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Seizure Susceptibility and Epileptogenesis in Depression-Sensitive Rats

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Seizure Susceptibility and Epileptogenesis in Depression-Sensitive Rats

By

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B.S., University of South Carolina, 2006

Advisor: David Weinshenker, Ph.D.

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Abstract

Seizure Susceptibility and Epileptogenesis in Depression-Sensitive Rats
By Sunshine Alisha Epps

A bi-directional comorbidity between depression and epilepsy has been established clinically, with patients with epilepsy being at an increased risk of developing depression, and patients with depression having an elevated incidence of epilepsy. Despite this bi-directionality, animal models of this comorbidity have primarily focused on epilepsy-related depression. Much less attention has been given to depression-related epilepsy, even though this direction of the comorbidity also has a negative impact on quality of life, treatment efficacy, and prognosis for the patient. In order to better understand this direction of the comorbidity, we assessed seizure- and epilepsy-related behaviors in a rat line selectively bred for a depression-like phenotype, the Swim Low-Active rat (SwLo). SwLo rats were selectively bred based on a depression-susceptible phenotype in the forced swim test (increased floating), and demonstrate other anhedonic-like phenotypes as well. Their counterparts, the Swim High-Active rats (SwHi), were selectively bred for a depression-resistant phenotype (increased struggling on the forced swim test). We have here demonstrated increased susceptibility to chemically- and electrically-induced seizures in the SwLo rats, as well as elevated susceptibility to the development of epilepsy (i.e. epileptogenesis) in certain parameters of the electrical kindling paradigm. We have thus validated these rats as a rodent model of comorbid epilepsy and depression from the direction of depression-related epilepsy, making them the first model of this kind. An additional goal in generating an animal model of this comorbidity was to provide a tool for screening novel therapies for safety and efficacy in treating comorbid epilepsy and depression. We have therefore assessed chronic aerobic exercise for antidepressant and anticonvulsant effects in the SwLo rats, allowing us to demonstrate the safety, efficacy, and selectivity of this therapeutic strategy in the comorbidity. These studies have characterized a rodent model of depression-related epilepsy, the first model of its kind, providing a novel tool for uncovering the mechanisms underlying this comorbidity and screening novel therapeutics for safety and efficacy in treating comorbid epilepsy and depression.

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Tables & Figures

Abbreviations

5-HT	serotonin
6 Hz	6 hertz model
ACh	acetylcholine
ACTH	adrenocorticotropic hormone
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BDNF	brain derived neurotrophic factor
C(t)	threshold cycle
CORT	cortisol in humans, corticosterone in rodents
CRH	corticotrophin releasing hormone
DA	dopamine
Dbh	dopamine-beta-hydroxylase
Dbh -/-	dopamine b-hydroxylase knockout mice
DOPS	L-threo-3,4-dihydroxyphenylserine
EEG	electroencephalography
FST	forced swim test
GABA	γ-aminobutyric acid
GAERS	Genetic Absence Epilepsy Rat from Strasbourg
GalR1	galanin receptor 1
GalR2	galanin receptor 2
GCOS	GeneChip Operating Software
GEPRs	Genetically Epilepsy-Prone Rats
HPA	hypothalamic-pituitary-adrenal axis
ICES	increasing current electroshock
ICSS	intracranial self-stimulation
IL	interleukin
IL-1ra	interleukin-1 receptor antagonist
KA	kainic acid
KD	ketogenic diet
LC	locus coeruleus
MDD	major depressive disorder
MES	maximal electroshock
mGluR	metabotropic glutamate receptor
NE	norepinephrine
NET	norepinephrine transporter
NET -/-	norepinephrine transporter knockout mice
NMDA	N-methyl-D-aspartate
PTZ	pentylenetetrazol
qRT-PCR	quantitative real-time PCR
QTL	quantitative trait loci
SE	status epilepticus
SWD	slow wave discharges
SwLo	Swim Lo-Active Rat
SwHi	Swim Hi-Active Rat
TLE	temporal lobe epilepsy

TrkB	tyrosine-related kinase B
TST	tail suspension test
VNS	vagus nerve stimulation
Wag/Rij	Wistar Albino Glaxo/from Rijswijk Rat
WDS	wet dog shake

RHYTHM AND BLUES: ANIMAL MODELS OF EPILEPSY AND DEPRESSION COMORBIDITY

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1.1 Abstract

Clinical evidence shows a strong, bi-directional comorbidity between depression and epilepsy that is associated with decreased quality of life and responsivity to pharmacotherapies. At present, the neurobiological underpinnings of this comorbidity remain hazy. To complicate matters, anticonvulsant drugs can cause mood disturbances, while antidepressant drugs can lower seizure threshold, making it difficult to treat patients suffering from both depression and epilepsy. Animal models have been created to untangle the mechanisms behind the relationship between these disorders and to serve as screening tools for new therapies targeted to treat both simultaneously. These animal models are based on chemical interventions (e.g. pentylenetetrazol, kainic acid, pilocarpine), electrical stimulations (e.g. kindling, electroshock), and genetic/selective breeding paradigms (e.g. Genetically Epilepsy-Prone Rats (GEPRs), Genetic Absence Epilepsy Rat from Strasbourg (GAERS), WAG/Rij rats, Swim Lo-Active rats (SwLo)). Studies on these animal models point to some potential mechanisms that could explain epilepsy and depression comorbidity, such as various components of the dopaminergic, noradrenergic, serotonergic, and GABAergic systems, as well as key brain regions, like the amygdala and hippocampus. These models have also been used to screen possible therapies. The purpose of the present review is to highlight the importance of animal models in research on comorbid epilepsy and depression and to explore the contributions of these models to our understanding of the mechanisms and potential treatments for these disorders.

1.2 Introduction

As early as 400 B.C., Hippocrates noted that "Melancholics ordinarily become epileptics, and epileptics, melancholics." Clearly, the ancients were aware of a bi-directional comorbidity between epilepsy and unipolar depression (Kanner, 2005), which has since been confirmed. Epileptics are 3-5 times more likely to develop unipolar depression than people without epilepsy. Given the higher incidence of depression in individuals with many serious diseases, this may not seem surprising; however, patients with active or past depression, or a family history of depression, are also at elevated risk of developing epilepsy, a risk that goes up several fold if there is also a history of suicide attempts (Hesdorffer *et al*, 2006). Thus, this bi-directional relationship cannot be explained away as a psychosocial phenomenon and suggests a shared neurobiological substrate. The comorbidity between epilepsy and unipolar depression is associated with worsened prognosis, negative impact on quality of life, and treatment resistance. Furthermore, several anticonvulsant medications can cause depressed mood, among them gabapentin, lamotrigine, oxcarbazepine, tiagabine, and valproate, whereas some antidepressants increase the risk of seizure, particularly in overdose situations, emphasizing the challenge of treating these patients (Hesdorffer and Kanner, 2009; Judge and Rentmeester, 2011). Recent epidemiological studies have indicated an increased suicidality risk in patients on antiepileptic medications, particularly levetiracetam, lamotrigine, and topiramate (Andersohn *et al*, 2010; Bagary, 2011; Fountoulakis *et al*, 2012; Machado *et al*, 2011; Olesen *et al*, 2010; Patorno *et al*, 2010; VanCott *et al*, 2010). Although some of these studies have been limited to retrospective assessments with low statistical power, have failed to account for the possible contribution of comorbid

psychiatric disorders, or did not distinguish between patients using antiepileptic drugs for epilepsy versus off-label uses, the potential for elevated suicide risk with antiepileptic medications is a serious concern that requires further investigation and improvements to current treatment of epilepsy in patients with comorbid epilepsy and depression. Indeed, the FDA has requested that a "black box" warning be placed on these drugs (Hesdorffer *et al*, 2009; Mula and Hesdorffer, 2011). While there have been many advances in our understanding of epilepsy and depression since the days of Hippocrates, it is still unclear what mechanisms underlie this comorbidity, which systems should be targeted to treat the disorders, and how best to test promising therapies for efficacy and safety.

Animal models are an important tool for studying and treating many complex disorders, and such models of epilepsy and unipolar depression comorbidity are now being developed. Here we provide a compilation of the current knowledge in the field and discuss potential mechanisms and therapeutic targets. Table 1.1 provides a summary of existing animal models of epilepsy and unipolar depression comorbidity. In the following sections, we offer a framework for understanding how each model reflects aspects of the diseases and discuss paradigms used to create the models, potential underlying neurochemical and neuroanatomical mechanisms, and directions for future studies.

1.3 Existing animal models of depression and epilepsy comorbidity

1.3.1 Paradigms

Several different types of epilepsy have been clinically associated with depression, including both partial and generalized epilepsies. Partial epilepsies have a defined, focal region of seizure onset, though generalization to other brain regions may occur secondarily. There is a particularly high correlation between depression and complex partial seizures, which feature impaired consciousness and may often be characterized by motor automatisms. Temporal lobe epilepsy (TLE), a complex partial epilepsy affecting the temporal lobe region of the brain, is strongly associated with depression. Pilocarpine, kainic acid, and kindling paradigms serve as good animal models of TLE because their use impacts temporal lobe structures and produces similar motor automatisms.

Generalized epilepsy involves multiple brain regions and is characterized by either tonic/clonic motor convulsions or brief losses of consciousness (absence seizures). While several studies suggest that depression is less common in patients with generalized epilepsies than partial epilepsies, a link between generalized convulsive or absence epilepsies and depression does exist (Shehata and Bateh Ael, 2009).

