizures (Becker and Hale, 1993; Duka et al., 2004). Interestingly, kindling via electrical stimulation (providing repeated subthreshold doses of electrical stimulation until those doses eventually sum to surpass the threshold for eliciting a seizure) has been used in animals as a model of epilepsy for decades (Racine, 1978; McNamara, 1986).

C. Mechanisms/Networks of Seizure

The neurochemicals and structures involved in epileptogenesis and in seizures are relatively well known and understood. Animal models of epilepsy have been incredibly useful in this respect, helping uncover the roles of various transmitters and brain regions in epilepsy.

1. Hyperexcitable States and the Balance of Glutamate and GABA

The epileptic brain and associated seizures are generally considered to result from an imbalance of the excitatory glutamatergic and inhibitory GABAergic neurotransmitter systems (Bradford, 1995; Chang and Lowenstein, 2003; Scharfman, 2007; Marmiroli and
Further, it has been known for some time that an initial seizure episode increases the likelihood for future seizures (Berg and Shinnar, 1991; Hauser and Lee, 2002; Ben-Ari et al., 2008), and that the propensity for future seizure activity increases with each seizure episode (Chang and Lowenstein, 2003; Morimoto et al., 2004; Jefferys, 2010). This is colloquially known as “seizures begetting seizures”, which is similar to a kindling effect itself, wherein each individual seizure can effectively sum to progressively worse seizures later on.

Exactly how this imbalance develops is unknown but research in rodent model systems offers many theories. Epileptic seizures can result in the excitotoxic death of neurons in various brain regions (Costa et al., 2004; André et al., 2007; Engrand and Crespel, 2009), and one thought is that the loss of these neurons as targets causes the targeting cells to vastly increase their branching fiber output in an attempt to reconnect to the original target (Borges et al., 2003; Cavazos et al., 2004; Sharma et al., 2007; Kuo et al., 2008; Zhang et al., 2012). The mechanism for the generation of these branching fibers is not precisely known, but has been hypothesized to involve the increased expression of genes responsible for regulating axonal growth (Lee et al., 2012a), NMDA-receptor dependency (Sutula et al., 1996), and certainly relies on new protein synthesis (Longo and Mello, 1999). However they come to be, the result is in an increase in aberrant synapses, some of which are glutamatergic in nature and only exacerbate the excessively excitatory balance of the system. The excitotoxic cell death and growth of abnormal excitatory circuitry not only occurs in experimental animals but also in human patients with epilepsy (De Lanerolle et al., 1989; Sutula et al., 1989; Houser et al., 1990; Mathern et al., 1996).
However the imbalance develops, once it exists other mechanisms come into play that sustain the dysfunctional circuits, including abnormal astrocytic function. Astrocytes play a critical role in the CNS in regulating glutamate uptake to keep the normal brain functioning properly (Hertz and Zielke, 2004; Coulter and Eid, 2012; Ransom and Ransom, 2012). When astrocytes themselves become dysfunctional, this can tip the balance even further in the excitatory direction. It has been demonstrated in rats that astrocytic hypertrophy begins to occur as soon as one day after seizure initiation (Shapiro et al., 2008) which can facilitate the growth of newly formed and abnormally connected granular cells after experimentally induced seizures by acting as an “ectopic glial scaffold” (Shapiro et al., 2005). Further, the glutamate degradation enzyme, glutamine synthetase, has been shown to be dysfunctional in the hippocampal astrocytes of human patients (De Lanerolle et al., 1989; Petroff et al., 2002; Eid et al., 2008) and in rats with experimental epilepsy (Represa et al., 1995; Shapiro et al., 2008; Estrada et al., 2012; Perez et al., 2012). Dysfunctional astrocytic enzymes could certainly be a primary contributor to excess glutamate in the brain and ensuing excitotoxicity (Schousboe and Waagepetersen, 2005; de Lanerolle et al., 2010). Thus, it appears the astrocytes themselves and their enzymatic contents can support the epileptogenic process in the human and experimental animal brain.

2. Channelopathies

Another mechanism by which the brain becomes seizure prone is through abnormal ion channel expression or function. Our lab has performed extensive investigation into the role of low threshold calcium channels (T channels) on epileptogenesis. T channels are upregulated following experimentally induced seizures,
and upregulation is maintained for at least 30 days after the initial seizure (Graef et al., 2009). Functionally, it was found that the epileptic thalamus has an increased propensity towards hyperexcitability as these channels were found to have a lower threshold for activation, and resultant calcium flux and bursts of activity (Graef et al., 2009; Graef and Godwin, 2010). Other studies have discovered similar T channel abnormalities that result in a hyperexcitable state in the hippocampus (Su et al., 2002; Becker et al., 2008). Becker et al, 2008 also demonstrated in a knockout mouse of one particular T channel isoform that seizure-induced neuropathological changes were drastically reduced and practically non-existent, providing strong support of the critical role of this particular channel in the development of epilepsy. Study in the WAG-Rij model of absence epilepsy has found that T channel expression, and the correlated Ca2+ conductance, is significantly increased in epilepsy prone rats compared to control animals (Broicher et al., 2008).

Acquired T channelopathies are only one vehicle for promoting epileptogenesis via altered and dysfunctional channel properties and expression. Potassium channelopathies are often pointed to as causal of various types of epilepsy (Noebels et al., 2012). Decreased expression of calcium-activated potassium channels has been found in a genetic model of epilepsy (N’Gouemo et al., 2009), providing evidence of their role in maintaining a hyperexcitable state for that seizure type. The expression of another potassium channel type, the inwardly rectifying potassium channel Kir4.1, is significantly reduced in the hippocampal astrocytes of human patients with mesial TLE (Heuser et al., 2012). These channels serve critical functions in astrocytes such as maintaining their typically strongly negative resting membrane potential and buffering potassium stores
during period of high neuronal activity; if such function is compromised, it is certain that neuronal excitability would be negatively impacted (Butt and Kalsi, 2006; Olsen and Sontheimer, 2008).

3. **Cortical Focus vs Thalamic Leader Theories**

In studies of epilepsy, two competing hypotheses have arisen as to the origin of hypersynchronous activity. One hypothesis, which may be referred to as the cortical focus hypothesis, posits that abnormal epileptic activity begins in the cortex and is then propagated and generalized to the rest of the brain through the thalamus. Research supporting this hypothesis has found in various models of epilepsy. For example, in the GAERS model of absence epilepsy, activity arising from the somatosensory cortex preceded ictal activity in other cortical areas and in the ventrobasal thalamus by over a full second. It was also found that the neurons within deep layers of the focal cortical area had a more depolarized resting state compared to other cortical neurons outside of the focal zone which certainly facilitated their seizure initiating capability (Polack et al., 2007). In a similar genetic model of absence, ictal activity localized within the perioral somatosensory cortex lead all other brain regions during a SWD in WAG/Rij rats, particularly during the first 500 ms of the seizure (Meeren et al., 2002; Coenen and Van Luijtelaar, 2003; van Luijtelaar and Sitnikova, 2006). Perhaps even more convincing, in cats with anesthesia-induced SWDs, those events persisted even after complete and total thalamectomy (Steriade and Amzica, 2003). There is also a wealth of evidence in human patients that show a clear leading role for the cortex as the focus for seizures (Benbadis, 2001; Sharma et al., 2007). By their very definition, partial epilepsies in humans arise
from a distinct focus (Dreifuss et al., 1989), the most common of which has a cortical origin (Engel, 1996, 2001).

The second major hypothesis is the “thalamic clock” hypothesis posits that it is the thalamus that initiates and propagates epileptic activity. In particular, it has been hypothesized that the thalamus serves as an epileptic “pacemaker”, setting the conditions necessary to tip the cortex into an ictal rhythm (Avanzini et al., 2000). Also, it had been previously demonstrated that rhythmic bursts in the thalamus preceded high voltage spindles in the cortex in awake and moving rats, and such temporal relationship was dissolved after microinjection of NMDA antagonists (Buzsáki, 1991); this came to be known as the “thalamic clock” hypothesis- that the thalamus regulates the rhythmicity of the cortex via voltage dependant mechanisms. Other findings that support the thalamus as the leader of epileptic activity have found that ibotenate lesions of the entire thalamic reticular nucleus and the lateral thalamus lead to the complete abolishment of SWDs in WAG-Rij rats (Meeren et al., 2009); thus it would appear this structure is as critical as the cortex in seizure generation.

The debate is not likely to be resolved soon. It is apparent from both camps that both the cortex and the thalamus are critical for the maintainance of epileptic seizures. Many studies have shown both the thalamus and cortex are necessary to sustain ictal activity, although clearly there are conflicting results as to which structure initiates such activity (Coenen and Van Luijtelaar, 2003; Meeren et al., 2009). In fact, we may find that these structures are hubs in a seizure network, and that the conflicting results reflect the interdependency between nodes within this network (Graef and Godwin, 2010).

4. Structures Involved
Regardless of whether it is the cortex or the thalamus that initiates and propagates seizures, both structures are critical elements of seizure generating networks. Particular seizure disorders may have foci of ictal origin in cortical regions, in the thalamus, in the hippocampus or in other limbic regions. Absence epilepsy in particular is thought to rely almost entirely on the thalamus for seizure generation, while the most common form of epilepsy, TLE, originates from the hippocampus or limbic cortices.

a. Hippocampal, Thalamic and Cortical Circuitry

The hippocampus, the primary structure in the brain responsible for the organization and consolidation of learning and memory, is arranged in a generally unidirectional loop. The flow of the circuit progresses in the following order: 1) inputs originate via perforant path fibers from the entorhinal cortex and synapse onto granular cells in the dentate gyrus. 2) The granular cells in turn project via the mossy fibers to the CA3 region. 3) The CA3 pyramidal cells then project axons, known as the Schaffer collaterals, onto the cells of the CA1 region. 4) These cells in turn project back out to the entorhinal cortex as well as other cortical areas, completing the cortico-hippocampal-cortical loop (Kandel et al., 2000; Amaral and Lavenex, 2006). There also exists a secondary excitatory pathway in the hippocampus, originating from Layer 3 cortical neurons in the entorhinal cortex which project directly to the distal dendrites of CA1 pyramidal cells (Steward and Scoville, 1976; Coulter et al., 2011). Because of the predominately excitatory and unidirectional pathway of the hippocampus, it is easy to imagine how the system could be hijacked into an abnormal and hyperexcitatory state. It is also well documented that changes occurring in the hippocampus after an initial human or experimental seizure can facilitate the occurrence of future seizures, and pathological
changes in the hippocampus are a common hallmark of epilepsy (De Lanerolle et al., 1989; Sutula et al., 1989; Engel, 1996; Mathern et al., 1996; Borges et al., 2003; André et al., 2007).

The hippocampus is enriched in many types of receptors for multiple transmitter systems, including Glu, GABA, acetylcholine and others (Amaral and Campbell, 1986; Pohorecki and Domino, 1987; Vizi and Kiss, 1998). Approximately 90% of the neurons in the hippocampus are principal pyramidal cells and granular cells, which are glutamatergic signalling cells. The remaining cells in the hippocampus are GABAergic interneurons (Freund and Buzsáki, 1996). Interspersed amongst these cell types are the terminals of extrahippocampal fibers that are noradrenergic, serotonergic and cholinergic (Vizi and Kiss, 1998).

The circuitry of the thalamus and cortex has also been extensively investigated. A greatly simplified version of the flow of information in this circuit is as follows: incoming sensory information enters the appropriate thalamic nucleus after passing through a GABAergic sheath known as the thalamic reticular nucleus (TRN) that encompasses the bulk of the thalamus. Upon leaving the relay nucleus of the thalamus, fibers travel to layer 4 of the cortex either directly or after passing again through the TRN. From layer 4 of the cortex, information then synapses on layer 6 cortical pyramidal cells, and is often generalized across the brain. In fact, both layer 4 and 6 cortical cells make extensive connections with widespread cortical brain regions, and it has been shown that not only does layer 6 (and layer 5 for that matter) project out of the cortex, but also back up to layer 4. This extensive and diffuse projection pattern contributes greatly to the generalization of multiple types of seizure disorders, including absence epilepsy.
In human patients, generalization of convulsive seizure types also occurs via cortico-thalamo-cortical projections (Blumenfeld et al., 2009). A similar circuitous path is followed by outgoing information. Signals that arise in cortical regions are passed to the TRN or directly to the relay nuclei via layer 6 cells. (for excellent reviews of cortico-thalamo-cortical circuitry, see Amaral, 2000; Sherman and Guillery, 2002; Clascá et al., 2012).

D. Rodent Models of Epilepsy

Animal models of human conditions are absolutely critical to understand the causes, consequences, and possible therapies for a vast number of diseases and disorders. Fortunately, there are numerous reliable and valid models to study epilepsy and other seizure disorders. The mechanisms responsible for the development of such neural abnormality have been much more understood because of these models. Further, treatment options for epilepsy exist because of work within the framework of such models. The ethical and reasonable use of animals in research is one of the greatest assets to science and must be conducted with the utmost care in order to push knowledge forward.

1. Animal Models: Necessary and Valid

Animals used to conduct scientific research are absolutely necessary to understand basic principles of various diseases. It is simply unethical to use human beings for some studies. In the pursuit of new efficacious treatment options, humans are necessary and some might be willing to sign up, but to understand the basic molecular mechanisms behind some conditions? Some may not be so willing to volunteer for that.
Yet, without such basic conceptual understanding, development of novel therapeutics would come to a grinding halt.

Beyond the necessity of their use, the epilepsy models used must be proven valid; this means various conditions are met. For example, the seizure model must predict the response of standard drug treatments. Also, the model must reflect some of neuropathological changes that occur in the human condition. Finally, the overt behavior of the animal must mirror those seen in humans with the disease.

2. Genetic Models

One of the greatest advantages of using mice in research is that the complete genetic sequence of the animal is understood. If one gene or a cluster of genes is thought responsible for a condition, it is relatively easy to manipulate that and create a subspecies knockout line wherein all of the offspring carry the deleted, silenced, or in some way mutated and quiescent version of the gene.

a. Mouse

i. Lethargic

Mice of the lethargic (lh/lh) strain have an inherited mutation of the calcium channel β4 subunit. This mutation truncates the C-terminus of the β subunit of the P/Q type calcium channel such that the binding site for α- subunit is missing (Burgess et al., 1997). This ultimately results in drastically altered calcium channel kinetics and the “lethargic” phenotype of the mice. Further, in vitro slice recordings of the somatosensory thalamus of these mice has demonstrated drastically reduced excitatory synaptic transmission, while GABAergic signalling is unencumbered compared to control littermates (Caddick et al., 1999). More specific work has pointed to the ventrolateral and
reticular nuclei of the thalamus as the generator network of the seizures in lh/lh mice (Hosford et al., 1995). Behaviorally, these mice are typically ataxic. When movements do occur, they are unstable, lurching and generally uncoordinated (Hosford and Wang, 1997). The EEG of lh/lh mice shows a periodic, spontaneous SWD that is typically 3 to 4 times the amplitude of the background EEG signal and falls within the 4 to 6 Hz frequency range (Hosford et al., 1992). While these oscillations are slightly higher than those occurring in human patients, it is known that these abnormal oscillations arise from thalamocortical circuitry (Hosford et al., 1995). Lethargic mice are known to respond well to the classic first-line therapeutic for absence epilepsy: ethosuximide (Aizawa et al., 1997).

**ii. Other Genetic Mouse Models**

Other mouse models of genetic absence epilepsy include the stargazer and tottering models, among others (Sarkisian, 2001). These mice are quite similar to the lh/lh model in terms of behavioral phenotype. Similar to lh/lh mice, stargazer mice also have an inherited mutation in calcium channel regulating genes, although it is in the gamma subunit (Letts et al., 1998; Osten and Stern-Bach, 2006). The tottering mouse has a quite pronounced absence-like phenotype, motor dystonia and calcium channel dysfunction similar to that of the lethargic mouse (Fletcher et al., 1996; Campbell and Hess, 1999).

**b. Rat**

Genetically epileptic rats are also available and useful to study. Genetic Absence Epilepsy Rats from Strasbourg (GAERS) exhibit SWDs in the 7 to 11 Hz frequency range (Charpier et al., 1999; Polack et al., 2007). GAERS exhibit behavioral arrest and impaired consciousness during these synchronous and bilateral events (Marescaux et al.,
The WAG-Rij rat, another model of absence, also has synchronous and bilateral SWDs in the 8 to 10 frequency range (Coenen and Van Luijtelaar, 2003). The EEG waveform of WAG-Rij SWDs is strikingly similar to the ictal waveform seen in human patients (Sitnikova and Van Luijtelaar, 2007), although the frequency of the oscillations is slightly higher in rodents than in human patients (Benbadis, 2001). Both of the rat models respond positively to drugs used in humans with absense, such as ethosuximide (Van Luijtelaar et al., 2002; Manning et al., 2003). Both the GAER and WAG-Rij rodent models of absence epilepsy meet the criteria to be considered a valid model of absense: the behavior of the epileptic animal is similar to epileptic humans, the neuropathology is similar and the response to first line drugs used is similar (Coenen et al., 1992; Snead, 1995).

