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A Rat Model of Depression and Epilepsy Co-morbidity

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A Rat Model of Depression and Epilepsy Co-morbidity

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B.S. Alabama State University, 2002

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An abstract of
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Abstract

A Rat Model of Depression and Epilepsy Co-morbidity

By KROSHONA D. TABB

Epidemiological evidence has shown that depression is the most common co-morbid psychiatric condition associated with epilepsy. More specifically, studies show that 20-55% of epileptic patients with temporal lobe epilepsy (TLE) and/or complex partial seizures suffer from depression. In addition, individuals with a history or even a family history of depression are at increased risk for developing epilepsy. Major depressive episodes and suicide attempts independently increase the risk for unprovoked seizures, and suicide occurs in the epilepsy patients five times more often than in the general population. These findings indicate that there exists a bidirectional linkage between the diseases that is more than coincidental.

Although there have been extensive epidemiological studies showing the correlation between depression and epilepsy, there is a lack of experimental evidence reinforcing or explaining the mechanisms underlying these observations. Creating an animal model exhibiting both depressive- and epileptic-like phenotypes is a way to evaluate the potential neurological underpinnings that connect these two diseases. Our overall objective was to characterize seizure susceptibility in rodent models of depression as a first step in creating an animal model of epilepsy and depression co-morbidity. Rats selectively bred for low and high basal activity in the forced swim test (FST) (SwLo and SwHi, respectively), low and high activity in the FST after stress (SUS and RES, respectively), hyperactivity after shock (HYPER), resistance to changes in motor activity after shock (MON RES), and non-selected (NS) controls were tested for seizure susceptibility using various classes of chemoconvulsants.

We found that rats selectively bred for susceptibility to depressive-like phenotypes (i.e. SwLO, SUS, and HYPER lines) had an increase in mortality rate following kainic acid seizure induction and a decreased latency to status epilepticus following pilocarpine administration than their depression-resistant counterparts. In addition, we found that SwLo rats tended to experience more spontaneous generalized seizures than SwHi rats one month after pilocarpine-induced status epilepticus.

Because some antidepressant drugs are proconvulsant and some anticonvulsant drugs negatively affect mood, treating patients with both epilepsy and depression can be problematic. A special high-fat diet, the ketogenic diet (KD), is effective in treating refractory epilepsy in children and also may have mood elevating properties, making it an intriguing therapeutic candidate for co-morbid individuals. In an attempt to elucidate the anticonvulsant and potential antidepressant properties of the KD, we investigated the effect of the KD on the expression of the anticonvulsant and anxiolytic neuropeptides neuropeptide Y (NPY) and galanin. Despite our findings that changes in the brain expression of these neuropeptides did not appear to underlie the therapeutic effect of the KD, with further experimentation this diet may still prove to be a viable treatment option for patients suffering from depression and epilepsy.

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CHAPTER 1:

INTRODUCTION

1.1 Preface

The term co-morbidity was originally coined by A.R. Feinstein in 1970 as a way to refer to the greater than coincidental association of two conditions in the same individual (Tellez-Zenteno et al., 2007). Currently, Merriam-Webster's dictionary defines the term co-morbid as "existing simultaneously with and usually independently of another medical condition." Hippocrates first documented the co-morbid occurrence of depression and epilepsy in 400 B.C. by noting "melancholics ordinarily become epileptics and epileptics melancholics: what determines the preference is the direction the malady takes; if it bears upon the body, epilepsy, if upon intelligence, melancholy" (Gilliam and Kanner, 2002). In the modern medical era, many examples have been documented where a family history of depression precedes seizure onset (Forsgren and Nyström, 1990; Hesdorffer et al., 2000; Hesdorffer et al., 2006) and where epileptic episodes manifest depressive behaviors (Trimble et al., 2004; Swinkels et al., 2005; Tellez-Zenteno et al., 2007). The following introduction will review relevant information on each disease state and present key evidence supporting our rationale for investigating the co-morbid occurrence of melancholy and epilepsy Hippocrates witnessed centuries ago.

1.2 Depression

Etiology. The World Health Organization (WHO) predicts that depression will be the second most important cause of the global health burden by the year 2020 (Hankin, 2006). Depression is quickly becoming one of the leading psychiatric disorders, affecting some 20 million people in the US according to the National

Institute of Mental Health (NIMH) [Heo et al., 2007]. Although researchers have not fully elucidated the neurobiological mechanisms that are involved in causing the disease, it has been speculated that an imbalance of monoamines in the brain may contribute to hallmark symptoms (Shah et al., 1999; Jones and Lucki, 2005; Dunlop and Nemeroff, 2007). Most clinically accepted antidepressant drugs (ADs) exert their effect by increasing the amount of monoamines that are available in the brain by blocking the reuptake from the synapse, inhibiting intraneuronal metabolism, or blocking the presynaptic inhibitory autoreceptors (Elhwuegi, 2004) [Figure 1.1].

Depressive disorders can be influenced by a combination of genetic heritability, environment, biochemical, and psychological factors, leading to interruptions of a person's normal daily activities. Many researchers believe that depression is not only genetically heritable, but also the result of multiple genes interacting with the environmental factors (El Yacoubi and Vaugeois, 2007). Some of these environmental triggers of depression can include personal trauma, loss of a loved one, relationship difficulties, and stressful situations. The cumulative effects of chronic stress may also play a critical role in burden of the disorder (Nemeroff, 2007). Some of the demographic characteristics associated with a high risk of the disease include being female, having a separated/widowed/divorced/never married status, and a person's employment classification (Shah et al., 1999).

Classification. There are several forms of depressive disorders that include, but are not limited to, major depressive disorder (MDD), dysthymic disorder (DD), and bipolar disorder. MDD, the most common disorder, is characterized by melancholy, feelings of worthlessness or guilt, anhedonia, suicide ideation, changes in weight,

insomnia or excessive sleepiness, and loss of interest (Kandel, Schwartz, and Jessell, 2000). Dysthymic disorder, on the other hand, is described as having long-term less severe symptoms that prevent a person from functioning normally but do not meet the criteria for MDD. People with this disorder can also experience an episode of major depression in their lifetime. Bipolar disorder is defined by cycling mood changes. These extreme mood changes are typically treated with mood stabilizers, which include lithium and anticonvulsant drugs, such as valproic acid. Bipolar disorder is not as common as the aforementioned depressive disorders, but can be just as debilitating, if not more so.

Diagnosis and Treatment. Depression can be a debilitating disease affecting the way a person eats, sleeps and performs his/her daily routine. Without proper diagnosis and treatment, and sometimes despite psychotherapy and pharmacotherapy, depression can last for weeks, months, or even years. Physicians perform physical examinations, laboratory testing, and in depth interviews as a routine way to assess psychological disorders. The Diagnostic and Statistical Manual of Mental Disorders fourth edition (DSM-IV) is a classification system allowing clinicians and researchers to evaluate their patients using standardized criteria. To meet the criteria for major depression a patient must exhibit either a melancholy mood or display a diminished interest or pleasure in usual activities and must have at least five symptoms in *Table 1* during the same 2-week period (Mondimore et al., 2007). Physicians often assess the patient's family history to establish if the patient has a genetic predisposition to the disorder and also investigate other drug-induced, medical, and psychiatric sources that may contribute to the patients' diminished mood. Depression is often co-morbid

with other psychiatric disorders (e.g. post traumatic stress disorder [PTSD]), neurological diseases (e.g. epilepsy) and medical conditions (e.g. chronic pain/infection). Antidepressant drugs are typically the first-line treatment and are effective in 60%-70% of depressed patients properly diagnosed and appropriately treated (Shah et al., 1999). In extenuating circumstances doctors will sometimes suggest other radical and more controversial treatment methods for patients exhibiting severe and refractory major depression. Electroconvulsive therapy (ECT) and vagus nerve stimulation (VNS) are two neurotherapeutic methods currently being used to treat people experiencing treatment-resistant depression. These techniques employ the use of electrode placements to send electrical impulses to the brain and vagus nerve, respectively, eliciting a change in brain neurotransmission and ultimately alleviating depression symptoms. While ECT and VNS are deemed to be successful treatments, researchers are continually searching for innovative ways to treat depression and novel AD targets.

Animal Models: Although depression is the most common psychiatric disorder, elucidating the mechanisms that underlie the disease can be hard to interpret and are often controversial. The use of animal models has been critical for the investigation of the neurobiological substrates of the disease and its treatments. In human patients, a diagnosis of depression heavily relies on the DSM-IV criteria and a physicians' objectivity, but inducing depression in an animal rests on imitating maybe one or two specific depressive behaviors listed in the DSM-IV criterion (e.g. anhedonia, weight loss). The Porsolt's forced swim test (FST), the chronic mild stress (CMS) paradigm, and the selective breeding of rodents are a few common techniques implemented to

mimic depressive-like behaviors and screen for antidepressant drug efficacy in a laboratory setting.

Porsolt's FST is the most widely used test for screening ADs (El Yacoubi and Vaugeois, 2007). The paradigm consists of placing a rodent in a cylinder of water from which there is no escape and measuring the animal's behavior for several minutes (Porsolt et al., 1977). In a modified version of the FST, struggling (or climbing), swimming and immobility are analyzed separately and are increased with serotonergic and catecholaminergic ADs, respectively (Weiss et al., 1998; and Cryan and Slattery, 2007). The length of time an animal is immobile was originally interpreted as 'behavioral despair' because the authors theorized that the animal had given up on trying to escape. Currently, the notion of 'behavioral despair' in the FST is controversial, and the test is mostly used more generally to assess a change in behavior in a stressful situation and AD efficacy (rodents struggle more and float less following AD administration).

In 1984, Paul Willner adapted the CMS paradigm as a way to develop an animal model of depression that would be both valid as a simulation of depression, and chronic in its duration (Willner, 1997). The model mimics the depressive symptoms of anhedonia, weight changes, and sleep disturbances, by subjecting an animal to a variety of unpredictable stressors that are reversible by AD treatment (Cryan and Mombereau, 2004). The effectiveness of this procedure is monitored by tracking, over repeated tests, a decrease in the consumption of a preference of a palatable sucrose solution. Willner originally reported that chronic sequential exposure of rats or mice to a chronic mild stress regime (CMS) caused a decrease in the

responsiveness to rewards, which is typically reported as a decrease in the consumption of, and preference for, palatable, dilute sucrose solutions, as well as a decrease in the rewarding properties of a variety of pharmacological and natural reinforcers in the place preference paradigm (Willner, 2005). While the CMS paradigm has a number of advantages over more "acute" paradigms such as the FST, the CMS procedure is very labor-intensive and replication reliability from lab to lab has been poor (see Appendix 1, page 89).

The selective breeding of animals has been used as a way to replicate the genetic heritability of depression. Animal models of depression produced from selective breeding may also be useful in illuminating gene-environment interactions that contribute to depression (Friedman et al., 2006). Selectively bred rodents are typically bred for how much they show, or display behaviors related to depression (e.g. inactivity in the FST). Over the last two decades, researchers have developed several selectively bred rodent models of depression (Scott et al., 1996; Weiss et al., 1998; El Yacoubi and Vaugeois., 2007; Will et al., 2003; Overstreet et al., 2005). The selectively bred rats utilized in my experiments will be subsequently discussed in detail.

1.3 Epilepsy

Etiology. The word *epilepsy* is derived from the Greek word *epilepsia*, which means *epi-* (upon) and *lepsis* (to take hold of, or seizure). It can be defined as having two or more unprovoked, recurrent seizures and is characterized by abnormal activity in the brain causing synchronous rather than asynchronous neuronal firing. In 400

B.C., Hippocrates referred to epilepsy as the "sacred disease" because he believed seizures were not the result of demonic possession but instead a disease of the brain treatable by diet and drugs. According to the Center for Disease Control (CDC) website, epilepsy affects approximately 2.7 million Americans in the United States and 1-2% of the population worldwide. In an effort to better diagnose and treat patients with epilepsy, clinicians and scientists are still attempting to understand how the disease is triggered and develops.

There are several ways a person can develop epilepsy. Head trauma greatly increases the likelihood of getting epilepsy. Stroke, very high fever, injury at birth, drug and alcohol withdrawal, and brain tumors are other documented causes of the disease. Extreme sleep deprivation, drug overdoses and electroconvulsive shock (ECT) are other extenuating circumstances that may cause seizures to occur. Rare mutations, mostly in genes encoding ion channels, have been identified as key players in heritable epilepsy (Yamakawa, 2006). In fact, some individuals can have a single seizure and do not go on to develop the disease.

Classification. Epilepsy can be broadly classified as *idiopathic*, *symptomatic*, or *cryptogenic*. Idiopathic epilepsies are generally benign in the sense that they are not associated with brain lesions, neurologic abnormalities other than seizures, or mental impairment, and that they tend to be self-limited or respond readily to antiepileptic drugs (Engel et al., 1998). Genetic factors also play an integral role when diagnosing idiopathic epilepsy and the manifestations that typically occur are age related (e.g. juvenile myoclonic epilepsy). Conversely, when an epilepsy syndrome can be identified based upon a specific etiology and/or identifiable lesion, it is classified as

symptomatic. Epilepsies that have unknown or ambiguous etiologies are called *cryptogenic*, an epidemiological term that has been recently replaced by a more accurate term meaning "probably symptomatic." Epilepsy syndromes can be further classified as generalized or partial. Generalized seizures begin with seizure discharge on both sides of the brain. In humans, generalized seizures manifest as grand mal or tonic-clonic seizures in which an affected individual typically loses consciousness and experiences full body convulsions. Absence seizures — also known as petit mal seizures — involve a brief, sudden lapse of conscious activity with no obvious motor component. Occurring most often in children, absence seizures may look like the person is merely staring into space for a few seconds. Partial seizures (i.e. complex partial seizures, temporal lobe epilepsy [TLE]), that may or may not cause loss of consciousness, are the result of focal trauma in which seizure discharges initiate in one area of the brain and can spread over to other areas, leading to secondary generalized seizures (see Table 1.2).

Diagnosis and Treatment. There are multiple factors speculated to contribute to a complete understanding of the plight of a person suffering from an epileptic syndrome. Over the last decade, novel diagnostic imaging tools such as intracranial monitoring, functional magnetic resonance imaging (fMRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET), and single-photon emission computed tomography (SPECT) have allowed researchers and clinicians to further understand the neural correlates of an epileptic brain (Leeman and Cole, 2008). The use of these tools, in addition to traditional diagnostic methods (e.g. electroencephalogram [EEG], electrocardiogram [ECG]), facilitate better diagnoses

and treatments for patients suffering from the disorder with the expectation of improving their quality of life.

Physicians typically prescribe antiepileptic drugs (AEDs) to patients suffering from the disease. Over last decade, pharmaceutical companies have taken advantage of novel targets for AEDs to improve medications that can often have adverse contraindications. Some of the first AEDs prescribed to patients with epilepsy included carbamazepine, phenytoin, primidone, ethosuximide, and valproic acid. These medications were effective and affordable but they carried a high risk of hepatic dysfunction, drug interactions, and severe side effects (Leeman and Cole, 2008). Recently, there have been new selections of AEDs available that have fewer side effects and drug interactions (e.g. pregabalin, oxcarbazepine, zonisamide, levetiracetam). Some alternative options to treat intractable epilepsy, other than pharmacologic management, include surgical approaches, implantable devices (e.g. vagus nerve stimulation), and the ketogenic diet (see Chapter 4).

The mechanism of action of anticonvulsant drugs fall into three major categories: 1) Drugs that limit the sustained repetitive firing of a neuron, typically promoting the inactivated state of voltage-activated Na^+ channels. These drugs are effective against the most common forms of epileptic seizures 2) Drugs that optimize seizure control is to enhance gamma-aminobutyric acid (GABA) mediated synaptic inhibition and 3) Drugs that limit the activation of voltage-gated Ca^{2+} channels (Leeman and Cole, 2008).

Animal Models. Scientific advancement in the epilepsy field has been greatly enhanced by our ability to use animal models of the disease to mimic the neurological

and behavioral manifestations seen in epileptic patients. Specifically, chemi-convulsants (e.g. flurothyl, pentylenetetrazol) are a common way to assess acute seizure threshold. Other chemi-convulsants (e.g. pilocarpine, kainic acid) can be used in the laboratory setting to replicate seizure occurrence and neuropathology seen in patients with temporal lobe epilepsy (TLE).

The kindling paradigm is one of the most widely used models of seizures and epilepsies, especially TLE (McIntyre and Gilby, 2007). Kindling, repeated electrical stimulation of the limbic regions resulting in progressive seizure generalization in an initially nonepileptic brain, has been employed as a tool to examine the effects of epileptogenesis and whether epilepsy development can be modified (Bertram, 2007). In addition to kindling, numerous genetic models have also been employed to investigate the neurobiological mechanisms of seizure disorders. One example is the genetically epilepsy-prone rats (GEPRs). These animals display convulsions and wild running following exposure to high decibel sounds. From this epileptic model, researchers were able to determine specific brainstem pathways mediating this type of audiogenic-induced seizure activity. Furthermore, selectively bred rat models of amygdala kindling such as the genetically seizure-prone or seizure resistant rats, have experimentally showed genetic and acquired predisposing factors do influence the evolution of the epileptogenesis process (Vinogradova, 2008). Animal modeling has also been explored in other animal species (e.g. rabbits, mice) as a way to imitate human epileptic syndromes (reviewed in Sarkisian, 2001).

The exact mechanisms involved in the progression of events causing unprovoked seizures has not been fully elucidated, but from the evidence previous presented it is

speculated that animal models of seizure disorders have significantly advanced research aiding in the development of better treatments for epilepsy patients (Curia et al., 2008; Martin and Pozo, 2006).

1.4 Depression and Epilepsy Co-morbidity

Etiology. The co-morbidity of epilepsy and depression was first documented by Hippocrates who noted, "melancholics ordinarily become epileptics and epileptics melancholics"(McConnell and Synder, 1998). Currently, there exists extensive epidemiological evidence suggesting a bidirectional relationship between epilepsy and depression (Kanner and Nieto, 1999; Lambert and Robertson, 1999). Depression is the most frequent psychiatric co-morbid condition in patients with epilepsy and significantly contributes to increased morbidity and mortality (Koh et al., 2007). The disease severely affects quality of life patients with epilepsy and as a consequence increases the suicide risk among these individuals (Kanner, 2003_a). The risk of patients with epilepsy developing depression and for patients experiencing clinical depression developing epilepsy is 4-5 fold higher than in the general population (Barry, 2003). In fact, higher rates of psychopathology are observed in individuals with epilepsy compared with the general population, other neurological control groups, and individuals with non-neurological disorders (Kanner, 2003_a). More specifically, at least 30% of epileptic patients with temporal lobe and/or complex partial seizures suffer from depression (Paciello et al., 2002).

There are several possible reasons why there is a failure to identify co-morbid mood disorders such as depression in epilepsy patients: 1) There is the common

misconception by patients, relatives, and clinicians of depression being "a normal reaction" to facing a life with seizures and the expected obstacles in a social, academic, professional, and economic domains. 2) Depressive disorders have an atypical clinical expression in patients with epilepsy and fails to meet DSM-IV criteria for MDD. Blumer coined the pleomorphic pattern of depressive symptom occurring in epilepsy patients as interictal dysphoric disorder (IDD) (Barry et al., 2008). IDD symptoms (e.g. anhedonia, fatigue, anxiety, irritability, poor frustration tolerance) occur intermittently and mimic DD; however, the symptom-free periods of IDD usually lasting a couple of days preclude DSM criteria for DD. 3) Patients become accustomed to living in a chronic depressed state to the point of where they are forget "what is was like to be happy or euphoric" 4) Patients and relatives misperceive that depressive symptomatology is "part of living epilepsy" and thus does not require special treatment and 5) The "don't ask, don't tell" phenomenon refers to the deliberate decision by nonpsychiatrists not to inquire about symptoms of depression because they do not know how to manage or where to refer their patients (Barry et al., 2008).

Depression and epilepsy co-morbidity is more than coincidental. Evidence suggests that a significant number of patients with new-onset epilepsy were already suffering from mood and anxiety disorders before they ever had their first seizures. In 1990, Forsgren and Nyström conducted a Swedish community-based study of incident cases with non-provoked epileptic seizures, using case-referent methodology. The authors explored the possible risk factors for epileptic seizures in patients with newly diagnosed epilepsy. Eighty-three cases, 67.4% whom of had seizures of localized onset, were each compared to 2 age- and sex-matched controls. Data showed that a history of

depression increased the risk of unprovoked seizures 7 fold. In 2000, Hesdorffer et al. looked at older (over 55 years) adults with new onset idiopathic/cryptogenic epilepsy and assessed depression by interviews using DSM-III criteria. For each case (n = 145), two age- and sex-matched controls (n = 290) were selected from the same community. They found that prior major depression increased the risk of epilepsy by ~ 4-fold. In a follow-up study in 2006, the same group conducted a population based case-control study using Icelandic children and adults. For each case identified by a national seizure surveillance system (n = 324), age-matched controls were selected from the Icelandic population registry as the next two same sex births (n= 647). Using telephone interviews with patients and their parents and DSM-IV criteria to diagnose MDD, the authors found that depression (1.5-fold), suicide attempt (13.3 fold) and depression accompanied by suicide attempt (4.2 fold) increased the risk for developing unprovoked seizures. Utilizing the Canadian Health Survey, Tellez-Zenteno and colleagues (2007) sampled 37,000 people in Canada, and found that 253 (0.6%) were epileptic. Using the Composite International Diagnostic Interview to assess depression, they found that the lifetime risk of major depression or suicide ideation was ~ 2-fold higher in PWE than the general population.

Hypothesized causes for the co-morbidity are 1) Neurobiological. Depressive disorders are contingent upon seizure type and location, severity and frequency. Also, the depletion of monoamines and GABA may contribute to the pathogenesis of both disorders. 2) Iatrogenic. The use of anticonvulsant drugs has been shown to have complications inducing both positive and negative effects on mood in people with epilepsy. 3) Psychosocial. Increased stressful life events, poor adjustment to epilepsy,

and lack of independence are thought to contribute to depression in epileptic patients (Jobe, 1999).

If some types of epilepsies are associated with behavioral disturbances, the question arises as to whether there are specific anatomical changes in the brain that correlate with their development. Many studies have examined TLE and identified the temporal lobe pathology that occurs including hippocampal sclerosis, and in some cases neuronal loss (McConnell and Synder, 1998). Recently, scientists have focused their attention on elucidating potential structures that are involved in the co-morbidity within the temporal lobe, specifically in the amygdala and the hippocampus (Richardson et al., 2007). With the aid of *in vivo* neuroimaging techniques, researchers have begun to define the structural, functional, and chemical abnormalities associated with depression (reviewed in Drevets, 2000) and epilepsy (McDonald, 2008). Brain regions involved in both TLE and depression include the temporal lobes with the hippocampus, amygdala, entorhinal and neocortex; the frontal lobes; subcortical structures such as the basal ganglia and thalamus; and the connecting pathways (Kondziella et al., 2007).