Animal models of depression and epilepsy comorbidity exhibit a wide range of epilepsy- and depression-related phenotypes. Several models evaluate differences in seizure susceptibility by measuring the latency to seizure onset following a single dose of chemical convulsant or the threshold of electrical stimulation required to initiate seizure onset. While these models are useful in the study of acute seizure behaviors characteristic

of epilepsy, they cannot be used to study epilepsy development (i.e. epileptogenesis). We therefore need animal models of epilepsy that also help us understand epileptogenesis and the underlying neural changes that may occur during this progression. Such models currently include paradigms that elicit spontaneous seizures several weeks after chemical convulsant-induced status epilepticus (SE, a defined period of continuous motor seizing) and kindling paradigms, which apply doses of chemical convulsant or electrical shock that are initially subthreshold, but eventually provoke behavioral seizures following repeated administration.

As with epilepsy, several different aspects of depression can be modeled using animal paradigms. In the forced swim test (FST), a rat or mouse is placed in a container of water, and the duration of struggling (active movement of all 4 paws, forepaws breaking surface of water) and floating (complete immobility, no limb movement) are measured. Because antidepressants increase struggling behavior, immobility is considered a depressive-like phenotype. The tail suspension test (TST), in which a mouse is suspended from a rod by its tail and assessed for immobility, is also commonly used and shows predictive validity.

Anhedonia, or a loss of pleasure in previously enjoyed activities, is another key characteristic of clinical depression that is modeled in rodents by testing for sucrose or saccharin preference compared to water in a 2-bottle choice paradigm. A lack of preference or decreased consumption of sucrose is considered an anhedonic-like phenotype associated with depression.

1.3.2 Chemical convulsant-induced seizures

Pentylenetetrazol

Several animal models of epilepsy and associated depression have been created by assessing depression-related behaviors following administration of chemical convulsants. Pentylenetetrazol (PTZ), a GABA-A receptor antagonist, induces generalized tonic-clonic seizures and can be administered as either one large dose or a series of subconvulsive doses (i.e. kindling). PTZ-kindled rats spent significantly more time immobile in the FST (Mortazavi *et al*, 2005); however, another study reported no difference in FST immobility in PTZ-kindled mice (Takechi *et al*, 2011). This discrepancy may be due to differences in dosage, time course, and/or species, which could be clarified by further studies of depression-related phenotypes, such as anhedonia, following PTZ.

Kainic acid

Kainic acid (KA) is an ionotropic glutamate (kainate) receptor agonist that induces primary limbic seizures with secondary generalization. Following acute KA-induced seizures, rats exhibited increased immobility in the FST, as well as increased immobility in the FST and decreased sucrose preference after repeated KA-induced seizures that persisted for months (Koh *et al*, 2007; Tchekalarova *et al*, 2011). We showed that rats selectively bred for high immobility in the FST (the SwLo rat, discussed in detail in section 1.3.4) exhibited increased mortality following KA-induced seizures (Tabb *et al*, 2007). However, other studies have reported decreases in FST immobility

following intrahippocampal infusion of KA in female mice (Groticke *et al*, 2008), suggesting future studies on the role of sex, species, and route of administration in this model are warranted.

Pilocarpine

Pilocarpine, a muscarinic acetylcholine receptor agonist that induces primary limbic seizures with secondary generalization, is the most commonly used chemical convulsant for studies of epilepsy and depression comorbidity. Following pilocarpine administration, we can measure latency to onset of acute limbic motor seizures, which often progress to SE. After a predetermined period in SE, seizures can be terminated using an anticonvulsant, such as diazepam or phenytoin, and the development of spontaneous seizures can be assessed following a several-week latency period. A number of studies reported more immobility in the FST several weeks after pilocarpine-induced SE in rats (Krishnakumar *et al*, 2009; Mazarati *et al*, 2008; Mazarati *et al*, 2010; Mazarati *et al*, 2009; Pineda *et al*, 2011, 2012; Smolders *et al*, 2008); researchers also saw a decrease in saccharin preference (when assessed), a further indication of a depression- and anhedonic-like phenotype in these epileptic rats.

Despite these results, several other studies failed to find a depression-like phenotype in pilocarpine-treated animals despite the appearance of spontaneous seizures following SE, and in fact noted decreased immobility in the FST and TST (Dos Santos *et al*, 2005; Groticke *et al*, 2007; Muller *et al*, 2009). Interestingly, while the studies that found an association between pilocarpine-induced seizures and immobility in the FST used male animals, all but one of the negative studies used female mice, pointing to the

possibility of sex or species differences. Additional studies that include animals of both genders and different species, as well as studies assessing other measures of depression, such as sucrose preference or intracranial self-stimulation, could clarify this issue.

1.3.3 Electrically induced seizures

Electrical kindling

In the kindling paradigm, electrical stimulation is applied via intracranial (depth) electrode to a specific brain region of interest, with some of these regions implicated in both depression and epilepsy (e.g. the amygdala and hippocampus). Initially, a "subthreshold" current is applied that induces an electrographic seizure, but not a behavioral seizure. Repeated stimulations at this current eventually produce behavioral seizures and serve as a model for the epileptogenic process. Stimulations are typically administered once or twice a day over multiple days, and an animal is considered "fully kindled" after the expression of one or more seizures characterized by rearing and/or falling behaviors (a stage 4 or 5 on the Racine scale) following 2 or more consecutive stimulations. There are also "rapid kindling" paradigms now that require many stimulations per day and greatly accelerate the kindling process.

Studies of depression-related behaviors following kindling have yielded mixed results. Standard amygdala or hippocampal kindling did not produce differences in immobility in the FST or preference for sucrose in rats (Helfer *et al*, 1996; Ma and Leung, 2004). FAST rats, which were selectively bred for accelerated amygdala kindling and showed a variety of anxiety-related phenotypes, actually had increased sucrose

preference versus their SLOW kindling counterparts (Runke *et al*, 2011). However, rapid kindling of the ventral hippocampus increased immobility in the FST and decreased saccharin preference, suggestive of a depressive-like phenotype (Mazarati *et al*, 2007). Combined, these findings suggest that the timing of stimulations, as well as the behavioral endpoint, are important modulators of depression-related behaviors and kindling. It is possible that the delayed time course of classical kindling experiments, which occur on the scale of weeks to months, may allow for the development of compensatory mechanisms that protect against depressive phenotypes, while the rapid kindling paradigm does not permit such compensation.

Other electrically induced seizure paradigms

Other electrically induced seizure models have also been developed. These paradigms include maximal electroshock (MES, a generalized tonic-clonic model involving stimulation through earclip or corneal electrodes), the 6 Hertz model (6 Hz, a model of treatment-resistant epilepsy through corneal electrode stimulation), and the increasing current electroshock model (ICES, a model of treatment-resistant limbic seizures induced via a train of electrical pulses of linearly increasing intensity). Although depression-related phenotypes have not been investigated in these models, they have been used to assess the efficacy and mechanism of action of various antidepressants at reducing seizure behaviors, as discussed further in sections 1.4.2 and 1.4.3.

1.3.4 Genetic/selective breeding models

Several inbred or selectively bred rat lines have been studied as models of comorbid epilepsy and depression, including the Wistar Albino Glaxo/from Rijswijk (Wag/Rij) rats, the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), the Genetically Epilepsy-Prone Rats (GEPR), and the Swim Lo-Active (SwLo) rats. All of these rat lines were generated based on an epilepsy-like phenotype, and then tested for behaviors relevant to depression, with the exception of the SwLo rats, which were selectively bred for immobility in the FST and then tested for seizure susceptibility and epileptogenesis. Also of interest is the dopamine β-hydroxylase knockout (Dbh -/-) mouse, which shows enhanced seizure susceptibility, but decreased responsivity to antidepressants.

Wistar Albino Glaxo/from Rijswijk (Wag/Rij) rats

The Wag/Rij rats are an inbred line displaying spontaneous slow wave discharges (SWD) in cortical EEGs. They are a well-established model of absence epilepsy and respond to absence pharmacotherapies (Sarkisova and van Luijtelaar, 2011). Numerous studies of these rats have revealed depression-like phenotypes. Wag/Rij rats exhibited elevated FST immobility compared to outbred Wistar rats, which was reversed by chronic, but not acute, treatment with the antidepressant imipramine. The Wag/Rij rats also showed decreased sucrose consumption, suggestive of anhedonia (Sarkisova *et al*, 2003).

Genetic Absence Epilepsy Rats from Strasbourg (GAERS)

The GAERS are another absence model selectively bred for SWD in the EEG from a subset of outbred Wistar rats (Danober *et al*, 1998). Similar to the Wag/Rij rats, GAERS were also found to consume significantly less sucrose (Jones *et al*, 2008). While this points to an anhedonic phenotype, it is difficult to draw conclusions from a single test.

Genetically Epilepsy-Prone Rats (GEPRs)

The GEPRs were selectively bred for susceptibility to audiogenic, generalized tonic-clonic seizures. This model has been subdivided further into the GEPR-3 and GEPR-9 lines, with the GEPR-9s having more severe seizures. Hyperthermia and general handling procedures are often sufficient to induce seizures in these rats, and they are also more susceptible than normal rats to seizures induced by several electrical and chemical seizure-inducing paradigms. Importantly, electrographic, behavioral, and pharmacological characteristics seen in both types of GEPRs are reminiscent of human tonic-clonic epilepsies (Jobe *et al*, 1999). The GEPRs also display depression- and anhedonic-like phenotypes. GEPR-3s exhibited increased immobility in the FST, decreased saccharin consumption, and disturbances in the sleep-wake cycle similar to those seen in human depression, supporting the validity of the GEPR-3s as a genetic model of generalized epilepsy and depression comorbidity (Jobe and Weber, 2006).