3. Chemical Models

Beyond the various genetic models of epilepsy, other useful models for the study of epilepsy exist that involve the inducement of seizures using an exogenous compound or stimulus. Genetic models may be thought to be superior and somehow “more valid” because of similarities in the pathology involved in the human and rodent epilepsies. However, induced models of epilepsy are fast, reproducible and often repeatable within subjects, making them an appealing option for investigation.

a. Pilocarpine

Multiple species, including rats and mice (Cavalheiro et al., 1996; Curia et al., 2008) and even some non-human primates (Perez-Mendes et al., 2011), can be injected with the muscarinic agonist pilocarpine to induce generalized, convulsive seizures. The pilocarpine model is a model of both status epilepticus (SE, continuous seizure activity)
and temporal lobe epilepsy, because of the neural structures involved and affected by pilocarpine (Turski et al., 1983; Cavalheiro et al., 1996; Curia et al., 2008). Animals are pretreated 30 minutes prior to pilocarpine with the muscarinic antagonist, scopolamine, to reduce potentially fatal peripheral effects of pilocarpine, such as pulmonary edema and excessive salivation (Clifford et al., 1987). Some investigators that use pilocarpine choose to use a lithium pretreatment regimen instead of scopolamine, which allows for a lower dose of pilocarpine to be used (Clifford et al., 1987). The goal is the same however, to reduce mortality from pilocarpine. Mortality is an immense consideration in this model; even mice of the same strain from different vendors can have drastically different mortality rates (Borges et al., 2003; Müller et al., 2009b).

Once pilocarpine is given, whether with lithium or scopolamine beforehand, a period of SE develops wherein the animal exhibits more or less continuous behavioral and electrographic seizures that last for at least 30 minutes (Treiman et al., 1990; Cavalheiro et al., 1996; Curia et al., 2008). There are bouts of clonic/tonic seizures that occur during this time, and it has been found that SE precedes a profound increase in glutamate release in the hippocampus, likely contributing the neurotoxicity of the model (Costa et al., 2004; de Oliveira et al., 2011). Following the initial bout of SE, the animal goes through a silent, or latent, period during which no behavioral seizures occur, but significant neurological changes are occurring that allow for the eventual development of spontaneous, recurrent seizures (SRS, Cavalheiro et al., 1996; Lehmann et al., 2001; Curia et al., 2008; Shapiro et al., 2008; Estrada et al., 2012). Pathological changes in the entorhinal and piriform cortices begin as soon as 6 hours after the onset of SE, while changes in the hippocampal hilus are not seen until 36 to 48 hours later (André et al.,
The molecular changes that underlie the development of SRS, including the upregulation of specific calcium channels (Becker et al., 2008; Graef et al., 2009; Graef and Godwin, 2010) begin almost as rapidly. While SE lasts on the order of hours and the silent period lasts a few weeks, the period of SRS is lifelong and the hallmark of the pilocarpine model (Turski et al., 1983, 1984, 1989; Curia et al., 2008).

Mice that have been given pilocarpine show some of the same neuropathological changes that are seen in human TLE patients, including mossy fiber sprouting, astrogliosis and hippocampal cell death (Cavalheiro et al., 1996; Borges et al., 2003; Cardoso et al., 2011). While the aberrant mossy fibers certainly contribute to the generalization and occurrence of future seizures, they are not critically necessary, as inhibition of their development with rapamycin does not inhibit SRS (Buckmaster and Lew, 2011). It remains to be seen if preventing some of the other changes, such as the reactive gliosis and cell death, can inhibit the development of SRS.

Perhaps most interesting as validation of the pilocarpine model, some of the cognitive issues seen in human patients with TLE have also been described in rodents given pilocarpine, such as spatial learning disruptions (Chauvière et al., 2009; Inostroza et al., 2011; Cardoso et al., 2011) and increased anxiety-related behavior (Müller et al., 2009a). Lastly, it has been shown that rodents given pilocarpine respond well to traditional AEDs, have fewer SRS, less hippocampal cell loss and gliosis (Oliveira et al., 2005; Cunha et al., 2009; Perucca, 2009; Chen et al., 2010; Zheng et al., 2010).

b. Gamma-Hydroxybutyrate (GHB)

While pilocarpine is a model of convulsive epilepsy, GHB, or its prodrug gamma-butyrolactone (GBL), is used to model non-convulsive seizures (Depaulis et al., 1988;
Snead, 1988; Maitre et al., 2000). Also in contrast to pilocarpine, GBL or GHB can be administered multiple times in the same animal because the effects are short lived and do not result in the development of spontaneous seizures (Pol et al., 1975; Snead, 1988; Goodwin et al., 2009). In support of the model’s validity, GBL or GHB-treated animals respond well to the classic antiepileptic drugs typically used for treatment of that seizure type, such as ethosuximide (Snead, 1988; Aizawa et al., 1997). GBL is typically given intraperitoneally, and is converted into GHB in the liver, resulting in a more rapid peak brain concentration of GHB in the brain than injection of GHB itself (Snead, 1991; Palmer, 2004); therefore most investigators choose GBL over GHB in their experiments.

GBL acts at both GHB and GABA receptors in the brain (Maitre et al., 2000; Wong et al., 2004; Crunelli et al., 2006; Ticku and Mehta, 2008). Behavioral studies have demonstrated that experimental animals are capable of discriminating GHB from other sedative/hypnotic compounds, and that baclofen, a GABA<sub>B</sub> agonist, often results in GHB-appropriate responding (Carter et al., 2009; Koek et al., 2009). Some GABA<sub>A</sub> active compounds also elicit GHB-appropriate responding, although less often than the GABA<sub>B</sub> active drugs and only at lower doses (Koek et al., 2004). Further, GABA<sub>B</sub> antagonists are capable of blocking the SWDs caused by GHB (Snead, 1996). These findings all suggest that not only is GHB active at its own receptor, but also at GABA<sub>B</sub> receptors across an array of doses, and at GABA<sub>A</sub> in smaller doses.

Thalamocortical circuitry has long been known to be crucial for the development of GHB-induced SWDs; within 1 hour of GHB injection, significant increases in fos expression is found throughout the thalamus (Zhang et al., 1991). One possible explanation of how GHB may induce absence-like SWDs is that in the ventrobasal
thalamus, GHB decreases both basal and K+-induced GABA and K+-induced Glu release, thereby creating an environment in which the development of absence-like seizures is likely (Banerjee and Snead, 1995). The same group had earlier found that increased GHB binding after GHB administration was restricted to areas that participate in SWDs, including the VPL, VPM, TRN and superficial cortex; no increased binding was observed in other areas, such as the hippocampus (Banerjee et al., 1993).

4. Other Models

There are other chemical models of epilepsy that are useful to study generalized epilepsy types. These include the pentylentetrazole (PTZ) model and the kainate acid model, in both of which mice receive an intraperitoneal bolus of one of those compounds and exhibit clonic/tonic seizures (Lösch, 1998; Sharma et al., 2007; Dhir, 2012). Kainate can also be administered intrahippocampally to produce seizures (Bouilleret et al., 1999). Some models rely on focal application of heavy metals to induce seizures, such as cobalt, although the use of these models is far less common today than previously (Bregman et al., 1985). There is also a penicillin model of generalized absence epilepsy, most commonly using cats or rodents (Chen et al., 1986; Avoli, 1995).

Beyond chemically induced models of epilepsy, there are many other models and variations of the models that have already been described herein. Some more common alternative models for human TLE include the electrical kindling model, wherein electrodes are placed intracranially into seizure prone regions, such as the amygdala and subthreshold stimulation applied with replication such that seizures are resultant (Fisher, 1989; Sharma et al., 2007). The audiogenic seizure model “primes” young rodents with an auditory stimulus and later exposure to a specific auditory stimulus elicits discrete
clonic/tonic seizures (Garcia-Cairasco et al., 1993). Another sensory driven model uses patterns of photic stimulation to elicit seizures (Szabó et al., 2012). Photically induced seizures and other reflexive seizure types can occur in some populations of sensitive patients (Wilkins et al., 1980; Guerrini et al., 1994; Trenité, 2006).

E. Therapeutics for Epilepsy

1. Antiepileptogenics

The ultimate goal of any drug therapy is to cure the patient of illness; whether the patient is then able to stop taking the medication may also be part of that goal. However, it seems our medical establishment is always a step behind illness, treating symptoms as they occur as opposed to treating the underlying cause of the illness or even better, being able to predict a particular illness and prevent it from occurring. This would be the goal of antiepileptogenesis; preventing the development of epilepsy in people who would otherwise have it. For example, it is known there is a likelihood of developing a seizure disorder in people who have had some sort of traumatic brain injury (TBI, (Pitkanen and Bolkvadze, 2012)). If those people were given some treatment or compound that would prevent the neuropathological changes that lead to the seizure disorder, this treatment would be called an antiepileptogenic (Löscher and Brandt, 2010). These treatments would be extremely difficult to develop, as prediction of what may cause a seizure disorder is nearly impossible. Not all of those who experience a TBI will develop a seizure disorder, and some people who have seizure disorders have absolutely no known cause. Therefore, physicians rely on AEDs to treat seizures.

2. Anticonvulsant and Anti-absence Drugs
Anticonvulsants are a first line treatment option for those who have a seizure disorder. While preventing the seizures from occurring in the first place is currently impossible, there are a wide variety of drugs on the market today that treat the seizures themselves. The mechanism of action of these drugs can vary significantly, but for those patients who respond well, an oral anticonvulsant may be the only necessary medical intervention for the disorder. There is a more detailed description of specific anticonvulsants and other AEDs in a later section of this review.

Anti-absence medications are similar to anticonvulsants, in that they treat the seizures themselves, but the mechanism of action of these medications is often quite different. It so happens that most typical anticonvulsants actually exacerbate non-convulsant seizures, and conversely, those medications that are used to treat non-convulsive seizures are ineffective against convulsive seizures (Panayiotopoulos, 1999; Rogawski and Löscher, 2004). Therefore, alternative medications that specifically treat non-convulsive seizures are necessary.

3. Other Therapies

Unfortunately for many people, traditional oral therapies do not work to control their seizures. These patients are considered treatment resistant, and must find a more effective alternative treatment. Options do exist, and for some, they are effective at regaining control of epileptic activity.

a. Diet

Mounting evidence is beginning to support what some patients have long known, that certain dietary manipulations are effective at regaining control over seizures, or at bolstering the effectiveness of concurrent therapies. Low carbohydrate diets are often
pointed to as beneficial for people with various types of epilepsy (Levy et al., 2012; Masino and Rho, 2012). The mechanism of action is unknown, but most likely is due to a variety of antiepileptic properties the diet elicits. Potential explanations range from the enhancement of GABAergic transmission in the ketonic brain (Dahlin et al., 2005) to the reduction of blood glucose via caloric restriction (Greene et al., 2001). As is the case with most scientific explanations, the true answer is likely a combination of all the explanations and effects of the diet on the body. Regardless of why and how it works, this method of dietary manipulation would not exacerbate a seizure condition and may be worth trying for some patients. One major concern remains, however, and that is the tolerability of the diet itself. Patient compliance is a considerable issue in medical interventions of all kinds, and if one isn’t willing to take a pill regularly, then the likelihood of getting on and staying on a quite restrictive diet is not high.

b. Surgery

Neurosurgery is significantly more invasive than any other intervention for epilepsy treatment, and is therefore considered a last line treatment option for most patients. Multiple neurological tests and scans are performed to attempt to locate the epileptic focus (or foci) in the brain so that it may be removed (Placantonakis and Schwartz, 2009). The extent of the ictal focus has been found to be a critical determinant of post-surgical prognosis; as might be expected a smaller focus paired with a larger yet still conservative resection often has the best outcome for patients (Guye et al., 2006). However, it is often difficult to pin down a specific epileptic focus in the brain, and even if one is found, it may be impossible to remove for a variety of reasons. It may be in an inoperable location or a part of the brain that the patient may not be comfortable losing.
due to concerns for postoperative cognitive dysfunction (Helmstaedter et al., 1998; Hamberger and Drake, 2006).

A more radical alternative to focal removal of epileptic brain tissue is to perform what is known as a “split brain” procedure (Spencer and Spencer, 1985). Quite simply, surgeons sever connections in the corpus callosum, the bundle of nerve fibers and connective tissue that links one hemisphere of the brain to the other, in an attempt to stop the generalization of seizure activity across the brain. This is considerably more drastic than focal removal of a small portion of tissue, and patients can have a wide array of post-surgical side effects such as memory disruption and visual field inattention (Sauerwein and Lassonde, 1997; Berlucchi, 2012).

One other surgical option is the implantation of a stimulating electrode into the brain, targeted to thalamic or subthalamic structures, which can emit electrical pulses to interrupt and reduce seizure frequency (Theodore and Fisher, 2004; Jones, 2010; Lee et al., 2012b). This procedure is known as deep brain stimulation (DBS), and while still considered an experimental therapy for the treatment of epilepsy, the procedure has shown extraordinary promise and efficacy in treating another neurological disorder: Parkinson’s disease (Volkmann, 2004).

c. Vagal Nerve Stimulation

The last treatment option that will be reviewed here is vagal nerve stimulation (VNS). With this therapy, electrodes that are tethered to and periodically stimulate the vagal nerve are connected to a generator that is surgically implanted in a patient’s chest, just under the clavicle (Theodore and Fisher, 2004). While there is still some debate as to the precise mechanism of action, the fact remains that many people with intractable
epilepsy benefit from this therapy and show pronounced reductions in the number of seizures they experience (Ben-Menachem, 2002).

F. Current Challenges in AEDs

1. Prevalence, Impact and Compliance

There are currently over 2 million people living with epilepsy in the United States today, with 150,000 new cases being diagnosed every year (Banerjee et al., 2009; Anon, 2012). Further, the associated health care costs are astronomical; some estimates put that number at $17.6 billion (Anon, 2012). Beyond the financial cost of epilepsy, the emotional and psychological cost for patients is also high. Epilepsy is often comorbid with a several psychological conditions, including depression and anxiety (Harden and Goldstein, 2002; Hoppe and Elger, 2011).

Not only does the condition itself cause considerable health issues, but often the drugs used to treat epilepsy are wrought with negative side effects. The use of AEDs is often associated with sleepiness, fatigue, perturbances of gross motor function, dizziness, metabolic issues, gastrointestinal upset, significant drug interaction and cognitive dysfunction (Bourgeois, 2004; Bootsma et al., 2006; Chung et al., 2007; Rodenburg et al., 2011; Perucca and Gilliam, 2012). Further, perhaps the worst problem associated with AEDs is that patient response is low, and often one must try multiple drugs or combinations of drugs until the efficacy is maximized (Perucca and Gilliam, 2012). Most cruelly, the patient population that is most commonly treatment resistant (and the population with the highest incidence of seizures) are neonatal children under 28 days old (Jensen, 2009). For adult patients, all of the treatment related complications often create an environment where patient compliance is low. If a patient is too negatively affected by
side effects, or feels the drug isn’t working for them, they are less likely to continue talking that drug.

2. Today’s Drugs: Mechanisms and Limitations

a. First Generation AEDs

The first generation antiepileptic drugs that were developed are carbamazepine (Tegretol), phenobarbital (Solfoton), phenytoin (Dilantin), and valproate (Depakote). These were the first drugs developed to deal with seizures effectively and are still in use today, though they often create the most significant side effects in patients (Loring et al., 2007; Kennedy and Lhatoo, 2008; Perucca and Gilliam, 2012). Phenobarbital, the earliest of these to be developed, is still used today but mainly to inhibit treatment-resistant SE that has failed to respond to benzodiazepines, which are faster to administer and reach effective levels in the brain (Treiman et al., 1998).

b. Second Generation AEDs

The second generation of antiepileptic drugs developed includes felbamate (Felbatol), gabapentin (Neurontin), lamotrigine (Lamictal), levetiracetam (Keppra), oxcarbazepine (Trileptal), tiagabine (Gabitril), topiramate (Topamax) and zonisamide (Zonegran). These drugs were developed in an attempt to combat the multiple side effects of the first generation drugs, and to attempt to achieve seizure control for a wider population of patients (Wilby et al., 2005; Chung et al., 2007). These drugs are all commonly used today and for some patients are extremely efficacious, though still wrought with side effects of their own (Onat and Ozkara, 2004; Chung et al., 2007). Gabapentin has been found to be therapeutic for other disease states beyond epilepsy, in particular, neuropathic pain (Gilron et al., 2005; Moore et al., 2011). Similar to epilepsy,
the mechanism behind neuropathic pain is thought to be hyperexcitation in the CNS (Willis, 2001).

c. Third Generation AEDs

Examples of third generation drugs include brivaracetam (Rikelta), carisbamate (Comfyde), eslicarbazepine (Zebinix), fosphenytoin (Cerebyx), lacosamide (Vimpat), pregabalin (Lyrica), retigabine (Trobalt), rufinamide (Banzel) and stiripentol (Diamomit). Also currently being investigated as third generation drugs are carabersat, DP-valproic acid (a prodrug of valproic acid), fluorofelbamate, ganaxolone, losigamone, remacemide, safinamide, seletracetam, soretolide, talampanel and valrocemide, all of which are pending brand names. These drugs are active at a very diverse array of targets (Rogawski, 2006; Patsalos and Berry, 2012), although the goal remains to increase efficacy for more patients. Currently, most research in novel antiepileptics is occurring at research institutions as opposed to big pharmaceutical companies, who have mostly divested themselves of AED development (Rogawski, 2006). One major concern however is that these novel agents may work for some resistant patients, but generally do not increase efficacy across the spectrum of the epilepsies (Perucca, 2002).