Typical symptoms of depression, including sleep disturbances, loss of appetite, and weight gain are often masked or confounded by the side effects of anticonvulsive drugs (Kuhn et al., 2003). Because depression is often under diagnosed and under treated in the epileptic population, adequate recognition and treatment of psychiatric conditions in epileptic patients is essential for patient management. Even with controlling for seizure frequency, patients experiencing temporal lobe epilepsy with co-morbid depression exhibit significantly poorer performance on measures of intelligence, language, visuoperceptual ability, memory and executive function than those with TLE

alone (Harden, 2002). With appropriate patient management and proper attention, physicians will begin to address the overwhelming burden of treating depressed epileptic patients.

Treating co-morbid patients has been exceptionally challenging because the central effects of AEDs are not restricted to just modulating cortical excitability; in addition, they can also modify systems that regulate mood and behavior (Engel, 1998). Because anticonvulsants can have psychotropic effects, the use of AEDs has increased seizure-related psychiatric complications. Several AEDs have been shown to increase psychiatric side effects in epileptic patients (e.g. depression and suicide ideation). Older anticonvulsant treatments (e.g. phenobarbital) have been linked to depression in both children and adults (Brent et al., 1986; Robertson et al., 1987). Currently, prescribed AEDs such as vigabatrin, topiramate, and zonisimide have all been shown to increase the risks of affective disorders (Levinson et al., 1999; Matsuura, 1999; Mula et al., 2003). Increased AED-related psychiatric risk likely results from a combination of the severity of the epilepsy, polytherapy, rapid titration, and the high doses of the AEDs.

The high incidence and impact of depression in epilepsy has become such a concern that an expert panel composed of neurologists and psychiatrists from the Epilepsy Foundation's Mood Disorders Initiative composed and published a "Consensus Statement" to improve the recognition and treatment of depressive disorders in patients with epilepsy (Barry et al., 2008). In this review, they discuss the idiosyncratic aspects of depressive symptoms in this population (e.g. doesn't always meet DSM-IV criteria) and other barriers in diagnosis and treatment, and lay out a set of recommendations that

includes the use of psychometric tools for diagnosis and a stepwise algorithmic approach to treatment.

Animal models of co-morbidity. In 1999, Phillip Jobe and colleagues suggested a need to provide animal models to support epidemiological evidence of co-morbidity. There are two intuitive ways to create a co-morbid epilepsy and depression model: 1) test epileptic animals for depressive-like phenotypes, or 2) test animals with pre-existing depressive-like phenotypes for seizure sensitivity and/or epilepsy. Several groups have taken the first approach using rodent models (McIntyre and Gilby, 2007; Mazarati et al., 2007; Mazarati et al., 2008).

Recently, Mazarati and colleagues examined whether rats subjected to lithium chloride and pilocarpine-induced SE displayed behavioral or biochemical alterations consistent with depression. Epileptic animals exhibited increase in immobility time in the forced swim test (FST) and decreased sucrose intake, behaviors that are indicative of anhedonia and a despair-like state. A Canadian group, led by Dan McIntyre, has spent their efforts investigating cognitive deficits in rats that are prone and resistant to kindling-induced epileptogenesis. These studies have provided data supporting a possible co-morbid relationship of epilepsy and depression, but they have not offered insight as to how genetic predisposition to depression influences epileptic syndromes.

To my knowledge, my experiments investigating the seizure characteristics of rat lines selectively bred for depressive-like behaviors that are described in this document represent the only example of the second approach. Attempting to improve upon available rodent models of depression, the Weiss lab selectively bred normal, outbred Sprague-Dawley rats for a number of phenotypes relevant to human depression (Weiss et

al., 1998; West et al., 1999). Rats were initially purchased from Charles River Breeding Laboratories in 1987 and tested for various phenotypes. Individual rats with extreme phenotypes were then bred together over many generations to produce the current lines used in experiments. The selectively-bred lines are as follows: 1) Swim-High Active (SwHI), 2) Swim-Low Active (SwLO), 3) Swim-test susceptible (SUS), 4) Swim-test resistant (RES), 5) Monitor Hyperactive (HYPER), 6) Monitor Resistant (MON RES), 7) Non-Selected Controls (NS). The first lines derived were the SwHI and SwLO lines, which were developed as a potential novel screen for antidepressant drugs. SwHIs show high motor activity in the forced swim test (FST). SwLOs, on the other hand, show low motor activity in the FST. The SUS are characterized by their ability to show normal basal activity in the FST but a reduced activity in the FST after a mild stressor of 95 dB of white noise. The RES are characterized by their resistance to a reduction of activity in the FST after the same mild stressor as the SUS. The HYPERs show high levels of hyperactivity beginning 3-5 days after a stressful event (i.e. 2-3 hours tailshock) compared to control rats, which show a 2-4 day reduction in locomotor activity following stress. MON RES rats do not show any change of spontaneous motor activity after stress. Controls used in all the following experiments are NS (randomly-bred) wild type rats. Creating an animal model of depression and epilepsy co-morbidity by employing the use of the Weiss animals will lead to novel data examining the role of heritable depression and epilepsy.

1.5 Summary

The main goal of this research is to establish an animal model of depression and epilepsy by combining animal models of depression and models of epilepsy. Development of such a model will serve several purposes. First, it will experimentally confirm what so far has been strictly an epidemiological phenomenon. Second, it will allow an investigation into the genes and molecules that may underlie the connection between these disorders. Finally, it will provide a screening tool for testing the efficacy of new therapies for the treatment of co-morbid individuals. In the following chapters I will describe novel data examining the relationship between depression and epilepsy utilizing a unique model of depression, (i.e. selectively-bred rodents) and common seizure inducing chemoconvulsants (i.e. flurothyl, kainic acid, and pilocarpine). I will also describe experiments aimed at defining the therapeutic mechanism of the ketogenic diet, a potential alternative treatment for epilepsy and depression co-morbidity.

Figures

Figure 1.1 The monoamine neuron and the site of action of antidepressants (Elhwuegi, 2004).

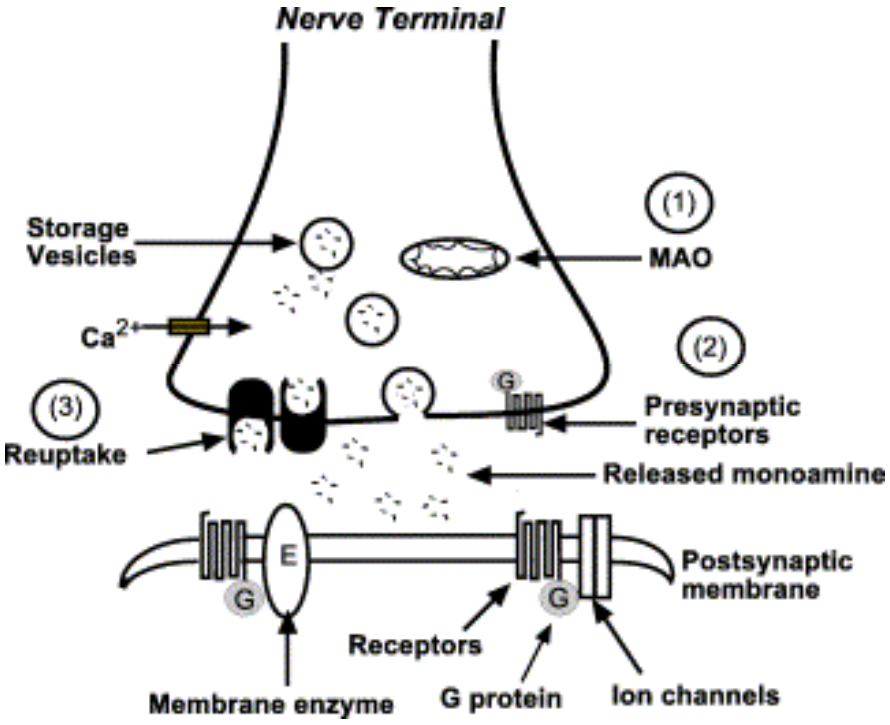

Figure 1.1 The monoamine neuron and the site of action of antidepressants. Monoamines are synthesized in the presynaptic nerve terminal, stored in the storage vesicles by vesicular monoamine transporter and released by Ca^{2+}-dependent exocytosis. After release, they act on postsynaptic or presynaptic receptors. Most of the monoamine receptors are likened to G proteins, the activation of which will open certain ion channel or either activate or inactivate certain membrane enzyme. The inactivation of the monoamine is done by active reuptake into the nerve terminal and/or glial cells using specific Na^{+}/Cl^{-}-dependent transporter. After reuptake into the nerve terminal, the monoamine is taken up again by the storage vesicles using the vesicular transporter or exposed to oxidation by MAO. The acute effect of antidepressants on the monoamine system is (1) inhibition of neuronal MAO (2) blockade of the presynaptic alpha2 receptors, or (3) inhibition of the reuptake of the monoamines. These three mechanisms will increase acutely the level of the monoamines at the synapse.

Tables

Table 1.1 DSM-IV criteria for depression (Mondimore et al., 2007).

Table

Chronic depression: symptoms and course of illness

Category	Symptoms	Course
Major depressive disorder	Low mood Anhedonia Changes in appetite, weight, sleep patterns, and/or psychomotor activity	Symptoms present continuously for 2 weeks Single episodes or recurrent Often without interepisode recovery
Dysthymic disorder	Depressed mood Changes in appetite, weight, sleep patterns Low energy Low self-esteem Cognitive problems Hopelessness	Symptoms present for 2 years Continuous symptoms for > 2 months No major depressive episodes

Table 1.1 DSM-IV criteria for depression. There are 2 major categories for depressive illness: Major depressive disorder (MDD), for which there are a number of subcategories and qualifiers; and dysthymic disorder (DD), conceptualized as a more chronic but less severe depressive illness.

Table 1.2 Seizure classifications.

1. **Partial seizures**
   - A. Simple partial seizures
     - i. With motor signs
     - ii. With somatosensory or special sensory symptoms
     - iii. With autonomic symptoms or signs
     - iv. With psychic symptoms
   - B. Complex partial seizures
     - i. Simple partial onset followed by impairment of consciousness
     - ii. With impairment of consciousness at onset ıset
   - C. Partial seizures evolving to secondarily generalized seizures
     - i. Simple partial seizures evolving to generalized seizures
     - ii. Complex partial seizures evolving to generalized seizures
     - iii. Simple partial seizures evolving to complex partial seizures evolving to generalized seizures
2. **Generalized seizures**
   - A. Absence seizures
     - i. Typical absence
     - ii. Atypical absence
   - B. Myoclonic seizures
   - C. Clonic seizures
   - D. Tonic seizures
   - E. Tonic-clonic seizures
   - F. Atonic seizures
   - G. Unclassified epileptic seizures

Table 1.2 Seizure classifications. An epileptic seizure classification has been developed by the Task Force on Classification and Terminology of the International League against Epilepsy (ILAE). The initial classification was created in 1970, and then revised in 1981. The classification of seizures is based on clinical history and manifestations as well as laboratory, neurophysiologic, and radiographic studies.

CHAPTER 2:

RATS BRED FOR SUSCEPTIBILITY TO DEPRESSION-LIKE PHENOTYPES ARE MORE SENSITIVE TO LIMBIC SEIZURES THAN THEIR DEPRESSION-RESISTANT COUNTERPARTS

Adapted from:

Tabb K, Boss-Williams KA, Weiss JM, Weinshenker D. (2007) Rats bred for susceptibility to depression-like phenotypes have higher kainic acid-induced seizure mortality than their depression-resistant counterparts. Epilepsy Research, Volume 74: 140-146.

2.1 Abstract

Epidemiological evidence suggests that epilepsy and depression are co-morbid diseases. In fact, depression is the most common neuropsychiatric disorder associated with epilepsy, particularly temporal lobe epilepsy, and individuals with a history of depression are at a higher risk for developing epilepsy than the general population. Despite the epidemiological evidence for this link, there has been little experimental evidence to support the connection or elucidate possible underlying mechanisms. In an effort to address this problem, we assessed seizure susceptibility and severity parameters in rats selectively bred for either susceptibility (the SwLo, SUS, and HYPER lines) or resistance (the SwHi, RES, and MON RES lines) to depression-like phenotypes. We found rats bred for susceptibility to depression-like phenotypes experienced higher mortality following kainic acid-induced seizures than their resistant counterparts. SwLo and SUS rats also had a shorter latency to clonic-tonic seizure following pilocarpine administration. In contrast, most line differences were not recapitulated when flurothyl was used to elicit seizures. Stress reduced kainic acid-induced mortality rates in all lines except the HYPER rats, supporting previously established indications that the stress response of HYPER rats is abnormal. These combined results support epidemiological evidence that a neurobiological link exists between depression and epilepsy, and that genetic factors may contribute to this relationship. Further investigation and validation of this potential animal model of epilepsy and depression may shed light on the mechanisms underlying the co-morbidity, suggest novel therapeutic targets, and provide a tool for screening new medications.

2.2 Introduction

Epidemiological evidence has demonstrated that depression is the most common co-morbid psychiatric condition associated with epilepsy, and the risk that an epileptic will develop major depression is approximately five-fold higher than average (Kanner and Nieto, 1999; Wiegartz et al., 1999; Hermann et al., 2000; Harden, 2002; Kanner and Balabanov, 2002; Gaitatzis et al., 2004; Swinkels et al., 2005). Individuals with temporal lobe epilepsy (TLE), in particular, are especially prone to develop depression (Kanner and Nieto, 1999; Paradiso et al., 2001; Harden et al., 2002). Depression remains underdiagnosed in the epileptic population, at least in part, because both patients and clinicians often assume that low spirits must be a "normal" response to living with a debilitating neurological disease. However, this explanation alone cannot fully account for the co-morbidity of epilepsy and depression, because it is bidirectional (Kanner and Balabanov, 2002). Epilepsy occurs with approximately five-fold greater frequency among individuals with a history of depression (or even a family history of depression) than among the general population, indicating that the bidirectional relationship is more than a psychosocial phenomenon and that the two disorders likely share common pathogenic mechanisms (Forsgren and Nyström, 1990; Kanner and Nieto, 1999; Hesdorffer et al., 2000; Hesdorffer et al., 2006; Kanner and Balabanov, 2002; Thapar et al., 2005; Kanner, 2005). In fact, major depressive episodes and suicide attempts independently increase the risk for developing unprovoked seizures and epilepsy (Hesdorffer et al., 2006), and depression scores and seizure frequency are significant predictors of each other both within and across time (Thapar et al., 2005).

Understanding the biological link between epilepsy and depression is desirable for a host of reasons. Depression has a more profound impact on an epileptic patient's quality of life than seizure severity or frequency (Johnson et al., 2004; Meldolesi et al., 2006). Furthermore, treating these co-morbid individuals presents problems, because some antidepressant drugs increase seizure susceptibility, and, conversely, some anticonvulsant drugs precipitate depressive episodes (Brent et al., 1987; Trimble, 1996; Alldredge, 1999; Kanner and Nieto, 1999; Pisani et al., 1999). Despite a clear epidemiological link between the two diseases, there is little experimental evidence to support a shared pathology, and even fewer animal models that could be implemented in order the reveal the potential underlying mechanisms, a deficiency much discussed in recent reviews (e.g. Kanner, 2003 $_{b}$ and Kanner, 2008).

In an attempt to experimentally confirm the striking epidemiological co-morbidity between seizure threshold and depression and to simultaneously create an animal model, we tested whether rats selectively bred for depression-like phenotypes are more susceptible to seizures produced by chemoconvulsants. Additionally, some of our rat lines were specifically bred for phenotypes that only manifest following a stressor, so we wanted to assess the effects of stress on their seizure latency. We hypothesized stress induction, specifically in these rats (the SUS, and HYPER lines), would further decrease their seizure latency.

2.3 Materials and Methods

Selectively bred rats. With the idea of expanding upon the rodent models of depression available at the time, Weiss and colleagues selectively bred normal Sprague–

Dawley rats for a number of phenotypes relevant to human depression (Scott et al., 1996; Weiss et al., 1998; Weiss et al., 2000; Weiss et al., 2005; West and Weiss, 2005; our unpublished data). These lines include: Swim-Lo active (SwLo), with low motor activity in a Porsolt forced swim test (FST); Swim-Hi active (SwHi), with high motor activity in the FST; Swim-test susceptible (SUS), which are highly susceptible to having their swim-test behavior disrupted by mild stress; Swim-test resistant (RES), which are highly resistant to having their swim-test behavior disrupted by mild stress; Monitor Hyperactive (HYPER), which experience long-lasting hyperactivity following stress; and Monitor Resistant (MON RES), which are resistant to changes in activity following stress. Randomly bred (nonselected; NS) rats served as controls. The following generations were used: SwLo (34, 39-41, 47), SwHi (34, 36, 41, 47), SUS (33, 34, 36, 41, 42), RES (32, 33, 35, 42), HYPER (30, 32, 33), MON RES (28, 32, 33, 35), and NS (29, 36, 38, 39, 47).

Male rats were used for all experiments, and each rat was used in a seizure experiment only once. Animals were housed in ventilated racks (2–3 rats per cage) at the Emory University Briarcliff vivarium. The colony room was maintained on a 12 h light–dark cycle (lights on from 7 am to 7 pm.), and the rats received standard laboratory chow and water ad libitum. Animals were treated in accordance with the Guidelines for Animal Care and Use of the National Institutes of Health, and the Emory University Institutional Animal Care and Use Committee approved all experiments.

Kainic acid seizure induction. Kainic acid (opika-1, Ocean Produce International, Shelburne, Nova Scotia, Canada) was administered to rats as previously described (Szot et al., 1999), but with slight modifications. Rats were injected

intraperitoneally (i.p.) with kainic acid (10 mg/kg), then placed in a clear container and observed closely for behavioral seizures. Latency to various seizure behaviors was scored using a modified version of the Racine scale (Racine, 1972): 0 = no seizure, 1 = staring, 2 = wet-dog shakes, 3 = forelimb clonus, 4 = rearing and falling, and 5 = clonic-tonic (CT) seizure. If a CT seizure was not observed in the first hour, rats were given booster injections of kainic acid (5 mg/kg) every hour until they experienced a CT seizure. Maximal seizure severity was assessed by mortality 24 h after the CT seizure.

Pilocarpine seizure induction. Pilocarpine hydrochloride (minimum 99% titration; Sigma Aldrich; St. Louis MO) was administered to rats as previously described (Borges et al, 2003; Raol et al., 2006), but with slight modifications. Rats were first injected subcutaneously (s.c.) with atropine bromide (N-methylatropinium bromide; VWR) at 2 mg/kg to block the peripheral effects of pilocarpine. After 30 minutes, rats were injected with pilocarpine (380 mg/kg, i.p.), and latency to CT seizure was measured. If a rat did not have a CT seizure after 1 hour, a booster dose of pilocarpine (190 mg/kg, i.p.) was administered.

Flurothyl Seizure Induction. Rats were placed in an airtight Plexiglas chamber (15 cm × 19. 5 cm × 35. 5 cm), and the volatile convulsant 2,2,2-trifluroethylether (flurothyl, Sigma–Aldrich, St. Louis, MO) was infused at a rate of 20 μ L/min onto filter paper, from which it evaporated. Flurothyl was infused until the occurrence of a CT seizure, after which the rat was removed from the chamber. Seizure susceptibility was assessed by measuring latency to CT seizure and by maximal seizure severity (whether or not the animals progressed to tonic extension and death). Tonic extension

was defined as when the hind limbs pass through a 90° angle with the body and then reach maximum extension pointing straight down, accompanied by full body rigidity.

Stress induction. Some of the selectively bred ratlines exhibited their depressive-like behaviors only following specific stressors. Therefore, seizure susceptibility was also assessed following exposure to the conditions that elicited those phenotypes. SUS and RES rats were exposed to 30 min of 90–95 dB white noise in a novel environment to elicit their depression-relevant phenotypes, and immediately thereafter were tested for seizure susceptibility. Similarly, HYPER and MON RES rats received 3 h of tail shock, as described (Scott et al., 1996), and were tested for seizure susceptibility 2 days later.

Statistics. For analysis of seizure threshold (the dose of chemoconvulsant required to produce a CT seizure) and latency (the time from the initial chemoconvulsant administration that a particular stage of seizure occurred), Student's t-tests were used for comparison between two groups with equal variance, and Mann–Whitney tests were used for comparison between two groups with unequal variance. Fisher's exact test was used for a comparison of seizure mortality incidence between two groups. Logistic regression was used for analysis of correlation between seizure threshold and mortality rate. GraphPad InStat (version 3.0) and Prism (version 4.0) software for Macintosh were used for all statistical analyses.

2.4 Results

*Increased kainic acid-induced seizure mortality in rats selectively bred for depression***:** To determine whether a genetic susceptibility to depression-related behavioral characteristics correlates with an increased susceptibility to limbic seizures,

we assessed the response to kainic acid in rats selectively bred for susceptibility or resistance to depression-like phenotypes. Rats of all strains followed a typical behavioral progression following kainic acid administration (10 mg/kg, followed by 5 mg/kg/hr booster doses until CT seizure occurred). All rats tested required at least one booster dose to produce a CT seizure, with the exception of one SwLo rat and three NS rats.

Within 30 min of injection, the rats began to stare, followed by increasingly severe seizure behaviors, including wet-dog shakes, forelimb clonus, rearing and falling, and CT seizures. We found no significant differences in latency to staring, wet-dog shakes, forelimb clonus, rearing and falling, or CT seizures (Figure 2.1). We also did not observe any differences in number of seizures of any stage per unit time. However, following the elicitation of seizures, there were striking differences in mortality among the lines. In general, all lines bred for susceptibility to depression-like phenotypes had higher mortality rates than their depression-resistant counterparts (significant difference for SwLo versus SwHi and SUS versus RES, trend for HYPER versus MON RES) (Figure 2.2A). The mortality rate for randomly bred control (NS) rats fell between the susceptible and resistant lines. Of the rats that died, many expired during the first CT seizure, while others continued to have sporadic seizures and died either later that day or were found dead the next morning. The dose of kainic acid required to produce a CT seizure was equivalent between each pair of lines, except for the SUS rats, for which the dose was slightly but significantly increased (Figure 2. 2B). However, the higher dose probably cannot account for the increased mortality of the SUS rats, because dose and mortality were not significantly correlated across all lines ($p > 0.05$). For example, some

NS control rats that received a cumulative dose of 20 mg/kg survived, while others that received 15 mg/kg or even as little as 10 mg/kg died, and similar patterns were also observed among the other lines.