Swim Lo-Active (SwLo) rats

So far, all the cases discussed have begun with a model of epilepsy or seizure susceptibility, and then assessed potential depressive-like behaviors. To our knowledge, the SwLo rats are the only model that represents the opposite approach. Originating from outbred Sprague-Dawley rats, the SwLo rat line has been selectively bred for nearly 60 generations for increased immobility in the FST. This phenotype can be reversed by chronic, but not acute, antidepressant treatment, strengthening the validity of these animals as a rodent model of depression and antidepressant drug efficacy (Weiss *et al*, 1998b; West and Weiss, 1998). Their counterparts, the SwHi rats, have been selectively bred for increased activity in the FST and represent a model of depression resilience. Although the SwLo rats were selectively bred using the FST, they demonstrate other depression and anhedonic-like phenotypes, including decreased response to dopaminergic drugs and increased intracranial self-stimulation threshold (Weiss *et al*, 2008; West *et al*, 1999a; West *et al*, 1999b) (C. West, personal communication).

To determine whether SwLo rats might be a useful model of comorbidity launched from the depression side of the equation, we tested them for seizure susceptibility and epileptogenesis using a battery of paradigms. In our first set of studies, we found that SwLo rats showed no difference in their response to flurothyl, which induces cortical, generalized seizures, but they had increased mortality following kainic acid-induced limbic seizures, potentially via enhanced generalization to brainstem areas (Tabb *et al*, 2007). These initial results suggested that SwLo rats might best model temporal lobe epilepsy and depression comorbidity. These findings argue that SwLo rats

may make a useful rodent model of depression-related TLE comorbidity, the only such model of its kind to date.

Dopamine β-hydroxylase knockout mice (Dbh -/-)

Dopamine β-hydroxylase (DBH) converts dopamine to norepinephrine in noradrenergic neurons, and DBH knockout (Dbh -/-) mice lack NE completely (Thomas *et al*, 1998). Dbh -/- mice demonstrated enhanced seizure susceptibility and/or severity in a variety of paradigms, including flurothyl, PTZ, kainic acid, and auditory stimulation (Szot *et al*, 1999); they were also resistant to the anticonvulsant effects of valproic acid and the ketogenic diet (Schank *et al*, 2005; Szot *et al*, 2001). Interestingly, while FST performance is normal in Dbh -/- mice, they showed a marked resistance to antidepressants (see subsection on norepinephrine in section 1.4.1) (Cryan *et al*, 2001). Thus, Dbh -/- mice may serve as a model for studying anticonvulsant/antidepressant treatment resistance.

1.3.5 Limitations of animal models

While animal models are useful and necessary for enhancing our understanding of clinical conditions, they are not without their limitations. For example, the FST is considered the "gold standard" for screening novel therapeutic approaches for treating depression. However, despite its high predictive validity, the FST differs from clinical treatment of depression in several ways. Antidepressant treatment typically increases struggling in the FST or TST within 30-60 minutes, while chronic treatment of at least 2-4 weeks is required for improvement of depression symptoms in clinical populations. The

SwLo rats were created in an attempt to address this limitation, and in fact only respond to antidepressant treatment following chronic administration (West *et al*, 1998). Despite this improvement, animal models of depression have thus far not proven especially useful for identifying the mechanistic underpinnings of the disorder. Part of the problem likely stems from difficulties attributing "depressive-like symptomology" to changes in motor behavior in rodents. Much of what is currently known about the causes of depression has been determined by identifying the pharmacological targets of medications that offer clinical efficacy. However, numerous studies specifically targeting these same mechanisms often fail to show antidepressant effects, and drugs targeting novel molecules that improve depressive-like symptoms in animal models have failed to exhibit clinical benefit, emphasizing the need for caution in generalizing the findings from animal models to the clinical condition.

Although the face and construct validity of animal models of seizure susceptiblilty and epilepsy tend to be better than those for depression, they are still plagued by limitations. While these models have been used to identify several therapeutic strategies to reduce seizure susceptibility, none of the therapies to date have been able to prevent epileptogenesis, or the development of epilepsy. Similarly, models such as the electrical kindling paradigm are useful for observing the progression of seizure severity, but do not typically elicit the spontaneous, unprovoked seizures necessary for a clinical diagnosis of epilepsy. Thus, despite the great promise demonstrated by these models and their utility as screening tools for novel therapeutics, further development of animal models of comorbid epilepsy and depression is essential to addressing these shortcomings and improving the translational impact of these models.

1.4. Potential mechanisms and therapeutic targets

1.4.1 Neurotransmitters of interest

A variety of neurotransmitters have been implicated in epilepsy and depression individually, including acetylcholine, dopamine, GABA, glutamate, norepinephrine, and serotonin. Because this information has been reviewed extensively elsewhere, this review will focus only on their roles in animal models of epilepsy and depression comorbidity. Likewise, many of these neurotransmitters have been implicated in clinical studies of epilepsy and depression. This information has also been reviewed elsewhere and is beyond the scope of this commentary (Kanner, 2008, 2009; Krishnan and Nestler, 2008; Noebels, 2003; Werner and Covenas, 2010).

Acetylcholine

Because pilocarpine is a muscarinic receptor agonist known to produce both epilepsy and depression-like phenotypes, acetylcholine is a logical candidate to mediate comorbidity. Indeed, acetylcholine levels were elevated in the cortex and hippocampus following pilocarpine-induced SE (Jope *et al*, 1987). Similarly, acetylcholine levels were also increased in the thalamus and striatum of GEPRs (Laird *et al*, 1986). However, administration of carbachol, another muscarinic agonist, decreased the number and duration of SWD in Wag/Rij rats (Berdiev and van Luijtelaar, 2009), indicating acetylcholine may play opposing roles in absence and partial epilepsies. Interestingly, carbachol can also be used to kindle motor seizures, and the wet dog shaking behavior associated with carbachol kindling may have muscarinic mechanisms (Turski *et al*,

1984). Combined, these findings suggest that muscarinic dysfunction in general may have opposing effects in partial and absence comorbidity models. A role for acetylcholine in the depression-like behaviors of these models has not been investigated yet and should be explored in the future.

Dopamine

Dopamine is known for its key role in mediating reward in the mesocorticolimbic system, and impairment of this pathway is believed to contribute to the anhedonic symptomatology of depression. Dopamine exerted complex effects on seizure generation: stimulation of D1 receptors was proconvulsant, whereas stimulation of D2 receptors was anticonvulsant (Starr, 1996). Using a variety of monoamine reuptake blockers and glutamatergic drugs, Smolders et al reported that compounds that significantly increased dopamine levels in the hippocampus acted as anticonvulsants in the pilocarpine model of limbic seizures and as antidepressants in both the FST and TST (Smolders *et al*, 2008). Moreover, KA administration that elicited seizures and depressive behaviors significantly lowered dopamine levels in the hippocampus (Tchekalarova *et al*, 2011). SwLo rats are also known to have decreased tissue dopamine levels and dopamine release in the prefrontal cortex, and reduced locomotor response following infusion of dopaminergic drugs into the nucleus accumbens (Weiss *et al*, 2008; West *et al*, 1999a). Interestingly, infusion of amphetamine into the nucleus accumbens decreased immobility in the FST (West *et al*, 1999b). Further studies to assess the contribution of dopamine to seizure susceptibility in the SwLo rat would be helpful.

Dopamine has also been implicated in rodent models of absence epilepsy and depression comorbidity. Wag/Rij rats exhibited elevated levels of cFos immediate early gene activation in multiple terminal regions for the dopaminergic system, including the frontal cortex, nucleus accumbens, and striatum (Sarkisova *et al*, 2003). Furthermore, administration of a D2/D3 agonist showed antidepressant activity in the Wag/Rij rats, whereas a D2/D3 antagonist exacerbated depressive phenotypes in the FST (Sarkisova *et al*, 2008). These compounds produced very little effect in Wistar control rats, indicating the dopaminergic system plays a key role in the Wag/Rij phenotypes; however, additional studies in the Wag/Rij rats did not implicate dopaminergic system dysfunction as a cause for SWD (Willoughby *et al*, 1993). These findings suggest that dopaminergic alterations in Wag/Rij rats are more strongly related to depression-like phenotypes than seizure-related SWD, meaning dopaminergic abnormalities may only underlie certain aspects of the comorbidity.

Collectively, the current results support alterations in the dopaminergic systems of rodent models of partial and absence epilepsy, and these alterations could be behind certain components of the depression and epilepsy comorbidity phenotypes. Furthermore, pharmacological elevation of dopaminergic function may have both antidepressant and anticonvulsant efficacy, as well.

GABA

$GABA_A$ receptors are ligand-gated Cl^- channels that hyperpolarize the cell, whereas $GABA_B$ receptors are metabotropic, G protein-coupled receptors that activate a signaling cascade that opens a K^+ channel, allowing for a slower but more prolonged

hyperpolarization of the cell. Decreases in GABA transmission have been implicated in the excess excitation that is characteristic of epilepsy. Accordingly, some models of generalized convulsive seizures and depression comorbidity were associated with decreases in GABA, and increases in GABA levels can be therapeutic. For example, androstenol, which is thought to potentiate $GABA_A$ receptor function (Kaminski *et al*, 2006), decreased immobility in the FST and protected against 6 Hz and PTZ-induced seizures in a dose-dependent fashion in mice. Although $GABA_B$ receptors have been implicated in seizure susceptibility and depression individually, little is known about the role of these receptors in convulsive comorbidity models.