II. Metabotropic Glutamate Receptors (mGluRs)

Due to the numerous negative side effects associated with typical AEDs, it is necessary to develop novel compounds that target novel receptors in the brain. Newer generation AEDs do not always increase clinical efficacy for resistant patients, so perhaps the typical targets are not the best. In contrast to the action of ionotropic glutamate receptors, activation of mGluRs is much more modulatory and potentially longer lasting because of the involvement of second messengers as opposed to direct
channel activity (Conn and Pin, 1997). For these reasons, and more outlined below, mGluRs have become attractive candidates.

A. G-Protein Coupled Receptors

There are seven families of G-protein-coupled receptors (GPCRs) that are grouped according to their structure and activity. All GPCRs have seven transmembrane (TM) spanning domains that interact with an intracellular G-protein, composed of alpha, beta and gamma subunits (Gether, 2000). Typically, a sequence on the extracellular domain or on one of the extracellular TM loops is the interaction site for binding ligands (Gether, 2000). When a ligand is bound, a conformational change occurs in the GPCR, thus allowing GTP to bind in place of GDP and the alpha subunit to dissociate from the beta and gamma subunits (Gilman, 1987; Johnston and Siderovski, 2007), and allowing both portions of the dissociated receptor to effect downstream signalling (Davis et al., 2005; Johnston et al., 2006). Exactly how the activity of a ligand binding to the receptor activates the conformational change is unknown, but appears to depend on the particular G-protein and associated intracellular effectors; the end product is typically the excitation or inhibition of the cell (Ulloa-Aguirre et al., 1999; Karnik et al., 2003).

B. mGluRs- Groups 1 and 3

1. Group 1

The Group 1 mGluRs are mGluR1 and mGluR5. These mGluRs are coupled to the Gq protein, and when activated will excite the cell in which they are expressed via increased phospholipase C activity.

a. Location of Expression
Group 1 mGluRs are found throughout the nervous system, both centrally and in the periphery. These receptors are typically expressed postsynaptically (Shigemoto et al., 1997; López-Bendito et al., 2002). One immunoreactivity study in particular has demonstrated the existence of Group 1 mGluRs in the human cortical plate as early as 9 weeks gestational age (Boer et al., 2010), so these receptors are certainly involved in developmental regulation of the human cortex. In the visual thalamus of fully developed experimental animals, mGluR5 appears to be localized to the dendritic arbor of thalamic interneurons, and mGluR1 is found in the postsynaptic density in thalamic relay cells (Vidnyanszky et al., 1996; Godwin et al., 1996; Govindaiah et al., 2012). Of the Group 1 receptors, only mGluR5 is expressed in glial cells (Van den Pol et al., 1995).

b. Second Messenger Signaling

When activated, Group 1 mGluRs stimulate the G_q protein. The activation of this G protein initiates inositol phosphate hydrolysis, in which IP_3 and DAG are generated. These molecules can then in turn activate PKC, which affects a multitude of cellular functions and can result in release of intracellular Ca^{2+} stores, thus having an excitatory effect on the cell (Chavis et al., 1995; Siegelbaum et al., 2000; Moldrich and Beart, 2003). This pathway also stimulates voltage-gated calcium channels (Stefani et al., 1996; Fagni et al., 2000; Schwartz and Alford, 2000) and calcium-sensitive K^+ channels (Anwyl, 1999; Siegelbaum et al., 2000; Moldrich and Beart, 2003; Fagni, 2012) that ultimately result in cellular excitation.

c. Ligand Efficacy in Models of Epilepsy

Multiple studies have demonstrated the antiepileptic activity of antagonists of Group 1 mGluRs. AIDA, MPEP and MTEP (all Group 1 antagonists) were shown to be
effective at increasing the latency to seizure and/or decreasing their occurrence altogether in both adult (Mares and Mikulecká, 2004) and immature rodents (Mares, 2009) in the PTZ model. MPEP in particular has been suggested to inhibit both convulsive and non-convulsive generalized seizures, in several models (Chapman et al., 2000; Barton et al., 2003). For example, MPEP has been found to increase the latency to the first pilocarpine-induced seizure and reduce the significant mortality associated with administration of the neurotoxin (Jesse et al., 2008). In addition to Group 1 mGluR antagonists being putative anticonvulsants, Group 1 agonists have also been used to model epileptogenesis (Camón et al., 1998; Chapman et al., 2000; Smolders et al., 2004); these compounds can induce long term increases in excitatory current in hippocampal cells, even hours after the agonist is washed out (Bianchi et al., 2009). However, there is certainly conflicting evidence: Group 1 mGluR antagonists were not demonstrated to be effective anticonvulsants in two models of partial epilepsy in rodents (Löscher et al., 2006). Further, week-long administration of two mGluR5 antagonists in control rats demonstrated that the gene expression relating to various cellular functions, including several glutamate transporters, is decreased, suggesting that perhaps chronic application of drugs of this type may actually increase extracellular glutamate (Gass and Olive, 2008), thus supporting hyperexcitability and potentially exacerbating seizures.

2. Group 3

The Group 3 mGluRs are mGluR 4, 6, 7 and 8. These receptors are coupled to $G_{i/o}$ and inhibit adenylyl cyclase. When active, they typically inhibit the cells on which they are expressed. Other than mGluR6, which is only expressed in the mammalian retina, these receptors are found throughout the CNS.
a. Location of Expression

mGluR6 has the most restricted expression; it is only found in the mammalian retina (Nomura et al., 1994). The remaining Group 3 mGluRs (4, 7 and 8) are expressed mostly in axonal areas presynaptically (Bradley et al., 1996; Shigemoto et al., 1996; Corti et al., 2002). mGluR4 shows a diffuse pattern of expression, with presynaptic localization in the cerebellum, basal ganglia and thalamic relay nuclei (Corti et al., 2002), and expression of mGluR4 has been shown to be enhanced in rodents with genetic predisposition to epilepsy (Ngomba et al., 2008). mGluR7 and 8 appear to have mostly overlapping expression in most brain regions such as the cortex, cerebellum, hippocampus and thalamus; the olfactory bulb however appears to exclusively express mGluR7 and not mGluR8 (Saugstad et al., 1997; Corti et al., 1998). Further, Group 3 mGluRs have been found on multiple cell types outside of the CNS, including in colon epithelial cells (Chang et al., 2005).

b. Second Messenger Signaling

When activated, Group 3 mGluRs stimulate the G_{i/o} protein and adenylyl cyclase activity is inhibited, thus preventing the formation of cAMP in the cell (Siegelbaum et al., 2000; Moldrich and Beart, 2003). The net result is inhibition of the cell on which these receptors are expressed. Possible explanations of the mechanism of this effect include the activation of an outward presynaptic K+ current which inhibits neurotransmitter release (Cochilla and Alford, 1998) or by supression of voltage-gated Ca2+ channels (Stefani et al., 1996; Takahashi et al., 1996; Anwyl, 1999; Dietrich et al., 2002). In the hippocampus, mGluR7 in particular regulates glutamate release via its association with P/Q calcium channels (Martín et al., 2007).
c. Ligand Efficacy in Models of Epilepsy

Agonists of mGluR4, 7, and 8 have been demonstrated to be antiepileptics. Studies have shown these effects in many models, and their role in epileptogenesis has been demonstrated in human patients as well. In hippocampal tissue from human patients with TLE, mGluR4 expression is increased greatly; it is almost entirely absent in the hippocampus of people with no history of TLE (Lie et al., 2000). An agonist of mGluR4 can potentiate the anticonvulsant effect of AMPA and NMDA blockade in a sound-induced model of epilepsy (De Sarro et al., 2002). Interestingly, some evidence suggests the inhibition of excitatory activity by Group 3 mGluR agonists is somewhat age-dependant and dependant on the particular mGluR. In hippocampal slices from rats, DCPG (an mGluR8-specific agonist) only inhibits glutamatergic signalling in neonatal rats; there is no effect in adult animals (Ayala et al., 2008). However, the same group also found that an mGluR7-specific agonist, L-AP4 did reduce signalling across all ages (Ayala et al., 2008). Certainly these receptors play very different roles in the regulation of excitatory transmission across the lifespan of an animal. In mice with seizures induced by a Group 1 mGluR agonist, L-AP4 potently prevented those events from occurring (Tizzano et al., 1995). Regarding the anticonvulsant ability of L-AP4, some controversy exists. The L-AP4 mediated reduction of transmission in the hippocampus was reduced during the latent phase after pilocarpine induced SE (Bough et al., 2004), and remained impaired 2 month after SE (Kral et al., 2003). This does not preclude the possibility of some anticonvulsant activity of L-AP4 if given during SE, or perhaps prophylactically and it certainly demonstrates the involvement of mGluR7 in the development of spontaneous seizures after a neurotoxic challenge.
C. Group 2 mGluRs

mGluR2 and 3 constitute the Group 2 mGluR family. These receptors are similar to the Group 3 receptors, in that they are expressed mostly presynaptically on neural cells and couple to the \( G_{i/o} \) protein.

1. Location of Expression

mGluR2 and 3 are found throughout the nervous system. mGluR2 is thought to be an entirely neuronal receptor, while mGluR3 has been found on neural and glial cells (Ohishi et al., 1993, 1998; Petralia et al., 1996; Mineff and Valtschanoff, 1999; Hetzenauer et al., 2008). mGluR2 is found almost exclusively presynaptically and acts as an autoreceptor (Testa et al., 1994; Neki et al., 1996; Ohishi et al., 1998; Kew et al., 2001; Alexander and Godwin, 2006a; Gu et al., 2008), while mGluR3 has also been localized to the postsynaptic density (Ohishi et al., 1993; Petralia et al., 1996; Tamaru et al., 2001). This distribution of expression has been found in human brain tissue as well (Phillips et al., 2000). Experiments in rodent thalamocortical slices has found evidence of mGluR2 localized to the preterminus of corticoreticular synapses and specifically not on GABAergic intra-reticular synapses (Alexander and Godwin, 2006a). mGluR3 however has been found in GABAergic reticular cells (Gu et al., 2008). Outside of the CNS, Group 2 mGluRs have been found in the enteric nervous sytem, or the network of cells that innervate the gut, where they serve the same functional roles as those in the CNS, including modulating calcium current kinetics (Choi and Lovinger, 1996; Chen and Kirchgessner, 2002). Group 2 mGluRs have also been localized to other peripheral organs in the body, including the liver, lungs, kidneys, spleen and testes (Gill et al., 2000).
2. Second Messenger Signaling

Group 2 mGluRs are coupled to the $G_{i/o}$ protein, just as the Group 3 mGluRs are. The same intracellular cascade is initiated when these receptors are activated, such as inhibition of adenylyl cyclase and reduction of cAMP activity (Siegelbaum et al., 2000; Antoni, 2000), the result being inhibition of the cell via a variety of mechanisms, including activation of an outward presynaptic K+ current (Cochilla and Alford, 1998; Anwyl, 1999) or supression of voltage-gated Ca2+ channels (Chavis et al., 1994, 1995; Takahashi et al., 1996; Dietrich et al., 2002). Stimulation of the human mGluR2 with the native ligand, L-glutamate was inhibited by pertussis toxin, providing support of the connection of this receptor in the human brain to the $G_{i/o}$ protein (Phillips et al., 1998).

3. Orthosteric and Allosteric Ligands

Like all receptors in the nervous system, mGluRs have both orthosteric and allosteric binding sites. The allosteric binding site is in a different location than the orthosteric site, and the effects of allosteric binding are typically to modulate the effects of the orthosteric ligand. In the case of positive allosteric modulators (PAMs), receptor activation is enhanced by various mechanisms, including increased binding affinity of the orthosteric ligand (Johnson et al., 2004, 2005). mGluR2 and mGluR3 are quite similar with respect to their affinities for agonists and antagonists, in fact, no specific orthosteric compound can differentiate between the two receptors. Thankfully, the development of mGluR2-specific modulators has smoothed some of this difficulty without necessitating the use of expensive genetically modified animals (Johnson et al., 2003; Pinkerton et al., 2004; Rudd and McCauley, 2005; Bonnefous et al., 2005). Still, studies in knockout mice have been useful, but often demonstrate the redundancy of action of these receptors in

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response to classic antagonism (Linden et al., 2009). Some evidence suggests that modulation of a receptor in combination with orthosteric agonism potentiates the effect of the agonist alone; this has been demonstrated in various sleep related measurements using an mGluR2/3 agonist and mGluR2-specific PAM (Ahnaou et al., 2009) and in the potentiated effect of Group 2 mGluR-induced inhibition of glutamatergic transmission in the hippocampus (Galici et al., 2006).

It has been demonstrated that two different agonists of mGluR2/3 inhibited excitatory but not inhibitory transmission in the barrel cortex of rats; neural responses to a single tactile stimuli were significantly depressed with either agonist applied, while cross-whisker inhibition was not affected by the drugs (Cahusac and Wan, 2007). Agonists of Group 2 mGluRs also reduced the amplitude of fEPSPs in hippocampal slices from rodents, an effect which is prevented by antagonist pretreatment (Kew et al., 2001), and hippocampal mEPSC frequency is also significantly reduced by mGluR2/3 agonist treatment (Scanziani et al., 1995). The same finding has been shown at the thalamocortical synapse as well: agonism of mGluR2/3 significantly lowers glutamate release in this pathway (Mateo and Porter, 2007) and in the corticoreticular pathway (Alexander and Godwin, 2006a). A study of multiple mGluR2/3 agonists and mGluR2-specific PAMs has shown they are all effective at inhibiting Ca2+ dependant oscillations in the cortex of both rats and mice (Sanger et al., 2012). Even in surgically resected hippocampal tissue from human TLE patients, an agonist of mGluR2/3 (DCG-IV) suppressed fEPSPs in that structure (Dietrich et al., 2002). Beyond reducing synaptically released glutamate, it appears that activation of mGluR3 receptors on glial cells can also restore the ability of dysfunctional astrocytes to increase their glutamate uptake, which
may explain some of the neuroprotective effects of Group 2 mGluR agonists (Zhou et al., 2006).

It has been demonstrated in various seizure models that mGluR2 and 3 orthosteric agonists have antiepileptic effects (Moldrich et al., 2001, 2003; Imre, 2007). In models of absence epilepsy, it has been shown that mGluR2 and 3 are involved in the brain networks and pathology of absence, and agonists of mGluR2/3 have been shown to have therapeutic effects for this seizure type (Ngomba et al., 2011). Administration of mGluR2/3 agonists have delayed the development of amygdala-kindled seizures (Attwell et al., 1998a), significantly increased the seizure threshold for fully kindled rodents (Attwell et al., 1998a, 1998b), and protected against the development of convulsive seizures in the homocysteic acid model of epilepsy (Folbergrová et al., 2001). Further, an mGluR2/3 agonist not only prevented seizures induced by PTZ and picrotoxin (Kłodzińska et al., 1999, 2000) but also potentiated the effect of the classic antiepileptic drug diazepam (Kłodzińska et al., 2000). Another mGluR2/3 agonist is effective at preventing the occurrence of seizures induced by kainic acid, though the administration must be a prolonged infusion and at low doses; higher doses induced toxicity to hippocampal cells and exacerbated these seizures (Miyamoto et al., 1997). The Group 2 mGluR agonist LY37928 is effective at protecting against seizures induced by 6 Hz corneal stimulation in a dose dependant manner (Barton et al., 2003). In contrast, some groups report only partial anticonvulsant activity of mGluR2/3 agonists when used in a model of seizures induced by mGluR1 agonists (Smolders et al., 2004) and in the maximal electroshock model (Barton et al., 2003). The timing of drug administration is certainly also a consideration; it has been shown that giving mGluR2/3 agonists after the
onset of a generalized convulsive seizure is not as neuroprotective and efficacious as when the drug is given as pretreatment (Folbergrová et al., 2009).

Beyond the efficacy of Group 2 mGluR active drugs as antiepileptics, these compounds have demonstrated potential as antipsychotic and anxiolytic agents, both in animal models and in clinical trials (Imre, 2007; Harrison et al., 2008; Conn and Jones, 2009; Fraley, 2009; Wischhof and Koch, 2011; Fell et al., 2012). Drugs active at these receptors reduce head-twitching in response to hallucinogenic agents (Benneyworth et al., 2007) and reduce motor behavior in response to PCP- (Cartmell et al., 1999; Johnson et al., 2005; Galici et al., 2006; Hackler et al., 2010) and ketamine- (Imre et al., 2006) induced hyperlocomotion. It appears then that activation of mGluR2/3 affords the brain a certain amount of protection against a variety of insults (Okamura et al., 2003; Corti et al., 2007).