Decreased pilocarpine-induced seizure latency in rats selectively bred for depression. To confirm and extend the results found with KA, another acute model of TLE was used. We determined latency to pilocarpine-induced status epilepticus (SE) in the SwLo, SwHi, SUS, RES, and NS rat lines. Pilocarpine (380 mg/kg, i.p.) was injected, and latency to CT seizure was measured. If a rat did not have a CT seizure within one hour, a booster dose (190 mg/kg, i. p.) was administered. Rats bred for susceptibility to depression-like phenotypes (SwLo and SUS) had a significantly shorter latency to seizure than their depression-resistant counterparts (SwHi and RES, respectively). NS control rats had latencies similar to, but slightly higher than, the depression-sensitive lines (Figure 2.3). These results provide further evidence that depression-sensitive lines are more susceptible to chemoconvulsants that target the limbic regions of the brains and model TLE.

*Rats selectively bred for depression are not more susceptible to flurothyl-induced seizures***.** To determine whether the SwLo, SUS, and HYPER rats were particularly susceptible to TLE-like seizures, or whether they were also more susceptible to seizures involving other brain areas (e.g. cortical seizures), we assessed their response to the generalized convulsant agent flurothyl. There were no differences in latency to CT seizure between depression-susceptible lines and their depression-resistant counterparts, with the exception of MON RES rats, which were more resistant (i.e. longer latency) than HYPER rats (Figure 2.4). None of the animals we tested progressed to tonic extension,

nor did any die. These findings suggest that the increased seizure severity leading to mortality observed in the depression-sensitive lines is unlikely the result of a general, widespread increase in neuronal excitability, but is at least partially restricted to limbic regions.

The effects of stress on kainic acid-induced seizures. The selectively bred phenotypes for some lines are observed only following stress. For example, the SUS rats have normal basal activity in the forced swim test, but they display increased immobility following stress (Scott et al., 1996; West and Weiss, 2005). Furthermore, the hyperactive phenotype of the HYPER rats is greatly increased by stress (Weiss et al., 2000; Weiss et al., 2005; our unpublished data). We wished to assess whether the stress paradigms used to elicit depression-like phenotypes also alter seizure susceptibility for each line. We found that stress (i.e. 30 min exposure to 90–95 dB white noise in a novel environment for SUS and RES rats, 3 h tail shock for HYPER, MON RES, and NS control rats) decreased kainic acid-induced seizure mortality in all lines, except the HYPER (Figure 2.5A). Stress did not consistently alter the dose of kainic acid required to produce a CT seizure (i.e. no effect in NS or HYPER, significantly decreased dose in SUS and RES, trend towards increased dose in MON RES) (Figure 2.5B). Again, the effects of stress on the dose were unrelated to the effects on mortality, because the two measures were not significantly correlated. For example, stress did not alter the kainic acid dose in control rats, but prevented lethality, and most of the SUS rats that received 15 mg/kg kainic acid following stress survived, while all the SUS rats that received 15 mg/kg kainic acid in the absence of stress died. For the flurothyl-induced seizures, stress

did not alter any parameters, except for increasing latency to CT seizure in MON RES rats (Figure 2.6).

2.5 Discussion

Epilepsy and depression appear to be co-morbid disorders, and depression in epilepsy is clearly much more than a mere "psychosocial" phenomenon (Kanner, 2005). Despite the wealth of epidemiological evidence linking these diseases, there is a paucity of experimental evidence to support the link, and few animal models have been developed for studying the possible underlying mechanisms. Contó et al. (2005) reported that Wistar rats that were susceptible to convulsions induced by methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM), a benzodiazepine inverse agonist, were more anxious than DMCM-resistant rats when tested on the elevated plus-maze, but they demonstrated normal depression-like behavior in the forced swim test. Sarkisova and Kulikov (2006) likewise found that rats susceptible to audiogenic seizures displayed increased levels of anxiety but, again, no consistent differences in the forced swim test. Perhaps the best characterized model of epilepsy and depression co-morbidity is the genetically epilepsy-prone rat (GEPR), developed by Jobe and colleagues (reviewed by Dailey et al., 1989). These rats were selectively bred for susceptibility to audiogenic seizures, but they have also been reported to display behavioral responses related to depression, such as increased immobility in the forced swim test and decreased saccharin consumption that is possibly indicative of anhedonia (Jobe et al., 1999; Jobe and Weber, 2005).

Relative to the studies described above, we have approached the question of co-morbidity from the opposite direction. That is, instead of asking whether seizure susceptibility leads to depression-like behaviors, we asked whether the predisposition to depression-like behaviors increases seizure susceptibility. Our model possesses two other salient features that are particularly relevant to the topic at hand. First, genetic load is a critical component of both epilepsy and depression, and a family history of depression is itself a risk factor for developing epilepsy (Robertson et al., 1987; Kanner and Nieto, 1999; Harden, 2002). The selective breeding approach embodies a strong genetic component, because we used rats selectively bred for more than 30 generations for susceptibility or resistance to behaviors associated with human depression. Of particular note, all three lines bred for depression-relevant behavioral phenotypes were also susceptible to kainic acid-induced seizure mortality, compared to their counterparts that are resistant to these phenotypes, which suggests the lines may share genetic changes that contribute to the phenotypes. Furthermore, both the depression-like and seizure phenotypes were stable over multiple generations, indicating co-heritability. Second, the incidence of major depression is particularly high in patients with TLE, ranging from ~20–70% (Kanner and Balabanov, 2002; Kuhn et al., 2003). We found that our rat lines differed in response to kainic acid and pilocarpine, which are commonly used to model TLE, but not to any seizure phenotype related to flurothyl, which produces generalized seizures involving more cortical structures (e.g. Sperber et al., 1999; Szot et al., 1999).

The most robust phenotype we observed was the decreased latency to CT seizure in SwLo and SUS rats compared to the SwHi and RES rats, indicating that the depression-sensitive lines were also more seizure susceptible. One alternative

explanation for this result is that the depression-sensitive lines have altered pilocarpine pharmacokinetics, although this seems unlikely. We also observed an increase in mortality following KA-induced seizures in the SwLo and SUS lines. While it is extremely difficult to determine exactly why an animal dies using any seizure model, it is generally thought that very severe seizures propagate to and impair regions of the brain essential for life, such as those controlling respiration and cardiovascular function (i.e. brainstem). It is important to note that while the origin of kainic acid-induced seizures is the limbic system, our rats experienced CT seizures, indicating seizure generalization. It is unclear why mortality resulted from "secondarily generalized" (kainic acid) seizures, but not "primarily generalized" seizures (flurothyl), but it may be that they involve different brain regions. Alternatively, sustained severe seizure activity following kainic acid administration may be more devastating than the single CT seizure induced by our flurothyl paradigm. Another possibility is that the depression-sensitive animals we used have cardiovascular impairment that is responsible for their deaths. However, we find it unlikely that all three depression-sensitive lines that we have studied have compromised cardiovascular or other organ systems, and simply died for this reason, while the depression-resistant lines happen to have particularly robust heart function. Also, as part of the selective breeding paradigm, these lines commonly undergo procedures that put significant stress on the cardiovascular system, such as forced swim or a prolonged session (i.e. 3 h) of tail shock, and the rats never die during such manipulations. Thus, we attribute the higher kainic acid-induced mortality and shorter pilocarpine-induced seizure latency of the depression-sensitive lines to an increase in seizure susceptibility,

propagation and severity. However, a study of heart physiology and chemoconvulsant pharmacokinetics in the ratlines will be required to rule out the other possibilities.

Some of the rat lines were bred for phenotypes that only emerge following stress. While chronic stress can certainly exacerbate seizures, acute stress is typically anticonvulsant, at least in animals (reviewed by Reddy, 2006). We found that stress reduced mortality following kainic acid-induced seizures in all lines except for HYPER rats. This would indicate that the HYPER rats have an abnormal response to stress, as previously shown using other paradigms (Weiss et al., 2000; Weiss et al., 2005; our unpublished data).

While we are encouraged by the promise of the new epilepsy and depression co-morbidity models presented in this paper, they have some important limitations. First and foremost, none of the selectively bred rats, even those from the "depression-susceptible" lines, are epileptic. The seizure phenotypes we observed were induced by acute administration of chemoconvulsants, and we have never observed an unprovoked, spontaneous seizure in these lines. It will be necessary to test these rats in models of epileptogenesis, such as kindling or the spontaneous seizures that result weeks or months following pilocarpine-induced SE. Second, it is doubtful that a rodent can experience anything truly analogous to full human depression. The general consensus in the field has become that modeling individual behaviors relevant to human depression will likely yield better results than simultaneously modeling the host of complex cognitive and behavioral features encompassed by depression itself. That said, the forced swim test is still the most widely used animal model of depression and antidepressant drug efficacy, and the SwLo/SwHi and SUS/RES rats have significantly improved on previous

incarnations of this model (Scott et al., 1996; West and Weiss, 1998; West and Weiss, 2005). While psychomotor hyperactivity is a feature of agitated/psychotic depression, the phenotype of the HYPER rats more closely resembles mania or bipolar disorder (Weiss et al., 2000; Weiss et al., 2005). Intriguingly, some anticonvulsant drugs, such as valproic acid and carbamazepine, are also first-line treatments for bipolar disorder (Spina and Perugi, 2004; Nasrallah et al., 2006). Finally, we presently know little about the genetics or the neurobiology of these rats. Although a genetic strategy (selective breeding) was used to generate these lines, we have only begun the process of thorough genetic analysis to identify those genes that contribute to the observed phenotypes (Weinshenker et al., 2005). Insofar as the depression-related behaviors and seizure susceptibility differences are co-inherited, we would predict that at least some genetic factors underlie both phenotypes.

The greatest value of any animal model of disease is as a tool for studying the disease mechanism(s); in this case, monoamine dysfunction in the hippocampus (and other limbic structures) is an appealing candidate, as originally proposed by Jobe and colleagues (Jobe et al., 1999; Jobe and Weber 2005). The hippocampus is heavily implicated in both epilepsy and depression (Hecimovic et al., 2003; Campbell and Macqueen, 2004; Kanner, 2005; Warner-Schmidt and Duman, 2006) and receives dense noradrenergic input from the locus coeruleus and serotonergic input from the dorsal raphe nucleus. Furthermore, norepinephrine and serotonin have both antidepressant and anticonvulsant properties, and an abnormality in either neurotransmitter system could produce both low mood and seizure susceptibility (Jobe et al., 1999; Kanner and Balabanov, 2002). Drugs that block norepinephrine and/or serotonin reuptake are the

most common antidepressant drugs used clinically, and these same drugs can also reverse depressive-like behavior in SwLo and SUS rats in the forced swim test (Weiss et al., 1998; West and Weiss, 1998; West and Weiss, 2005). Interestingly, both the GEPR rats and our selectively bred rats have monoamine abnormalities (Jobe et al., 1999; Scott et al., 1996; Weiss et al., 2008). Thorough characterization of monoamine and other systems in these rats, as well as their neuronal and synaptic organization, may yield clues about the underlying mechanisms of epilepsy and depression co-morbidity, leading eventually to improved therapy for individuals who suffer from both disorders.

Figures

Figure 2.1 Kainic acid-induced seizure latency in rats bred for susceptibility and resistance to depression-like phenotypes.

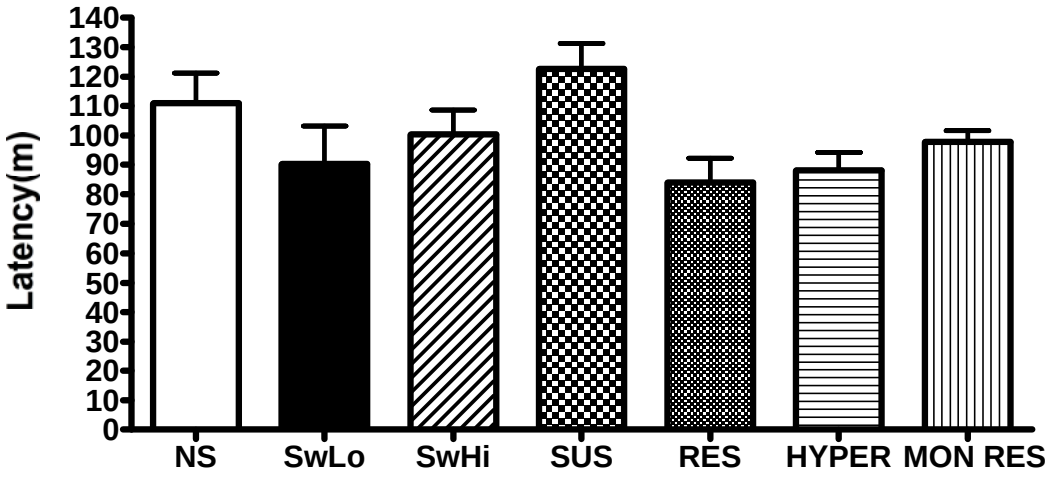

Figure 2.1 Kainic acid-induced seizure latency in rats bred for susceptibility and resistance to depression-like phenotypes. Kainic acid was administered to rats at a dose of 10 mg/kg, followed by 5 mg/kg booster injections until a CT seizure was observed. Shown is latency to CT seizure (mean ± S.E.M.) for each line. n = 6–14 per group.

Figure 2.2 Increased kainic acid-induced seizure mortality in rats selectively bred for depression.

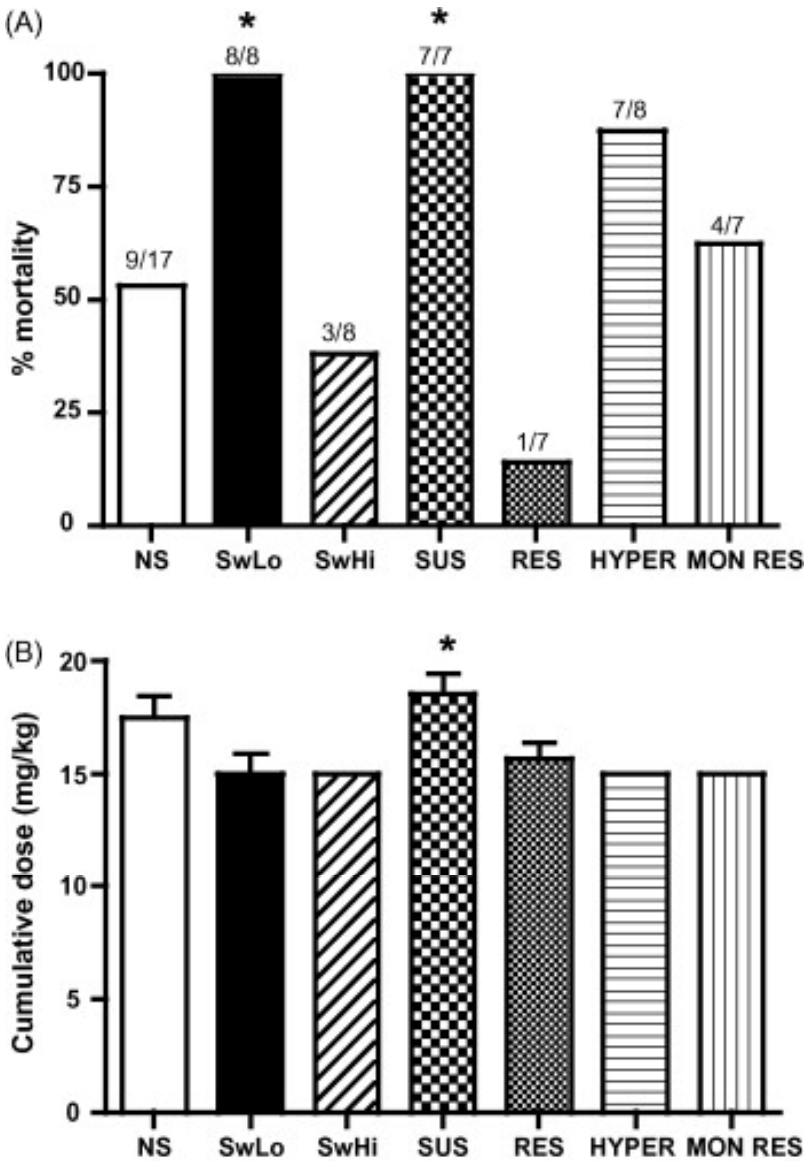

Figure 2.2. Increased kainic acid-induced seizure mortality in rats selectively bred for depression. Kainic acid was administered to rats at a dose of 10 mg/kg, followed by 5 mg/kg booster injections until a clonic-tonic seizure was produced. Shown is (A) mortality, with the number of animals that died over the total tested above each bar, and (B) the cumulative kainic acid dose (mean ± S.E.M.) required to produce a CT seizure. n = 7-17 per group. *$p < 0.05$ for the depression-susceptible line compared to its depression-resistant counterpart (SwLo vs. SwHi, SUS vs. RES, HYPER vs. MON RES, respectively).

Figure 2.3 Decreased pilocarpine-induced seizure latency in rats selectively bred for depression-sensitive phenotypes.

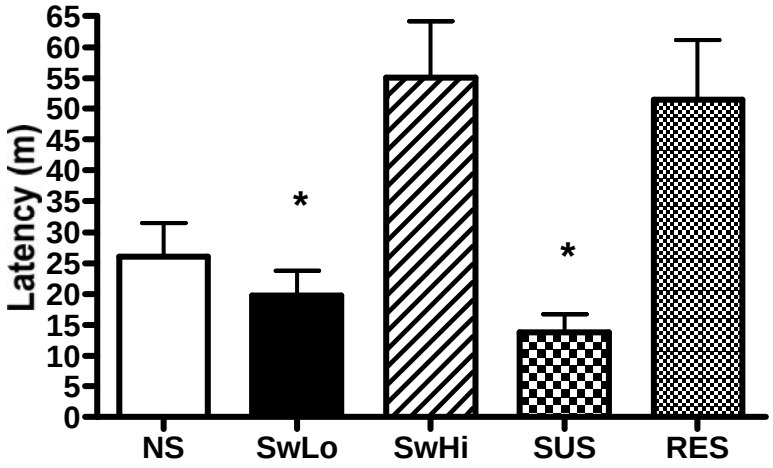

Figure 2.3 Decreased pilocarpine-induced seizure latency in rats selectively bred for depression-sensitive phenotypes. Shown is the latency to CT seizure following pilocarpine (380 mg/kg, i.p. followed by 190 mg/kg, i.p. booster dose if no CT seizure occurred after 1 hr). n= 8-15 per group. *$p < 0.05$ for the depression-susceptible line compared to its depression-resistant counterpart (SwLo vs. SwHi, SUS vs. RES, respectively).

Figure 2.4 Rats selectively bred for depression are not more susceptible to flurothyl-induced seizures**.**

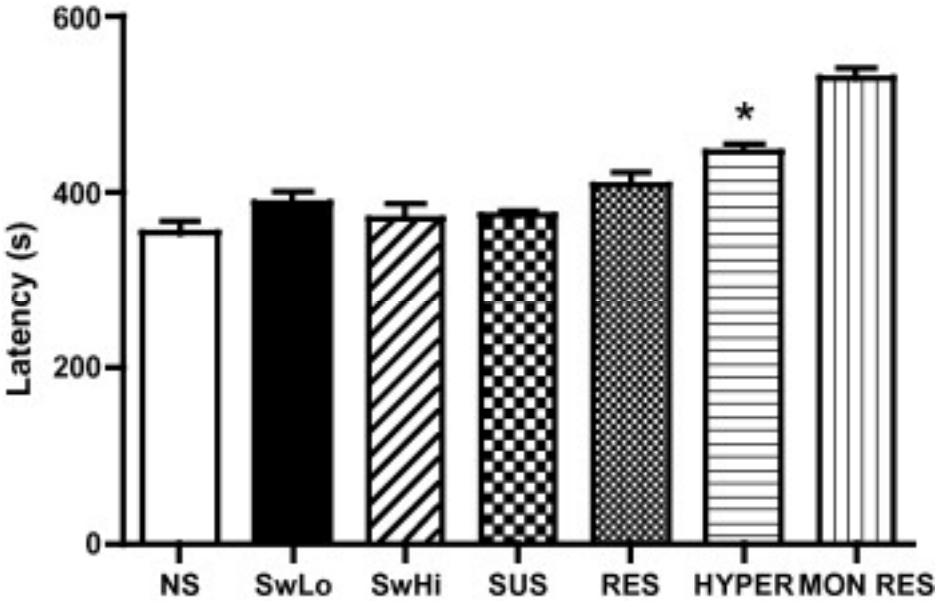

Figure 2.4 Rats selectively bred for depression are not more susceptible to flurothyl-induced seizures. Flurothyl was administered to rats at a rate of 20 μ L/min, and latency to CT seizure was observed. Shown is latency to CT seizure (mean ± S. E. M.) for each line. n = 6–16 per group. *$p < 0.05$ for the depression-susceptible line compared to its depression-resistant counterpart (SwLo vs. SwHi, SUS vs. RES, HYPER vs. MON RES, respectively).

Figure 2.5 The effects of stress on kainic acid-induced seizures.