In animal models of absence epilepsy and depression comorbidity, GABA has complex effects that depend on the particular receptor subtype and brain region involved. Wag/Rij rats have decreased cortical $GABA_B$ receptor function (Inaba *et al*, 2009), and antagonism of $GABA_A$ receptors by bicuculline administration in the ventrobasal thalamic complex increased the magnitude of SWD and was alleviated by coadministration of a $GABA_B$ antagonist. However, administration of the $GABA_B$ antagonist alone had no effect on SWDs, suggesting $GABA_B$ receptors serve primarily as modulators of $GABA_A$ function (Staak and Pape, 2001). Vigabatrin and ethosuximide, two anticonvulsants that increase GABA levels, were found to exhibit both anticonvulsant and antidepressant effects in Wag/Rij rats, supporting a role for GABA in this comorbidity model (Russo *et al*, 2011; Sarkisova *et al*, 2010). In GAERS, $GABA_A$ inhibitory postsynaptic currents in the nucleus reticularis thalami had an increased amplitude and slower decay, as well as decreased paired-pulse depression, reminiscent of human epilepsy and computer-generated models of absence epilepsy (Bessaih *et al*,

2006). Because these changes were present prior to the onset of seizures, they are unlikely to represent a compensatory response to absence epilepsy. Collectively, these results imply that subtle, region-specific alterations in GABAergic function may underlie the absence seizure phenotype, and perhaps the depressive phenotype of these animals, with alterations in $GABA_A$ in some brain regions potentially increasing seizure susceptibility, and alterations in $GABA_B$ playing a modulatory role in neural excitation (Bessaih *et al*, 2006). Further research on the role of GABA in depression-related behaviors of these models is needed.

Glutamate

As the primary excitatory neurotransmitter in the brain, changes in the levels and function of glutamate are often a component of epilepsy. Ionotropic glutamate receptors, consisting of NMDA, AMPA, and kainate subtypes, are ligand-gated cation channels that depolarize the cell. Metabotropic glutamate receptors, or mGluRs, are G protein-coupled receptors that signal via second messenger pathways and allow for slower, more prolonged actions.

Ionotropic glutamate receptors are clearly implicated in epilepsy and depression separately, but have rarely been evaluated for simultaneous seizure and depression-like phenotypes in comorbidity models. For example, in the kainic acid model of comorbid epilepsy and depression, kainate induced seizures by activating kainate receptors. Antagonism of NMDA or AMPA receptors had a therapeutic effect on the progression of amygdala kindling (Gilbert, 1988) and self-sustaining SE induced by electrical stimulation of the perforant pathway (Mazarati and Wasterlain, 1999). NMDA

antagonists such as ketamine decreased immobility in the FST and protected against stress-induced depression phenotypes in the sucrose preference test (Autry *et al*, 2011). In regards to metabotropic glutamate receptors, administration of AIDA, an mGluR1 antagonist, decreased seizure severity following pilocarpine administration and decreased immobility in the TST (but not the FST) (Smolders *et al*, 2008).

In the GAERS absence seizure model, cortical NMDA responses were much more widely distributed and of longer duration, perhaps contributing to their hyperexcitable synchronicity (Pumain *et al*, 1992). Additionally, ionotropic glutamatergic receptors were found to interact in the Wag/Rij model. Administration of NMDA increased the frequency of SWD, but this increase could be blocked by co-administration of an AMPA antagonist. Similarly, administration of AMPA alone increased the frequency of SWD (Peeters *et al*, 1994), but co-administration of an NMDA receptor antagonist was sufficient to block this increase. In GEPRs, inhibition of glutamate synthesis, NMDA, or AMPA receptors attenuated seizure severity (Faingold *et al*, 1992). Contrary to the pilocarpine-SE model, where decreases in mGluR 2/3 activation increased with seizure sensitivity, mGluR 2/3 antagonists decreased seizure frequency in Wag/Rij rats (Ngomba *et al*, 2005).

Combined, these observations hint at a role for both ionotropic and metabotropic glutamate receptors in comorbid epilepsy and depression and point to a therapeutic role for antagonists of these receptors. However, determining the contribution of various glutamate receptors and their therapeutic potential in seizure and depression phenotypes simultaneously will require more research.

Norepinephrine (NE)

As part of the monoamine hypothesis of depression, noradrenergic dysfunction has been implicated in the etiology and treatment of that disorder, and NE has less well known but potent anticonvulsant properties (Weinshenker and Szot, 2002) as well, meaning alterations in this system may both underlie the comorbidity and serve as a novel therapeutic target.

NE function has been studied in some animal models of depression and epilepsy comorbidity. Perhaps the best characterized of these is the GEPR model, which has profound and widespread deficits in NE transmission in both GEPR-3s and GEPR-9s, including impairments in the telencephalon, thalamus/hypothalamus, midbrain, pons/medulla, and cerebellum (Jobe *et al*, 1982). 6-OHDA lesions of the noradrenergic system in the GEPR model exacerbated seizure severity, whereas increasing NE signaling using NE reuptake inhibitors or α1, β1, or β2 agonists dose-dependently relieved seizures (Ko *et al*, 1984; Wang *et al*, 1994). SwLo rats have a specific decrease in NE in the hippocampus (Weiss *et al*, 2008), and chronic, but not acute, treatment with NE reuptake inhibitors rescued the depression-like phenotype of these rats in the FST (West *et al*, 1998). It would be interesting to see whether noradrenergic drugs are also antidepressant in GEPR rats and anticonvulsant in SwLo rats.

Mice that lack norepinephrine (Dbh -/-) showed decreased seizure thresholds, enhanced seizure severity, and increased mortality in response to flurothyl-, PTZ-, kainic acid- and sound-induced seizures, and seizure susceptibility is rescued by restoring NE transmission (Szot *et al*, 2004; Szot *et al*, 1999; Weinshenker *et al*, 2001). Somewhat surprisingly, Dbh -/- mice had no baseline immobility differences in the FST or TST;

however, they were resistant to the effects of multiple classes of antidepressant drugs. Restoration of NE levels with L-threo-3,4-dihydroxyphenylserine (DOPS) reinstated the antidepressant effect of desipramine in FST, demonstrating the importance of NE transmission for antidepressant efficacy (Cryan *et al*, 2001).

Norepinephrine transporter (NET) knockout (NET -/-) mice have increased basal extracellular NE levels and show decreased immobility in the FST and TST and decreased PTZ and kainic acid seizure threshold (Kaminski *et al*, 2005; Xu *et al*, 2000). These results indicate that NET -/- mice may be a model of comorbidity resilience, although other studies have seen a proconvulsant phenotype under some conditions (Ahern *et al*, 2006).

Additional support for the role of NE in epilepsy and depression comorbidity comes from rats kindled with PTZ; they exhibited decreased immobility in the FST and TST following treatment with NET inhibitors (Takechi *et al*, 2011). NE transporter abundance was significantly increased in the perirhinal cortex following PTZ kindling, which would presumably decrease extracellular NE levels, suggesting that the noradrenergic dysfunction may be directly involved in this model of the comorbidity (Takechi *et al*, 2011). Finally, NE depletion attenuated the anticonvulsant activity of the ketogenic diet, vagus nerve stimulation, and valproic acid (Fornai *et al*, 2011; Schank *et al*, 2005; Weinshenker, 2008). Taken as a whole, these findings argue strongly that NE plays a role in epilepsy and depression comorbidity, as well as treatment efficacy.

Serotonin

Serotonin, the other monoamine implicated in depression, also modulates seizure susceptibility and may be involved in at least some aspects of depression and epilepsy comorbidity in a variety of models.

The pilocarpine-induced SE paradigm, which produced spontaneous seizures and increased immobility in the FST, decreased hippocampal serotonin concentration, turnover, and release (Mazarati *et al*, 2008; Pineda *et al*, 2012). Furthermore, several compounds that produce anticonvulsant and antidepressant effects in the pilocarpine model, such as citalopram, imipramine, MK-801, and MPEP, also increased hippocampal serotonin release (Smolders *et al*, 2008).

Several studies have assessed the role of various serotonergic subtypes. Specifically, a number of studies have revealed increases in the function of the presynaptic serotonin 1A autoreceptor and expression of the serotonin 2C receptor following pilocarpine-induced seizures (Krishnakumar *et al*, 2009; Pineda *et al*, 2011). The depression-like phenotypes associated with these models were reversed by the 5-HT1A agonist 8-OH-DPAT, and b. monnieri, an herbal remedy which increases hippocampal serotonin levels. Expression of the 5-HT5b gene and hippocampal 5-HT levels were reduced following KA-induced seizures and were associated with increased immobility in the FST and decreased sucrose preference (Koh *et al*, 2007; Tchekalarova *et al*, 2011).

GEPR-9s exhibited deficits in serotonergic transmission, including impaired tryptophan hydroxylase activity, widespread decreases in concentration and uptake of 5-HT, and alterations in 5-HT receptor abundance (Statnick *et al*, 1996a, b).

Carbamazepine, which increases serotonin levels, also decreased seizure severity in a dose-dependent fashion in both GEPR-3s and GEPR-9s (Dailey *et al*, 1997). Co-administration of a 5-HT 1A receptor antagonist (pindolol or LY 206130) with fluoxetine made fluoxetine more effective at reducing seizures in GEPR-9s (Browning *et al*, 1997). Thus, studies with KA and the GEPRs point to the involvement of deficits in serotonergic tone in multiple animal models of comorbid epilepsy and depression and reveal candidate targets for treatment.

1.4.2 Other mechanisms of interest

Beyond the classical neurotransmitters, there are several other classes of molecules and systems that are worthy of discussion. Although some of these have been implicated in epilepsy or depression individually, they deserve consideration for future investigation in the context of comorbidity.

Ion channels

Many different ion channels have been implicated in epilepsy, including voltage-gated Na^+, Ca^{2+}, and K^+ channels. Rare mutations in ion channels cause familial epilepsy, and mood disorders are included in the phenotypic spectrum of some epilepsy-associated ion channel mutations (Mahoney *et al*, 2009), which means that ion channel dysfunction may also contribute to comorbidity.