4. Of Particular Interest: mGluR2

While it is clear that most mGluRs hold potential as targets for antiepileptic drug development (Conn, 2003; Alexander and Godwin, 2006b), mGluR2 is of particular interest. Due to the unique pre- and extrasynaptic location of mGluR2, this receptor has great potential as a target for antiepileptic drug development, since only during times of extraordinarily high glutamatergic signalling would this receptor become active (Cartmell and Schoepp, 2000; Knöpfel and Uusisaari, 2008). It is most unfortunate then that no orthosteric agonist yet exists that has differential affinity for mGluR2 over mGluR3 (Kew and Kemp, 2005). Therefore the use of a PAM is necessary to parse out the perhaps unique antiepileptic roles for the two Group 2 receptors. It is possible that combining a Group 2 agonist with an mGluR2-specific PAM may enhance any anti-seizure effects of
drugs active at these sites. This would be an added benefit to the finding that mGluR2 may be resistant to homologous desensitization (Iacovelli et al., 2009).

It could be that drugs active at mGluR2 alone may serve as novel antiepileptics. Even better, they may only become active when enough endogenous ligand is on board as well, as would be the case during a seizure. Thus, an exemplary situation could exist, inasmuch that someone could take a drug daily, but the effects of that drug would only become apparent once a seizure began, and would otherwise be without side effects. This would be an immense advantage over today’s standard antiepileptics, a class of drugs which are almost universally fraught with deleterious side effects.

In sum, I have endeavored to investigate the utility of Group 2 mGluRs as targets for antiepileptic drug development using the pilocarpine and the GBL models of epilepsy. Both of these models have many positive facets, including the animal’s extraordinary responsiveness to typical AEDs, prototypical motor behavior during seizure and an EEG signature that is extremely similar to that seen in human patients with various types of epilepsy. In the studies presented herein, both behavioral measures of seizure inhibition and recordings of intraictal EEG were made in an attempt to fully characterize the behavioral and neurological effect these drugs have on the initial development and progression of seizures. The novelty of these experiments lies in the fact that the PAM BINA has not been tested in either of these models of seizure before, and hence it is not known what antiepileptic effect may exist within the context of the GBL and the pilocarpine models. These two models were chosen because they are among the most well established models of convulsive and non-convulsive seizures types; compared to
other models, both of thee have reasonably predicted the efficacy of most standard antiepileptic drugs, and certainly reflect the behavioral state of the human condition.
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CHAPTER TWO: Activation of Group 2 Metabotropic Glutamate Receptors Reduces the Severity of Acute Pilocarpine Induced Status Epilepticus

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1.1 INTRODUCTION

Temporal lobe epilepsy (TLE) is a chronic condition characterized by recurrent seizures that involve the medial or lateral temporal lobe. Antiepileptic drugs (AEDs) can be effective, but nearly 30% of patients are refractory to them, and some medications possess negative side effects that reduce patient compliance. There is also a wide range of individual responsiveness to AEDs, therefore the development of novel pharmacological targets remains an important goal.

The pilocarpine model of TLE mimics the process of epileptogenesis, and possesses many characteristics of the human disorder. Pilocarpine administration in mice results in an acute period of status epilepticus (SE) that is defined by continuous seizure activity lasting at least 30 minutes. After the initial period of SE, there is a “latent period” during which significant neural reorganization occurs followed by chronic life-long susceptibility to spontaneous, recurrent seizures (Cavaloheiro et al., 1996; Curia et al., 2008; Müller et al., 2009; Perez-Mendes et al., 2011; Turski et al., 1989, 1984, 1983). The maintenance and generalization of SE and the development of spontaneous recurrent seizures (SRS) is thought to occur through hyperglutamatergic activity via NMDA receptors in the hippocampus (Nagao et al., 1996; Priel and Albuquerque, 2002; Smolders et al., 1997). Therefore, pilocarpine administration in wild-type mice provides the opportunity to assess novel therapies that interfere with excessive glutamate signaling.

A potential target for such novel therapies are the Group 2 metabotropic glutamate receptors (mGluRs), mGluR2 and mGluR3 (Alexander and Godwin, 2006a; Moldrich et al., 2003). Unlike ionotropic glutamate receptors, mGluRs do not transmit
fast synaptic responses and are less active during each synaptic release event (Conn, 2003). mGluRs tend to produce longer lasting effects than ionotropic glutamate receptors due to their G-protein involvement (Conn and Pin, 1997). The Group 2 mGluRs are coupled to the $G_{i/o}$ protein and may limit glutamate release via inactivation of high threshold calcium channels, activation of potassium channels and/or by interfering with neurotransmitter release (Anwyl, 1999; Cochilla and Alford, 1998; Scanziani et al., 1995; Takahashi et al., 1996). In particular, mGluR2 appears to be exclusively positioned outside of the active zone of synapses where it may only be activated during high frequency neuronal activity (Alexander and Godwin, 2006b, 2005; Cartmell and Schoepf, 2000; Knöpfel and Uusisaari, 2008; Shigemoto et al., 1997), similar to that which occurs during SE (Blumenfeld et al., 2009; Chen and Wasterlain, 2006; Morimoto et al., 2004; Racine, 1972). In most systems studied to date, mGluR2 is specifically expressed presynaptically (Petralia et al., 1996; Shigemoto et al., 1997), which may allow for interrupting hyperexcitable activity before it spreads across the synapse and brain. Thus, mGluR2 exhibits a distinctive localization that may lend itself to abolishing or reducing the activity at hyperexcitable synapses.

Several Group 2 mGluR agonists, such as LY354740, LY389795 and LY379268, have been found to be anticonvulsant in limbic and generalized motor seizure models (Attwell et al., 1998a, 1998b; Klodzińska et al., 2000; Miyamoto et al., 1997; Moldrich et al., 2001a, 2001b; Monn et al., 1997). Also, the antiepileptic effects of Group 2 agonists can be abolished by pretreatment with Group 2 antagonists, revealing the specificity of the drugs and receptor system against seizures (Folbergrová et al., 2001). While it is difficult to specifically target mGluR2 because of a lack of specific agonists, the positive
allosteric modulators 3’-[(2-cyclopentyl-2,3-dihydro-6,7-dimethyl-1-oxo-1H-inden-5-yl)oxy]methyl]-[1,1’-biphenyl]-4-carboxylic acid (BINA) and N-4’-cyano-biphenyl-3-yl)-N-(3-pyridinylmethyl)-ethanesulfonamide hydrochloride) (CBiPES) have pharmacological specificity at mGluR2 (Ahnaou et al., 2009; Benneyworth et al., 2007; Bonnefous et al., 2005; Fell et al., 2010; Galici et al., 2006; Johnson et al., 2005, 2003; Sanger et al., 2012). In these experiments, we used the Group 2 mGluR agonist LY379268, BINA and CBiPES in treating mice before and after pilocarpine-induced SE. We tested the hypothesis that administration of Group 2 mGluR active compounds prior to pilocarpine administration (pretreatment) can ameliorate the behavioral and electroencephalographic progression of acute SE, providing a form of prophylaxis. A second hypothesis we tested was that these drugs may be capable of arresting the progression of SE after animals are given pilocarpine (posttreatment), thus providing rescue from SE. Briefly, we found that mGluR2-active compounds (including LY379268 alone and a cocktail of LY379268+BINA) were capable of reducing the severity of SE if given prior to pilocarpine. We also observed that these drugs could decrease the severity of SE if given after pilocarpine administration, though higher doses were necessary. CBiPES alone was also capable of reducing some of the behavioral manifestations of seizure when given prior to pilocarpine.

1.2 METHODS

1.2.1 Rotarod Pilot Study

All animal procedures were approved by the Wake Forest School of Medicine Institutional Animal Care and Use Committee. We performed rotarod trials in pilot animals (n = 36) in order to assess gross motor performance in normal mice after
administration of mGluR2 active drugs. There were six groups, with six mice in each group: saline (0.25 mls), diazepam (5 mg/kg), LY379268 (10 mg/kg), LY379268 (20 mg/kg), BINA (100 mg/kg), and cocktail (LY379268 (20 mg/kg) + BINA (100 mg/kg)). Mice were randomly assigned to a group and given four habituation trials on the rotarod apparatus (SDI Inc., San Diego, CA) on day 1. Trials lasted up to 180 seconds or until the mouse fell from the rod.

On test day (day 2), mice were given a preinjection trial and then tested again 10 minutes, 30 minutes, 1 hour, and 5 hours after injection. Time spent on the rotarod was measured and postinjection performance was compared to the preinjection trial within each group. The diazepam group was included as a positive control for a compound that is commonly known to interfere with motor performance and ability (Savić et al., 2009; Willerslev-Olsen et al., 2011).

1.2.2 Surgery

A tethered electroencephalography/electromyography (EEG/EMG) acquisition system (Pinnacle Technologies Inc, Lawrence KS) was used for these studies. For surgical implantation of EEG electrodes, a subset of male C57Bl/6 mice (n = 48) were anesthetized using ketamine and xylazine (100 mg/kg and 10 mg/kg, respectively). Supplemental oxygen was provided and atropine (0.04 mg/kg) was given preoperatively to suppress bronchial secretions. Once areflexia was apparent, a 1 cm incision was made and the skull exposed. Four pilot holes were drilled through the skull for placement of stainless steel screws. These screws terminated in a preamplifier headmount that was affixed to the skull using dental acrylic. Silver epoxy was used to cover each screw and maintain electrical continuity with the headmount. Two EMG leads were placed into the
neck musculature. The incision was sutured around the headmount and topical antibiotic was applied. Mice were moved to a recovery cage and given ketoprofen (5 mg/kg) for pain management. Mice recovered at least one week before initiating any subsequent experiment. Systemic antibiotics were not given, as post-operative infection was considered exclusionary criteria for the study according to our protocol.

1.2.3 Behavior

During the pre- (treatment given before pilocarpine) and posttreatment (treatment given after pilocarpine) studies, we measured the onset and severity of the behavioral response to pilocarpine administration. The measures taken included: the latency to the first stage 5 clonic/tonic (C/T) seizure, bouts of individual C/T seizures during SE, and the maximum Racine score. When an animal failed to reach a C/T seizure in response to pilocarpine, they were automatically assigned a latency score of 180 min, which was the maximum length of observation. These animals were assumed to have not developed a C/T response to pilocarpine, but still allowed for them to be included in their group for statistical comparisons. In the posttreatment studies, treatment was given when an animal reached a stage 3 Racine score, which is a readily apparent behavioral response to pilocarpine. Seizure scoring was performed using an adapted Racine scale (Racine, 1972). The scale is as follows: 0 (lack of any apparent seizure activity), 1 (oral automatisms), 2 (head nodding), 3 (forelimb clonus), 4 (rearing), 5 (clonic/tonic seizure with rearing and falling), 6 (wild running/bouncing), to 7 (death as a consequence of pilocarpine and resulting seizures).

1.2.4 Pilocarpine Administration
Pilocarpine (330 mg/kg) was administered to mice (n = 114) 30 min after an injection of methyl-scopolamine (1 mg/kg), which was used to inhibit peripheral effects of pilocarpine and reduce mortality. This dose of pilocarpine was chosen because it has been demonstrated in the literature to be the lowest concentration of pilocarpine that most reliably elicits seizures (Curia et al., 2008; Turski et al., 1983). Pilocarpine was given outside of the home cage while mice were individually housed in monitoring cages. Animals were monitored for three hours for behavioral scoring. In a subset of animals, EEG monitoring was also performed during the first hour. EEG sampling occurred at a rate of 200 Hz with a preamplifier applied band pass filter from 0.5 to 40 Hz. For the pretreatment studies, drugs were given 15 minutes prior to pilocarpine. The groups in that study included saline (referred to as “Pilo Only”, n = 14), LY379268 (10 mg/kg, n = 14), BINA (100 mg/kg, n = 14), and cocktail, which received both LY379268 and BINA (10 and 100 mg/kg respectively, n = 14). For the posttreatment studies, drugs were given immediately after the animal had the first stage 3 Racine seizure, which is characterized by forelimb clonus. The groups in that study were saline (“Pilo Only”, n = 12), LY379268 (20 mg/kg, n = 12), BINA (100 mg/kg, n = 10), and cocktail, which received both LY379268 and BINA (20 and 100 mg/kg respectively, n = 12). Finally, a second mGluR2-specific positive allosteric modulator (PAM), CBiPES (30 mg/kg), was tested for efficacy in both pre- and posttreatment behavioral experiments (n = 6 in both studies).

1.2.5 Drugs

LY379268 was a kind gift from Eli Lilly and Company and was dissolved in 0.9% saline. CBiPES was also kindly provided by Eli Lilly and Company and was dissolved in a vehicle containing 1% carboxymethylcellulose, 0.25% Tween 80 and
0.05% Dow Antifoam, with the pH adjusted to 7.4. BINA was a gift by Dr. Jeffrey Conn (Vanderbilt University, Nashville, TN) and was dissolved in a vehicle containing 10% Tween 80 and 10% NaOH with the pH adjusted to 7.4. Diazepam was manufactured by Hospira, Inc (Lake Forest, IL). Methyl-scopolamine and pilocarpine were purchased from Sigma (St. Louis, MO) and both dissolved in 0.9% saline. All drugs were given intraperitoneally in a volume range of 0.25 to 0.5 mls.

1.2.6 Data Analysis

Rotarod data were analyzed using a repeated-measures ANOVA with a Dunnett’s post hoc to test for significant differences within each group’s performance before and after injection. Digitized EEG signals were transformed into power spectral data using a custom-written Matlab protocol. Analysis of that transformed data was performed using a Kruskal-Wallis test with Dunn’s post hoc analysis. The spectral bands analyzed were defined as follows: delta (0.5 to 3 Hz), theta (4 to 7 Hz), alpha (8 to 12 Hz), and beta (13 to 25 Hz). If normality could be assumed for data that were continuous in nature, analysis was performed using a one-way ANOVA with Tukey’s post hoc test. If behavioral data were discrete in nature, a Kruskal-Wallis test with a Dunn’s post hoc was used. If the behavioral data were categorical in nature, then a Chi-square test of homogeneity was used to test for statistically significant differences.

1.3 RESULTS

1.3.1 Rotarod Pilot Study

A repeated-measures ANOVA was performed within each experimental group with a Dunnett’s post hoc to determine if any group’s postinjection performance was significantly different from their preinjection performance on the rotarod. The diazepam
group spent significantly less time on the rotarod 10 and 30 minutes after the injection (p < 0.01, n = 6, Fig 1) compared to before the injection. No other group exhibited differences in gross motor ability at any time point after injection compared to before the injection. The means ± SEM for the time (in seconds) spent on the rotarod for each group at every time point are presented in Table 1. The mGluR-active drugs used in this study do not appear to significantly affect motor performance on the rotarod test.

1.3.2 Pretreatment Study

Figure 2 shows the power spectral data 60 minutes after pilocarpine administration when Group 2 mGluR-active drugs were given as pretreatment. Power (shown on the Y axis) has been normalized to the average power occurring in that bandwidth during baseline, which was recorded prior to any drug administration. Power in each frequency bandwidth (delta- 0 to 3 Hz, theta- 4 to 7 Hz, alpha- 8 to 12 Hz, and beta-13 to 25 Hz) was averaged, normalized to baseline activity, and a Kruskal-Wallis test with Dunn’s post hoc was performed across groups within each frequency band.

Sixty minutes after pilocarpine administration (Fig 2), the LY379268 group had significantly less power than the Pilo Only, BINA and Cocktail groups (p < 0.001) in the delta bandwidth. In theta bandwidth, the LY379268 group continued to demonstrate less power than the Pilo Only and BINA groups (p < 0.001), as well as the cocktail group (p < 0.01). Also in the theta range, the cocktail group had significantly less power than the Pilo Only group (p< 0.05) and the BINA group (p < 0.01). Finally in the alpha and beta bandwidths, the LY379268 and cocktail groups had significantly less power than the Pilo Only group (p < 0.001). The EEG data suggests that LY379268 and the cocktail
mitigated the abnormal power increases that were seen in control mice that received only pilocarpine with saline as pretreatment.

The latency to the first C/T seizure was significantly increased for the LY379268 and cocktail groups compared to the Pilo Only group (one-way ANOVA with Tukey’s post hoc, p < 0.001, n = 14 for all groups, mean ± SEM for LY379268 = 148.2 ± 14.61 min, cocktail = 163.5 ± 11.63 min, Pilo Only = 61.29 ± 13.76 min, Fig 3a). Also, the LY379268 and cocktail groups had a significantly increased latency to the first C/T seizure compared to the BINA group (one-way ANOVA with Tukey’s post hoc, p < 0.001 for the cocktail group, p < 0.01 for the LY379268 group, mean ± SEM for BINA = 76.07 ± 15.44 min, Fig 3a). Mice pretreated with LY379268 had fewer bouts of C/T seizures during SE (Kruskal-Wallis test with Dunn’s post hoc analysis; p < 0.01, mean ± SEM = 0.71 ± 0.44 bouts) than Pilo Only mice (mean ± SEM = 5.36 ± 1.27 bouts, Fig 3b). Mice pretreated with the cocktail also had significantly fewer bouts of C/T seizures compared to Pilo Only mice (p < 0.001, mean ± SEM for cocktail = 0.21 ± 0.15, Fig 3b). Lastly, using a chi square test for homogeneity, it was found that there were significant differences between all of the groups Racine scores (p = 0.0008, mean ± SEM Racine score for Pilo Only = 5.07 ± 0.29, LY379268 = 2.70 ± 0.52, BINA = 4.79 ± 0.46, cocktail = 1.57 ± 0.54, Fig 3c).