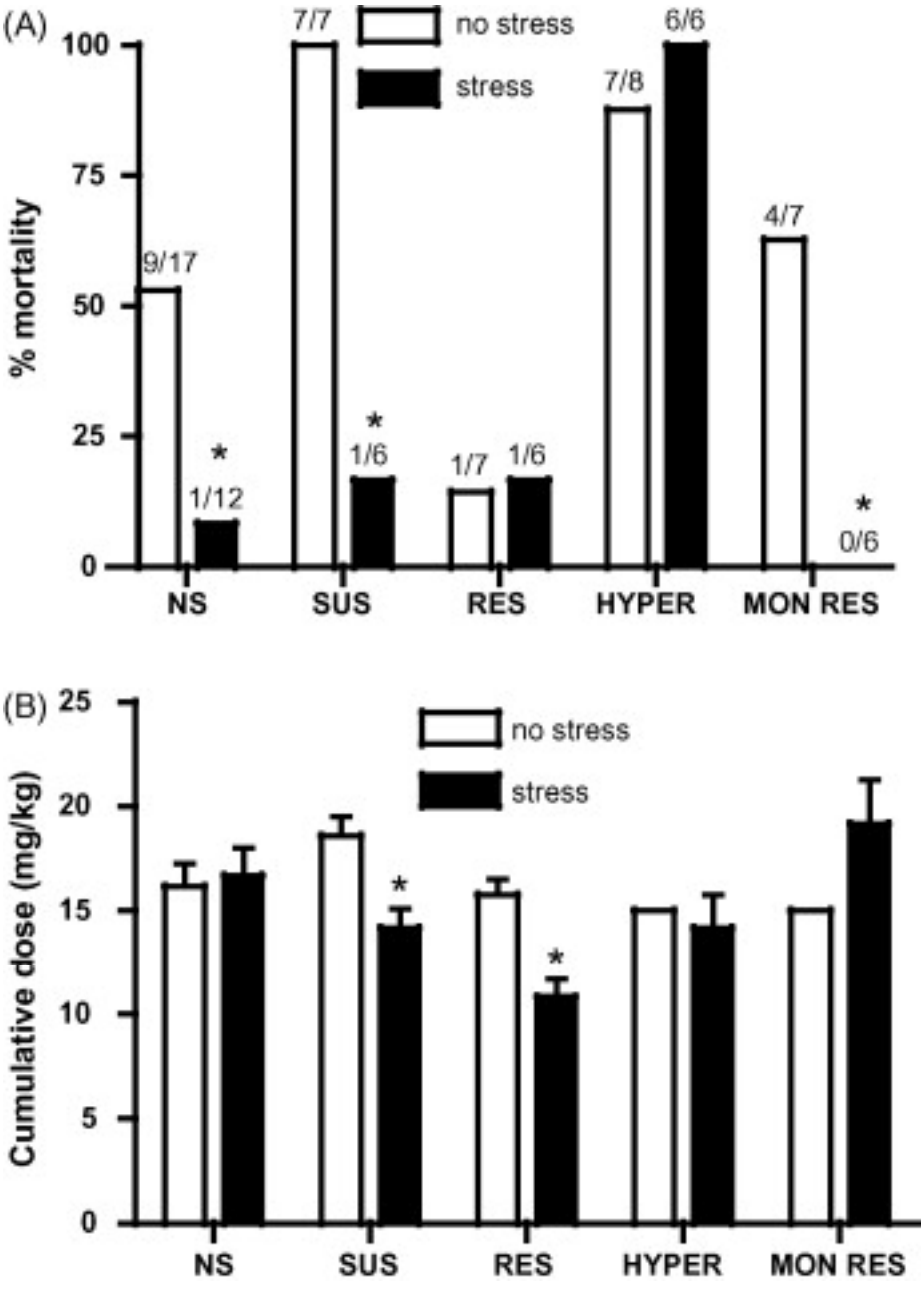

Figure 2.5 The effects of stress on kainic acid-induced seizures. Kainic acid-induced seizures were assessed following exposure of the rats to stress paradigms used to produce the depression-like phenotype in each line. SUS and RES rats were exposed to 30 min of 90–95 dB white noise in a novel environment, while HYPER, MON RES, and NS control rats received 3 hours of tail shock. Shown is (A) mortality, with the number of animals that died over the total tested above each bar, and (B) the cumulative kainic acid dose (mean ± S.E.M.) required to produce a CT seizure. n = 6-17 per group. *$p < 0.05$ compared to the no-stress condition for each line.

Figure 2.6 The effects of stress on flurothyl-induced seizures

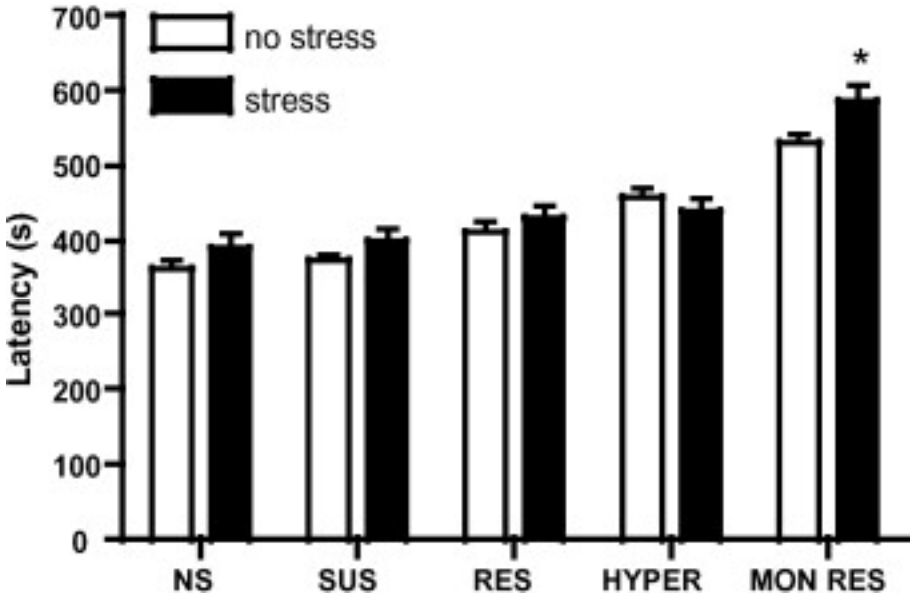

Figure 2.6 The effects of stress on flurothyl-induced seizures. Flurothyl-induced seizures were assessed following exposure of the rats to stress paradigms used to produce the depression-like phenotype in each line. SUS and RES rats were exposed to 30 min of 90–95 dB white noise in a novel environment, while HYPER, MON RES, and NS control rats received 3 h of tail shock. NS control rats also received 3 h of tail shock. Shown is latency (mean ± S.E.M.) to CT seizure following flurothyl administration (20 μ L/min). n = 6–18 per group. *p < 0. 05 compared to the no-stress condition for each line.

CHAPTER 3:

PILOCARPINE-INDUCED EPILEPTOGENESIS TRIGGERS GENERALIZED SEIZURES IN DEPRESSION-SENSITIVE RATS

3.1 Abstract

Temporal lobe epilepsy (TLE) is an epileptic disorder that is often associated with several psychological and cognitive co-morbidities. Depression is particularly common in refractory TLE patients, and has a greater impact on their quality of life than seizure frequency and seizure severity. However, few studies have addressed the association between depression-like disorders and TLE experimentally. Utilizing animal models of TLE, such as the pilocarpine model of epilepsy, is a useful way to further understand the relationship between these diseases. In a previous study, we showed that rats bred for depression-like phenotypes were also more susceptible to acute seizures induced by kainic acid and pilocarpine. To further validate our model of epilepsy and depression co-morbidity, we investigated the relationship between heritable depression and TLE using the pilocarpine model of epileptogenesis in rats bred for depression sensitivity (SwLo rats) and resilience (SwHi rats). Status epilepticus (SE), a period of sustained seizure activity, was induced using the chemiconvulsant pilocarpine. Five weeks following SE, we assessed the number of spontaneous seizure behaviors that occurred over two 12-hr dark cycle periods, one prior to a swim stress and one following a swim stress. SwLo and SwHi rats experienced a similar number of spontaneous seizure behaviors (e.g. myoclonic jerks, wet dog shakes, twitching), and the frequency of these seizure behaviors increased following swim stress in both lines. In contrast, only the SwLo rats displayed robust spontaneous generalized seizures (2/9 SwLo rats during the pre-stress observation period, 4/9 during the post-stress observation period); spontaneous generalized seizures were never observed in SwHi rats (0/6) during either observation period. These findings indicate that SwLo rats, in addition to their acute seizure

sensitivity, also develop more severe spontaneous seizures, further validating these animals as a model of epilepsy and depression co-morbidity. Future studies using SwLo rats may elucidate the mechanisms underlying the relationship between these diseases.

3.2 Introduction

Temporal lobe epilepsy (TLE) is a seizure disorder characterized by distinct pathology: hippocampal atrophy, induration, distinctive neuronal loss, and astrogial proliferation seen prominently in area CA1 of the hippocampus (Lee et al., 2007). Epidemiological evidence suggests that depression is the most common psychological disorder associated with epilepsy, particularly in patients suffering from TLE (Harden, 2002; Harden and Goldstein, 2002; Kanner, 2006; Spencer, 2007). Limbic system dysfunction increases the risk for depression (Altshuler et al., 1999) and TLE has been linked to higher incidences of suicide ideation (Baker, 2006; Pompili et al., 2007) and memory impairment (Groticke et al., 2007). Currently, there is an urgency to develop animal models of epilepsy and depression co-morbidity, elucidate the underlying neurobiological mechanisms, and develop new treatments (Briellmann et al., 2007; Richardson et al., 2007; Groticke et al., 2008). In an effort to achieve these goals, we previously tested seizure susceptibility in rats selectively bred for sensitivity or resilience to depression-like phenotypes. We found that the depression-sensitive rats experienced greater kainic-induced seizure-induced mortality and had a shorter latency to clonic-tonic seizure following pilocarpine administration than their depression-resistant counterparts (Tabb et al., 2007; see Chapter 2). The goal of the present study was to further explore

the relationship between depression and TLE by examining spontaneous seizure activity following pilocarpine-induced epileptogenesis in these selectively bred rats.

3.3 Materials and Methods

Selectively bred rats. A subset of male rats from the experiments assessing latency to CT seizures following acute pilocarpine administration (Chapter 2) was used (SwLo line, *n* =9, SwHi line, *n* =6) in these epileptogenesis experiments. Animals were housed in ventilated racks (2 rats per cage) at the Emory University Briarcliff vivarium. The colony room was maintained on a 12 h light–dark cycle (lights on from 7 a.m. to 7 p.m.), and the rats received standard laboratory chow and water ad libitum. Animals were treated in accordance with the Guidelines for Animal Care and Use of the National Institutes of Health, and the Emory University Institutional Animal Care and Use Committee approved all experiments.

Pilocarpine seizure induction. Pilocarpine hydrochloride (minimum 99% titration; Sigma Aldrich; St. Louis, MO) was administered to rats as previously described (Borges et al, 2003; Raol et al., 2006), but with slight modifications. Rats were first injected with atropine bromide (N-methylatropinium bromide; VWR) (2 mg/kg, s.c.) to prevent the peripheral effects of pilocarpine. Thirty minutes later, rats were injected with pilocarpine hydrochloride (380 mg/kg, i.p.), and latency to clonic-tonic (CT) seizure was recorded. If a CT seizure was not observed in the first hour, rats were given booster injections of pilocarpine (190 mg/kg, i p.) every hour until a CT seizure was observed. The data for latency to CT seizure is shown in Chapter 2. Once a CT seizure occurred, rats continued to seize, which marked the beginning of status epilepticus (SE), defined as

continuous seizure activity. After 1 hour of SE, diazepam (5 mg/kg, i.p.; Hospira; Lake Forest, IL) was administered to terminate seizure activity, and booster doses (2.5 mg/kg, i.p.) were administered every hour if SE persisted. Animals were monitored for signs of malady and unresponsiveness for the first 24 hr after pilocarpine administration, and were given marshmallow certified supreme mini-treats (Bios-Serv; Frenchtown, NJ) and saline (1-2 mg/kg, s. c.) (Sigma Aldrich; St. Louis, MO), as needed.

Monitoring of Spontaneous Seizure Activity. Five weeks following pilocarpine administration, rats were videotaped for 24 hours using GeoVision Surveillance System V (Geovision Inc., Irvine, CA) software, and videotapes were scored for spontaneous seizure behaviors during the 12-hr dark cycle (i.e. 1800h–700h). During the dark cycle, the room was weakly illuminated by dim red light to allow video recording. TLE is known as a seizure disorder provoked by stress and acute stress can elicit changes in seizure frequency (Potschka et al., 2000). In addition, the SwLo and SwHi rats were selectively bred based on performance in the forced swim test, which is a form of acute stress. To determine whether stress affected the expression of spontaneous seizure activity in the rats, each rat was exposed to a 15-minute swim stress utilizing a modified version of Porsolt's forced swim test (Weiss et al., 1998) following the first 24 hr of videotaping. Directly following the swim stress, rats were placed back in their cages and videotaped for an additional 24 hr. Video was again scored for seizure activity during the dark cycle. Spontaneous seizure behavior was defined as any behavior not consistent with normal rat behavior (e. g. eating, drinking, grooming, locomoting). These behaviors included, but were not limited to myoclonic jerks, twitches, jumps, and wet

dog shakes. Spontaneous generalized seizures were defined as continuous clonic-tonic seizures.

Statistics. Student's t-tests were used for comparison between two groups with equal variance, and Mann–Whitney tests were used for comparison between two groups with unequal variance. GraphPad InStat (version 3.0) and Prism (version 4.0) software for Macintosh were used for all statistical analyses.

3.4 Results

The effect of pilocarpine-induced epileptogenesis on spontaneous seizure behaviors: To determine whether a genetic susceptibility to depression-related behavioral characteristics correlates with an increase in spontaneous seizures, we assessed seizure behaviors (i.e. tremors, myoclonic jerks, jumping, rearing and falling, tonic-clonic generalized seizures, etc) in SwLo and SwHi rats five weeks following pilocarpine-induced SE. As shown in Figure 3.1A, all rats in both lines displayed some seizure behaviors prior to the swim stress (Day 1), and the number of seizure-like events was significantly increased following the swim stress (Day 2) (Figure 3.1B). There were no differences in the total number of seizure behaviors observed between SwLo and SwHi rats for either time point. However, only rats of the SwLo line experienced spontaneous generalized convulsions (Figure 3.2A). During Day 1 of observation, 2/9 of the SwLo rats had generalized convulsions, and one animal had five generalized seizures in during the 12-hr period. On Day 2, 4/9 SwLo rats had at least one spontaneous generalized seizure. None of the 6 SwHi rats in the experiment had a generalized seizure on either day.

3.5 Discussion

Pilocarpine is a widely used and validated chemiconvulsant model of TLE in animals (Curia et al., 2008). It has been extensively utilized to determine pathological changes and gene expression patterns involved in epileptogenesis (Borges et al., 2003; Lee et al., 2006), and as a screening tool for anticonvulsant efficacy (Martin and Pozo, 2006). Currently, the pilocarpine model of TLE is being implemented as a way to evaluate the association of depression and TLE (Mazarati et al., 2008; Kondziella et al., 2007; Groticke et al., 2007). When administered systemically to rodents, pilocarpine causes SE acutely, which, after a quiescent incubation period, develops into epilepsy with spontaneous recurrent seizures (SRS) and hippocampal alterations that are reminiscent of hippocampal sclerosis in patients with TLE (Groticke et al., 2007). Pilocarpine-induced epileptogenesis results in synaptic organization, neuronal damage, and gliosis in hippocampal pyramidal neurons, similar to that observed in the kindling model of TLE (Curia et al., 2008) and produces epilepsy that in many ways is reminiscent of human TLE pathogenesis, although it is highly variable from animal to animal and is associated with high levels of mortality (Borges et al., 2003; Curia et al., 2008).

We observed no differences between SwLo and SwHi rats in total number of seizure behaviors, although we did find that a 15-minute swim stress increased seizure incidence over the following dark cycle. Our most significant finding was that some SwLo rats, which were bred for depression-susceptibility, had very severe spontaneous generalized seizures weeks following pilocarpine, while SwHi rats, which were bred for depression resilience, did not. Combined with our data showing that SwLo rats are also more sensitive to acutely-induced seizures (Tabb et al., 2007; Chapter 2), these results

further validate these rats as a model of epilepsy (particularly TLE) and depression co-morbidity. Our study does have some limitations. We only observed the animals during dark cycle phases because we reasoned that seizures would be more frequent when the rats were awake and active. However, it is possible that the SwHi rats were having generalized seizures during the light cycle. Another caveat is that because we scored all seizures behaviorally, we may have missed some mild seizures that could be detected only by EEG monitoring. An additional limitation to our experiment is the fact that the SwHi rats initially required more pilocarpine to achieve SE than their SwLo counterparts. The cumulative pilocarpine dose administered to the SwHi rats averaged ~507 mg/kg compared to 380 mg/kg for SwLo rats. Thus, the spontaneous seizure differences we observed may be underestimated.

Several groups have measured depressive-like behaviors in epileptic animals. Recently, Mazarati and colleagues examined whether rats were subjected to lithium chloride and pilocarpine-induced SE displayed behavioral or biochemical alterations consistent with depression. The development of chronic epileptic state was confirmed by the presence of spontaneous seizures and by enhanced brain excitability. Post-SE animals exhibited increase in immobility time in the forced swim test (FST) and a loss of taste preference in a saccharin solution consumption test, behaviors that are indicative of anhedonia and a despair-like state. The data also revealed compromised serotonergic transmission (impaired 5-HT concentration, turnover, and release) in the hippocampus. Administration of the selective serotonin reuptake inhibitor fluoxetine (20 mg/kg/day for 10 days) significantly shortened immobility time in the FST in naïve, but not post-SE animals, indicative of treatment-resistant depression-like behavior. The study by

Groticke and colleagues (2007) examined behavioral and cognitive alterations in mice following pilocarpine-induced SE. Surprisingly, these authors observed a decrease in depression-like behavior using the FST and tail suspension test following SE, although they did display cognitive impairment and an increase in anxiety-like behaviors. In a follow-up study, the same authors used a single unilateral injection of kainate into the dorsal hippocampus to induce nonconvulsive status epilepticus and the development of spontaneous seizures (Groticke et al., 2008). Similar to the results with the pilocarpine-treated mice, a decrease in depression-like behavior was observed in the FST, although this model was not anxiogenic. Finally, the induction of epileptogenesis in immature rats has been shown to result in depressive-like behaviors later in life (Koh et al., 2007; Mazarati et al., 2007).

Although the aforementioned studies examine the role of depression and TLE, there are some differences when compared to the rationale and findings of our study. The main difference is that these other groups induced an epilepsy-like state and then assessed depressive behaviors. Because pre-existing depression is a risk factor for developing epilepsy, we took the opposite approach by examining seizure susceptibility and epileptogenesis in rats with a pre-existing depressive-like state. One advantage to utilizing selective bred animals in our experiments is that we have employed a heritable model of depression in our pursuit to understand the relationship between depression and TLE. This is a salient feature when one considers many individuals diagnosed with depression have a family history of the disease. Also, some of the epileptic rodents noted above displayed less depressive-like behavior, which is not consistent with the clinical co-morbidity under consideration.

Depression is a relatively frequent co-morbid psychiatric disorder in chronic epilepsy, especially, TLE (Richardson et al., 2007). The data of the present study supports similar findings that TLE and depression may have a causal relationship (Kuhn et al., 2003; Groticke et al., 2007; Kondziella et al., 2007). The relationship between epilepsy and depression continue to be a topic of great interest. Recently, a panel of expert psychiatrists, neurologists, social workers, and psychologists assembled to bring recognition to the plight of people with epilepsy suffering from co-morbid psychiatric disorders, specifically depression. They published a Consensus Statement describing the clinical manifestations of the co-morbidity and developed psychometric tools for diagnosis and a stepwise algorithmic approach to treatment (Barry et al., 2008). Clearly, there is still a need to further investigate mood disorders affecting epileptic patients. Our animal model may be useful for determining the mechanism underlying the relationship between the two diseases, particularly the genetic influences, and for screening novel treatment strategies.

Figures

Figure 3.1 The effect of pilocapine-induced epileptogenesis on spontaneous seizure behavior.

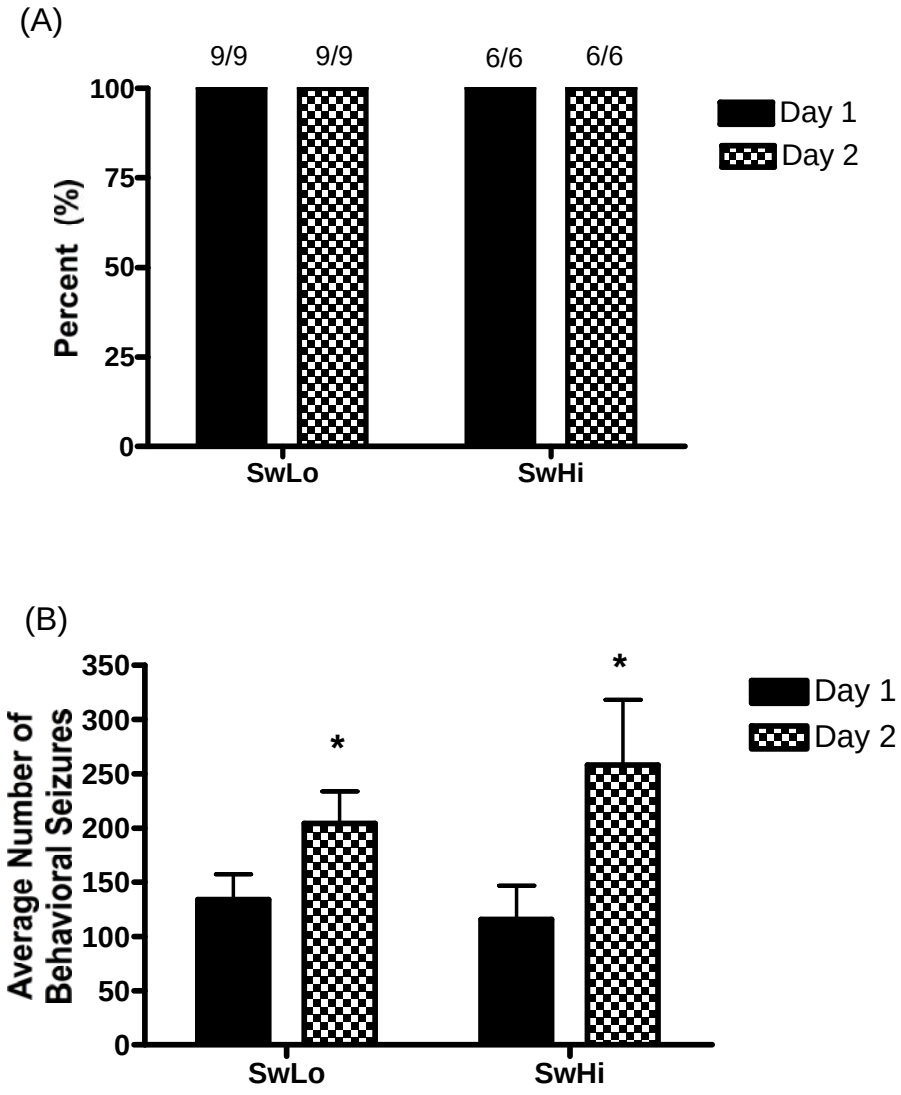

Figure 3.1 The effect of pilocapine-induced epileptogenesis on spontaneous seizure behavior. Spontaneous seizure behavior was defined as any behavior not consistent with normal rat behavior (e.g. eating, drinking, grooming, locomoting, etc). These behaviors included myoclonic jerks, twitches, jumps, and wet dog shakes. Shown is (A) the percent of SwLo and SwHi rats displaying seizure behaviors (number of rats displaying seizure behaviors over the total number of rats tested is shown over each bar), and (B) the mean ± SEM seizure behaviors in SwLo and SwHi rats during the 12 hour dark cycle prior to (Day 1) or following (Day 2) a 15 min swim stress (n = 6-9). *$p < 0.05$, Day 1 compared to Day 2. Day accounts for 25% of total variance.