Amiloride, which blocks epithelial Na^+ channels, was an effective antidepressant in the FST and anticonvulsant in the PTZ and ICES paradigms (Ali *et al*, 2004). JPZ-4, a dual Na^+ and Ca^{2+} channel blocker, also decreased immobility in the FST and increased

seizure thresholds in the 6 Hz, MES, and PTZ models (Foreman *et al*, 2008). However, another Na^+ channel blocker, lamotrigine, has been associated with increased risk of suicidality in the clinical population (Bagary, 2011; Fountoulakis *et al*, 2012; Machado *et al*, 2011; Olesen *et al*, 2010; Patorno *et al*, 2010; VanCott *et al*, 2010). These results highlight the necessity of improvements in our understanding of the role of Na^+ activity in these phenotypes and their treatment.

Ca^{2+} channels are also implicated in rodent models of comorbid epilepsy and depression, and Ca^{2+} channel blockers have been put forward as effective treatment strategies. In the GEPR model, Ca^{2+} channel current and subunit abundance were increased in the inferior colliculus (N'Gouemo *et al*, 2009), and a variety of Ca^{2+} channel antagonists had anticonvulsant properties (De Sarro *et al*, 1990). Additionally, in Wag/Rij rats, expression of Cav2.1 channels in the reticular thalamic nucleus increased along with the appearance of SWD (van de Bovenkamp-Janssen *et al*, 2004). Treatment with ethosuximide, which likely acts by blocking Ca^{2+} channels, decreased both SWD and FST immobility. Na^+ channel blockers, such as zonisamide or carbamazepine, did not impact FST performance, suggesting that Ca^{2+} channel activity may be more relevant for the comorbidity in this model, with more therapeutic potential (Sarkisova *et al*, 2010). Interestingly, an increase in suicidality has been suggested following treatment with levetiracetam (Andersohn *et al*, 2010; Fountoulakis *et al*, 2012; Patorno *et al*, 2010; VanCott *et al*, 2010), which may act through inhibition of Ca^{2+} channels (Vogl *et al*, 2012). Additional studies are therefore clearly warranted to elucidate the role of Ca^{2+} channels in epilepsy, depression, and/or suicidality.

Entry of K^+ helps the neuron return to its resting potential, thereby terminating the action potential, and K^+ channel dysfunction can cause neuronal hyperexcitability. Several K^+ channel subunits and channel function were reduced in the hippocampus and cortex of GEPR rats (Verma-Ahuja *et al*, 1995); similar changes in K+ channel subunits could be blocked by treatment with ethosuximide in Wag/Rij rats (Blumenfeld *et al*, 2008). Additionally, certain K^+ channel knockout mice demonstrated depression-like phenotypes. Knockout of the Kv4.2 K^+ channel produced fluoxetine-resistant FST immobility in mice (Lockridge *et al*, 2010). TASK3 knockout mice, which lack expression of the Kcnk9 K^+ channel, also displayed increased immobility in both the FST and TST (Gotter *et al*, 2011). Importantly for the comorbidity of depression with epilepsy, mutations in the *TASK3* gene have also been identified in the GAERS model of absence epilepsy (Holter *et al*, 2005). Interestingly, the deletion of other Trek-1 (Kcnk2) K^+ channels was associated with depression resistance (Heurteaux *et al*, 2006). Together, these findings hint at an intriguing role for K^+ channel dysfunction in both depression and epilepsy, but determining whether K^+ channels are a viable therapeutic target for comorbidity awaits further studies targeting these phenotypes simultaneously.

Hypothalamic-Pituitary-Adrenal Axis

Stress plays a major role in the development of depression and is also a common trigger for seizures in epileptic patients. Stress exerts many of its effects on the brain and body via regulation of the hypothalamic-pituitary-adrenal axis (HPA). Stress triggers the release of corticotrophin releasing hormone (CRH) from the hypothalamus, which stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior

pituitary, which in turn elicits the release of glucocorticoids (cortisol in humans, corticosterone in rodents; CORT) from the adrenal cortex. A negative feedback loop decreases the activity of this pathway when glucocorticoid levels are high by acting at the level of the pituitary and hypothalamus.

In general, the HPA axis is overactive in several animal models of depression and epilepsy comorbidity. For example, exposure to early life stress increased CORT levels and lowered the number of stimulations required to achieve amygdala kindling in Wistar rats (Kumar *et al*, 2011). Rats exposed to pilocarpine-induced SE showed an elevation of CORT levels that was not suppressed with dexamethasone administration, and a positive correlation was seen between the degree of CORT elevation and immobility in the FST (Mazarati *et al*, 2009). In this model, administration of CRH increased CORT levels and immobility in the FST. Blocking glucocorticoid receptors with intra-raphe (but not intra-hippocampal) mifepristone improved FST scores only in the most severely depressed rats (Pineda *et al*, 2011).

HPA axis activation has also been linked to the severity of seizure phenotypes in models of absence epilepsy. Wag/Rij rats exhibited an elevation in foot shock stress-induced CORT levels. Interestingly, SWD were significantly decreased in the first 15 minutes following foot shock, while CORT levels were still elevated, and then SWD were increased after CORT levels return to baseline, suggesting that the period of HPA hyperactivity may worsen seizure frequency (Tolmacheva *et al*, 2012). While the connections between HPA axis activity and comorbid epilepsy and depression are still unclear, there does appear to be a relationship that merits further investigation.

Neuroinflammation

Neuroinflammatory responses are frequently linked with depression and epilepsy clinically and have also been assessed in rodent models of their comorbidity. In the pilocarpine-induced SE model, intrahippocampal delivery of an interleukin-1 receptor antagonist (IL-1ra) decreased FST immobility and increased preference for saccharin solution. Basal CORT levels were normalized, and serotonin release and response to dexamethasone were both partially restored following IL-1ra; however, there were no effects on seizure frequency (Mazarati *et al*, 2010). Coadministration of fluoxetine and IL-1ra, but not fluoxetine alone, was also effective at improving the depression-like phenotypes, but again had no effect on seizure frequency (Pineda *et al*, 2012). Other studies using animal models of comorbid epilepsy and depression have found varying effects of IL-1 alterations on seizure frequency. For example, IL-1B activation was elevated in GAERS, and blockade of IL-1β synthesis decreased SWD duration and number (Akin *et al*, 2011). In contrast, IL-1β administration was anticonvulsant in an amygdala kindling paradigm (Sayyah *et al*, 2005). These studies indicate that neuroinflammatory molecules like IL-1β may contribute to some aspects of comorbid epilepsy and depression, but the precise nature of these effects remains a mystery for now.

Neurotrophins

Neurotrophins, particularly brain derived neurotrophic factor (BDNF) and its receptor, TrkB, likely contribute to depression and epilepsy and mediate therapeutic efficacy. Indeed, decreases in BDNF are seen in adults with epilepsy (LaFrance *et al*,

2010) and depression, and these abnormalities can be reversed following antidepressant treatment (Kozisek *et al*, 2008). TrkB and BDNF have also been implicated in animal models of epilepsy and depression. For example, overexpression of hippocampal BDNF decreased the severity of spontaneous seizures following pilocarpine-induced SE, pointing to an anticonvulsant effect of BDNF signaling (Paradiso *et al*, 2011). By contrast, in a kainic acid-induced SE paradigm, transgenic mice with increased TrkB expression showed accelerated development of spontaneous seizures, while mice expressing a dominant negative form of TrkB showed delayed development of spontaneous seizures, indicating TrkB signaling has a proconvulsant effect (Heinrich *et al*, 2011). Likewise, deletion of TrkB in the hippocampus inhibited amygdala and hippocampal kindling epileptogenesis, suggesting a proconvulsant effect of TrkB activation (Kotloski and McNamara, 2010). Interestingly, this effect of TrkB knockout was not phenocopied by BDNF knockout, implicating another TrkB ligand.

BDNF and TrkB have also been assessed in rodent models of depression. Exercise, which has antidepressant and anticonvulsant properties, causes an increase in BDNF expression (Sartori *et al*, 2011) (discussed further in section 1.4.4). Mice expressing a Val66Met mutation that leads to decreased BDNF exhibited decreased sucrose preference and increased FST immobility following restraint stress (Yu *et al*, 2012), implying BDNF may serve as a protective mechanism against the harmful effects of stress on depression phenotypes. Similarly, blockade of TrkB with K252a prevented lamotrigine from exerting antidepressant effects in a variety of tests, suggesting that TrkB is also important for antidepressant drug efficacy (Li *et al*, 2011). Despite these studies on the role of neurotrophic factors in depression and epilepsy separately, more work is

needed to uncover the role of BDNF and TrkB in the comorbidity of these disorders, especially in terms of the seemingly contradictory findings concerning seizure susceptibility. Moreover, the dual antidepressant and proconvulsant effects of BDNF/TrkB signaling highlight the difficulties of finding treatment strategies that are not contraindicated.

1.4.3 Brain regions of interest

Several regions of the brain have been implicated in animal models of comorbid epilepsy and depression. Because the hippocampus and amygdala show structural and metabolic abnormalities in patients with depression and epilepsy, can be electrically kindled, and experience profound neuronal loss following pilocarpine- or KA-induced SE, these regions are of interest for TLE models (Groticke *et al*, 2007, 2008). The vast majority of animal models of comorbid limbic epilepsy and depression are associated with changes in structure, neurotransmitters, ion channels, and other mechanisms discussed above that lead to dysfunction of the hippocampus and/or amygdala.

Other neuroanatomical substrates are also implicated in depression and epilepsy comorbidity. A large body of evidence has demonstrated a role for the inferior colliculus and other midbrain regions in the comorbid phenotype of the GEPRs (N'Gouemo *et al*, 2009; N'Gouemo *et al*, 2010). In Wag/Rij rats, the frontocortical regions, striatum, and nucleus accumbens appear to be important for the seizure and depression-related phenotypes (Sarkisova *et al*, 2008). Of these regions, the prefrontal cortex and nucleus accumbens are also implicated in the SwLo rat phenotypes (Weiss *et al*, 2008; West *et al*,

1999b), while the thalamus appears to be a key region in GAERS and GEPRs (Bessaih *et al*, 2006; Jobe *et al*, 1982).