Taken together, the behavioral and spectral data suggest that LY379268 (10 mg/kg) alone is protective against SE when given prior to pilocarpine. LY379268 lessened pilocarpine-induced power changes and also increased the time until initial seizure onset, decreased the number of individual C/T seizures during SE and lowered the average maximum Racine score. BINA does not appear to provide protection against
pilocarpine-induced power changes in EEG or in behavioral expression of SE. BINA alone did not increase the latency to the first C/T seizure, reduce the bouts of C/T seizures, or lower the average Racine score. The cocktail of both LY379268 and BINA does not seem to provide any more protection from SE than LY379268 does alone. In general it appears there is no added benefit of administering the cocktail as opposed to the mGluR2/3 agonist alone as pretreatment against pilocarpine-induced SE.

1.3.3 Posttreatment Study

Figure 4 shows the power spectral data 60 minutes after pilocarpine administration when mGluR-active drugs were given after SE began. Latency to a stage 3 seizure, and thus latency to treatment administration, was not significantly different between groups in the posttreatment study (Fig 5a). A stage 3 seizure occurred and treatment was given on average 23.74 ± 2.69 (mean ± SEM) minutes after pilocarpine. A Kruskal-Wallis with Dunn’s post hoc analysis was performed on all of the spectral data within each frequency range after it was normalized to baseline activity.

Sixty minutes after pilocarpine (Fig 4), the LY379268, BINA and cocktail groups all exhibited significantly less power compared to the Pilo Only group (p < 0.001 for the LY379268 and cocktail groups, p < 0.05 for the BINA group) in the delta frequency range. Also in the delta range, the LY379268 group had significantly less power than both the BINA group (p < 0.001) and the cocktail group (p < 0.01). In the theta and alpha ranges, the LY379268 group showed less power compared to the Pilo Only and the BINA groups (p < 0.001), as well as the cocktail group (p < 0.05). Also in the theta and alpha ranges, the cocktail group showed significantly less power than the Pilo Only group (p < 0.001) and the BINA group (p < 0.01). Finally, in the beta range, all groups exhibited less
power than the Pilo Only group (p < 0.001), and the LY379268 and cocktail groups showed less power than the BINA group (p < 0.001).

A one-way ANOVA with Tukey’s post hoc analysis determined there was a significantly increased latency to the onset of the first C/T seizure for the LY379268 (n = 12) and the cocktail (n = 12) posttreatment groups as compared to the Pilo Only group (n = 12, p < 0.001, latency mean ± SEM for LY379268 = 180 ± 0 min, cocktail = 165.7 ± 9.68 min, Pilo Only = 61.78 ± 15.65 min, Fig 5b). Also, the cocktail and LY379268 groups had significantly increased latencies to the first C/T seizure compared to the BINA group (n = 10, p < 0.001, latency mean ± SEM for BINA = 74.2 ± 17.93 min, Fig 5b). LY379268 posttreated mice had fewer bouts of C/T seizures during SE than Pilo Only mice (p < 0.001, Kruskal-Wallis test with Dunn’s post hoc analysis, bouts mean ± SEM for LY379268 = 0 ± 0, bouts mean ± SEM for Pilo Only = 2.22 ± 0.66, Fig 5c). The cocktail posttreated mice had significantly fewer bouts of seizures than the Pilo Only group (p < 0.01) and the BINA group (p < 0.05, bouts mean ± SEM for cocktail = 0.17 ± 0.11, bouts mean ± SEM for BINA = 2.2 ± 0.61). Lastly, using a chi square test for homogeneity, all of the posttreatment groups had significantly different Racine scores (p = 0.0034, Racine score mean ± SEM for Pilo Only = 5.44 ± 0.29, LY379268 = 3.5 ± 0.15, BINA = 5.4 ± 0.3, cocktail = 3.67 ± 0.22, Fig 5d).

The posttreatment data suggest that Group 2 mGluR activation with LY379268 reduces behavioral seizures, as evidenced by increased latency to a C/T seizure, reduced bouts of C/T seizures and decreased Racine scores compared to the Pilo Only group. The BINA group was never significantly different from the Pilo Only group, and the LY379268 group was never significantly different from the cocktail group on any
behavioral measure. The EEG data generally demonstrated that after treatment was given, any activation of mGluR2/3 with an agonist or in combination with a PAM, some protection would be provided in the form of reduced power compared to Pilo Only animals. Since LY379268 was not found to be different from the cocktail group, there may be no added benefit of a PAM in treating C/T seizures with mGluR2 active drugs.

1.3.4 CBiPES Study

We performed an additional round of behavioral studies using CBiPES (30 mg/kg, n = 6 in both the pre- and posttreatment groups), a recently created PAM that has been shown to potentiate the effect of LY379268 (Johnson et al., 2005) as well as mimic the antipsychotic effects of LY379268 (Fell et al., 2010). The same behavioral measures were recorded as in the previous experiments, including latency to the first C/T seizure, bouts of C/T seizures, and the maximum Racine score.

CBiPES (30 mg/kg, n = 6) given as pretreatment before pilocarpine resulted in significantly longer latency to the first C/T seizure compared to Pilo Only pretreatment (one-way ANOVA with Tukey’s post hoc, p < 0.05, latency mean ± SEM for CBiPES = 132.3 ± 23.84 min, Table 2). When given as posttreatment, CBiPES still significantly increased the latency to the first C/T seizure compared to the Pilo Only posttreatment group (one-way ANOVA with Tukey’s post hoc, p < 0.05, latency mean ± SEM for CBiPES = 135.3 ± 28.33 min, Table 2). CBiPES also significantly reduced the bouts of C/T seizures during SE compared to Pilo Only when given as pretreatment (Kruskal-Wallis with Dunn’s post hoc, p < 0.01, Table 2). When given as posttreatment, CBiPES did not significantly reduce the bouts of C/T seizures during SE compared to the Pilo Only posttreatment group (Kruskal-Wallis with Dunn’s post hoc, p > 0.05, Table 2).
Finally, although differences were found in the latency to the first seizure and bouts of seizures during SE, no significant difference was found between these groups in either the pre- or posttreatment studies (chi square test for homogeneity, p > 0.05, Table 2).

1.4 DISCUSSION

Our results indicate that activation of Group 2 mGluRs may provide both prophylaxis against and rescue from SE. Administration of Group 2 mGluR drugs either before or after pilocarpine diminished the severity of acute SE, as evidenced by reduced EEG spectral power and reduced behavioral seizure in experimental animals. Pilo Only animals in both the pre- and posttreatment experiments that received only saline as an intervention demonstrated a typical progression through SE; average latency to the first C/T seizure was 61.54 minutes, the number of bouts of C/T seizures during SE was 3.79, and the average Racine score was 5.26. The SE protective effects of the compounds were not due to any gross motor disturbances as evidenced by performance on the rotarod (Fig 1). In that experiment, mice demonstrated consistent motor ability even after administration of the drugs used in this study. Our findings of a lack of motor disruption and concurrent positive effect on EEG power and seizure related behavior by the Group 2 mGluR drugs is in contrast to studies in which a classic AED, diazepam, was shown to reduce the behavioral manifestation of seizure but not its neurological correlate (Morita et al., 1982; Turski et al., 1989). We did not study the effect of diazepam on our SE mice; however, we note that it significantly limited motor performance on the rotarod and that none of the mGluR active drugs had a similar effect. Thus, any demonstration of decrease of seizure by these drugs is not due to an inability to manifest gross motor behavior. Some studies have noted reduced motor ability with higher doses of LY379268 (Cartmell
et al., 1999; Feinberg et al., 2002), but it is important to note that while it appears in Fig 1 that mice given higher doses of LY379268 have reduced activity, their preinjection performance was similarly reduced; thus this effect on motor performance has nothing to do with the higher dose of LY379268 but may be due to cohort effects out of our control.

When given as pretreatment, the cocktail of LY379268 and BINA did not provide greater protection against pilocarpine-induced SE than when LY379268 was given alone. The mice in the cocktail and LY379268 groups exhibited similar latencies to the first C/T seizure, similar bouts of those seizures and similar Racine scores; they were not different on any behavioral measure. This suggests the anti-seizure effect of the cocktail is most likely due to the presence of the agonist, since no added benefit occurred when BINA was added. It has been previously hypothesized that with simultaneous orthosteric and allosteric ligand binding at mGluR2 there may be increased receptor sensitivity to the endogenous and/or orthosteric ligand (Johnson et al., 2003). Mechanistically, allosteric modulation of mGluRs may increase receptor sensitivity, receptor availability, and/or efficacy of receptor homodimerization (Johnson et al., 2005; Kew and Kemp, 2005), however, in our study, it does not appear that any of these allosteric modulations could overcome the changes produced by pilocarpine without the orthosteric ligand on board as well. We were unable to demonstrate that BINA alone could reduce seizure severity. In contrast, with CBiPES alone pretreatment, we did find a significant increase in the latency to the first C/T seizure and a reduction in the bouts of C/T seizures compared to Pilo Only mice. This supports previous work in anxiety models, such as stress-induced hyperthermia, in which CBiPES was found to mimic the effect of LY379268 when administered alone (Fell et al., 2010; Johnson et al., 2005; Sanger et al., 2012).
When posttreatment was given after mice reached a stage 3 Racine seizure, we found that LY379268 was capable of limiting the severity of SE when compared to the Pilo Only posttreatment group. However, this required a higher dose of LY379268 than was used in the pretreatment experiment. A pilot group of posttreatment mice given the same dose of LY379268 used in the pretreatment study was not significantly different from the Pilo Only mice on any behavioral measure (data not shown). However, when the dose was doubled, the LY379268 group was significantly protected against SE compared to the Pilo Only group. Sixty minutes after pilocarpine was given, LY379268 and the cocktail reduced the power of pilocarpine-induced changes compared to the Pilo Only and BINA group in both the pre- and posttreatment studies. One explanation for BINA not adding any benefit when used in combination with LY372968 was that the dose used was not in the effective range, though previous studies that examined the effect of BINA in several models of psychosis and anxiety used much lower doses and found significant effects on their measure of interest (Benneyworth et al., 2007; Galici et al., 2006). Therefore, we believe an appropriate dose of BINA was used in our seizure model. When we administered CBiPES as posttreatment, we did find a significant behavioral effect in that the latency to the first C/T seizure was significantly increased compared to the Pilo Only group. Interestingly, CBiPES was more easily dissolved than BINA under our conditions, and while both drugs were entirely dissolved for injection, this cannot be excluded as a possible explanation of the difference in efficacy between these two PAMs.

The protective effect of the cocktail within the posttreatment paradigm is most likely due to LY379268 and not synergy between the modulator and the agonist, as there is no additional protective effect seen with the addition of BINA. It may be that once an
animal has reached a stage 3 seizure, the protective effect of mGluR2/3 stimulation may be due to activation of mGluR3 alone. More studies are needed, but one plausible explanation of this effect may be that LY379268 activation of mGluR3 on glial cells tonically reduces pilocarpine-induced glutamate release, and the addition of BINA does not potentiate the effect as it is not pharmacologically active at mGluR3.

Ermolinsky et al (2008) demonstrated that after pilocarpine administration, endogenous levels of mGluR3 gene expression are reduced 24 hours after SE, and then rebound to control levels one month later. mGluR2 follows a similar expression pattern, with reductions the first day and a rebound by one month later. However, two months after SE, mGluR2 levels were reduced again (Ermolinsky et al., 2008). Similarly, Garrido-Sanabria et al (2008) also found reduced expression of mGluR2/3 24 hours after pilocarpine-induced SE. This group also demonstrated that DCG-IV, an mGluR2/3 agonist, did not reduce the amplitude of fEPSPs in chronically epileptic rats, suggesting a long-term loss of these targets in epileptic animals (Garrido-Sanabria et al., 2008), the consequence of which could be increased excitability in the brains of these animals. The results of these two studies suggest that delaying mGluR2/3 drug administration by 24 hours or more after pilocarpine would yield little to no effect on hyperexcitability in epileptic rodents. However, the data we obtained provide support for the idea that early treatment may slow or entirely abolished the seizure related neurological changes that occur after pilocarpine administration. The effect of our treatment schedule on the development (or lack thereof) of subsequent SRS in the chronic period of pilocarpine treated mice remains to be determined.
Benzodiazepines are currently the first line treatment for controlling SE in adults and children, although these drugs may only prevent behavioral seizures and may not provide complete protection against the development of future seizures or other neurological consequences after an initial bout of SE (Bleck, 1999; Rabinstein, 2010; Saz et al., 2011; Treiman et al., 1998). Certainly, treating acute SE is most critical in a clinical setting, but preventing future seizures precipitated by an episode of SE should also be of consideration. Further studies are needed to demonstrate if the drugs used in these experiments may curtail the development of SRS after pilocarpine, as well as the resulting neuropathological changes that occur with epileptogenesis. If it could be demonstrated that these drugs also prevent or reduce the incidence of SRS, it would further support the development of drugs that target Group 2 mGluRs for the treatment of epilepsy.

Acknowledgements

The authors wish to express our gratitude to P. Jeffrey Conn at Vanderbilt University for providing BINA. We would also like to thank Eli Lilly and Company for kindly providing the LY379268 and CBiPES. Also, we would like to express our gratitude to Hong Qu Shan, David Klorig and Walter Wiggins for their helpful comments and suggestions during the writing phase of this manuscript.

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Figure 1. **Drugs active at Group 2 mGluRs do not perturb gross motor ability.**

C57Bl/6 mice were used (n = 6 per group) to determine whether performance on a rotarod apparatus would be altered by injection with either saline, diazepam (5 mg/kg), LY379268 (10 mg/kg), LY379268 (20 mg/kg), BINA (100 mg/kg), or a cocktail of both BINA and LY379268 at the highest dose. Diazepam was used to demonstrate the effect of an antiepileptic drug that is also known to inhibit motor function. Time spent on the rotarod was measured prior to injection, and then 10 min, 30 min, 1 h, and 5 h postinjection. A repeated measures ANOVA with Dunnett’s post hoc analysis was used to determine which time points postinjection were significantly different from the preinjection performance within each particular group. The only significant differences were found in the diazepam group, at 10 min post- and 30 min postinjection. *, p < 0.01, n = 6 per group. Abbreviations: 10 m, 10 minutes postinjection; 30 m, 30 minutes postinjection; 1 h, 1 hour postinjection; 5 h, 5 hours postinjection.
Figure 2. Pretreatment with Group 2 mGluR drugs reduces power of pilocarpine-induced SE in EEG recordings 60 minutes after pilocarpine administration. In the delta range, the LY379268 group shows decreased power compared to all other groups (p < 0.001, n = 6 in all groups). In the theta frequency range, the LY379268 group shows decreased power compared to the Pilo Only group (p < 0.001), the BINA group (p < 0.001) and the cocktail group (p < 0.01). The power is also decreased in the theta range for the cocktail group compared to the Pilo Only group (p < 0.05) and the BINA group (p < 0.01). In the alpha and beta range, the LY379268 and cocktail groups show decreased power compared to the Pilo Only and BINA groups (p < 0.001 for both comparisons). The Pilo Only group received saline as a control pretreatment prior to pilocarpine. The frequency ranges were defined as delta (0.5-3 Hz), theta (4-7 Hz), alpha (8-12 Hz) and beta (13-25 Hz). Power has been normalized to the average power at baseline in each frequency range. Compared to Pilo Only: *, p < 0.05; ***, p < 0.001. Compared to BINA: ##, p < 0.01; ###, p < 0.001. Column abbreviations: Pilo: Pilo Only, LY68: LY379268 (10 mg/kg), BINA: BINA (100 mg/kg), Cocktail: Cocktail (LY379268 (10 mg/kg) + BINA (100 mg/kg)).
Figure 3. **Pretreatment with Group 2 mGluR active drugs reduces behavioral severity of pilocarpine induced SE.**

**A.** Latency to the first C/T seizure during SE was significantly increased for the LY379368 and the cocktail groups compared to the Pilo Only group (n = 14 in all groups, one-way ANOVA with Tukey’s post hoc, p < 0.01). The LY379268 and cocktail groups also had an increased latency to the first C/T seizure compared to the BINA group (one-way ANOVA with Tukey’s post hoc, p < 0.01 for the LY379268 group, n = 14; p < 0.001 for the cocktail group).

**B.** Average bouts of C/T seizures during SE were significantly reduced with pretreatment with LY379268 compared to the Pilo Only group (Kruskal-Wallis test with Dunn’s post hoc, p < 0.01). Pretreatment with the cocktail also significantly reduced bouts of C/T seizures compared to the Pilo Only group (Kruskal-Wallis test with Dunn’s post hoc, p < 0.001).