Figure 3.2 The effect of pilocarpine seizure induction on the development of spontaneous generalized seizures.

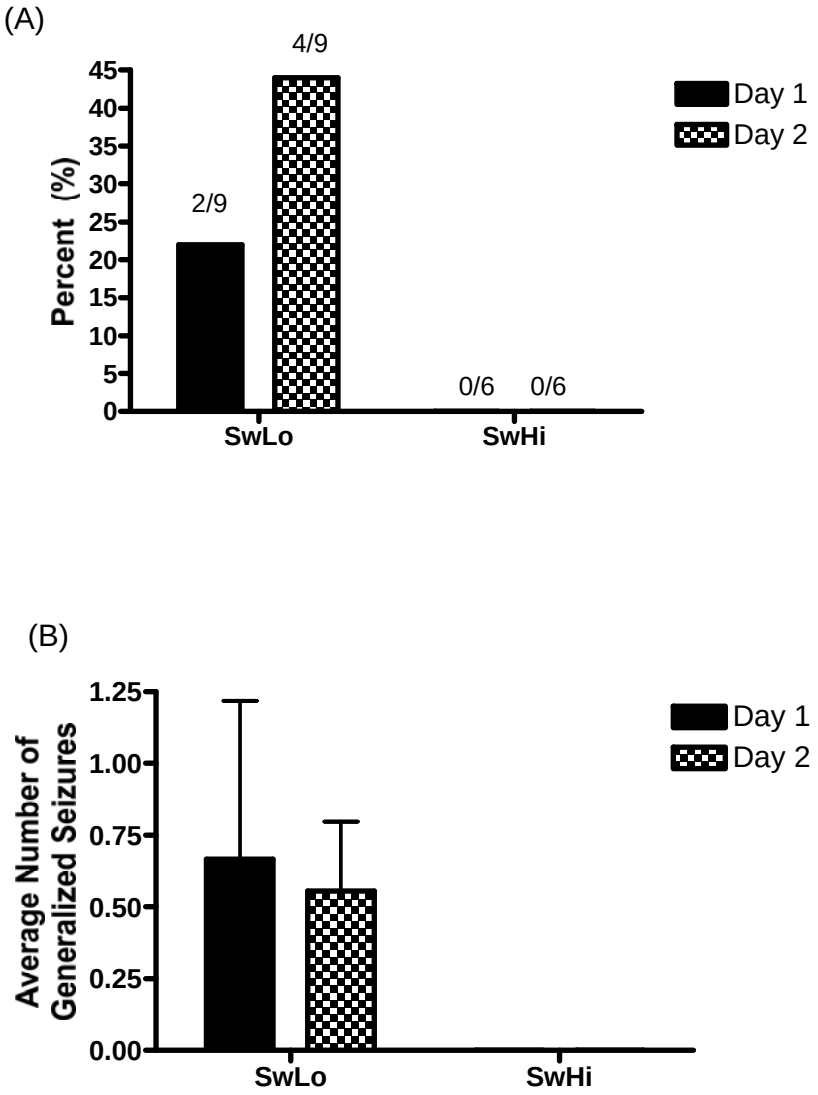

Figure 3.2 The effect of pilocarpine seizure induction on the development of spontaneous generalized seizures. Spontaneous generalized seizures were defined as continuous CT seizures. Shown is (A) the percent of SwLo and SwHi displaying seizure behaviors (number of rats displaying generalized seizures over the total number of rats tested is shown over each bar), and (B) the mean ± SEM generalized seizures in SwLo and SwHi rats during the 12 hour dark cycle prior to (Day 1) or following (Day 2) a 15 minute swim stress.

CHAPTER 4:

THE KETOGENIC DIET: A POSSIBLE TREATMENT FOR PATIENTS WITH EPILEPSY SUFFERING FROM DEPRESSION

Adapted from:

Tabb, K., P. Szot, et al. (2004). "The ketogenic diet does not alter brain expression of orexigenic neuropeptides. " Epilepsy Res **62**(1): 35-9.

4.1 Abstract

The ketogenic diet (KD) is a high fat, low protein, and low carbohydrate diet that is widely used treatment for refractory epilepsy and has been successfully useful in treating patients with various epilepsy syndromes. Recently, there has been emerging evidence suggesting the possibility of prescribing the KD to treat other neurological and psychological diseases, even depression. It is known that treating epileptics diagnosed with depression can often be a difficult feat given that some traditional antidepressants have been shown to exacerbate seizure activity. Although the anticonvulsant mechanism of the KD is still being elucidated, there is speculation that the KD may possess mood-stabilizing properties. In an effort to further explore the mechanistic properties of the KD, we examined neuropeptide Y (NPY) and galanin as possible targets mediating its anticovulsant effects. NPY and galanin are neuropeptides that are regulated by energy states, possess anticonvulsant activity, and may regulate mood. Specifically, we tested the hypothesis that the anticonvulsant efficacy of the KD is mediated by increased expression of NPY and galanin via alterations in food intake and energy metabolism. In situ hybridization experiments revealed no effect of the KD on NPY or galanin mRNA expression, suggesting that increased expression of NPY and galanin do not contribute to the anticonvulsant effect of the KD. Although these results did not elucidate the mechanism of KD efficacy, the KD may still prove to be a feasible treatment option for epilepsy patients suffering from depression.

4.2 Introduction

The ketogenic diet (KD) is a high fat, low protein, and low carbohydrate diet shown to be effective in treating refractory childhood epilepsy, although its anticonvulsant mechanism is still unknown (Vining, 2002; Thiele, 2003). The KD was first introduced in the 1950's and its implementation slowly declined through the years. Recently, the KD has regained popularity as viable treatment option for adults and children suffering from intractable epilepsy. The KD is capable of providing anticonvulsant relief with an efficacy that rivals newer antiepileptic drugs (LeFevre et al., 2000). There are several animal studies that have assessed the efficacy of the diet and found that the KD is effective in a variety of seizure paradigms comparable to clinical correlates (Table 4.1) (Stafstrom et al., 2004).

Although the KD has been used to treat epilepsy, there is evidence it may be useful in treating neurodegenerative diseases such as Parkinson's disease, (Vanitallie et al., 2005), Alzheimer's disease (Van der Auwerea et al., 2005) and it has been shown to inhibit tumor growth in a mouse model of astrocytoma (Seyfried et al., 2003). More importantly for the present discussion, it has been speculated that the KD may provide some relief to patients suffering from psychiatric disorders including depression and bipolar disorder (El-Mallakah and Paskitti, 2001; Murphy et al., 2004; Freeman et al., 2007). This hypothesis was based on the observation that there are several "alternative" anticonvulsant interventions being used to treat depression (e.g. electroconvulsive therapy [ECT]), vagus nerve stimulation [VNS]). In addition, the KD has efficacy in the forced swim test, a rodent model of depression and antidepressant drug efficacy (Murphy et al., 2004).

A hallmark feature of the KD treatment is the production of the ketone bodies: acetoacetate, β-hydroxybutyrate, and acetone (Bough and Rho, 2007; see Figure 4.1). Ketones provide an alternate substrate to glucose for energy utilization and can cross the blood brain barrier during fasting or KD administration. Because the KD causes a shift from the use of glucose to fats as a primary energy source, it has been hypothesized that changes in energy balance may underlie the anticonvulsant effect of the KD (Schwartzkroin, 1999; Sheth and Stafstrom, 2002; Vining, 2002; Greene et al., 2003). Therefore, the ideal candidate to mediate the anticonvulsant effects of the KD would link energy balance and seizure susceptibility. The expression of NPY and galanin are regulated by nutritional status. Under conditions of satiety (high glucose, insulin, and leptin), NPY and galanin expression is suppressed, while starvation conditions (low glucose, insulin, and leptin) induce the expression of these neuropeptides (Williams et al., 2001; Gundlach, 2002). Galanin expression is also enhanced by high-fat intake (Leibowitz et al., 1998). Genetic, pharmacological, and physiological studies have demonstrated that NPY and galanin have potent anticonvulsant activity (Vezzani et al., 1999; Mazarati et al., 2001).

The KD was developed to mimic the effects of starvation on epilepsy, and decreases circulating glucose, insulin, and leptin levels while maintaining high fat intake (Sankar and Sotero de Menezes, 1999; Fraser et al., 2000; Vining, 2002; Greene et al., 2003). The KD may therefore increase the expression of NPY and galanin, leading to an anticonvulsant effect. An increase in NPY and galanin expression may also have a positive effect on mood. Both NPY and galanin are co-localized with the monoamine neurotransmitters norepinephrine (NE) and serotonin (5-HT), which have been identified

as key players in depression (Sheline et al., 1998; Gilliam et al., 2004). Secondly, both neurotransmitters appear to have antidepressant/anxiolytic-like activity under certain conditions. Galanin, acting via the GalR2 receptor, attenuates depression-like behavior, although it is important to note that the GalR1 and GalR3 receptors appear to exacerbate depression-like behavior (Kuteeva et al., 2008). NPY also has potent antidepressant and anxiolytic properties (Heilig, 2004; Eaton et al., 2007). Ascertaining how the KD provides this anticonvulsant effect may concomitantly reveal the mechanisms responsible for the KD's antidepressant properties.

In the present study, we assessed the role of NPY and galanin in providing the anticonvulsant effect of the KD. We tested this hypothesis by measuring the expression of NPY and galanin by in situ hybridization in various brain regions in mice fed a normal diet (ND) or a KD. Ascertaining how the KD provides this anticonvulsant effect may concomitantly reveal the mechanisms responsible for the KD's antidepressant properties.

4.3 Materials and Methods

Mice: Wild-type C57BL6/J mice were generated from a breeding pair of wild-type control mice from the norepinephrine transporter knockout colony of Marc Caron (Duke University). Mice were bred and maintained in a specific pathogen free facility with a 12-h light/12-h dark cycle at Emory University. Adult (3–4 months) male mice were used for the in situ hybridization experiment, and both male and female mice were used for the flurothyl seizure susceptibility experiment. No differences were found between males and females, and results were combined. Animals were treated in accordance with the Guidelines for Animal Care and Use of the National Institutes of

Health (NIH), and the Emory University Institutional Animal Care and Use Committee (IUCAC) approved all experimental protocols.

Administration of the ketogenic diet: Mice were fasted overnight and were then fed standard rodent chow or a ketogenic diet (KD; Harlan Teklad TD 96355; 4. 3:1 [fat]:[carbohydrate + protein] ratio) for 2 weeks. One cohort from each diet group was euthanized by CO2 asphyxiation, and brains were removed, frozen on dry ice, and processed for in situ hybridization. Another cohort from each diet group was tested for flurothyl-induced seizure susceptibility. All experiments were performed during the light cycle between 10:00 and 14:00.

Flurothyl-induced Seizures: Mice were placed individually in an airtight Plexiglas chamber, and flurothyl (bis-2,2,2-trifluroethyl ether, Sigma-Aldrich) was infused at a rate of 20 μl/min onto filter paper from which it vaporized. Latency to first myoclonic jerk (MJ) and generalized clonic-tonic (CT) seizure was used as a measure of seizure threshold, while the progression to tonic extension was used as a measure of seizure severity.

β-Hydroxybutyrate blood levels: Trunk blood levels of β-hydroxybutyrate (BHB) were measured from all animals used in the in situ hybridization experiment and a subset of animals used in the seizure susceptibility experiment using the Precision Xtra system and ketone strips (MediSense).

In situ hybridization: Eighteen-micrometer of coronal brain sections were cut on a cryostat and mounted onto Superfrost slides (Fisher Scientific). Slides were postfixed in 4% paraformaldehyde and washed in phosphate-buffered saline. The slides were then treated with acetic anhydride (0.25% in 0.1 M triethanolamine), dehydrated in graded

series of alcohol (70, 95, and 100%), delipidated in chloroform, slightly rehydrated (100 and 95% alcohol), and air-dried. In situ hybridization was performed as described (Szot et al., 1997). The NPY oligonucleotide probe was a 51-base probe complementary to nucleotides 1671–1722 of the NPY mRNA (Larhammar et al., 1987). The galanin oligonucleotide probe was a 51-base probe complementary to nucleotides 155–206 of the galanin mRNA (Vrontakis et al., 1987). Briefly, [33P]dATP labeled probes were applied to the tissue with silanized coverslips, placed in a moist chamber, and incubated overnight at 37 °C. Sections were washed in 1× SSC at 65 °C and dehydrated through a graded series of alcohol containing 300 mM ammonium acetate. Hyperfilm (Amersham) was exposed to slides for 5 days, and the films were developed in Kodak D-19 developer, rinsed in water, and fixed in Kodak Rapid Fix. Optical densities were obtained from films using the MicroComputer Imaging Device (MCID; Imaging Research Inc., Ont., Canada). Separate optical density measurements were made on the left and right sides of at least three anatomically matched sections (Paxinos and Franklin, 1997). For each animal and in each region studied, an average of six optical density readings after background subtraction were used to generate a mean ± S.E.M.

Statistics: Statistical analysis and graphing were performed using Graphpad Instat and Prism.

4.4 Results

The ketogenic diet is anticonvulsant in mice: As previously reported, the KD produced a modest but significant anticonvulsant effect (Szot et al., 2001); KD-fed mice had a longer latency to both first MJ and generalized CT seizure (Fig. 4.2). Seizure

severity was also reduced in KD-fed mice; 10/10 ND-fed mice progressed to tonic extension, while 6/11 KD-fed mice experienced seizures of that severity ($p < 0.05$ by Fisher's Exact Test). Administration of the KD-induced ketosis compared to the ND group (BHB levels: ND 0.27 ± 0.03 mM, KD 1.08 ± 0. 11 mM, $p < 0.001$ by Mann–Whitney U-test). This level of ketosis is somewhat lower than that seen during successful seizure control by the KD in humans (not, vert, similar2–6 mM; Huttenlocher, 1976 and Gilbert et al., 2000), but typical for an anticonvulsant effect in mice (Szot et al., 2001).

The ketogenic diet does not alter brain expression of NPY or galanin: To determine whether the anticonvulsant effect of the KD was associated with increases in the expression of NPY or galanin, hybridization of antisense probes to NPY (Fig. 4.3A) and galanin (Fig. 4.3B) was assessed in brain sections from ND- and KD-fed mice. There was a main effect of brain region for both neuropeptides (NPY: $F(5, 48) = 208.\ 49$, $p< 0.0001$; galanin: $F(4, 39) = 9.\ 8$, $p < 0.0001$), but no main effect was found for diet. The KD did not increase the expression of either neuropeptide, although there was a trend towards reduced NPY expression in KD-fed mice (NPY: $F(1, 48) = 3.\ 82$, $P = 0.\ 06$; galanin: $F(1, 39) = 0.\ 14$, $P = 0.\ 71$.

4.5 Discussion

It is believed that the shift from carbohydrate to fat as an energy source is the source of the KD's anticonvulsant effect. Our study set out to further elucidate the clinical mechanism of action of the KD by assessing the role of NPY and galanin. Because NPY and galanin are regulated by nutritional status and energy balance and are

anticonvulsant, we hypothesized that the KD might enhance the expression of these anticonvulsant neuropeptides. We found no effect of the KD on NPY or galanin mRNA in the brain of mice despite a significant anticonvulsant effect of the KD. Although the starvation-like conditions of the KD have been reported to result in low insulin and leptin levels, which in theory should increase NPY and galanin expression, the KD is inherently different from true starvation in that exogenous rather than endogenous fuels are being consumed for energy. This distinction may manifest as a different response of central satiety systems in which NPY and galanin expression are not up-regulated. Another important element of these findings is that the KD does not significantly affect the expression of two major brain neuropeptides involved in weight regulation and energy balance, which supports the clinical safety of the KD.

Because a high-fat diet increases galanin expression in the paraventricular nucleus of the hypothalamus (PVN; Leibowitz et al., 1998), it is somewhat surprising that the KD did not mimic this effect, although our study differed in species (mice versus rats), length of diet exposure (2 weeks versus 4 weeks), and diet composition (4.3:1 versus 1.7:1 [fat]:[carbohydrate + protein] ratio). Any one or a combination of these differences could account for the failure of the KD to increase PVN galanin expression in our study.

Although these results do not support our hypothesis, we cannot exclude a role for NPY and galanin in the anticonvulsant effect of the KD. Low insulin and leptin levels enhance both the expression of NPY and galanin and the firing properties of NPYergic and galaninergic neurons (Williams et al., 2001; Gundlach, 2002). Therefore, the KD could increase NPY and galanin release while leaving expression levels unaffected.

Future experiments investigating a role for NPY and galanin in the anticonvulsant effect of the KD could include quantification of neuropeptide levels in the brains of KD-fed animals and assessment of the anticonvulsant effect of the KD in NPY or galanin knockout mice.

Although ADs are commonly prescribed to treat depression, physicians will often suggest alternative treatment methods for patients refractory to drug treatment. ECT and VNS are two neurothereupeutic methods currently being used to treat people experiencing treatment-resistant depression. These techniques employs the use of electrode placements to send electrical impulses to the brain (ECT) and vagus nerve (VNS), eliciting a change in neurotransmission and ultimately alleviating depression symptoms in 30-50 % of depressed patients (Prudic et al., 2004; Milby et al., 2008). While ECT and VNS are deemed to be successful treatments, they are quite invasive, and public often views them as harsh and inhumane. Alternative methods for treating refractory depression, such as the KD, could offer a less invasive way to treat depression that is just as efficacious. Consumers are becoming more interested in natural and holistic therapies, and the effect of nutrition on mental health states is currently being investigated (Lakhan and Vieira, 2008). Understanding how nutritional supplementation plays a role in mood disorders may lead to novel ways to treat them and also bring relief to patients plagued by the adverse side effects of conventional drug therapy.

Treating patients with epilepsy and depression co-morbidity has been challenging for physicians. Many traditional antiepileptic medications can exacerbate depressive symptoms in epilepsy patients (Levinson et al., 1999; Mula et al., 2003). Conversely, pharmacological treatment of depression and other psychological disorders in epilepsy

patients can often worsen seizure occurrence and severity (Curran et al., 1998; Kanner et al., 2000). The use of the KD for the treatment of epilepsy and depression co-morbidity warrants further mechanistic and clinical investigation. Although we did not assess depression-like phenotypes in the present study, we did not observe an effect on galanin or NPY expression, suggesting that these neuropeptides do not play a primary role in KD efficacy. The KD does appear to increase NE in the brain (P. Szot and D. Weinshenker, unpublished data), which in theory could underlie, at least in part, both anticonvulsant and antidepressant properties. Interestingly, an Atkins diet, which has some similarities to the KD, not only reduced seizure frequency in some patients, but also improved mood in a pilot trial (Carrette et al., 2008). It would be interesting to further assess the ability of the KD to alleviate both depressive- and epilepsy-like phenotypes in animal models of co-morbidity (e.g. Tabb et al., 2007; see Chapter 2) and to alleviate depression in patients suffering from both diseases.

Figures

Figure 4.1 Production of ketone bodies in (Bough and Rho, 2007).

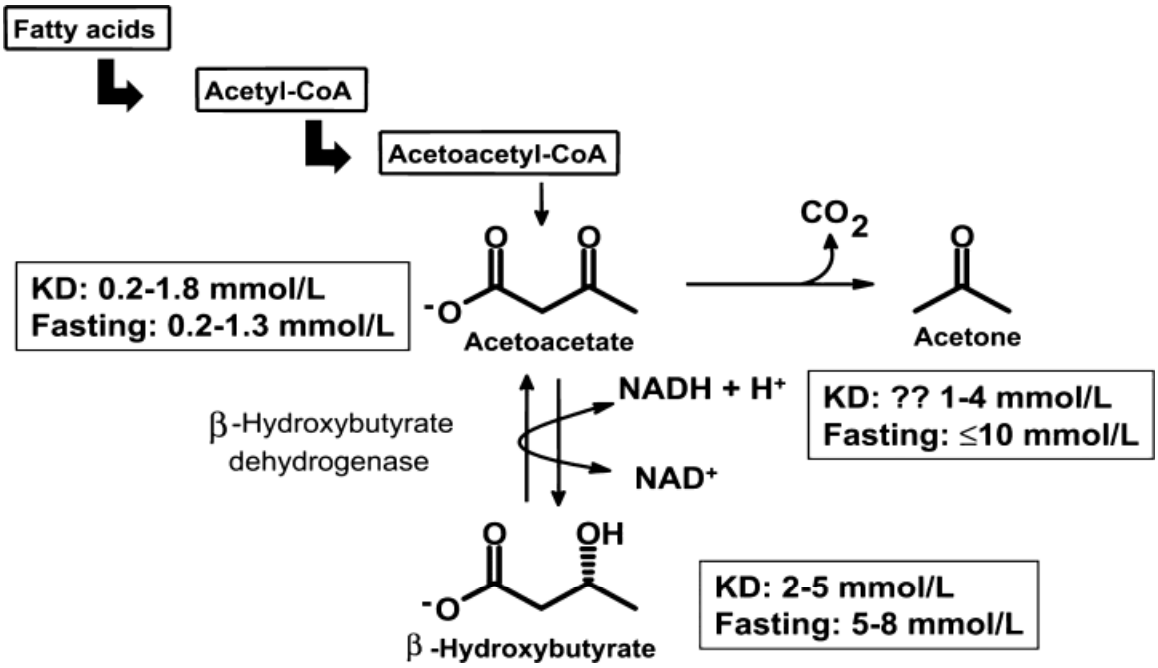

Figure 4.1 Production of ketone bodies. Metabolic pathways highlighting the production of ketone bodies fatty acids during fasting or treatment with the ketogenic diet (KD). Estimated fasting- or KD-induced concentrations of beta-hydroxybutyrate, acetoacetate, and acetone in blood are listed (large boxes). Measures of beta-hydroxybutyrate levels in blood are most commonly used as the clinical indicator of successful KD treatment.

Figure 4.2 The ketogenic diet is anticonvulsant in mice.