Each of these brain regions has been discussed in depth in the individual sections on mechanisms underlying comorbid epilepsy and depression (for further detail, see sections 1.4.1 and 1.4.2). Though not reiterated here, it is important to note that a contribution of these same regions in multiple paradigms is suggestive of a causal role or potential therapeutic target for depression and epilepsy comorbidity.

1.4.4 Non-pharmacological therapies

Difficulties with safe and efficacious treatment are a major clinical problem in depression and epilepsy comorbidity; patients are often refractory to treatment, and many anticonvulsants have depressant effects, while several antidepressants have proconvulsant effects (Hesdorffer *et al*, 2009; Judge *et al*, 2011), meaning non-pharmacological therapies may be especially useful for this clinical population. Three such therapies, vagus nerve stimulation, exercise, and the ketogenic diet, have demonstrated both anticonvulsant and antidepressant properties in animal models. Because these therapies appear to have the potential for clinical efficacy while simultaneously lacking adverse effects on one disease or the other, they may represent the best therapeutic strategies to explore in the future for the treatment of epilepsy and depression comorbidity.

Vagus nerve stimulation

Vagus nerve stimulation (VNS) is used clinically to treat otherwise refractory depression and epilepsy as separate conditions. Stimulation of the vagus nerve decreased

immobility in the FST in mice in a 5-HT-dependent manner (Furmaga *et al*, 2011), while it also reduced PTZ-induced cortical electrographic spiking in rats (Zhang *et al*, 2008). Consistent with the antidepressant and anticonvulsant properties of NE discussed in section 1.4.1, multiple effects of VNS were dependent upon an intact locus coeruleus (Fornai *et al*, 2011), suggesting the augmentation of noradrenergic function underlies its therapeutic efficacy. Despite these promising results, little is known about the effects of VNS on comorbid epilepsy and depression symptoms, which should be explored for their therapeutic potential.

Aerobic exercise

Aerobic exercise has both anticonvulsant and antidepressant effects clinically and in animals. Rats exposed to 3 weeks of voluntary wheel running showed decreased KA-induced seizure severity associated with increased expression of galanin mRNA in the locus coeruleus, and the anticonvulsant effects were blocked by pretreatment with a galanin receptor antagonist (Reiss *et al*, 2009). Additionally, 28 days of voluntary wheel running increased hippocampal BDNF levels and decreased immobility in the FST and TST in mice (Sartori *et al*, 2011). While research in this area is just taking off, exercise nevertheless shows great promise as a non-pharmacological therapy for comorbid epilepsy and depression.

Ketogenic diet

The ketogenic diet (KD) is a high-fat, low-carbohydrate, low-protein diet used for nearly 100 years to control refractory epilepsy. Interestingly, rats fed a ketogenic diet

displayed decreased immobility in the FST, indicating the diet may also have antidepressant properties (Murphy *et al*, 2004). The anticonvulsant effects of the KD may be mediated at least in part by NE, as demonstrated by loss of efficacy in Dbh -/- mice (Weinshenker, 2008). Whether the ketogenic diet can alleviate both depression- and seizure-related phenotypes in an animal model of comorbidity has yet to be determined.

1.5 Conclusion

The bi-directional comorbidity between depression and epilepsy has become recognized as a serious clinical problem due to its negative impact on patients' quality of life and challenges to successful treatment and prognosis. The high incidence and impact of depression in epilepsy has raised enough alarm that an expert panel of neurologists and psychiatrists from the Epilepsy Foundation's Mood Disorders Initiative wrote and published a "Consensus Statement" to improve the recognition and treatment of depressive disorders in patients with epilepsy (Barry *et al*, 2008). The establishment of several chemical, electrical, and genetic animal models of this comorbidity is a crucial step toward a better understanding of the underlying neurobiological substrates and potential therapeutics.

Though these animal models have revealed some useful information, this field is still in its infancy, with many issues that need to be addressed. Despite the wealth of animal models of epilepsy that also show depression-related phenotypes, there is currently only one model of depression that also shows enhanced seizure susceptibility and epileptogenesis: the SwLo rat. More models addressing this direction of the comorbidity are needed for a better understanding of the similarities and differences in

causes, mechanisms, and treatment strategies for this half of the comorbidity equation. Additionally, many of the current models focus on seizure susceptibility rather than true epilepsy (i.e. the development of unprovoked, spontaneous seizures). Because the clinical comorbidity is between depression and epilepsy, rather than depression and seizure susceptibility per se, a stronger emphasis on animal models of epileptogenesis may be more clinically relevant.

Future studies on the genetic underpinnings of this comorbidity are also needed. Since both diseases are known to have a large genetic component, it seems likely that genetic risk factors also contribute to comorbidity (Kanner and Nieto, 1999). Genetic/selective breeding models of comorbidity represent valuable resources for the identification of susceptibility genes. Quantitative trait loci (QTL) and linkage mapping studies, microarrays, and/or analysis of protein expression could all be used to uncover candidates for predictive screening or targets for novel therapeutics. Targeting these candidates with site-specific genetic manipulations and/or viral vectors and assessing the effect on the phenotypes of interest would also contribute a great deal to both our understanding of and ability to treat epilepsy and depression comorbidity.

In conclusion, further investigation of current animal models and the development of entirely new animal models are critical for the discovery of neurobiological substrates underlying epilepsy and depression comorbidity, for identifying novel therapeutic targets, and as a screening tool for testing the safety and efficacy of such new treatments.

The experiments discussed in this dissertation have aimed to address several of these concerns and advance our knowledge in this field through further validation of the SwLo rat model of depression and epilepsy comorbidity. We have therefore

characterized the SwLo rat line using a variety of seizure- and epileptogenesis-susceptibility paradigms, and utilized this model to test a non-pharmacological therapy for both antidepressant and anticonvulsant efficacy. The validation of this rodent model has provided an important new tool for studying depression-related epilepsy, the only such tool of its kind, and will allow for additional studies into the mechanisms underlying this comorbidity and further screening of novel therapeutic strategies.

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Table 1.1 Characterization of animal models of epilepsy and depression comorbidity.

Model	Seizure Phenotype	Depression Phenotype	Comments	Reference
Partial Epilepsy				
Kainic Acid (Acute and SE)	Primary limbic seizures with secondary generalization	↑ FST immobility ↓ Sucrose preference	↓ Hippocampal DA ↓ 5-HT and *Htr5b* gene expression are proconvulsant and/or prodepressant ↑ TrkB signaling is proconvulsant; ↓ TrkB signaling is anticonvulsant	(Tchekalarova *et al*, 2011) (Koh *et al*, 2007; Tchekalarova *et al*, 2011) (Heinrich *et al*, 2011)
Pilocarpine (Acute and SE)	Primary limbic seizures with secondary generalization	Mixed results on FST ↓ Sucrose preference Sex & species may be important	↑ ACh in cortex & hippocampus following seizure ↑ Hippocampal DA is anticonvulsant & antidepressant ↓ mGluR 1 activation is anticonvulsant & antidepressant ↓ 5-HT hippocampal concentration, turnover, release, evoked cortical release ↑ 5-HT is anticonvulsant & antidepressant ↑ 5-HT 1A function & 5-HT 2C levels following seizures ↑ CORT, CRH administration are proconvulsant ↓ Glucocorticoid receptor activation is antidepressant ↓ IL-1 activation is antidepressant but has no effect on seizures ↑ BDNF expression is anticonvulsant	(Jope *et al*, 1987) (Smolders *et al*, 2008) (Smolders *et al*, 2008) (Mazarati *et al*, 2008; Pineda *et al*, 2011) (Krishnakumar *et al*, 2009; Smolders *et al*, 2008) (Krishnakumar *et al*, 2009; Pineda *et al*, 2011) (Mazarati *et al*, 2009) (Pineda *et al*, 2011) (Mazarati *et al*, 2010; Pineda *et al*, 2012) (Paradiso *et al*, 2011)
Electrical Kindling	Primary limbic seizures with secondary generalization	Mixed results on FST & sucrose preference Time course may be important	↓ NMDA/AMPA activation is anticonvulsant ↑ CORT is proconvulsant ↑ IL-1B is anticonvulsant ↓ TrkB expression is anticonvulsant	(Gilbert, 1988) (Kumar *et al*, 2011) (Sayyah *et al*, 2005) (Kotloski and McNamara, 2010)
SwLo	Enhanced susceptibility to primarily limbic seizures	Selectively bred based on ↑ immobility in FST	↓ Prefrontal DA levels & release ↑ DA is antidepressant ↓ Hippocampal NE ↑ NE is antidepressant	(Weiss *et al*, 2008) (West *et al*, 1999) (Weiss *et al*, 2008) (West and Weiss, 1998)
Generalized Convulsive Epilepsy				
Pentylenetetrazol (PTZ)	Generalized tonic-clonic seizures	Mixed results on FST	↑ $GABA_A$ potentiation (Androstenol) is anticonvulsant & antidepressant ↑ NET in perirhinal cortex; antidepressant effect of NE & DA reuptake inhibition ↓ Na^+ channel activation is anticonvulsant & antidepressant	(Kaminski *et al*, 2006) (Takechi *et al*, 2011) (Ali *et al*, 2004; Foreman *et al*, 2008)
GEPRS	Selectively bred based on susceptibility to audiogenic seizures; generalized tonic-clonic seizures	↑ FST immobility ↓ Saccharin consumption Altered diurnal motor activity	↑ ACh in thalamus and striatum ↓ Glutamate synthesis, ↓ Activation of NMDA/AMPA are anticonvulsant ↓ NE throughout the brain ↑ Activation of α1, β1, β2 receptors is anticonvulsant ↓ NE via 6-OHDA lesion of the locus coeruleus, A1, or noradrenergic dorsal bundle is proconvulsant ↓ 5-HT concentration, uptake, tryptophan hydroxylase, 5-HT 1A receptors (in hippocampus) ↑ 5-HT (carbamazepine) is anticonvulsant ↓ 5-HT 1A receptor activation is anticonvulsant when coadministered with fluoxetine ↑ 5-HT 1B receptors in hippocampus ↑ Ca^{2+} current & subunit abundance in inferior colliculus ↓ Ca^{2+} activation is anticonvulsant ↓ K^+ subunit levels & function in hippocampus & cortex	(Laird *et al*, 1986) (Faingold *et al*, 1992) (Jobe *et al*, 1982) (Ko *et al*, 1984) (Wang *et al*, 1994) (Statnick *et al*, 1996) (Dailey *et al*, 1997) (Browning *et al*, 1997) (Statnick *et al*, 1996) (N'Gouemo *et al*, 2009; N'Gouemo *et al*, 2010) (De Sarro *et al*, 1990) (Verma-Ahuja *et al*, 1995)
Dbh -/-	↑ seizure susceptibility	↓ antidepressant responsivity	↓ NE is proconvulsant & confers resistance to antidepressants ↑ NE restores antidepressant effect of desipramine; ↑ NE or adrenergic receptor activation is anticonvulsant against flurothyl- and PTZ-induced seizures	(Cryan *et al*, 2001; Schank *et al*, 2005; Szot *et al*, 2004; Szot *et al*, 2001; Szot *et al*, 1999; Weinshenker *et al*, 2001)