**C.** The Racine scores were also significantly different between all pretreatment groups (Chi square, p = 0.0008). Seizures were considered C/T if they reached at least a stage 5 Racine score. Compared to Pilo Only: **, p < 0.01; ***, p < 0.001. Compared to BINA: #, p < 0.01; ###, p < 0.001.
Figure 4. **Posttreatment with Group 2 mGluR drugs reduces power of pilocarpine induced SE in EEG recordings 60 minutes after pilocarpine administration.** In the delta frequency range, the LY379268 and cocktail groups show decreased power compared to the Pilo Only group (p < 0.001, n = 6 in all groups), as does the BINA group (p < 0.05). Also in the delta range, the LY379268 group shows decreased power compared to the BINA group (p < 0.001) and the cocktail group (p < 0.01). In the theta and alpha ranges, the LY379268 group demonstrated decreased power compared to the Pilo Only group (p < 0.001), the BINA group (p < 0.001) and the cocktail group (p < 0.05). Also in the theta and alpha bandwidths, the cocktail group had significantly decreased power compared to the Pilo Only group (p < 0.001) and the BINA group (p < 0.01). Finally in the beta range, all other groups had less power than the Pilo Only group (p < 0.001), and the LY379268 and cocktail groups also had less power than the BINA group (p < 0.001). The Pilo Only group received saline as a control posttreatment after animals reached a stage 3 Racine seizure. The frequency ranges were defined as delta (0.5-3 Hz), theta (4-7 Hz), alpha (8-12 Hz) and beta (13-25 Hz). Power has been normalized to the average power at baseline in each frequency range. Compared to Pilo Only: **, p < 0.01; ###, p < 0.001. Compared to BINA: ##, p < 0.01; ###, p < 0.001. Compared to cocktail: ^, p < 0.05; ^^, p < 0.01. Column abbreviations: Pilo: Pilo Only, LY68: LY379268 (20 mg/kg), BINA: BINA (100 mg/kg), Cocktail: Cocktail (LY379268 (20 mg/kg) + BINA (100 mg/kg)).
Figure 5. **Posttreatment with Group 2 mGluR active drugs reduces severity of pilocarpine induced SE behavioral measures.** The average latency to stage 3 Racine seizures did not vary between groups (one-way ANOVA with Tukey’s post hoc, \( p > 0.05 \), \( n \geq 10 \) in all groups, mean latency for treatment administration was 24 min). **B.** Latency to the first C/T seizure during SE was significantly increased for the LY379368 and the cocktail groups compared to the Pilo Only group and the BINA group (one-way ANOVA with Tukey’s post hoc, \( p < 0.001 \) for both comparisons, \( n = 12 \) in these three groups). No mouse that received LY379268 posttreatment ever reached a stage 5 C/T seizure, therefore that entire group was scored as 180 min latency (see Methods), as that was the maximum length of observation time for this study. **C.** Average bouts of C/T seizures during SE were significantly reduced with posttreatment with LY379268 and the cocktail compared to the Pilo Only group that received saline posttreatment (Kruskal-Wallis test with Dunn’s post hoc, \( p < 0.01 \) for the cocktail group, \( p < 0.001 \) for the LY379268 group, \( n \geq 10 \) in all groups). The LY379268 and cocktail groups also experienced fewer bouts of seizures than the BINA group (\( p < 0.05 \) for the cocktail group, \( p < 0.01 \) for the LY379268 group). **D.** The Racine scores were also significantly different between all posttreatment groups (Chi square, \( p = 0.0034 \), \( n \geq 10 \) in all groups). Seizures were considered C/T if they reached at least a stage 5 Racine score. The Pilo Only group received saline as a control posttreatment after animals reached a stage 3 Racine seizure. Compared to Pilo Only: **, \( p < 0.01 \); ***, \( p < 0.001 \). Compared to BINA: #, \( p < 0.05 \); ##, \( p < 0.01 \); ###, \( p < 0.001 \).
Table 1. **Time spent on rotarod (Mean ± SEM)**. Time the experimental groups spent on the rotarod apparatus prior to injection of various drugs, and then 10 minutes, 30 minutes, 1 hour and 5 hours after injection. Habituation trials were performed the day prior to testing and maximum trial length was 180 seconds.

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<th>30 min post</th>
<th>1 hr post</th>
<th>5 hr post</th>
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<td>124.16 ±</td>
<td>117.72 ±</td>
<td>117.40 ±</td>
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<td>20.84</td>
<td>21.79</td>
</tr>
<tr>
<td><strong>Diazepam (5 mg/kg)</strong></td>
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<td>49.48 ±</td>
<td>38.18 ±</td>
<td>90.62 ±</td>
<td>147.22 ±</td>
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<tr>
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<td>42.9 ±</td>
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<td><strong>BINA (100 mg/kg)</strong></td>
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<td>101.98 ±</td>
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<td></td>
<td>18.47</td>
<td>14.27</td>
<td>22.42</td>
<td>19.20</td>
<td>20.28</td>
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<td><strong>Cocktail (LY379268 (20 mg/kg) + BINA)</strong></td>
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<td>93.34 ±</td>
<td>98.68 ±</td>
<td>108.90 ±</td>
<td>96.04 ±</td>
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<td></td>
<td>21.45</td>
<td>25.74</td>
<td>24.20</td>
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### TABLE 2

Pretreatment

<table>
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<th>Latency to C/T seizure</th>
<th>Bouts of C/T seizures</th>
<th>Racine Score</th>
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<td>Pilo Only</td>
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<td>5.07 ± 0.29</td>
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<td>CBiPES (30 mg/kg)</td>
<td>132.3 ± 23.84 min*</td>
<td>0.56 ± 0.29*</td>
<td>3.67 ± 0.57</td>
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Posttreatment

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<th>Racine Score</th>
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<td>Pilo Only</td>
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<tr>
<td>CBiPES (30 mg/kg)</td>
<td>135.3 ± 28.33 min*</td>
<td>0.67 ± 0.49</td>
<td>4.5 ± 0.56</td>
</tr>
</tbody>
</table>

Table 2. Pre and posttreatment behavioral differences between the CBiPES and Pilo Only groups (Mean ± SEM). Latency to the first C/T seizure, bouts of C/T seizures and Racine score are presented for the experimental groups receiving pilocarpine only or CBiPES as pre- or posttreatment. Asterisks indicate significant differences between those groups.
CHAPTER THREE: Group 2 metabotropic glutamate receptors as targets for absence epilepsy treatment in the GHB model

Erin H. Caulder, M.A. and Dwayne W. Godwin, PhD

A manuscript in preparation for submission to the journal Epilepsy Research for publication. Stylistic variations within are appropriate for the requirements of this journal.
1.1 INTRODUCTION

Absence epilepsy is characterized by sudden and brief loss of consciousness, behavioral arrest and synchronous, bilateral spike wave discharges present on the electroencephalogram (EEG, Anon, 1989). This seizure type is most common in children, although it can affect adults and may also occur as part of a different seizure disorder (Eriksson and Koivikko, 1997; Panayiotopoulos, 1999). The standard treatment for absence is either ethosuximide or valproic acid; both treatments have been in use for several decades and novel pharmacological treatments have been slow in coming (Panayiotopoulos, 1999; Manning et al., 2003; Gören and Onat, 2007). Ethosuximide and valproate are both effective when the patient is responsive and compliant, although a growing number of absence patients are neither compliant nor responsive for a variety of reasons. Often, negative side effects associated with the treatments reduce compliance and unfortunately, some patients do not respond to the treatments at all (Posner et al., 2005; Penovich and Willmore, 2009). These issues highlight the need for novel targets and treatments for absence epilepsy.

Gamma-hydroxybutyrate (GHB) is often used to model absence epilepsy in animals because of the behavioral arrest, SWD events on the EEG and responsiveness to standard antiabsence treatments (Snead, 1988, 1991; Banerjee et al., 1993; Sarkisian, 2001; Koek et al., 2009). The GHB prodrug, gamma-butyrolactone (GBL) is more commonly used than GHB itself because of a more rapid onset of action (Snead, 1991). GBL is rapidly converted to GHB in vivo via liver and serum lactonase activity and reaches peak concentration in the blood within a few minutes (Roth et al., 1966; Wong et al., 2004; Goodwin et al., 2009). The action of GHB in the brain at both the GABA\textsubscript{B} and
the GHB receptors results in the absence-like phenotype and EEG profile that is associated with this model (Banerjee et al., 1993; Wong et al., 2004; Crunelli et al., 2006; Carter et al., 2009; Koek et al., 2009). Due to the utility of the GHB model in predicting the efficacy of ethosuximide and valproate, it is used to predict the efficacy of other novel antiepileptic drugs.

Much recent evidence has suggested metabotropic glutamate receptors (mGluRs) may be relevant targets for antiepileptic drug development (Miyamoto et al., 1997; Attwell et al., 1998; Kłodzińska et al., 1999; Moldrich et al., 2001; Folbergrová et al., 2001; De Sarro et al., 2002; Mares and Mikulecká, 2004; Mares, 2009). Group 2 mGluRs (mGluR2 and 3) exhibit unique and mutually exclusive localizations in the central nervous system; mGluR2 is found on presynaptic cells in the thalamus and cortex outside of the active zone, while mGluR3 has been found within the active zone of postsynaptic densities, and on glial cells (Ohishi et al., 1993, 1998; Petralia et al., 1996; Shigemoto et al., 1997; Mineff and Valtschanoff, 1999; Cartmell and Schoepp, 2000; Alexander and Godwin, 2006a; Gu et al., 2008; Hetzenauer et al., 2008). When activated, these receptors reduce glutamate release and signalling in the pathways in which they are expressed, thereby reducing excitatory neurotransmission (Conn and Pin, 1997; Cartmell and Schoepp, 2000).

It has been shown that drugs active at these receptors in particular have antiepileptic properties (for review, see Alexander and Godwin, 2006b; Ngomba et al., 2011), as well as anxiolytic properties (for review, see Conn and Jones, 2009). The mGluR2/3 agonist LY379268 specifically has been shown to reduce epileptic activity in several models (Moldrich et al., 2001; Imre, 2007). In our own lab, we have found
evidence that LY379268 might be an excellent candidate as an anticonvulsant therapy. Interestingly, some evidence exists that suggests that antagonism of mGluR2/3 with LY341495 may provide better therapy against absence-like, nonconvulsive seizures (Ngomba et al., 2005).

Beyond the antiepileptic properties inherent to mGluR2/3 active compounds, these drugs are also known to affect gross motor behavior. For example, evidence exists that doses of LY379268 can inhibit basal levels of both gross and fine motor movements in the home cage behavior of rats (Cartmell et al., 1999; Feinberg et al., 2002), although forced motor behavior on a rotarod apparatus was unaffected (Cartmell et al., 1999). Conversely, the mGluR2/3 antagonist LY341495 has been demonstrated to be pro-motor in various studies. Specifically, LY341495 has been shown to increase exploratory behavior in an open field while significantly decreasing rest behavior (O’Neill et al., 2003), reducing locomotor habituation in a novel arena (Bespalov et al., 2007), and increasing mobility time in a forced swim task (Bespalov et al., 2008).

The goal of this study was to test the hypothesis that mGluR2/3 active drugs may suppress absence-like seizures in the GHB model. Further, we wished to describe the effect of mGluR2/3 active drugs on unforced motor behavior, as measured by total distance and time spent engaging in movement in an open field.

1.2 EXPERIMENTAL PROCEDURES

1.2.1 Animals

All animal procedures were approved by the Wake Forest Graduate School of Arts and Sciences Institutional Animal Care and Use Committee and in accordance with NIH and USDA guidelines, including measures to keep animal numbers to a minimum.
and reduce any potential suffering. Adult male C57Bl/6 mice (n = 60, Harlan, Indianapolis, IN) were used in these experiments. All mice were group-housed for the duration of the study, fed and watered ad libitum and kept on a 12 h dark and light cycle, with lights on at 7 am and off at 7 pm.

1.2.2 Surgery

Pinnacle Technologies, Inc (Lawrence, KS) tethered electroencephalography/electromyography (EEG/EMG) acquisition system was used for these studies. Surgical implantation of EEG electrodes was performed on a group of mice (n = 24). Mice were anesthetized using a ketamine and xylazine (100 mg/kg and 10 mg/kg respectively, i.p.) cocktail and then placed into a small animal stereotaxic frame. Atropine (0.04 mg/kg, s.c.) was given to suppress bronchial secretions. The hair covering the skull was removed, a 1 cm incision was made and the skull exposed. Four pilot holes were drilled to allow placement of stainless steel screws that make contact with the surface of the brain. Silver epoxy was coated over each screw to maintain electrical continuity with the headmount. The headmount was then affixed to the skull using dental acrylic. Two EMG leads attached to the back of the headmount were placed into the neck musculature. The incision was flushed with sterile saline, sutured around the headmount and covered with topical antibiotic. Mice were then removed from the stereotaxic, placed in a recovery cage and given ketoprofen (5 mg/kg) for pain management. Mice were placed back into their home cage once ambulation was restored, and allowed to recover at least one week before initiating any experiments. Systemic antibiotics were not administered as post-operative infection was an exclusionary condition for our study as per the protocol.

1.2.3 Electroencephalography
The design of the EEG study was such that each animal received a saline injection as well as drug injections. EEG data collected during drug conditions was normalized to the data collected during the saline condition by dividing drug condition spectral power by the power during the saline condition. Mice that had been implanted with the EEG system were given a saline injection and recorded for two hours prior to any other drug injection. Mice were then randomly assigned to the following drug conditions: GBL (100 mg/kg), LY379268 (10 mg/kg), LY341495 (3 mg/kg), GBL+LY379268 (100 mg/kg and 10 mg/kg respectively), and GBL+LY341495 (100 mg/kg and 3 mg/kg respectively) with 8 mice in each group. Each mouse had at least three days of washout between each injection. If the mouse received another drug or drugs in combination with GBL, those were given 15 min prior to GBL (which was given at Time 0). Mice were immediately plugged into the EEG system after the final drug was given and recorded for two hours.

1.2.4 Open Field Task

Mice (n = 6 per group, 36 total) were placed individually into a 45.72 cm square cube made of white plexiglass (Triad Plastics Inc, Winston Salem, NC) and video monitored for 45 minutes. Mice were randomly assigned to one of 6 groups for these studies: saline, GBL (100 mg/kg), LY379268 (10 mg/kg), LY341495 (3 mg/kg), GBL+LY379268 (100 mg/kg and 10 mg/kg respectively), and GBL+LY341495 (100 mg/kg and 3 mg/kg respectively). Injection of other compounds occurred 15 min prior to GBL, and immediately after GBL injection mice were placed into the field. After 50 minutes, mice were removed and placed back into their home cage.

Two arenas in the open field were defined (Fig 1). The first was the surround, which was an approximately 10 cm wide area along all four sides of the field that abutted
the outer walls. The second defined arena was the center, which was the remaining portion of the field that did not include any area adjoining the walls. These regions were used to analyze the behavior of mice in the field in terms of where the animals spent time and made various movements.

1.2.5 Drugs

LY379268 was a kind gift from Lilly USA, Inc., and was dissolved in 0.9% saline. LY341495 was purchased from Tocris Bioscience (Bristol, United Kingdom) and dissolved in saline. GBL was purchased from Sigma Aldrich (St. Louis, MO) and diluted to the appropriate concentration with saline. All injections were given i.p. in a volume of 0.25 mls.

1.2.6 Data Analysis

Each animal’s spectral data was normalized to the baseline recording and then analyzed using a Kruskal-Wallis test with Dunn’s post hoc analysis. The spectral bands we analyzed were delta (0.5 to 3 Hz), theta (4 to 7 Hz), alpha (8 to 12 Hz), and beta (13 to 16 Hz). Behavioral data that were continuous in nature (i.e. total distance moved, time spent moving, etc) were analyzed with a one-way ANOVA with Tukey’s post hoc test.

1.3 RESULTS

1.3.1 EEG Analysis

The spectral data were normalized to a baseline recording where only saline was given and averaged for every subject (n = 8 per group) to perform statistical analysis using a Kruskal-Wallis test with Dunn’s post hoc to test for distinct group differences. This analysis was performed within the delta (0.5 to 3 Hz), theta (4 to 7 Hz), alpha (8 to...
12 Hz) and beta (13 to 16 Hz) ranges 30 minutes after drug injection and is shown in Figure 2.

30 minutes after drug injection (Fig 2) in the delta range, the LY379268 group had reduced power compared to the GBL group ($p < 0.01$). In the theta range, the GBL+LY341495 group had less power compared to the GBL group ($p < 0.05$) and the GBL+LY379268 group ($p < 0.01$). In the alpha range, the LY341495 group had reduced power compared to the GBL+LY379268 group ($p < 0.05$), and the GBL+LY341495 group had less power than both the GBL+LY379268 ($p < 0.01$) and the LY379268 ($p < 0.05$) groups. Finally in the beta range, the LY341495 group had reduced power compared to the GBL+LY379268 group ($p < 0.05$). The GBL+LY341495 group also had reduced power compared to the GBL+LY341495 group ($p < 0.01$).