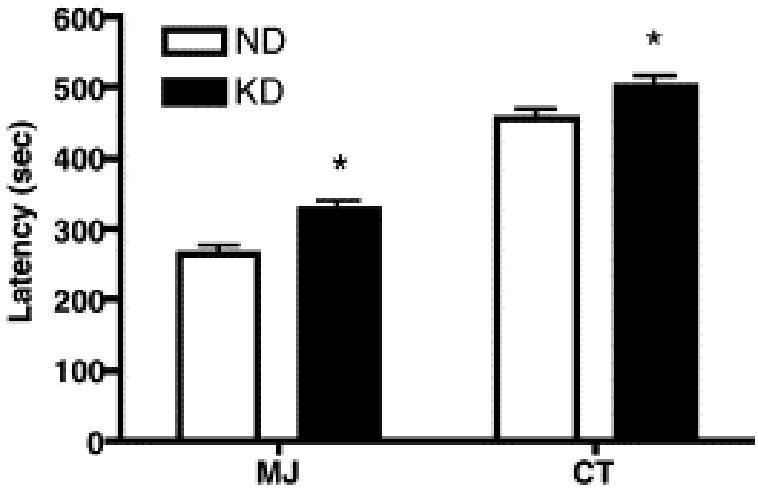

Figure 4.2 The ketogenic diet is anticonvulsant in mice. The anticonvulsant effect of the KD against flurothyl-induced seizures. Flurothyl-induced seizure susceptibility was assessed in C57BL6/J mice fed on a normal diet (ND; $n = 10$) or a ketogenic diet (KD; $n = 11$). Shown are the latencies (mean and S.E.M.) to first myoclonic jerk (MJ) and generalized clonic-tonic seizure (CT). * $p < 0.05$ compared to the ND control (t-test).

Figure 4.3 The ketogenic diet does not alter brain expression of NPY or galanin.

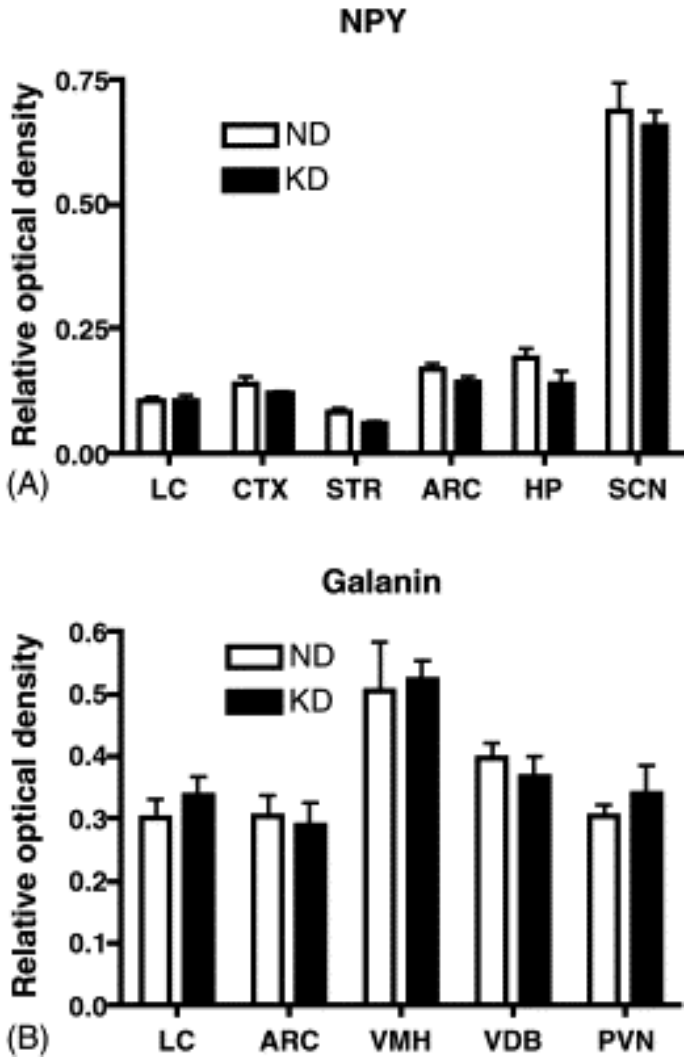

Figure 4.3 The ketogenic diet does not alter brain expression of NPY or galanin.

The KD has no effect on NPY or galanin expression. (A) NPY and (B) galanin mRNA expression was assessed in mice fed a normal diet (ND; $n = 5$) or a ketogenic diet (KD; $n = 5$) by in situ hybridization. Shown is the relative optical density (the hybridization densities minus background; mean and S.E.M.) for each brain region examined. LC: locus coeruleus, CTX: cortex, STR: striatum, ARC: arcuate hypothalamic nucleus, HP: hippocampus, SCN: superchiasmatic nucleus, VMH: ventral medial hypothalamus, VDB: ventral diagonal band, and PVN: paraventricular nucleus of the hypothalamus.

Tables

Table 4.1 Animal models of the ketogenic diet: observations and clinical correlates

	Observation in animal models	Clinical correlate
Age relationship	Younger animals respond better to KD	Children extract and utilize ketones from blood better than older individuals
Diet type	Classic and MCT diets both increase seizure threshold	In patients, classic and MCT KDs are equally efficacious, but the MCT has more gastrointestinal side effects, which limits its clinical usefulness
Latency to KD effectiveness	There is a latency period of several days to KD effect in animals	During the pre-KD fast, seizure reduction is often noted; in other children, there is a latency of days to weeks before seizures decrease
Ketosis required for anticonvulsant effect?	Unclear whether relationship is causal; 'ketosis necessary but not sufficient'	Unclear whether relationship is causal; 'ketosis necessary but not sufficient'
Reversal of protective effect when KD is discontinued	Rapid (as soon as ketosis lost?) in animals	Children who lose ketosis have a rapid return of seizures and EEG abnormalities (Huttenlocher, 1976)
Seizure type	KD is effective in models employing a wide variety of seizure paradigms	KD is effective in a wide variety of seizure types and epilepsy syndromes; the diet is clearly indicated in certain conditions, such as glucose transporter deficiency (De-Vivo et al., 1991)

Adapted from Stafstrom CE, et al., 2003
KD, ketogenic diet; MCTs, medium-chain triglycerides; PUFA, polyunsaturated fatty acid.

Table 4.1 Animal models of the ketogenic diet: observations and clinical correlates.

CHAPTER 5:

FUTURE DIRECTIONS

Understanding the neurobiological mechanisms of depression and epilepsy co-morbidity will ultimately provide more comprehensive diagnoses and lead to better treatment therapies for persons afflicted with both disease states. Traditionally, the diagnosis and treatment of seizure disorders revolve primarily around achieving good seizure control, but in recent years attention has also been drawn to the importance of psychosocial adjustment in patient management and their quality of life (Polisher et al., 2006). However, with the increased cost of living and severely diminished reimbursements for non-procedure patient encounters, having a dedicated psychiatrist integrated into an epilepsy program is considered a luxury (Ettinger, 2004), thus it becomes imperative that neurologists are aware of psychiatric conditions affecting their patients.

In addition, it must be recognized that the co-morbidity of depression and epilepsy is not simply a psychosocial reaction to having a chronic debilitating disorder, but is rather a complex compilation of biological, seizure, and medication-related influences, and deserving of special attention when evaluating a patient with epilepsy (Ettinger, 2004). The evidence presented is this dissertation supports the existence of common, possibly genetic, pathogenic mechanisms operant in both conditions facilitating the development of one disorder in the presence of the other**.**

It seems fairly intuitive for physicians and researchers in the epilepsy field to recognize and make haste to resolve the psychiatric burdens that accompany living with chronic seizure disorders. In 2008, the Epilepsy Foundation as part of its Mood Disorder Initiative developed a consensus statement. This expert panel provided insight into the plight of epileptics suffering from affective disorders using depression literature as well

as epilepsy-specific studies (Barry et al., 2008). In an attempt to increase and improve the recognition of affective disorders in people with epilepsy continuing clinical and experimental investigations of the neurobiological correlates responsible for depression and epilepsy co-morbidity are still necessary. The development of novel therapies will continue to rely on basic research and the innovative animal modeling of these disorders; the selectively bred rats described in this document could be an invaluable tool for the further study of the mechanisms underlying epilepsy and depression co-morbidity and its future treatment.

Establishment and validation of animal models of co-morbidity between depression and epilepsy is instrumental for both understanding the mechanisms of the condition, and for preclinical development of effective therapies (Mazarati et al., 2008). Conferring the importance of model validity is critical for discussion of creating an animal model of epilepsy and depression co-morbidity. Animal model validity criteria are a set of guidelines researchers use to adequately assess how well an animal model correlates to the clinical manifestations of a human occurrence of the disease. The guidelines include construct validity, etiological validity, face validity, and predictive validity. Construct validity is defined as the extent to which the model represents the true nature of the disease including the cognitive and neurobiological aspects. The extent to which the same conditions or agents that cause the disease in humans also cause it in the model is known as etiological validity. Face validity is the extent to which abnormalities occurring in the model resemble those occurring in the disease. Predictive validity describes the extent to which effective clinical therapies are also effective in the model. Our proposed animal model of depression and epilepsy co-morbidity best meets

some criteria for etiological and predictive validity, while face and construct validity is weak in some cases, and in others will require further investigation.

Since the depression that manifests in patients with epilepsy has a significant genetic component, etiological validity is met because of the genetic heritability of depressive and seizure-susceptible phenotypes that occur in the selectively bred rats. Each line has been bred for a particular behavioral phenotype (i.e. SwLO, increased immobility in the FST) that has been recapitulated for generations. In addition, cross-fostering studies have demonstrated that the genotype of the rat being tested, and not environmental influences such as differences in maternal care, produces most of the behavioral differences under study (J.Weiss, personal communication). Although we do not yet know the exact nature of the genetic contribution to traits in these rats, our lab has recently begun to further address the question of etiological validity by identifying genes that are responsible for both depression-like and seizure susceptible phenotypes in the Weiss inbred rat lines. Proposed experiments will use quantitative trait loci (QTL) analysis to discover chromosomal regions responsible for depression-like behaviors and seizure susceptibility, followed by expression microarray analysis to identify which genes are differentially expressed between lines and located on chromosomal regions important to both behaviors. Some candidate genes that show differential hippocampal expression between SwLo and SwHi rats (e.g. GABA receptors) are promising, as they have been implicated in both epilepsy and depression (A. Epps, personal communication). After candidate genes have been identified, the next step will be to experimentally test their contribution in vivo. For example, suppose we find a gene that maps to a chromosomal region associated with low activity in the forced swim test (FST) and seizure

susceptibility that shows lower expression in SwLo rats compared to SwHi rats. One approach would be to rescue the SwLo phenotypes by pharmacological or genetic activation/over expression of the target gene in SwLo animals, and to conversely induce a SwLo-like phenotype by antagonism/knockdown of the target gene in SwHi animals. It will also be interesting to compare our list of candidate genes with those generated by other groups in various models of epilepsy and depression.

Aside from the genetic component, we still do not know most of the factors that cause epilepsy and depression. Certainly humans do not develop depression after being forced to swim. Inasmuch as the FST is probably stressful to a rat, childhood trauma and neglect combined with other stressors later in life likely contribute to depressive episodes (e.g. Nemeroff, 2007). Symptomatic epilepsy is caused by factors such as brain malformation and severe head trauma, which our rats were never exposed to. Idiopathic/cryptogenic epilepsy is thought to be caused, at least in part, by mutations/polymorphisms in multiple susceptibility genes, which could also underlie the seizure susceptibility in our rats.

In terms of antidepressant treatments, the predictive validity of the selectively bred rats is well established and quite good. Most clinically effective antidepressant therapies increase struggling and decrease floating in the FST in SwLo and SUS rats, while many of the false positives observed in the classic FST (e.g. amphetamine, caffeine) are not effective (West and Weiss, 1998; 2005). The clinical time course of antidepressant efficacy is typically on the order of weeks. While acute antidepressant administration shows activity in the congenital FST in normal animals, only chronic (a~ 2 weeks) administration of antidepressant drugs rescues the behavioral phenotype of SwLo

and SUS rats (West and Weiss, 1998; 2005). The predictive validity of these rats as it pertains to seizure susceptibility is unknown. In a pilot experiment, we found that chronic antidepressant administration did not rescue the increased kainic acid-induced mortality in the SwLo rats. It would be very useful and informative to test the effect of several classes of antidepressants and on other seizure phenotypes (e.g. acute pilocarpine-induced seizure sensitivity), as well as testing the effect of anticonvulsant therapies on swim test activity and seizure susceptibility. As discussed in Chapter 4, non-drug therapies, such as the ketogenic diet, might be effective in the treatment of both phenotypes while lacking the confounding side effects of many canonical antidepressants and anticonvulsants.

Our animal model is very weak in causative or construct validity. Construct validity revolves around how well does the factor, which induces the modeled behavior, correspond to current pathophysiological theories of the modeled disease (Kondziella et al., 2007). Is often an impossible feat to mimic the pathology of a disease in an animal due to scientists limited knowledge of the underlying causes of epilepsy and depression co-morbidity. Improving construct validity in our model will heavily rely on future studies further elucidating the neurochemical abnormalities and biochemical markers responsible for depression and epilepsy co-morbidity. Recent, imaging studies have revealed 5-HT and the 5-HT receptors (Witkin et al., 2007) and hippocampal activation (Richardson et al., 2007) as potential links between the two disorders.

Co-expression of epilepsy and depressive-like behaviors is an understudied area of epilepsy. My findings along with future projects provide empirical evidence that epilepsy and depression co-morbidity exist in rodents. Expanding on our rodent model

of epilepsy and depression will allow testing of novel anticonvulsants and antidepressants in a co-morbid model of epilepsy and depression eventually leading to increased quality of life for patients afflicted with both disorders.

REFERENCES

Alldredge, B. K. (1999). "Seizure risk associated with psychotropic drugs: clinical and pharmacokinetic considerations." Neurology 53(5 Suppl 2): S68-75.

Altshuler, L., R. Rausch, et al. (1999). "Temporal lobe epilepsy, temporal lobectomy, and major depression." J Neuropsychiatry Clin Neurosci 11(4): 436-43.

Baker, G. A. (2006). "Depression and suicide in adolescents with epilepsy." Neurology 66(6 Suppl 3): S5-12.

Barry, J. J. (2003). "The recognition and management of mood disorders as a comorbidity of epilepsy." Epilepsia 44 Suppl 4: 30-40.

Barry, J. J., A. B. Ettinger, et al. (2008). "Consensus statement: the evaluation and treatment of people with epilepsy and affective disorders." Epilepsy Behav 13 Suppl 1: S1-29.

Bertram, E. (2007). "The relevance of kindling for human epilepsy." Epilepsia 48 Suppl 2: 65-74.

Borges, K., M. Gearing, et al. (2003). "Neuronal and glial pathological changes during epileptogenesis in the mouse pilocarpine model." Exp Neurol 182(1): 21-34.

Bough, K. J. and J. M. Rho (2007). "Anticonvulsant mechanisms of the ketogenic diet." Epilepsia 48(1): 43-58.

Brent, D. A. (1986). "Overrepresentation of epileptics in a consecutive series of suicide attempters seen at a children's hospital, 1978-1983." J Am Acad Child Psychiatry 25(2): 242-6.

Brent, D. A., P. K. Crumrine, et al. (1987). "Phenobarbital treatment and major depressive disorder in children with epilepsy." Pediatrics 80(6): 909-17.

Briellmann, R. S., M. J. Hopwood, et al. (2007). "Major depression in temporal lobe epilepsy with hippocampal sclerosis: clinical and imaging correlates." J Neurol Neurosurg Psychiatry 78(11): 1226-30.

Campbell, S. and G. Macqueen (2004). "The role of the hippocampus in the pathophysiology of major depression." J Psychiatry Neurosci 29(6): 417-26.

Carrette, E., K. Vonck, et al. (2008). "A pilot trial with modified Atkins' diet in adult patients with refractory epilepsy." Clin Neurol Neurosurg.

Conto, M. B., J. G. de Carvalho, et al. (2005). "Behavioral differences between subgroups of rats with high and low threshold to clonic convulsions induced by DMCM, a benzodiazepine inverse agonist." Pharmacol Biochem Behav 82(3): 417-26.

Cryan, J. F. and C. Mombereau (2004). "In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice." Mol Psychiatry 9(4): 326-57.

Cryan, J. F. and D. A. Slattery (2007). "Animal models of mood disorders: Recent developments." Curr Opin Psychiatry 20(1): 1-7.

Curia, G., D. Longo, et al. (2008). "The pilocarpine model of temporal lobe epilepsy." J Neurosci Methods 172(2): 143-57.

Curran, S. and K. de Pauw (1998). "Selecting an antidepressant for use in a patient with epilepsy. Safety considerations." Drug Saf 18(2): 125-33.

Dailey, J. W., C. E. Reigel, et al. (1989). "Neurobiology of seizure predisposition in the genetically epilepsy-prone rat." Epilepsy Res 3(1): 3-17.

Drevets, W. C. (2000). "Neuroimaging studies of mood disorders." Biol Psychiatry 48(8): 813-29.

Dunlop, B. W. and C. B. Nemeroff (2007). "The role of dopamine in the pathophysiology of depression." Arch Gen Psychiatry 64(3): 327-37.

Eaton, K., F. R. Sallee, et al. (2007). "Relevance of neuropeptide Y (NPY) in psychiatry." Curr Top Med Chem 7(17): 1645-59.

El Yacoubi, M. and J. M. Vaugeois (2007). "Genetic rodent models of depression." Curr Opin Pharmacol 7(1): 3-7.

El-Mallakh, R. S. and M. E. Paskitti (2001). "The ketogenic diet may have mood-stabilizing properties." Med Hypotheses 57(6): 724-6.

Elhwuegi, A. S. (2004). "Central monoamines and their role in major depression." Prog Neuropsychopharmacol Biol Psychiatry 28(3): 435-51.

Engel J, P. T., Ed. (1998). Epilepsy: a comprehensive textbook. Philadelphia, Lippincott-Raven.

Ettinger, A. B. (2004). "Commentary on "Personality changes following temporal lobectomy for epilepsy"." Epilepsy Behav 5(4): 601-2.

Forsgren, L. and L. Nyström (1990). "An incident case-referent study of epileptic seizures in adults." Epilepsy Res 6(1): 66-81.

Fraser, D. A., J. Thoen, et al. (2000). "Reduction in serum leptin and IGF-1 but preserved T-lymphocyte numbers and activation after a ketogenic diet in rheumatoid arthritis patients." Clin Exp Rheumatol 18(2): 209-14.

Freeman, J. M., E. H. Kossoff, et al. (2007). "The ketogenic diet: one decade later." Pediatrics 119(3): 535-43.

Friedman, E., M. Berman, et al. (2006). "Swim test immobility in a genetic rat model of depression is modified by maternal environment: a cross-foster study." Dev Psychobiol 48(2): 169-77.

Gaitatzis, A., Carroll, K., Majeed, A.W., Sander, J., (2004). "The Epidemiology of the comorbidity of epilepsy in the general population." Epilepsia 45: 1613-1622.

Gilbert, D. L., P. L. Pyzik, et al. (2000). "The ketogenic diet: seizure control correlates better with serum beta-hydroxybutyrate than with urine ketones." J Child Neurol 15(12): 787-90.

Gilliam, F. and A. M. Kanner (2002). "Treatment of depressive disorders in epilepsy patients." Epilepsy Behav 3(5S): 2-9.

Gilliam, F. G., A. J. Fessler, et al. (2004). "Systematic screening allows reduction of adverse antiepileptic drug effects: a randomized trial." Neurology 62(1): 23-7.

Greene, A. E., M. T. Todorova, et al. (2003). "Perspectives on the metabolic management of epilepsy through dietary reduction of glucose and elevation of ketone bodies." J Neurochem 86(3): 529-37.

Groticke, I., K. Hoffmann, et al. (2007). "Behavioral alterations in the pilocarpine model of temporal lobe epilepsy in mice." Exp Neurol 207(2): 329-49.

Groticke, I., K. Hoffmann, et al. (2008). "Behavioral alterations in a mouse model of temporal lobe epilepsy induced by intrahippocampal injection of kainate." Exp Neurol.

Gundlach, A. L. (2002). "Galanin/GALP and galanin receptors: role in central control of feeding, body weight/obesity and reproduction?" Eur J Pharmacol 440(2-3): 255-68.

Hankin, B. L. (2006). "Adolescent depression: description, causes, and interventions." Epilepsy Behav 8(1): 102-14.

Harden, C. L. (2002). "The co-morbidity of depression and epilepsy: epidemiology,etiology, and treatment." Neurology 50(Suppl 4): S48-S55.

Harden, C. L. and M. A. Goldstein (2002). "Mood disorders in patients with epilepsy: epidemiology and management." CNS Drugs 16(5): 291-302.

Hecimovoc, H. G., J.D., Sheline, Y.I., Gilliam, F.G., (2003). "Mechanisms of depression in epilepsy from a clinical perspective." Epilepsy Behav 4(Suppl. 3): S25-S30.

Heilig, M. (2004). "The NPY system in stress, anxiety and depression." Neuropeptides 38(4): 213-24.

Heo, M., C. F. Murphy, et al. (2007). "Relationship between the Hamilton Depression Rating Scale and the Montgomery-Asberg Depression Rating Scale in depressed elderly: a meta-analysis." Am J Geriatr Psychiatry 15(10): 899-905.

Hermann, B. P., M. Seidenberg, et al. (2000). "Psychiatric comorbidity in chronic epilepsy: identification, consequences, and treatment of major depression." Epilepsia 41 Suppl 2: S31-41.

Hesdorffer, D. C., W. A. Hauser, et al. (2000). "Major depression is a risk factor for seizures in older adults." Ann Neurol 47(2): 246-9.

Hesdorffer, D. C., W. A. Hauser, et al. (2006). "Depression and suicide attempt as risk factors for incident unprovoked seizures." Ann Neurol 59(1): 35-41.

Huttenlocher, P. R. (1976). "Ketonemia and seizures: metabolic and anticonvulsant effects of two ketogenic diets in childhood epilepsy." Pediatr Res 10(5): 536-40.

Jobe, P. C., J. W. Dailey, et al. (1999). "A noradrenergic and serotonergic hypothesis of the linkage between epilepsy and affective disorders." Crit Rev Neurobiol 13(4): 317-56.

Jobe, P. C. W., R.W. (2005). "Affective Disorder and epilepsy comorbidity in the genetically epilepsy prone-rat (GEPR). In Gilliam, F. Kanner, A.M., Sheline, Y.I. (Eds.) Depression and Brain Dysfunction." Taylor and Francis Medical Books, London: 121-157.

Johnson, E. K., J. E. Jones, et al. (2004). "The relative impact of anxiety, depression, and clinical seizure features on health-related quality of life in epilepsy." Epilepsia 45(5): 544-50.

Jones, M. D. and I. Lucki (2005). "Sex differences in the regulation of serotonergic transmission and behavior in 5-HT receptor knockout mice." Neuropsychopharmacology 30(6): 1039-47.

Kandel, E. R., J. H. Schwartz, et al. (2000). Principles of Neural Science. New York, McGraw-Hill

Kanner, A. M. (2003)$_{a.}$ "Depression in epilepsy: prevalence, clinical semiology, pathogenic mechanisms, and treatment." Biol Psychiatry 54(3): 388-98.