Generalized Absence Epilepsy				
Wag/Rij	Inbred line with spontaneous SWD in cortical EEG	↑ FST immobility ↓ Sucrose consumption	↑ ACh via carbachol is anticonvulsant	(Berdiev and van Luijtelaar, 2009)
			↑ cFos activation in DA terminals; not related to SWD	(Sarkisova *et al*, 2003; Willoughby *et al*, 1993)
			↑ D2/D3 activation (parlodel) is antidepressant	(Sarkisova *et al*, 2008)
			↓ D2/D3 activation (raclopride) is prodepressant	(Sarkisova *et al*, 2008)
			↓ $GABA_A$ activation (bicuculline) is proconvulsant; attenuated by coadministration of $GABA_B$ antagonist	(Staak and Pape, 2001)
			↓ $GABA_B$ function in neocortex	(Inaba *et al*, 2009)
			↑ GABA (vigabatrin, ethosuximide) is anticonvulsant & antidepressant	(Russo *et al*, 2011; Sarkisova *et al*, 2010)
			↑ Activation of NMDA/AMPA is proconvulsant; blocked by ↓ activation of AMPA/NMDA, respectively	(Peeters *et al*, 1994)
			↓ mGluR 2/3 activation is anticonvulsant	(Ngomba *et al*, 2005)
			↓ Na^+ channel activation had no effect on depression	(Sarkisova *et al*, 2010)
			↑ Cav2.1 Ca^{2+} channel expression with SWD	(van de Bovenkamp-Janssen *et al*, 2004)
			↓ Ca^{2+} channel activation is anticonvulsant & antidepressant	(Sarkisova *et al*, 2010)
			↓ K^+ subunit levels & function in hippocampus & cortex; reversed by Ca^{2+} channel blockade	(Blumenfeld *et al*, 2008)
			↑ CORT following stress associated with ↓ in SWD	(Tolmacheva *et al*, 2012)
GAERS	Selectively bred for spontaneous SWD	↓ sucrose consumption	↑ $GABA_A$ postsynaptic current amplitude	(Bessaih *et al*, 2006)
			↑ NMDAR distribution, longer duration	(Pumain *et al*, 1992)
			Mutation in *TASK3* (TWIK-related acid-sensitive K^+ channel 3) gene	(Holter *et al*, 2005)
			↑ IL-1B activation; ↓ IL-1B synthesis is anticonvulsant	(Akin *et al*, 2011)

Table 1.1 Characterization of animal models of epilepsy and depression comorbidity. Abbreviations: 5-HT, serotonin; ACh, acetylcholine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; BDNF, brain-derived neurotrophic factor; CORT, corticosterone; CRH, corticotropin-releasing hormone; DA, dopamine; Dbh, Dopamine-beta-hydroxylase; EEG, electroencephalography; FST, forced swim test; GABA, γ-aminobutyric acid; GAERS, Genetic Absence Epilepsy Rats from Strasbourg; GEPRS, Genetically Epilepsy Prone Rats; IL, interleukin; mGluR, metabotropic glutamate receptor; NE, norepinephrine; NET, norepinephrine transporter; NMDA, N-methyl-D-aspartate; SE, status epilepticus; SWD, slow wave discharge; SwLo, Swim Lo-Active; TrkB, Tyrosine-related kinase B; Wag/Rij, Wistar Albino Glaxo/from Rijswijk; WDS, wet dog shake

CHAPTER 2:

SEIZURE SUSCEPTIBILITY AND EPILEPTOGENESIS IN A RAT MODEL OF EPILEPSY AND DEPRESSION COMORBIDITY

Adapted from:

Epps SA, Tabb KD, Lin SJ, Kahn AB, Javors MA, Boss-Williams KA*, et al* (2012a). Seizure susceptibility and epileptogenesis in a rat model of epilepsy and depression co-morbidity. *Neuropsychopharmacology* **37**(13): 2756-2763.

2.1 Abstract

Although a strong comorbidity exists clinically between epilepsy and depression, the cause of this comorbidity remains unknown, and a valid animal model is crucial for the identification of underlying mechanisms and the development of a screening tool for novel therapies. While some rodent models of epilepsy have been reported to display behaviors relevant to affective disorders, the seizure susceptibility of animals prone to depression-like behavior has not been characterized. Towards this end, we assessed several forms of seizure sensitivity and epileptogenesis in rats selectively bred for vulnerability (Swim Lo-Active; SwLo) or resilience (Swim High-Active; SwHi) to depression-like phenotypes. The SwLo rats exhibit decreased motor activity in a swim test and other depression-like phenotypes, whereas the SwHi rats display increased motor activity in a swim test. SwLo rats exhibited a decreased latency to limbic motor seizures following acute pilocarpine administration in the absence of differences in pilocarpine pharmacokinetics, and also had a decreased threshold to tonic seizures induced by electroshock. Approximately half of the SwLo rats, but none of the SwHi rats, had spontaneous limbic motor seizures 5 weeks following pilocarpine-induced status epilepticus. While the number of stimulations required to achieve full amygdala and hippocampal electrical kindling were similar in the two rat lines, SwLo rats had a lower final hippocampal kindling threshold and more wet dog shakes during both amygdala and hippocampal kindling. Combined, these results indicate that SwLo rats are a model of epilepsy and depression comorbidity that can be used for investigating underlying neurobiological and genetic mechanisms and screening novel therapeutics.

2.2 Introduction

A wealth of clinical data has established a strong comorbidity between epilepsy and depression (Hesdorffer *et al*, 2006; Kanner, 2003; Kanner and Balabanov, 2002). This comorbidity is bi-directional; that is, patients with epilepsy are 3-5 times more likely to develop depression, and patients with active depression, a history of depression, or a family history of depression are nearly twice as likely to develop epilepsy, a risk which rises to 4.2 fold if they have a history of suicide attempt (Hesdorffer *et al*, 2006; Morgan *et al*, 2012). Depression has a more profound impact on the quality of life of individuals with epilepsy than seizure frequency or severity (Boylan *et al*, 2004; Cramer *et al*, 2003; Jehi *et al*, 2011; Johnson *et al*, 2004; Kanner, 2006; Pulsipher *et al*, 2006). Importantly, this comorbidity is also highly detrimental to overall prognosis and outcome, as patients with both disorders exhibit higher rates of re-hospitalization and decreased success with treatment. Indeed, several antiepileptic medications, such as gabapentin, lamotrigine, oxcarbazepine, tiagabine, and valproate, can cause depressed mood, while some antidepressants increase seizure risk, particularly in overdose situations (Hesdorffer *et al*, 2009; Judge *et al*, 2011).

Several reasons for this comorbidity have been posited, including shared neurobiological pathways, neuroanatomical regions, and common genetic mechanisms (Harden, 2002; Kanner *et al*, 2002; Sankar and Mazarati, 2010; Wiegartz *et al*, 1999). The high incidence and impact of depression in epilepsy has become such a concern that an expert panel of neurologists and psychiatrists from the Epilepsy Foundation's Mood Disorders Initiative wrote and published a "Consensus Statement" to improve the recognition and treatment of depressive disorders in patients with epilepsy (Barry *et al*,

2008), and a valid animal model is needed to identify underlying mechanisms and develop a screening tool for novel therapeutics (Jobe *et al*, 1999).

While several studies have assessed depression-like symptoms in epilepsy-prone animals (Jobe, 2003; Jones *et al*, 2008; McIntyre and Gilby, 2007; Pineda *et al*, 2011), there is only one study in which seizure susceptibility was assessed in an animal model of depression, the SwimLo-Active (SwLo) rats (Tabb *et al*, 2007), which were selectively bred for low activity in a swim test and also exhibit other depression-like and anhedonic behaviors such as decreased response to dopaminergic drugs and increased intracranial self-stimulation threshold (Weiss *et al*, 1998b; Weiss *et al*, 2008; West *et al*, 1999a; West *et al*, 1999b) (C. West, personal communication). Their counterparts, the SwHi-Active (SwHi) rats, were selectively bred for high struggling activity in the forced swim test, and serve as a model of depression resilience (Weiss *et al*, 1998b).