1.3.2 Behavioral Analysis

1.3.2.1 Distance Moved in the Open Field

A one-way ANOVA with Tukey’s post hoc was used to test for differences in the total distance moved during the open field trial between all groups ($n = 6$ in all groups). As Figure 3 shows, multiple significant differences were found. The GBL group traveled a shorter distance in the open field than the saline ($p < 0.05$), LY341495 ($p < 0.05$) and the GBL+LY341495 ($p < 0.01$) groups. The LY379268 group also traveled a shorter total distance in the open field compared to the saline ($p < 0.01$), LY341495 ($p < 0.05$) and the GBL+LY341495 ($p < 0.001$) groups. Finally, the GBL+LY379268 group traveled less distance than the saline group ($p < 0.01$) as well as the LY341495 ($p < 0.01$) and GBL+LY341495 ($p < 0.001$) groups.
The mean ± SEM for all of the groups' total distance moved in the open field were as follows: saline (16660 ± 1588 cm), GBL (6030 ± 2312 cm), LY379268 (5683 ± 1961 cm), LY341495 (15747 ± 1501 cm), GBL+LY379268 (3890 ± 2560 cm) and GBL+LY341495 (19174 ± 2231 cm). Essentially, the groups stratified into two categories: the longer distance movers (saline, LY341485 and GBL+LY341495), and the short distance movers (GBL, LY379268 and GBL+LY379268).

1.3.2.2 Time Moving and Not Moving in the Open Field

Another metric of motility in the open field that was assessed was total time spent moving and not moving in the open field. To determine the significance of group differences in total time moving in the open field, a one-way ANOVA with Tukey’s post hoc was used. Figure 4a shows the total time spent moving in the open field during the 50 minute trial. The GBL and LY379268 groups spent less time moving in the open field compared to the saline (p < 0.01), LY341495 (p < 0.05) and GBL+LY341495 (p < 0.01) groups. Also, the GBL+LY379268 group spent less time moving than did the saline and LY341495 groups (p < 0.01) and the GBL+LY341495 groups (p < 0.001).

The mean ± SEM for total time spent moving in the open field for each group were as follows: saline (5.9 ± 0.5 min), GBL (2.1 ± 0.8 min), LY379268 (2.0 ± 0.7 min), LY341495 (5.6 ± 0.5 min), GBL+LY379268 (1.4 ± 0.9 min), GBL+LY341495 (6.7 ± 0.7 min). Consistent with the findings of total distance moved in the field, these groups appear to stratify into two categories of active movers (saline, LY341495 and GBL+LY341495) and non-movers (GBL, LY379268 and GBL+LY379268).

We also assessed total time spent not moving in the open field over the entire duration of the trial, using a one-way ANOVA with Tukey’s post hoc (Figure 4b). The
GBL and LY379268 groups both spent significantly more time not moving compared to the saline (p < 0.01), LY341495 (p < 0.05) and GBL+LY341495 (p < 0.001) groups. The GBL+LY379268 group also spent significantly more time stationary when compared to the saline and LY341495 groups (p < 0.01) and the GBL+LY341495 group (p < 0.001).

The mean ± SEM for each group’s total time spent not moving were as follows: saline (39 ± 0.5 min), GBL (42.8 ± 0.8 min), LY379268 (42.8 ± 0.7 min), LY341495 (39.3 ± 0.4 min), GBL+LY379268 (43.5 ± 0.9 min), and GBL+LY341495 (38.1 ± 0.8 min). As would be expected, this data mirrors the total time moving data, in which groups separate into two categories of those who spend more or less time not moving.

1.3.2.3 Time Immobile, Mobile and Highly Mobile in the Open Field

Motility in the open field was also analyzed by categorizing the velocity of movement as immobile, mobile or highly mobile. This measure takes into consideration not only if the center point of the animal is moving or not during a trial, but how fast that point is moving. This can distinguish time spent moving into two distinct categories, time spent mobile, and time spent highly mobile, which could differentiate between drugs that may have a highly excitatory effect on an animal’s motor behavior.

The total time spent immobile in the open field (Fig 5a) was assessed using a one-way ANOVA with Tukey’s post hoc. It was found that the GBL group exhibited significantly more total time immobile in the open field than did the saline (p < 0.01), LY341495 (p < 0.05) and the GBL+LY341495 (p < 0.001) groups. The LY379268 group also spent significantly more time immobile than the saline (p < 0.05), LY341495 (p < 0.05) and the GBL+LY341495 (p < 0.001) groups. Lastly, the GBL+LY379268 group
spent more time immobile when compared to the saline (p < 0.01), LY341495 (p < 0.01) and the GBL+LY341495 (p < 0.001) groups.

The mean ± SEM for each groups’ total time spent immobile in the open field were as follows: saline (41 ± 0.4 min), GBL (43.6 ± 0.5 min), LY379268 (43.5 ± 0.4 min), LY341495 (41.2 ± 0.3 min), GBL+LY379268 (44 ± 0.6 min) and GBL+LY341495 (40.4 ± 0.6 min).

The total time spent mobile in the open field was assessed with a one-way ANOVA and Tukey’s post hoc test. Figure 5b shows that the GBL and LY379268 groups spent significantly less time mobile than either the saline, LY341495 and GBL+LY341495 groups (p < 0.01 for all comparisons). Also, the GBL+LY379268 group spent less time mobile in the open field than did the saline and GBL+LY341495 groups (p < 0.001) as well as the LY341495 group (p < 0.01).

The mean ± SEM for each groups’ total time mobile in the open field were as follows: saline (2.6 ± 0.2 min), GBL (0.9 ± 0.4 min), LY379268 (0.9 ± 0.3 min), LY341495 (2.5 ± 0.2 min), GBL+LY379268 (0.6 ± 0.4 min) and GBL+LY341495 (2.7 ± 0.3 min).

The final motility measure that was analyzed was total time spent highly mobile. A one-way ANOVA with Tukey’s post hoc was used to test for group differences for this measure. Figure 5c shows that the GBL and LY379268 groups both spend significantly less total time being highly mobile in the open field compared to the GBL+LY341495 group (p < 0.01 for both comparisons). Also, the GBL+LY379268 group spent significantly less total time highly mobile than both the saline (p < 0.05) and the GBL+LY341495 (p < 0.01) groups.
The mean ± SEM for each groups’ total time highly mobile in the open field were as follows: saline (1.3 ± 0.3 min), GBL (0.4 ± 0.1 min), LY379268 (0.5 ± 0.2 min), LY341495 (1.2 ± 0.2 min), GBL+LY379268 (0.3 ± 0.2 min) and GBL+LY341495 (1.7 ± 0.3 min).

1.4 DISCUSSION

In this study, we found clear spectral power differences after mice were given mGluR2/3 active drugs in combination with GBL. Generally speaking, the GBL group had increased power in the delta and theta ranges, which is expected given those are the bandwidths in which GBL-induced seizures occur (Depaulis et al., 1988; Snead, 1991). The particular effect of each mGluR2/3 active drug appeared to be unique to specific frequency bands. In the delta range, the administration of LY379268 alone resulted in significant power decrease compared to GBL. Interestingly, the GBL+LY341495 had reduced power in the higher ranges compared to the GBL+LY379268 group. In the theta range, the addition of LY341495 to GBL had significantly reduced power compared to the GBL only group, suggesting a positive effect on EEG power that is typically wrought by GBL. In the delta range, while the finding was not significant, there is certainly a trend toward a reduction in power in the GBL+LY341495 group compared to GBL alone. It generally appears that the presence of an mGluR2/3 antagonist onboard with GBL reduces the power shift typically seen with GBL alone (Fig 2), in the frequency range in which the GBL induced seizures occur (4 to 6 Hz, Snead, 1988).

The spectral data were supportive of continuing to study mGluR2/3 antagonists as treatments for absence-like seizures. However, mice given GBL exhibit a global increase in power in the 4 to 6 Hz range and continuous synchronous spike and wave discharges in
the EEG (Snead 1984; 1991); thus this model does not allow for the counting of discrete ictal events. Because we were not able to discriminate individual GBL-induced spike wave discharges, an alternative model may be best to better understand the different roles of these compounds. Perhaps study in a genetic model of absence where obvious and discernable SWDs occur such as the GAER or WAG-Rij models (Marescaux et al., 1992; Coenen et al., 1992) would be a future step for this investigation. Then, counts of individual seizure events could be measured, and the time course of drug efficacy could be better understood. Beyond the spectral differences that were discovered, there were also considerable differences in motor behavior. This was done in part to understand the effect of mGluR2/3 active compounds in an unforced motor task. Previous work in our lab has demonstrated the lack of effect of LY379268 (even at 20 mg/kg, twice the dose used in this study) on a rotarod task. Previous work has suggested mGluR2/3 agonists inhibit motor behavior (Cartmell et al., 1999; Feinberg et al., 2002) and antagonist facilitate motor behavior (O’Neill et al., 2003; Bespalov et al., 2007, 2008). It was of interest to know what the effect of these drugs would be in conditions in which animals were allowed to move spontaneously and voluntarily.

In terms of the total distance moved in the open field, the drug groups tended to cluster into two groups. There were those that moved long distances, including the saline, LY341495 and GBL+LY341495 groups. There were also the short distances movers, including the GBL, LY379268 and GBL+LY379268 groups. It was most interesting to discover the GBL+LY341495 group performed similarly to the other long distance moving groups; LY341495 seemed to have completely reversed the typical motor-sedating effect of GBL. Groups that received GBL either alone or in combination with
LY379268 fell into the short distance moving category. It was intriguing to note the LY379268 group fell into the short distance moving category; a hypolocomotor effect has been previously described in animals who have received this drug with no other experimental manipulation, even at much lower doses (i.e. 1 mg/kg, Imre et al., 2006). This is also consistent with the findings of other groups who have demonstrated that LY379268 inhibits home cage behavior in rodents in a dose-dependant manner (Cartmell et al., 1999; Feinberg et al., 2002).

The time spent moving and not moving also divided these drugs groups into two distinct categories with the same groups falling onto each side. Administration of either saline, LY341495 and GBL+LY341495 resulted in more time spent moving and less time not moving. The remaining groups, GBL, LY379268 and GBL+LY379268 all spent less time moving and more time not moving. Consistent with the finding in the total distance measure, it was found that the GBL+LY341495 group behaved like a high mover and the LY379268 group performed like a low mover. The same pattern also held consistent when movement was further spilt into various states of mobility. Importantly, it was determined that LY341495 had no hyperlocomotor effect, when high mobility in the open field was measured (Fig 5c), no differences were found between the LY341495 group and the saline group; thus the mGluR2/3 antagonist did not exacerbate locomotion over normal activity levels. Further, the administration of LY341495 with GBL reversed the sedating effect of that drug on motor activity on all states of mobility (Fig 5).

One possible mechanism for motor rescue after GBL injection by LY341495 is based on what is known about the action of LY341495 to potentiate glutamatergic signalling in those cells which express mGluR2/3. It is possible that while GBL potently
activates both GABA\textsubscript{B} and GHB receptors found throughout the cortex and thalamus (Banerjee et al., 1993; Crunelli et al., 2006; Ticku and Mehta, 2008; Carter et al., 2009) and causes a reduction in overt motor behavior (Snead, 1982, 1991), the presence of LY341495 may override that effect by increasing glutamate release and facilitating the expression of motor behavior (i.e. the findings of O’Neill et al., 2003; Bespalov et al., 2007, 2008).

In summary, two quite interesting findings immediately became clear. The first, that the antagonist of mGluR2/3 eliminated the behavioral effects of GBL. In fact, in no behavioral measure was the GBL+LY341495 group found to be different from saline. In effect, although the animals in this group had received quite a powerful sedative/hypnotic, the presence of the mGluR2/3 antagonist completely reversed the typical motor depressant effects of GBL. This was not entirely unexpected given the mechanism of LY341495 in blocking mGluR2/3 and thus, increasing glutamatergic signaling in neuronal and glial cells (Cochilla and Alford, 1998; Cartmell and Schoepp, 2000). Animals given LY341495 in combination with GBL moved as far (Fig 3), for roughly the same amount of time (Fig 4) and spent similar times in different mobility states (Fig 4) as animals that received only saline before being placed into an open field. The second interesting finding was that the LY379268 group did not differ from the GBL group on any of the motor measures. There were some minor differences in the EEG in lower bandwidths, but the overt motor behavior was the same in both groups. Given that the mGluR2/3 agonist reduces glutamatergic signalling across multiple corticothalamic pathways, including those used for initiation and execution of motor movements (Cartmell et al., 1999; Cartmell and Schoepp, 2000) this was not entirely unexpected.
It has been previously hypothesized that mGluR2/3 might be a valid novel target for antiabsence drugs, and this study provides further support of that. An important review has previously discussed how mGluRs, and in particular mGluR2/3, have unique physiological properties and are localized in cortico-thalamocortical circuitry wherein their activation may interrupt the initiation of absence seizures, and data supporting that role in multiple animal models was thoroughly reviewed (Ngomba et al., 2011). The finding that LY341495 was effective at reversing the behavioral arrest characteristic of this seizure type was not surprising given the antithetical mechanisms of GBL and LY341495: GBL enhances GABA_B and GHB mediated signalling, to globally potentiate inhibitory signalling, while LY341495 enhances excitatory, glutamatergic signalling. Therefore, this class of compounds may prove efficacious for treating absence epilepsy and other non-convulsive seizure types. To continue this line of investigation would provide the possibility of an alternative treatment for absence epilepsy; a new treatment with higher compliance rates and fewer side effects.

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Figure 1. The open field for behavioral assessment of GBL and mGluR2/3 drug effects. Mice (n = 36) were randomly assigned to one of 6 groups for these studies: saline, GBL, LY379268, LY341495, GBL+LY379268, or GBL+LY341495 and then placed individually into an approximately 45 cm square cube arena and monitored for 45 minutes. The white box defines the center of the open field, while the gray shaded area defines the surround of the field.
Figure 2. **mGluR2/3 active compounds change the spectral power that is typically exhibited after GBL injection.** C57Bl/6 mice (n = 8 per group) were implanted with EEG acquisition head mounts and injected with one of the various drugs or drug combinations. EEG data collection began after drug injection, was analyzed with a Kruskal Wallis test with Dunn’s post hoc and has been normalized to a baseline, saline only recording. 30 min after injection, the GBL group had higher spectral power than the LY379268 group in the delta range. In the theta range, the GBL+LY341495 group had lower power than the GBL group (p < 0.05) and the GBL+LY379268 group (p < 0.01). In the alpha range, the GBL+LY379268 group showed higher power than the LY341495 group (p < 0.05) and the GBL+LY341495 group (p < 0.01). Also in the alpha range, the GBL+LY341495 group had less power than the GBL+LY379268 group (p < 0.05). Finally in the beta range, the GBL+LY379268 group showed higher power than the LY341495 group (p < 0.05) and the GBL+LY341495 group (p < 0.01). The frequency ranges studied were defined as: delta (0.5 to 3 Hz), theta (4 to 7 Hz), alpha (8 to 12 Hz) and beta (13 to 16 Hz). Compared to GBL: *, p < 0.05; **, p < 0.01. Compared to GBL+LY379268: #, p < 0.05; ##, p < 0.01; ###, p < 0.001. Compared to LY379268: ^, p < 0.05.
Figure 3. Administration of GBL alone or in combination with LY379268 reduced distance moved in an open field, LY341495 reversed this effect. Mice (n = 6 per group) were individually placed into an open field for a 50 minute trial. All drug injections occurred 15 minutes prior to placement in the open field, with the exception of GBL which was given immediately before placement in the field. A one-way ANOVA with Tukey’s post hoc was used to test for significant differences in these experiments. The GBL group moved a significantly shorter total distance in the open field than did the saline group (p < 0.05), the LY341495 group (p < 0.05), and the GBL+LY341495 group (p < 0.001). The LY379268 group also moved a significantly shorter total distance in the open field compared to the saline group (p < 0.01), the LY341495 group (p < 0.05), and the GBL+LY341495 group (p < 0.001). Finally, the GBL+LY379268 group moved significantly shorter total distance than the saline group (p < 0.01), the LY341495 group (p < 0.01), and the GBL+LY341485 group (p < 0.001). Compared to saline: *, p < 0.05; **, p < 0.01. Compared to LY341495: #, p < 0.05; ##, p < 0.01. Compared to GBL+LY341495: ^, p < 0.01; ^^^, p < 0.001.
Figure 4. **GBL alone or in combination with LY379268 decreased time spent moving and increased time spent not moving in an open field, LY341495 reversed this effect.**

**A. Total time spent moving in an open field.** The GBL group spent significantly less time moving in the open field than the saline group (p < 0.01), the LY341495 group (p < 0.05), and the GBL+LY341495 group (p < 0.01). The LY379268 group also spent significantly less time moving in the open field than the saline group (p < 0.01), the LY341495 group (p < 0.05), and the GBL+LY341495 group (p < 0.01). Finally, the
GBL+LY379268 group spent less time moving than the saline (p < 0.01), LY341495 (p < 0.01), and GBL+LY341495 (p < 0.001) groups. **B. Total time spent not moving in an open field.** As would be expected, those same groups that spent less time moving in the open field were thus spending more time not moving in the field. Compared to saline: **p < 0.01. Compared to LY341495: #, p < 0.05; ##, p < 0.01. Compared to GBL+LY341495: ^, p < 0.01; ^^^, p < 0.001.
Figure 5. Administration of GBL alone or in combination with LY379268 decreased time spent mobile and highly mobile and increased time spent immobile in an open
field, LY341495 reversed this effect. **A. Total time spent immobile in the open field.**

A one way ANOVA with Tukey’s post hoc analysis determined that the GBL group spent significantly more time immobile in the open field compared to the saline (p < 0.01), the LY341495 (p < 0.05) and the GBL+LY341495 (p < 0.001) groups. The LY379268 group also spent more time immobile compared to the saline (p < 0.05), LY341495 (p < 0.05), and the GBL+LY341495 (p < 0.01) groups. The GBL+LY372968 group also spent more time immobile than the saline (p < 0.01), LY341495 (p < 0.01), and the GBL+LY341495 (p < 0.001) groups.