Kanner, A. M. (2003)$_{b}$. "The Various Neuropathological "Faces" of Temporal Lobe Epilepsy." Epilepsy Curr 3(6): 212-213.

Kanner, A. M. (2005). "Depression in epilepsy: a neurobiologic perspective." Epilepsy Curr 5: 21-27.

Kanner, A. M. (2008). "Psychiatric comorbidity in children with epilepsy ... Or is it: epilepsy comorbidity in children with psychiatric disorders?" Epilepsy Curr 8(1): 10-2.

Kanner, A. M. and J. C. Nieto (1999). "Depressive disorders in epilepsy." Neurology 53(5 Suppl 2): S26-32.

Kanner, A. M. B., A. (2002). "Depression and epilepsy:how closely related are they?" Neurology 58(Suppl 5): S7-S29.

Koh, S., R. Magid, et al. (2007). "Depressive behavior and selective down-regulation of serotonin receptor expression after early-life seizures: reversal by environmental enrichment." Epilepsy Behav 10(1): 26-31.

Kondziella, D., S. Alvestad, et al. (2007). "Which clinical and experimental data link temporal lobe epilepsy with depression?" J Neurochem 103(6): 2136-52.

Kuhn, K. U., B. B. Quednow, et al. (2003). "Antidepressive treatment in patients with temporal lobe epilepsy and major depression: a prospective study with three different antidepressants." Epilepsy Behav 4(6): 674-9.

Kuteeva, E., T. Hokfelt, et al. (2008). "Galanin - 25 years with a multitalented neuropeptide : Galanin, galanin receptor subtypes and depression-like behaviour." Cell Mol Life Sci 65(12): 1854-63.

Lakhan, S. E. and K. F. Vieira (2008). "Nutritional therapies for mental disorders." Nutr J 7: 2.

Lambert, M. V. and M. M. Robertson (1999). "Depression in epilepsy: etiology, phenomenology, and treatment." Epilepsia 40 Suppl 10: S21-47.

Larhammar, D., A. Ericsson, et al. (1987). "Structure and expression of the rat neuropeptide Y gene." Proc Natl Acad Sci U S A 84(7): 2068-72.

Lee, M. C., J. Y. Kang, et al. (2006). "Clinical features and epileptogenesis of dysembryoplastic neuroepithelial tumor." Childs Nerv Syst 22(12): 1611-8.

Lee, T. S., S. Mane, et al. (2007). "Gene expression in temporal lobe epilepsy is consistent with increased release of glutamate by astrocytes." Mol Med 13(1-2): 1-13.

Leeman, B. A. and A. J. Cole (2008). "Advancements in the treatment of epilepsy." Annu Rev Med 59: 503-23.

Lefevre, F. and N. Aronson (2000). "Ketogenic diet for the treatment of refractory epilepsy in children: A systematic review of efficacy." Pediatrics 105(4): E46.

Leibowitz, S. F., A. Akabayashi, et al. (1998). "Obesity on a high-fat diet: role of hypothalamic galanin in neurons of the anterior paraventricular nucleus projecting to the median eminence." J Neurosci 18(7): 2709-19.

Levinson, D. F. and O. Devinsky (1999). "Psychiatric adverse events during vigabatrin therapy." Neurology 53(7): 1503-11.

Martin, E. and M. Pozo (2006). "Animal models for the development of new neuropharmacological therapeutics in the status epilepticus." Curr Neuropharmacol 4(1): 33-40.

Matsuura, M. (1999). "Epileptic psychoses and anticonvulsant drug treatment." J Neurol Neurosurg Psychiatry 67(2): 231-3.

Mazarati, A., U. Langel, et al. (2001). "Galanin: an endogenous anticonvulsant?" Neuroscientist 7(6): 506-17.

Mazarati, A., D. Shin, et al. (2007). "Kindling epileptogenesis in immature rats leads to persistent depressive behavior." Epilepsy Behav 10(3): 377-83.

Mazarati, A., P. Siddarth, et al. (2008). "Depression after status epilepticus: behavioural and biochemical deficits and effects of fluoxetine." Brain.

McConnell, H. W. and P. Synder (1998). Psychiatric Comorbidity in Epilepsy: Basic Mechanisms, Disagnosis, and Treatment. Washington, D.C., American Psychiatric Press, Inc.

McDonald, C. R. (2008). "The use of neuroimaging to study behavior in patients with epilepsy." Epilepsy Behav 12(4): 600-11.

McIntyre, D. C. and K. L. Gilby (2007). "Genetically seizure-prone or seizure-resistant phenotypes and their associated behavioral comorbidities." Epilepsia 48 Suppl 9: 30-2.

Meldolesi, G. N., A. Picardi, et al. (2006). "Factors associated with generic and disease-specific quality of life in temporal lobe epilepsy." Epilepsy Res 69(2): 135-46.

Milby, A. H., C. H. Halpern, et al. (2008). "Vagus nerve stimulation for epilepsy and depression." Neurotherapeutics 5(1): 75-85.

Mondimore, F. M., P. P. Zandi, et al. (2007). "A comparison of the familiality of chronic depression in recurrent early-onset depression pedigrees using different definitions of chronicity." J Affect Disord 100(1-3): 171-7.

Mula, M., M. R. Trimble, et al. (2003). "Topiramate and psychiatric adverse events in patients with epilepsy." Epilepsia 44(5): 659-63.

Murphy, P., S. Likhodii, et al. (2004). "The antidepressant properties of the ketogenic diet." Biol Psychiatry 56(12): 981-3.

Nasrallah, H. A., T. A. Ketter, et al. (2006). "Carbamazepine and valproate for the treatment of bipolar disorder: a review of the literature." J Affect Disord 95(1-3): 69-78.

Nemeroff, C. B. (2007). "Prevalence and Management of Treatment-Resistant Depression." Journal of Clinical Psychiatry 68: 17-25.

Overstreet, D. H., E. Friedman, et al. (2005). "The Flinders Sensitive Line rat: a selectively bred putative animal model of depression." Neurosci Biobehav Rev 29(4-5): 739-59.

Paciello, N., M. Mazza, et al. (2002). "[Depression in epilepsy: symptom or syndrome?]." Clin Ter 153(6): 397-402.

Paridiso, S., Hermann, B.P., Blumer, D., Davies, K., Robinson, R.G. (2001). "Impact of depressed mood on neuropsychological status in temporal lobe epilepsy." J. Neurol. Neurosurg. Psychiatry 70: 180-185.

Paxinos, K. B. J. F. a. G. (1997). "The Mouse Brain in Stereotaxic Coordinates." Academic Press, New York.

Pisani, F., E. Spina, et al. (1999). "Antidepressant drugs and seizure susceptibility: from in vitro data to clinical practice." Epilepsia 40 Suppl 10: S48-56.

Pompili, M., N. Vanacore, et al. (2007). "Depression, hopelessness and suicide risk among patients suffering from epilepsy." Ann Ist Super Sanita 43(4): 425-9.

Porsolt, R. D., A. Bertin, et al. (1977). "Behavioral despair in mice: a primary screening test for antidepressants." Arch Int Pharmacodyn Ther 229(2): 327-36.

Potschka, H., K. Schwabe, et al. (2000). "Development of kindling and spontaneous seizures after massed stimulation of different loci in the rat piriform cortex." Brain Res 855(2): 252-9.

Prudic, J., M. Olfson, et al. (2004). "Effectiveness of electroconvulsive therapy in community settings." Biol Psychiatry 55(3): 301-12.

Racine, R. J. (1972). "Modification of seizure activity by electrical stimulation. II. Motor seizure." Electroencephalogr Clin Neurophysiol 32(3): 281-94.

Raol, Y. H., I. V. Lund, et al. (2006). "Enhancing GABA(A) receptor alpha 1 subunit levels in hippocampal dentate gyrus inhibits epilepsy development in an animal model of temporal lobe epilepsy." J Neurosci 26(44): 11342-6.

Reddy, D. S. (2006). "Physilogical role of adrenal deoxycorticosterone-derived neuroactive steroids in stress-sensitive conditions." Neuroscience 138: 911-920.

Richardson, E. J., H. R. Griffith, et al. (2007). "Structural and functional neuroimaging correlates of depression in temporal lobe epilepsy." Epilepsy Behav 10(2): 242-9.

Robertson, M. M., M. R. Trimble, et al. (1987). "Phenomenology of depression in epilepsy." Epilepsia 28(4): 364-72.

Sankar, R. and M. Sotero de Menezes (1999). "Metabolic and endocrine aspects of the ketogenic diet." Epilepsy Res 37(3): 191-201.

Sarkisian, M. R. (2001). "Overview of the Current Animal Models for Human Seizure and Epileptic Disorders." Epilepsy Behav 2(3): 201-216.

Sarkisova, K. Y. and M. A. Kulikov (2006). "Behavioral characteristics of WAG/Rij rats susceptible and non-susceptible to audiogenic seizures." Behav Brain Res 166(1): 9-18.

Schwartzkroin, P. A. (1999). "Mechanisms underlying the anti-epileptic efficacy of the ketogenic diet." Epilepsy Res 37(3): 171-80.

Scott, P. A., M. A. Cierpial, et al. (1996). "Susceptibility and resistance of rats to stress-induced decreases in swim-test activity: a selective breeding study." Brain Res 725(2): 217-30.

Seyfried, T. N., T. M. Sanderson, et al. (2003). "Role of glucose and ketone bodies in the metabolic control of experimental brain cancer." Br J Cancer 89(7): 1375-82.

Shah, N., T. Eisner, et al. (1999). "An Overview of SSRIs for the Treatment of Depression." Journal of the Pharmacy Society of Wisconsin(July/August 1999): 33-46.

Sheline, Y. I., M. H. Gado, et al. (1998). "Amygdala core nuclei volumes are decreased in recurrent major depression." Neuroreport 9(9): 2023-8.

Sheth, R. D. and C. E. Stafstrom (2002). "Intractable pediatric epilepsy: vagal nerve stimulation and the ketogenic diet." Neurol Clin 20(4): 1183-94.

Spencer, S. (2007). "Epilepsy: clinical observations and novel mechanisms." Lancet Neurol 6(1): 14-6.

Sperber, E. F., K. Z. Haas, et al. (1999). "Flurothyl status epilepticus in developing rats: behavioral, electrographic histological and electrophysiological studies." Brain Res Dev Brain Res 116(1): 59-68.

Spina, E. and G. Perugi (2004). "Antiepileptic drugs: indications other than epilepsy." Epileptic Disord 6(2): 57-75.

Stafstrom, C. E. (2004). "Dietary approaches to epilepsy treatment: old and new options on the menu." Epilepsy Curr 4(6): 215-22.

Stafstrom, C. E. and K. J. Bough (2003). "The ketogenic diet for the treatment of epilepsy: a challenge for nutritional neuroscientists." Nutr Neurosci 6(2): 67-79.

Swinkels, W. A., J. Kuyk, et al. (2005). "Psychiatric comorbidity in epilepsy." Epilepsy Behav 7(1): 37-50.

Szot P.D., et al. (1997). "Effect of pentylenetetrazol on the expression of tyrosine hydroxylase mRNA and norepinephrine and dopamine transporter mRNA." Mol. Brain Res. 44: 46-54.

Szot, P., D. Weinshenker, et al. (2001). "Norepinephrine is required for the anticonvulsant effect of the ketogenic diet." Brain Res Dev Brain Res 129(2): 211-4.

Szot, P., D. Weinshenker, et al. (1999). "Norepinephrine-deficient mice have increased susceptibility to seizure-inducing stimuli." J Neurosci 19(24): 10985-92.

Tabb, K., K. A. Boss-Williams, et al. (2007). "Rats bred for susceptibility to depression-like phenotypes have higher kainic acid-induced seizure mortality than their depression-resistant counterparts." Epilepsy Res 74(2-3): 140-6.

Tabb, K., P. Szot, et al. (2004). "The ketogenic diet does not alter brain expression of orexigenic neuropeptides." Epilepsy Res 62(1): 35-9.

Tellez-Zenteno, J. F., S. B. Patten, et al. (2007). "Psychiatric comorbidity in epilepsy: a population-based analysis." Epilepsia 48(12): 2336-44.

Thapar, A., M. Roland, et al. (2005). "Do depression symptoms predict seizure frequency--or vice versa?" J Psychosom Res 59(5): 269-74.

Thiele, E. A. (2003). "Assessing the efficacy of antiepileptic treatments: the ketogenic diet." Epilepsia 44 Suppl 7: 26-9.

Trimble, M. R. (1996). "Anticonvulsant-induced psychiatric disorders. The role of forced normalisation." Drug Saf 15(3): 159-66.

Trimble, M. R. (2004). "The end of the seizure can be the beginning of the problem: Epilepsy is more than just the ictus." Neurology 62(5): 683.

Van der Auwera, I., S. Wera, et al. (2005). "A ketogenic diet reduces amyloid beta 40 and 42 in a mouse model of Alzheimer's disease." Nutr Metab (Lond) 2: 28.

Vanitallie, T. B., C. Nonas, et al. (2005). "Treatment of Parkinson disease with diet-induced hyperketonemia: a feasibility study." Neurology 64(4): 728-30.

Vezzani, A., G. Sperk, et al. (1999). "Neuropeptide Y: emerging evidence for a functional role in seizure modulation." Trends Neurosci 22(1): 25-30.

Vining, E. P. (2002). "The ketogenic diet." Adv Exp Med Biol 497: 225-31.

Vinogradova, L. V. (2008). "Audiogenic kindling in Wistar and WAG/Rij rats: Kindling-prone and kindling-resistant subpopulations." Epilepsia.

Vrontakis, M. E., L. M. Peden, et al. (1987). "Isolation and characterization of a complementary DNA (galanin) clone from estrogen-induced pituitary tumor messenger RNA." J Biol Chem 262(35): 16755-8.

Warner-Schmidt, J. L. and R. S. Duman (2006). "Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment." Hippocampus 16(3): 239-49.

Weinshenker, D., M. M. Wilson, et al. (2005). "A new method for identifying informative genetic markers in selectively bred rats." Mamm Genome 16(10): 784-91.

Weiss J. M., D., M.K., McCurdy, P.M., West, C.H.K., Bonsall, R.W., (2000). "Depression seen through an animal model: an expanded hypothesis of pathophysiology and improved models."

Weiss, J. M., K. A. Boss-Williams, et al. (2005). "Testing the hypothesis that locus coeruleus hyperactivity produces depression-related changes via galanin." Neuropeptides 39(3): 281-7.

Weiss, J. M., M. A. Cierpial, et al. (1998). "Selective breeding of rats for high and low motor activity in a swim test: toward a new animal model of depression." Pharmacol Biochem Behav 61(1): 49-66.

Weiss, J. M., M. K. Demetrikopoulos, et al. (2000). Depression seen through an animal model: an expanded hypothesis of pathophysiology and improved models. Anxiety, Depression, and Emotion. R. J. Davidson. Oxford, Oxford University Press: 3-35.

Weiss, J. M., C. H. West, et al. (2008). "Rats selectively-bred for behavior related to affective disorders: Proclivity for intake of alcohol and drugs of abuse, and measures of brain monoamines." Biochemical Pharmacology 75: 134-159.

West, C. H., R. W. Bonsall, et al. (1999). "Rats selectively bred for high and low swim-test activity show differential responses to dopaminergic drugs." Psychopharmacology (Berl) 146(3): 241-51.

West, C. H. and J. M. Weiss (2005). "A selective test for antidepressant treatments using rats bred for stress-induced reduction of motor activity in the swim test." Psychopharmacology (Berl) 182(1): 9-23.

Wiegartz, P., M. Seidenberg, et al. (1999). "Co-morbid psychiatric disorder in chronic epilepsy: recognition and etiology of depression." Neurology 53(5 Suppl 2): S3-8.

Will, C. C., F. Aird, et al. (2003). "Selectively bred Wistar-Kyoto rats: an animal model of depression and hyper-responsiveness to antidepressants." Mol Psychiatry 8(11): 925-32.

Williams, G., C. Bing, et al. (2001). "The hypothalamus and the control of energy homeostasis: different circuits, different purposes." Physiol Behav 74(4-5): 683-701.

Willner, P. (1997). "Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation." Psychopharmacology (Berl) 134(4): 319-29.

Willner, P. (2005). "Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS." Neuropsychobiology 52(2): 90-110.

Willner, P. and P. J. Mitchell (2002). "The validity of animal models of predisposition to depression." Behav Pharmacol 13(3): 169-88.

Witkin, J. M., M. Baez, et al. (2007). "Constitutive deletion of the serotonin-7 (5-HT(7)) receptor decreases electrical and chemical seizure thresholds." Epilepsy Res 75(1): 39-45.

Yamakawa, K. (2006). "Na channel gene mutations in epilepsy--the functional consequences." Epilepsy Res 70 Suppl 1: S218-22.

APPENDIX:

Adapted from:

Weinshenker D, Tabb K, Szot P. (2007) Norepinephrine: a molecular link between epilepsy and depression? In Encylopedia of Basic Epilepsy Research (P. Schwartzkroin, ed.), Elsevier (in press).

Abstract

Epilepsy and depression are co-morbid diseases. Depression is the most prevalent neuropsychiatric disorder among epileptic patients, and individuals with a history of depressive illness are at increased risk for developing epilepsy. Despite the convincing epidemiological evidence for the link between these diseases, the underlying mechanisms are unknown. Because the neurotransmitter norepinephrine has both antidepressant and anticonvulsant properties, it is an appealing mechanistic candidate for providing a link between epilepsy and depression. In this article, we review the literature describing modulation of seizure susceptibility and mood by norepinephrine, and highlight recent attempts to generate an animal model of epilepsy and depression co-morbidity.

Introduction

Epilepsy and depression co-morbidity. The link between epilepsy and depression was first described by Hippocrates, who noted that a relatively high frequency of epileptic patients experienced "melancholia", and that melancholics were prone to develop epilepsy. Since this noteworthy observation, a preponderance of the epilepsy and depression literature reveals that the co-morbidity is bidirectional. Epileptic patients are at high risk for depression, and patients with major depression have a higher frequency of epilepsy than the general population. As evidenced by the abundance of published reviews over the past few years, the association between these two diseases, and the challenges encountered in attempting to treat them simultaneously, have recently become topics of great interest. There is some debate about the magnitude of the risk factors for epilepsy and depression co-morbidity because of variance in study design and diagnostic criteria. However, most estimates place the risk for epileptic patients developing depression, and for depressed patients developing epilepsy, at 4-5 fold higher than the general population. Nearly all reviews caution that depression is severely under-diagnosed and under-treated in the epileptic population. Raising awareness among clinicians of this link, and developing experimental models and therapies by researchers, are critical for the future of treating co-morbid individuals.

Many theories have been put forth to explain the co-morbidity between epilepsy and depression, including arguments based on neurobiology, genetics, social environment, and drug treatment side effects. It is likely that all of these factors contribute to the connection to some extent. Why is the link between these two diseases under-recognized and under-treated clinically? One reason could be that despite the

numerous hypotheses concerning the basis for the co-morbidity, none have been empirically tested or convincingly demonstrated. Although epidemiological data support co-morbidity between epilepsy and depression, other types of experimental data – and particularly the identification of a common underlying mechanism -- would substantially reinforce general acceptance of this phenomenon. Because many studies that would be definitive are unethical to perform in humans, animal models appear to be the best experimental alternative to study the biological basis of epilepsy and depression co-morbidity. To date, very few animal models designed to test these hypotheses have been described.

Background

Norepinephrine: a molecular link between epilepsy and depression? Epilepsy and depression are very complicated neurological disorders, and it is generally accepted that no one single neurotransmitter is responsible for the manifestations of either epilepsy or depression. Of all the candidates that may represent a molecular link between epilepsy and depression, a particularly strong case can be made for norepinephrine (NE) because of the overwhelming evidence of its ability to modulate both seizure susceptibility and mood, as first proposed by Jobe and colleagues.

NE is one of the three most widely recognized neurotransmitters (serotonin [5-HT] and dopamine [DA] being the others) in the etiology and treatment of depression. The evidence for the involvement of NE in depression comes from both human and animal studies, and can be summarized as follows: (1) NE reuptake inhibitors and monoamine oxidase inhibitors (MAOIs) that increase NE levels are among the most

widely used and efficacious antidepressant drugs available. (2) Depletion of NE via neurotoxins or dietary restriction leads to recurrence of symptoms of depression in patients in remission. (3) Postmortem studies reveal multiple alterations in the noradrenergic system of depressed individuals, including changes in NE biosynthetic enzymes, the NE transporter (NET), and NE receptors. (4) Reduced NE levels have been detected in the cerebrospinal fluid of depressed individuals. (5) NE dysfunction contributes to aberrant behaviors in animals that in some ways resemble depressive symptoms. (6) NE is critical for the efficacy of antidepressant drugs in some behavioral tests in rodents and (7) Chronic treatment of rats with antidepressant drugs changes the firing properties of locus coeruleus (LC) neurons, the major noradrenergic nucleus in the brain. Therefore, the etiology of depression involves a reduced function of the noradrenergic system, and treatment involves increasing extracellular NE levels. It should be noted, however, that there is also strong evidence for the roles of DA and 5-HT in depression and its treatment, indicating that depression likely involves changes in multiple neurotransmitter systems.

Despite the particular focus of modern epilepsy research on amino acid neurotransmitters (i.e. GABA, glutamate) and ion channels, NE is a very potent endogenous anticonvulsant neurotransmitter in the brain. It is important to note that the function of NE in epilepsy appears to be of a modulatory nature, since a loss of NE function does not result in spontaneous seizures. Therefore, the evidence demonstrating a relationship between NE and epilepsy isn't as overwhelming as that between NE and depression. The strongest evidence supporting an involvement of NE in epilepsy is the following: (1) A reduction in central NE levels, induced either by neurotoxins or genetic

manipulation, increases susceptibility of animals to a large variety of convulsant agents. (2) Stimulation of LC neurons, and the subsequent release of NE at their terminals, reduces susceptibility of animals to convulsant agents. (3) LC noradrenergic neurons are activated when a seizure occurs. (4) NE is required for the efficacy of some antiepileptic therapies and (5) Noradrenergic receptor agonists can suppress seizures. A common link between epilepsy and depression, therefore, may be reduced noradrenergic function.