**B. Time spent mobile in the open field.**

The GBL group and the LY379268 groups both spent significantly less time mobile in the open field than the saline (p < 0.01), the LY341495 (p < 0.01) and the GBL+LY341495 (p < 0.01) groups. The GBL+LY372968 group also spent less time mobile than the saline (p < 0.001), LY341495 (p < 0.01), and the GBL+LY341495 (p < 0.001) groups.

**C. Total time spent highly mobile in an open field.**

The GBL group and the LY379268 groups both exhibited significantly less time highly mobile in the open field compared to the GBL+LY341495 group (p < 0.01). The GBL+LY379268 group also spent significantly less time highly mobile in the open field than did the saline (p < 0.05) and the GBL+LY341495 (p < 0.01) groups. Compared to saline: *, p < 0.05; **, p < 0.01; ***, p < 0.001. Compared to LY341495: #, p < 0.05; ##, p < 0.01. Compared to GBL+LY341495: ^, p < 0.05; ^, p < 0.01; ^^^, p < 0.001.
CHAPTER FOUR: DISCUSSION

My studies in the pilocarpine model of SE suggest that targeting mGluR2/3 with the agonist, LY379268 or a cocktail of LY379268 and the PAM BINA can partly ameliorate the severity of the SE that develops after pilocarpine administration. This was demonstrated by increased latency to the first C/T seizures, fewer bouts of C/T seizures and a reduced average Racine score in animals that received those treatments compared to control animals that received only saline in addition to pilocarpine. The use of the PAM CBiPES also inhibited some of the behavioral expression and severity of SE. The PAM BINA alone was unable to reduce the severity of SE in any measure studied, and did not confer any additional benefit to LY379268 when it was administered in combination with that agonist as a cocktail. It is likely that the effectiveness of the cocktail was entirely due to LY379268, especially considering that BINA alone was unable to provide any protection from pilocarpine-induced SE.

Importantly, the positive effects of the cocktail and LY379268 were not only demonstrated when the treatments were given prophylactically prior to pilocarpine but also after SE had begun, suggesting that not only would these drugs be effective if already on board when a seizure began but when one was already occurring. This could be clinically relevant for two reasons: first, if a patient is considered at high risk for developing a seizure, such as in the case of massive head trauma or during drug withdrawal, these drugs may be used to prevent the genesis of a seizure and the consequent problems associated with one (Hughes, 2009; Pitkanen and Bolkvadze, 2012; Beleza, 2012). Second, it suggests that these drugs could be used to rescue patients who are already experiencing one of the most devastating seizure disorders that is the most
frequently fatal if treatment fails. In fact, one report found that up to 27% of patients with convulsive SE will die within 30 days of the seizure episode (Treiman et al., 1998).

What either of these indications means in terms of the development of seizures later on is unknown. However, if efficacy is demonstrated during the most critical phase of an initial seizure, a reasonable hypothesis is that the later effects of that seizure, such as the increased propensity for the development of future seizures, would be reduced (Chang and Lowenstein, 2003; Morimoto et al., 2004). Increasing the latency to the first seizure and reducing bouts of seizures during SE may suggest inhibition of neurotoxicity in the system that may also delay or inhibit the onset of spontaneous seizures. The finding of a reduced average Racine score is also suggestive of a protective effect of these drugs against pilocarpine induced SE and might suggest inhibited neural damage.

These findings are supportive of a wealth of previous work that suggests Group 2 mGluRs may be valid targets for antiepileptic drugs. Antiepileptic effects have been demonstrated by LY379268 in many animal models, including the amygdala kindling model (Attwell et al., 1998a, 1998b), the homocysteic acid model (Folbergrová et al., 2001), the picrotoxin model (Kłodzińska et al., 1999), and the 6 Hz corneal stimulation model (Barton et al., 2003) among others. In the present studies, CBiPES was the only mGluR2 PAM effective at reducing severity of SE when administered alone, and this is supportive of previous work that suggest CBiPES mimics the effect of the agonist LY379268 (Johnson et al., 2005). While no evidence was found that the mGluR2 PAM BINA was effective when administered alone in our studies, it has been found to potentiate the effect of an mGluR2/3 agonist (Galici et al., 2006; Ahnaou et al., 2009).
Given that pilocarpine provides a neurotoxic insult in the brain (especially in the hippocampus (Costa et al., 2004; de Oliveira et al., 2011)) that is rapidly generalized, and the enriched expression of mGluR2 and 3 in the hippocampus (Gu et al., 2008; Hetzenauer et al., 2008), these drugs are well suited for effectiveness in this model. Human TLE is known to originate from the hippocampus or other limbic cortices (Engel, 1996; Blumenfeld et al., 2009) and thus these drugs would likely prove effective in that condition as well. Group 2 mGluRs exhibit somewhat diffuse expression across the brain, including cortical and thalamic areas (Ohishi et al., 1998; Alexander and Godwin, 2006); hence targeting them may prove useful in a variety of epilepsies that hijack that circuitry to generalize.

The experiments in the GBL model also suggest that targeting mGluR2/3 might be effective for treatment of non-convulsive, absence seizures. In contrast to the findings in the pilocarpine model, in which the agonist for mGluR2/3 was most effective, it was demonstrated that the antagonist, LY341495, had the most positive effect at reducing the EEG power and behavioral sedating effects wrought by GBL. When given GBL, mice exhibited a typical change in EEG in which power was increased in the delta and theta bandwidths. When given LY341495 prior to GBL, those changes were not exhibited, and EEG power did not increase to the extent induced by GBL. Also, mice given GBL exhibit very characteristic motor sedation, as evidenced by significantly decreased time spent moving and distance moved in an open field. Similar to the finding in the EEG measure, those mice that received LY341495 prior to GBL did not experience the sedating effects. Importantly, when LY341495 was administered alone, there was no effect on either EEG power or motor behavior in the open field compared to control. It was further determined
that the mGluR2/3 agonist LY379268 had no effect in reducing the EEG power changes or reversing the antimotor effects that GBL caused.

The findings from the GBL study are consistent with other work that points toward the antagonism of mGluR2/3 for the treatment of non-convulsive seizures. Specifically, one group had found considerable evidence in support of that phenomenon; LY341495 has antiepileptic effects in the WAG-Rij model of absence model, in that it reduces the frequency of ictal events in those rats (Ngomba et al., 2005, 2011). Other data have come to light supporting the use of antagonists to treat other seizures types, including those induced by PTZ (Watanabe et al., 2011) and other disease states that involve dysfunctional glutamatergic signaling, including schizophrenia and depression (Matrisciano et al., 2007; Amitai and Markou, 2010).

GBL is known to act at both the GABA\textsubscript{B} and GHB receptors in the brain (Maitre et al., 2000; Crunelli et al., 2006; Ticku and Mehta, 2008), potentiating inhibitory signaling in corticothalamic circuits. These same circuits are recruited into the absence seizure process (Fuentealba and Steriade, 2005; Meeren et al., 2005), in a manner similar to how these circuits facilitate the generation and spread of convulsive seizure types (Blumenfeld et al., 2009). As it is known that the expression pattern of mGluR2/3 is relatively diffuse across cortical and thalamic circuits, it is intuitive to consider these receptors as targets for not only convulsive seizures as discussed above, but also for non-convulsive seizure types. Animal models of human epilepsy (convulsive SE, TLE and non-convulsive absence epilepsy) rely on the same network in which the mGluR2/3 receptors are poised, and the hypothesis that targeting them may inhibit the initiation and generalization of seizures of all types holds great promise as a novel treatment strategy.
The finding that an agonist for mGlur2/3 was effective at reducing seizure severity in one model and the antagonist to those receptors was effective in an alternative model was not entirely unexpected. There are numerous AEDs in existence that have very diverse mechanisms of action that are effective for some forms of epilepsy and ineffective for many others (Rogawski, 2006). The disease state of epilepsy is so varied (see Introduction) that it would be naive to assume that one drug or even one class of drugs would be capable of treating all types. Certainly, there is not one type of chemotherapy to treat cancer; there are hundreds of cancers and hundreds of treatments. Epilepsy is no different. It may be that no single drug will treat or cure all forms of seizures.

More specifically, there are cases of AEDs that are effective for one seizure type, and actually make other seizure types worse. Take for example benzodiazepines. This class of drugs is often used to treat emergent seizures such as those that occur during SE or acute drug withdrawal. When used to treat either one of those types, benzodiazepines are quite effective. In fact, benzodiazepines in combination with another common AED are the first line treatment for convulsive SE (Treiman et al., 1998; Rabinstein, 2010). However, some models demonstrate that if benzodiazepines are given to an experimental animal that is having non-convulsive absence seizure, that seizure is exacerbated (Coenen et al., 1992), and that is certainly not the goal of treatment. Besides the possibility of making a particular seizure type worse, it is also true that some AEDs that are effective for one form of epilepsy are completely ineffective for another form. This is the case for ethosuximide and valproate, arguably the most effective antiabsence drugs on the market today. Both are similarly ineffective for convulsive seizure types (Panayiotopoulos,
What is the explanation for two antithetical ligands for one particular class of receptors having opposite effects in two seizure models? The mechanism of the ligands and expression pattern of these receptors points toward the answer. Histological and electrophysiological studies have shown that mGluR2/3 are predominately neuronal, presynaptic autoreceptors that inhibit glutamate release from the cells on which they are expressed (Shigemoto et al., 1997; Ohishi et al., 1998; Alexander and Godwin, 2006; Mateo and Porter, 2007). There are some exceptions however; mGluR3 is also expressed on glial cells and on neural cells in the postsynaptic density (Ohishi et al., 1993). Given this expression pattern and mechanism, is it is simple to hypothesize that agonists at this receptor system would suppress glutamate release in the pilocarpine model; wherein the excess spillover of glutamate during a seizure is the reason the seizure so readily generalizes across the brain and causes extensive excitotoxic damage (Cavaiello et al., 1996; Curia et al., 2008). Also, the activity of an mGluR2/3 ligand on receptors expressed on glial cells may potentiate the function of those cells, especially given that there is considerable seizure-induced upregulation of the receptors (Aronica et al., 2000) in a model where glial function is known to be significantly compromised (Borges et al., 2003). Perhaps the activity of the agonist provides some neuroprotection to astrocytes within the context of the pilocarpine model, as has been suggested by previous work in other models of induced neurotoxicity (Zhou et al., 2006; Ciccarelli et al., 2007; Durand et al., 2010, 2011).

In the GBL model of absence epilepsy, the mGluR2/3 antagonist may be effective at reducing the seizure-associated EEG power and the behavioral correlate of GBL administration due to its role in blocking the reduction of glutamate release (Kew and
Kemp, 2005). Thus, the antagonist of mGluR2/3 is potentiating glutamatergic transmission to such an extent that it may reverse the potentiation of the GABAergic system wrought by GBL. It is important to note that LY341495 had no effect by itself on EEG power or on motor activity, and only returned those measures to baseline when GBL was also applied; hence there were no hyperglutamatergic effects with the antagonist alone.

No discussion of these studies would be complete without addressing potential future directions for the work, limitations and how future studies may address those limitations. If this work were to continue, there would be two avenues that must be explored. The first is that within the context of the pilocarpine model, it would be of special interest to know how the development of spontaneous recurrent seizures is effected by the inhibition of seizure early the early phase of SE. If that proves to be the case, then not only would these compounds have some antiseizure effect, but may also have some antiepileptogenic effect as well. The second avenue of investigation would be perhaps transitioning to an alternative model of absence epilepsy. The GBL model showed real promise for these compounds, but it is was not possible to measure discrete seizure events. Using a genetic model, such as the GAER or WAG-Rij model, would allow for the quantification of discrete numbers of events, and not just a power shift in a frequency band relevant for this seizure type. Therefore one could understand if seizures were reduced in number, which is perhaps more indicative of an antiabsence effect than simply a reversal of GBL-induced EEG power, which doesn’t speak to the behavioral state of the animal at all.
Future work can address these limitations by expanding the models under which these compounds are tested. Also, investigating the antiepileptic effects of mGluR3 specific drugs could parse out the possibility that glial expression of that receptor is responsible for the antiepileptic effect demonstrated in the pilocarpine model. Lastly, it would be important to determine the receptor responsible for the antiabsence effects seen in the GBL model. Understanding the underlying circuitry responsible for this seizure type will provide some direction for where the compounds may exerting their effect.

The experiments in these two models of convulsive and non-convulsive epilepsies provide further evidence to support the use of mGluR2/3 as a target for antiepileptic drug development. There are far too many patients who experience no relief from their seizures, regardless of the drugs or interventions they try. There are patients who have tens to hundreds of seizures every day of their lives, creating not only an extremely difficult situation for themselves, but for their families, their caregivers, their medical team and all of society in general. The cost associated with intractable epilepsy is incalculable, because it isn’t only the wallet that is affected, but the psychological and emotional state of patients and everyone around them as well. For all of these reasons, research must continue along this line to find better targets and better and more effective treatments. Group 2 mGluRs may not be the perfect target for all epilepsies, but may very well prove beneficial for some and that is another step in the right direction for those directly affected by epilepsy and seizures.
References


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Education

Wake Forest University Graduate School of Arts and Sciences, Department of Neurobiology and Anatomy, PhD Candidate, 2006-current

Appalachian State University, M.A., General / Experimental Psychology, 2006
  Major Area: Behavioral Neuroscience
  Thesis: The Effects of Frequent Generalized Audiogenic Seizures on Some Aspects of Memory and Exploratory Behavior

Appalachian State University, B.S., Psychology, 2004

L.C. Ragsdale High School, 2000

Employment

2006- Appalachian State University, Instructor, Introduction to Psychology
2005- Appalachian State University, Instructor, Introduction to Psychology
2005- Appalachian State University, Rankin North Animal Facility employee
2004-2005- Appalachian State University, Research Assistant
2002-2004- Mast General Store, Valle Crucis, North Carolina

Research Interests

Behavioral Neuroscience
Sensory Neuroscience
Epilepsy
Antiepileptic Drug Development
Neuroscience Outreach and Educational Strategy Development for K-12 students

Academic Positions

2010-2011- Western North Carolina chapter of the Society for Neuroscience Executive Committee member, Graduate student representative
2010-present- Annual Science Day presenting scientist, “Brains!”, Aldert Root Elementary School, Raleigh NC

2008-2010- Executive Committee member, WFU Brain Awareness Council

2006-present- Member, Wake Forest University Health Sciences Brain Awareness Council

2006-present- MMARS (Matching Matriculating And Returning Students) participant

2004-present- Student member, Society for Neuroscience

Awards and Honors

2010- Recipient of the Mary K. Bell award for excellence in research
2008- Recipient of the Fine Science Tools travel award to attend Society for Neuroscience meeting

Other Educational Coursework and Experience

Fall 2012- Enrollment in the Pre-Award Certificate program at WFUHS
Sept 2012- Budget Basics course
Oct 2012- Contracts- Start to Finish course
Oct/Nov 2012- Human Subjects Research 101 course
October 2012- Contractual work for Preclinical Surgical Services- data collection, data entry, spreadsheet generation, data and electronic form quality control for a Good Laboratory Practices (GLP) study targeted to the FDA.

Volunteer and Community Outreach Activities

2012-present- Second Harvest Food Bank of NC volunteer
2012-present- WFBMC Angel Tree Committee volunteer
2012-present- Ardmore United Methodist Church youth counselor
2012-present- Open Arms Community Center volunteer, Winston Salem
2011-present- Administrative Council Member, Ardmore United Methodist Church
2010-present- Planning Committee member for the Leukemia and Lymphoma Society’s annual Light the Night walk in Triad area
2009-present- Ardmore United Methodist Church Food Pantry, Community Garden and Clothing Closet volunteer
2006-present- Visiting scientist for BAC school trips in Forsyth and Wake Counties
Graduate Research

Caulder EH and Godwin DW (2012) Group 2 metabotropic glutamate receptors as targets for absence epilepsy treatment in a GHB model. Manuscript submitted to Epilepsy Research for publication.

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