Animal models of epilepsy and depression co-morbidity. Perhaps the best-characterized model of reduced noradrenergic function contributing to the co-morbidity of epilepsy and depression is the genetically epilepsy-prone rat (GEPR). These rats were selectively bred for susceptibility to audiogenic seizures, but they have also been reported to display behavioral responses related to depression, such as increased immobility in the forced swim test, and decreased saccharin consumption that is possibly indicative of anhedonia. Importantly for the subject of this article, noradrenergic dysfunction appears to be at least partially responsible for the seizure phenotype of these rats, and antidepressant drugs that block the NE transporter and increase extracellular NE prevent seizures in GEPRs. Thus, a noradrenergic deficit in NE may underlie both the susceptibility to seizures and depression-like behaviors in this model. A critical test of this hypothesis would be to determine whether drugs that augment NE signaling could attenuate the depressive-like behaviors as well as the seizure susceptibility.

Methods

Rat model of depression-like phenotypes. We have recently developed a new animal model of epilepsy and depression co-morbidity. With the idea of expanding upon the available rodent models of depression, Weiss and colleagues selectively bred normal Sprague-Dawley rats for a number of phenotypes relevant to human depression. These lines include: Swim-Lo active (SwLo), with low motor activity in a Porsolt forced swim test (FST); Swim-Hi active (SwHi), with high motor activity in the FST; Swim-test susceptible (SUS), which are highly susceptible to having their swim-test behavior disrupted by mild stress; Swim-test resistant (RES), which are highly resistant to having their swim-test behavior disrupted by mild stress; Monitor Hyperactive (HYPER), which experience long-lasting hyperactivity following stress; and Monitor Resistant (MON RES), which are resistant to changes in activity following stress. Randomly bred (non-selected; NS) rats served as controls. To determine whether genetic susceptibility to depression-like phenotypes also influences limbic seizure susceptibility, adult rats of each of the 6 selectively bred lines, plus non-selected (NS) controls, were tested for maximal seizure severity after administration of kainic acid (10 mg/kg, then 5 mg/kg/hr until tonic-clonic seizure).

*Dopamine β-hydroxylase knockout (Dbh -/-) mouse***.** The previously described models used genetically susceptible animals, and we wondered whether an environmentally-induced depressive-like state could also enhance seizure susceptibility. Specifically, we tested whether mice subjected to a chronic mild stress (CMS) paradigm would be more susceptible to kainic acid-induced seizures. The CMS paradigm uses a

random series of multiple daily unpredictable mild stressors (e.g. forced swim, food or water deprivation, physical restraint, exposure to white noise, reversed light/dark cycle, etc) to elicit a depressive state. At the same time, we investigated further the possibility that a reduction in NE may be a potential link between epilepsy and depression by utilizing a mouse model of NE deficiency, the dopamine β-hydroxylase knockout (*Dbh* -/-) mouse. DBH converts DA to NE in noradrenergic cells; thus, *Dbh -/-* mice completely lack NE. We have previously shown that *Dbh -/-* mice are hypersensitive to many seizure-inducing agents. We predicted that *Dbh -/-* mice might be particularly prone to changes in seizure susceptibility following CMS exposure. Adult male *Dbh -/-* and control (*Dbh* +/-) mice that have normal NE content were exposed to 5 weeks of CMS. Following 5 weeks of CMS, the mice were assessed for seizure severity following KA administration. Mice were injected with KA (20 mg/kg, i.p.) and observed for one hour. During this hour, seizure behavior was scored using a modified version of the Racine scale: 1= staring, 2= heading nodding, 3= forelimb clonus, 4= rearing and falling, 5= generalized clonic-tonic seizure, 6= death.

Recent Results

Seizure-related mortality in rats bred for depression-like phenotypes. The strains selected for susceptibility to depression-like phenotypes (SwLo, SUS, and HYPER) had significantly higher mortality following kainic acid seizures than the strains selected for resistance to depression-like phenotypes (SwHi, RES, and MON RES) (Fig. 1A). Non-selected control rats fell roughly in between the seizure susceptible and seizure resistant strains. The dose of kainic acid required to produce a clonic-tonic (CT) seizure was

equivalent between each pair of strains, except for the SUS rats, for which the dose was slightly but significantly increased (Fig. 1B). However, the higher CT threshold dose probably cannot account for the increased mortality of the SUS rats, because dose and mortality were not significantly correlated across all lines. For example, some NS control rats that received a cumulative dose of 20 mg/kg survived, while others that received 15 mg/kg or even as little as 10 mg/kg, died; similar patterns were also observed among the other strains. The kainic acid seizure-induced mortality phenotype was evident across multiple generations of these rat lines, indicating that the depression and seizure mortality phenotypes are co-inherited and may be controlled by the same genes. In addition, the seizure mortality phenotype is unlikely to derive from a brain-wide change in excitability, as there were minimal strain differences in susceptibility to flurothyl, which induces generalized seizures that are likely cortical in origin (Fig. 2). These results demonstrate that rats selectively bred for susceptibility or resistance to depression-like phenotypes are also susceptible or resistant, respectively, to kainic acid-induced seizure mortality. Consistent with this result, and perhaps more directly related to the issue of linking depression with seizure susceptibility, we recently completed a pilot study and found that SwLo rats have a shorter latency to seizure following administration of the muscarinic convulsant agent pilocarpine than SwHi rats (unpublished data).

Our model possesses two other salient features that are particularly relevant to the topic at hand. First, genetic load is a critical component of both epilepsy and depression, and a family history of depression is itself a risk factor for developing epilepsy. The selective breeding approach embodies a strong genetic component, because we used rats

selectively bred for more than 30 generations for susceptibility or resistance to behaviors associated with human depression. Of particular note, all three lines bred for depression-relevant behavioral phenotypes were also susceptible to kainic acid-induced seizure mortality (compared to their counterparts that are resistant to these phenotypes), which suggests the lines may share genetic changes that contribute to the phenotypes. Furthermore, both the depression-like and seizure-related mortality phenotypes were stable over multiple generations, indicating co-heritability. Second, the incidence of major depression is particularly high in patients with temporal lobe epilepsy (TLE), ranging from ~20-70%. We found that our rat lines differed in response to kainic acid and pilocarpine, which are commonly used to model TLE, but not to any seizure phenotype related to flurothyl, which produces generalized seizures.

Strikingly similar to the GEPR strains, the SwLo and SUS rats have lower NE levels than their SwHi and RES counterparts, particularly in the hippocampus, and the depression-related phenotypes of both SwLo and SUS rats can be reversed by treatment with noradrenergic antidepressant drugs. The low NE levels in SwLo and SUS rats suggested to us that the reduction of NE in the depression-sensitive lines might underlie both the swim test and kainic acid phenotypes, and provide a potential mechanistic link between epilepsy and depression. However, initial attempts to attenuate kainic acid-induced seizure mortality in the depression-sensitive lines with these antidepressant drugs have been unsuccessful (unpublished data). Further experiments will be required to fully test this hypothesis.

Chronic mild stress in dopamine β-hydroxylase knockout mice. As shown in Fig. 3, two-way ANOVA revealed that KA-induced seizure severity was generally higher in *Dbh -/-* mice (main effect of genotype; $F[1,41] = 6.7$, $P = 0.01$), as we have previously shown. However, CMS did not increase seizure severity in mice of either genotype. Thus, the "depression" induced by CMS in normal or NE-deficient mice does not appear to render them more seizure-sensitive, at least using the KA model. However, some caveats should be noted. While the mice were exposed to a CMS paradigm previously shown to elicit depressive-like behaviors, we did not conduct such measures in these experiments. Thus, it is possible that our CMS conditions were not severe enough to result in a depressive state and an increase in seizure susceptibility. In addition, KA-induced seizure severity is already quite high in unstressed *Dbh -/-* mice, so there may have been a "ceiling effect". Modified studies of this kind may provide further insight.

Treatments for epilepsy and depression co-morbidity. Many cases of depression are successfully treated with pharmacotherapy (antidepressants such as 5HT and/or NE reuptake inhibitors for depression). Similarly, many individuals with epilepsy are successfully treated with anticonvulsants (e.g., valproic acid, phenytoin, lamotrigine, or gabapentin). However, it is unclear how best to treat individuals suffering from both diseases simultaneously. This issue is particularly important and complex because some antidepressant drugs (e.g. bupropion) are reported to increase seizure risk, and some anticonvulsant drugs (e.g. phenobarbital) can precipitate depressive episodes.

Both NE and 5HT have antidepressant as well as anticonvulsant properties, and it has been suggested that increasing the signaling within these neurotransmitter pathways

could be useful for the treatment of co-morbid individuals. We set out to test this idea by administering antidepressant drugs to mice, either acutely (single i.p. injection) or chronically (3 weeks via osmotic minipump) and assessing susceptibility to seizures induced by flurothyl. Importantly, our chronic administration paradigm was as therapeutically relevant as possible, taking into account both the delay between the start of administration, and the antidepressant effect and doses that produce serum drug levels comparable to those seen clinically. Surprisingly, we found that many of the antidepressant drugs tested had a bidirectional effect on flurothyl seizures; i.e., they were both proconvulsant (reduced the latency to seizure) and anticonvulsant (reduced seizure severity). This was particularly true of the selective NE reuptake inhibitors, and was confirmed using NE transporter (NET) knockout mice. Interestingly, the dual 5HT-NE reuptake inhibitor, venlafaxine, was the exception; it had no proconvulsant effect on seizure latency while retaining some of the anticonvulsant effect on seizure severity.

Our results suggest that, contrary to our hypothesis, pure NE reuptake inhibitor antidepressant drugs may not be the best choice for treating individuals with concurrent epilepsy and depression. One caveat to our experiments is that we tested these drugs only on flurothyl-induced seizures, which model generalized epilepsy. Given the prevalence of depression in TLE patients and the results with our selectively bred rats, it will be important to repeat these experiments using a limbic seizure model such as kainic acid.

What are some good candidates for epilepsy and depression pharmacotherapy? Our results in mice indicate that venlafaxine warrants further investigation. Other serotonergic drugs may also be of value. Although this article focuses on NE, there is growing evidence that changes in 5-HT could contribute to epilepsy and depression co-

morbidity. The involvement of 5-HT in depression is well established, and mice lacking the 5-HT2C receptor or the 5-HT7 receptor have increased seizure sensitivity. Furthermore, early life KA-induced seizures in rats lead to increased depressive-like behavior in adulthood, and evidence suggests an involvement of the 5-HT5B receptor in this process. Finally, it was recently shown that 5-HT1A receptor abundance in the brain is reduced in TLE patients, and this reduction was significantly more pronounced in TLE patients with co-morbid major depression.

The anticonvulsant drug lamotrigine has shown some promise in early trials to treat this co-morbidity. In addition to its known therapeutic effect on seizures, it also appears to improve mood in epileptic individuals with depression. In initial studies, there was no correlation between improvement on mood and seizure frequency, suggesting that the effects of lamotrigine on depressive symptoms were not a secondary consequence of its anticonvulsant activity. Other intriguing possibilities include non-drug therapies such as the ketogenic diet and vagus nerve stimulation. Both of these treatments were originally developed for treating epilepsy, and later found to have a positive effect on mood. Interestingly, both the ketogenic diet and vagus nerve stimulation increase extracellular NE in the hippocampus and require NE for their anticonvulsant efficacy in animals, while lamotrigine inhibits monoamine reuptake *in vitro*. It remains to be seen whether the mood-enhancing properties of lamotrigine treatment also involves NE.

Conclusions and Directions for Future Research

Despite the prevalence of depression in epilepsy and the mounting interest in the subject, the neurobiological mechanisms underlying the co-morbidity remain unknown.

NE and other monoamines remain the best candidates for a common mechanism, although the relationship tying epilepsy and depression appears to be complicated. The role of NE and 5-HT in depression is very well documented, but detailed studies of potential changes in these transmitter systems in epileptic and co-morbid individuals are needed. Further investigation of animal models, such as the GEPR rats and the Weiss selectively-bred lines, may yield clues that could evolve into new therapies for treating individuals suffering from both diseases. Treatments aimed at these neurotransmitters should be carefully considered.

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Fig. 1 Kainic acid-induced seizure mortality.

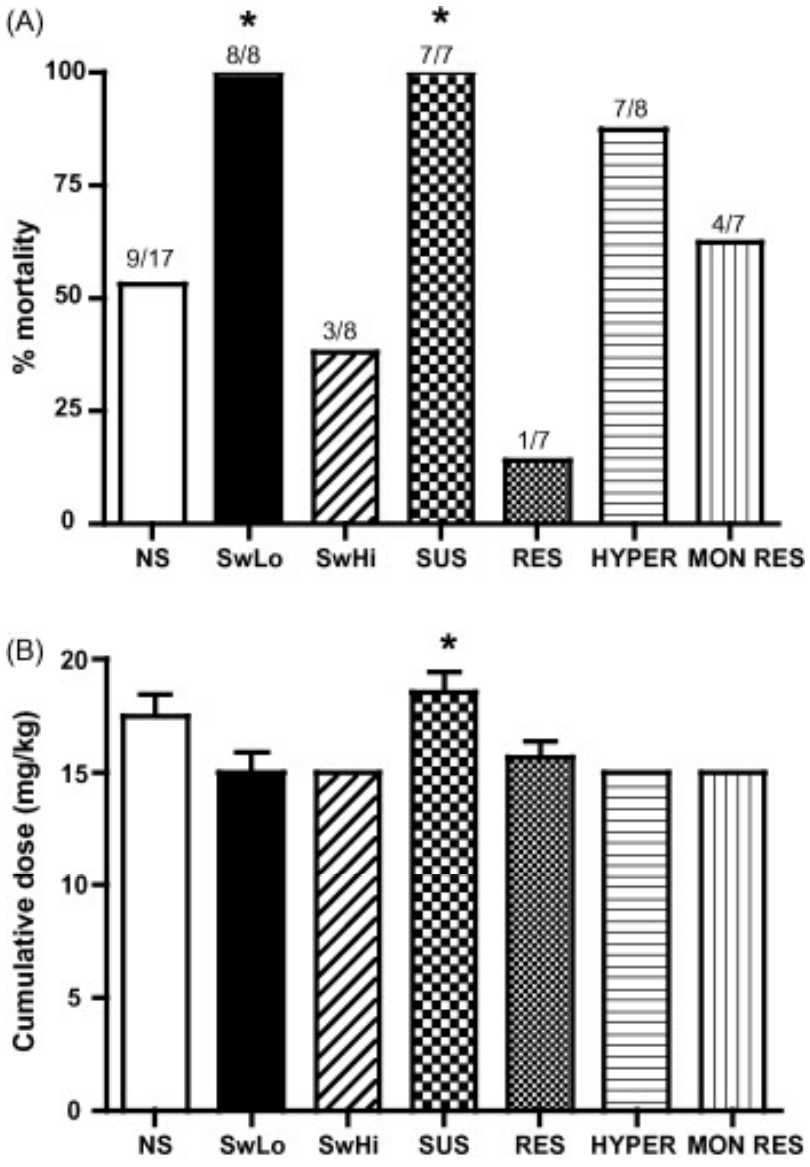

Fig. 1 Kainic acid-induced seizure mortality. Kainic acid was administered to rats at a dose of 10 mg/kg, followed by 5 mg/kg booster injections until a clonic-tonic seizure was produced. Shown is (A) mortality, with the number of animals that died over the total tested above each bar, and (B) the cumulative kainic acid dose (mean±SEM) required to produce a CT seizure. *p <0.05 for the depression-susceptible line compared to its depression-resistant counterpart (SwLo vs SwHi, SUS vs RES, HYPER vs MON RES, respectively). Reproduced from Tabb et al., Epilepsy Res 74:140-146, 2007 with permission from Elsevier.

Fig. 2 Flurothyl-induced seizure susceptibility.

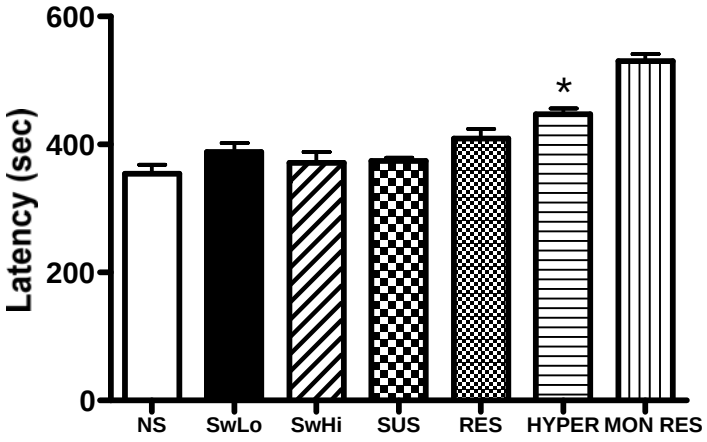

Fig. 2 Flurothyl-induced seizure susceptibility. Flurothyl was administered to rats at a rate of 20 μL/min, and latency to CT seizure was observed. Shown is latency to CT seizure (mean±SEM) for each line. *n*= 6-16 per group. **p*<0.05 for the depression-susceptible line compared to its depression-resistant counterpart (SwLo vs SwHi, SUS vs RES, HYPER vs MON RES, respectively). Reproduced from Tabb et al., Epilepsy Res 74:140-146, 2007 with permission from Elsevier.

Fig. 3 Effects of chronic mild stress on kainic acid-induced seizures in *Dbh* +/- and *Dbh* -/- mice.

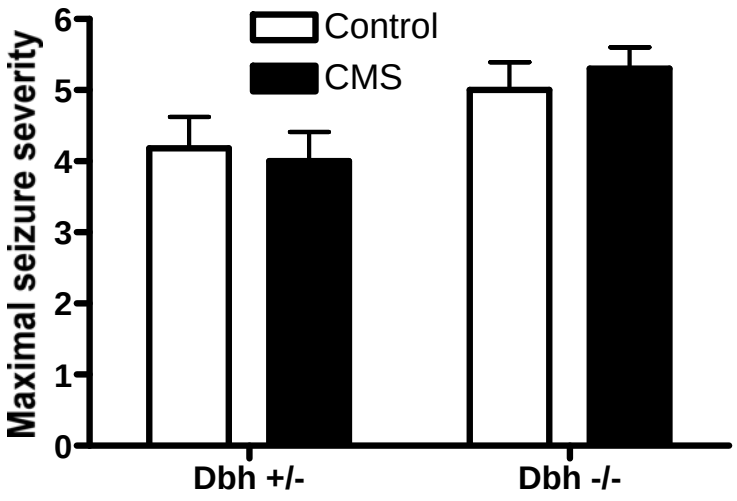

Fig. 3 Effects of chronic mild stress on kainic acid-induced seizures in *Dbh +/-* and *Dbh -/-* mice. Following 5 weeks of CMS, mice were injected with kainic acid (20 mg/kg, i.p.). Shown is maximal seizure severity (1= staring, 2= heading nodding, 3= forelimb clonus, 4= rearing and falling, 5= generalized clonic-tonic seizure, 6= death). *n* = 10-14 per group.

References

Ahern, T., Javors, M. A., Eagles, D. A., Martillotti, J., Mitchell, H. A., Liles, L. C., Weinshenker, D. (2006). The effects of chronic norepinephrine transporter inactivation on seizure susceptibility in mice. *Neuropsychopharmacology* **31**,730-738.

Brunello, N., Mendlewicz, J., Kasper, S., Leonard, B., Montgomery, S., Nelson, J., Paykel, E., Versiani, M., Racagni, G. (2002). The role of noradrenaline and selective noradrenaline reuptake inhibition in depression. *Eur Neuropsychopharmacol* **12**, 461-475.

Dailey, J. W., Reigel, C. E., Mishra, P. K., Jobe, P. C. (1989). Neurobiology of seizure predisposition in the genetically epilepsy-prone rat. *Epilepsy Res* **3**, 3-17.

Harden, C. L. (2002). The co-morbidity of depression and epilepsy: epidemiology, etiology, and treatment. *Neurology* **59(Suppl 4)**, S48-S55.

Jobe, P. C., Daily, J. W., Wernicke, J. F. (1999). A noradrenergic and serotonergic hypothesis of the linkage between epilepsy and affective disorders. *Crit Rev Neurobiol* **13**, 317-356.

Jobe, P. C., Weber, R. W. (2005). Affective disorder and epilepsy comorbidity in the genetically epilepsy prone-rat (GEPR). In Gilliam, F., Kanner, A. M. & Sheline, Y. I. (Eds.) Depression and brain dysfunction. Pp 121-157. London: Taylor & Francis Medical Books.

Kanner, A. M., Balabanov, A. (2002). Depression and epilepsy: how closely related are they? *Neurology* **58 (Suppl 5)**, S27-S39.

Ressler, K. J., Nemeroff, C. B. (1999). Role of norepinephrine in the pathophysiology and treatment of mood disorders. *Biol Psychiatry* **46**,1219-1233.

Scott, P. A., Cierpial, M. A., Kilts, C. D., Weiss, J. M. (1996). Susceptibility and resistance of rats to stress-induced decreases in swim-test activity: a selective breeding study. *Brain Res* **725**, 217-230.

Szot, P. (2004). Role of Norepinephrine the Anticonvulsant Mechanism of Action of the Ketogenic Diet. In: Stafstrom, C. E. & Rho, J. M. (eds.) Epilepsy and the Ketogenic Diet: Clinical Practice and Scientific Basis. pp 265-278. Totowa, NJ: Humana Press, Inc.

Tabb, K. D., Boss-Williams, K. A., Weiss, J. M., Weinshenker, D. (2007). Rats bred for susceptibility to depression-like phenotypes have higher kainic acid-induced seizure mortality than their depression-resistant counterparts. *Epilepsy Res* **74**, 140-146.

Thomas, S. A., Matsumoto, A. M., Palmiter, R. D. (1995). Noradrenaline is essential for mouse fetal development. *Nature* **374**, 643-646.

Weinshenker, D. Szot, P. (2002). The role of catecholamines in seizure susceptibility: new results using genetically engineered mice. *Pharmacol Ther* **94**, 213-233.

Weiss, J. M., Cierpial, M. A., West, C. H. (1998). Selective breeding of rats for high and low motor activity in a swim test: toward a new animal model of depression. *Pharmacol Biochem Behav* **61**, 49-66.

Weiss, J.M., West, C.H., Emery, M.S., Bonsall, R.W., Moore, J.P., Boss-Williams, K.A. (2008). Rats selectively-bred for behavior related to affective disorders: proclivity for intake of alcohol and drugs of abuse, and measures of brain monoamines. *Biochem Pharmacol* **75**, 134-159.

West, C.H., Weiss, J.M. (1998). Effects of antidepressant drugs on rats bred for low activity in the swim test. *Pharmacol Biochem Behav* **61**, 67-79.

West, C.H., Weiss, J.M. (2005). A selective test for antidepressant treatments using rats bred for stress-induced reduction of motor activity in the swim test. *Psychopharmacology (Burl)* **182**, 9-23.

Willner, P. (1997). Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* **134**, 319-329.