In that paper, we reported that SwLo rats have increased mortality following kainic acid-induced limbic seizures, but no differences in flurothyl-induced generalized seizure susceptibility, suggesting that the SwLo rats are specifically sensitive to limbic seizures (Tabb *et al*, 2007). This was of particular interest because the rate of depression is higher for patients with temporal lobe epilepsy (TLE), who suffer from recurrent limbic seizures, than other forms of epilepsy (Kondziella *et al*, 2007; Piazzini *et al*, 2001). While these initial results in the SwLo rats were provocative, they had several major limitations; mortality rate in the 24 hr following kainic acid administration was not a specific measure of seizure susceptibility in so far as we did not have direct evidence that the animals died from seizures. Also, brain levels of kainic acid were not measured and epileptogenesis was not assessed. Thus, we undertook the present studies to further

investigate limbic seizure susceptibility and epileptogenesis in the SwLo rat to determine its utility as a model of epilepsy and depression comorbidity.

2.3 Method

Selectively bred rats

SwLo and SwHi rats were selectively bred as described (Weiss *et al*, 1998b). Two to four month old rats were subjected to a 15 min swim in a tank of 25°C water. Duration of struggling (active movement of all 4 paws, forepaws breaking surface of water) and floating (complete immobility, no limb movement) were measured. All experiments used male rats of generations 34-56. A subset of rats in each experiment were exposed to a swim test to confirm the persistence of this phenotype in each line, while the remaining littermate animals were experimentally naive. On average, SwLo rats typically float for a minimum of 600 sec and struggle for no more than 10 sec of a 15 min test, while SwHi rats float for less than 20 sec and struggle in excess of 200 sec (Weiss *et al*, 2008). No differences in seizure susceptibility were observed between the swim test-exposed and naive rats, and data were combined. Each rat was used only once in a seizure experiment, and a period of at least one week was given following exposure to the swim test before seizure induction. Rats were maintained on a 12h light-dark cycle, with standard rat chow and water available *ad libitum*. All experiments were approved by the Emory University Institutional Animal Care and Use Committee.

Pilocarpine-induced seizures

Rats (approximately 2-4 months of age) were injected with the peripheral muscarinic antagonist atropine methyl bromide (2 mg/kg, s.c., Sigma-Aldrich, St. Louis, MO) 30 min prior to pilocarpine hydrochloride administration (380 mg/kg, i.p., Sigma-Aldrich, St. Louis, MO). Rats were placed in a clear chamber with continuous video monitoring, and latency to limbic motor seizure, defined as bilateral forelimb clonus with rearing and falling behaviors, was measured. Rats that did not achieve a limbic motor seizure within 1 hr following pilocarpine administration received a booster dose of pilocarpine (190 mg/kg).

A subset of rats from the acute pilocarpine study was allowed to sustain 1 hr of status epilepticus before seizures were terminated with diazepam (5 mg/kg, i.p., Sigma-Aldrich, St. Louis, MO). Five weeks later, these rats were returned to their individual test chambers and continuously video-recorded during a 12-hr dark cycle, and video was scored for spontaneous limbic motor seizures. During the following light-cycle, rats were subjected to a 15 min swim stress, as described above. No seizures occurred during the swim test. Rats were then returned to the test chambers and continuously video-recorded during the next dark cycle, and videos were again scored for spontaneous seizures. This allowed us to observe any delayed effects of stress on spontaneous seizure incidence, an important consideration given the known effects of stress on depression and epilepsy in humans (Frucht *et al*, 2000; Haut *et al*, 2003; Heim and Binder, 2012; Sperling *et al*, 2008).

Pilocarpine pharmacokinetics

Rats were administered atropine and pilocarpine as described above, then euthanized 5, 10, or 15 min later. The dorsal hippocampus was dissected on ice, and tissue pilocarpine levels were measured by HPLC.

All reagents used in this assay were HPLC grade quality. Milli-Q water was used for preparation of all solutions (Millipore, Bedford, MA, USA). Pilocarpine was obtained from Sigma Chemical Co. (St. Louis, MO, USA). For the brain calibrator curve, 30 mg of control (untreated) rat brain tissue was homogenized in 0.5 ml of 0.9% saline and spiked to achieve the desired concentrations of 0, 25, 150, and 300 µg/gm brain tissue. Thirty mg of unknown brain samples were weighed and homogenized in 0.5 ml saline. Brain homogenate samples and calibrators were mixed with 2 ml of dichloromethane in 16 x 100 mm polypropylene test tubes. Samples were shaken for 10 minutes on an Eberbach shaker and then centrifuged at 2100 *g* in a Beckman Coulter Allegra X15R for 10 minutes at 23^{o}C. The samples were frozen in a -80°C freezer for 10 min to facilitate separation of the two phases. The liquid organic supernatants were then transferred to 13 x 100 mm glass test tubes and evaporated to dryness at 40°C under a nitrogen stream. The residues were dissolved in 200 µl of HCl (1 mM) by vortex mixing for 1 min. The reconstituted samples were washed with 2 ml of diethyl ether by vortex mixing for 2 min and then centrifuged (2100 g, 10 min). After discarding the ether supernatant the aqueous samples were exposed to vacuum (20 s) to remove residual ether.

The HPLC system included a Waters model 510 pump, Waters model 717 sample injector, Waters model 2487 UV detector, and an Altima C18 analytical column (5 µ; 4.6 x 150 mm). Samples were analyzed at 214 nm and the flow rate of the mobile phase was

1.2 ml/min. The mobile phase contained 35% acetonitrile and 65% of a solution of 7 mM KH_2PO_4 (pH 4.0). Drug concentrations were quantified by comparing sample peak areas against the linear regression of calibrator sample peak areas from a four point standard curve (0, 25, 150, and 300) µg pilocarpine/gm brain. The limit of detection for the assay was 5 µg/gm. Pilocarpine levels were expressed in µg/gm.

Increasing current electroshock-induced seizures

Electroshock seizures were induced by application of electrical stimulation via earclip electrodes using an Ugo Basile ECT Unit #57800 (Ugo Basile North America, Collegeville, PA). The initial stimulation was a 100 Hz, 5 msec square wave with an intensity of 10 mA; the intensity of successive stimulations was linearly increased by 1 mA/1 sec (Kitano *et al*, 1996), with successive stimulations separated by 1 min. This procedure was repeated until tonic hindlimb flexion was observed, allowing for the identification of a seizure current threshold for each individual rat.

Electrical kindling

Rats were anesthetized with isoflurane and implanted with a bipolar recording and stimulating electrode in the right amygdala (2.6 mm posterior, 4.6 to 5.0 mm lateral, and 7.1 to 8.1 mm below dura, relative to bregma) or right dorsal hippocampus (3.7 mm posterior, 2.5 mm lateral, and 2.7 to 3.2 mm below dura, relative to Bregma) (Paxinos and Watson, 1998). The electrode and a ground screw (placed over the ipsilateral cortex) were fitted into a head cap (Plastics One, Roanoke, VA) and anchored in place using dental cement.

One week following electrode implantation, two Grass S44 stimulators and two Grass PSIU6 constant current stimulus isolators (Grass Technologies, West Warwick, RI) were used to deliver a 1.0 sec train of 1.0 ms biphasic rectangular pulses at 60 Hz. Electrographic seizure activity was recorded using a Grass 78D Polygraph (Grass Technologies, West Warwick, RI) for the entire duration of the seizure. The initial kindling stimulation determined the seizure threshold to be used for kindling stimulations. For each rat, seizure threshold was determined by applying a starting stimulation of 50 uA. If an electrographic seizure was not induced, the stimulation was increased by 25 uA every 60 sec until seizure spiking was observed. This threshold stimulation, which initially evoked an electrographic seizure but no behavioral seizure activity, was then used for all further tests of the rat. Kindling stimulations were applied twice daily (4 hr inter-stimulation interval). Behavioral seizures were classified using a modified Racine scale (He *et al*, 2004): 0, normal activity; 1, facial clonus, immobility, wet dog shakes, and/or stiffened tail; 2, head nodding/bobbing; 3, unilateral forelimb clonus, continuous body clonus (without loss of posture); 4, rearing with bilateral forelimb clonus, severe continuous whole body clonus; 5, rearing and falling (loss of postural control); 6, tonic-clonic seizure. Additionally, the number of wet dog shakes observed during each stimulation was recorded. If at any point during the kindling stimulations a rat failed to show both electrographic seizure and seizure behaviors for two consecutive stimulations, the threshold value was redetermined, beginning with the previously-defined threshold value and increasing intensity by 25 uA every 1 min until an electrographic seizure was obtained. This new threshold value was then used for all further stimulations.

A rat was considered to have reached a fully kindled state after 3 consecutive Class 4 or higher seizures. Stimulations were terminated in any rats that had not achieved kindled status by 40 stimulations in the amygdala or 80 stimulations in the hippocampus. At the conclusion of the kindling experiment, proper electrode placement was confirmed by cresyl violet staining.

Statistical analysis

Data were analyzed using a Student's t-test for the acute pilocarpine, increasing current electroshock, and kindling studies, a two-way ANOVA for the pilocarpine pharmacokinetics, and a Fisher's Exact Test for the chronic pilocarpine experiments. All data analysis was conducted using Prism GraphPad 5.0 and IBM SPSS 17.0.

2.4 Results

Acute pilocarpine-induced seizure susceptibility

Following acute administration of pilocarpine (380 mg/kg, i.p.), SwLo rats displayed a decreased latency to limbic motor seizure compared to SwHi rats ($t_{20} = 3.528$, $p < 0.01$) (Figure 2.1A) (Tabb, 2008).

Pilocarpine pharmacokinetics

To verify that the increased pilocarpine-induced seizure susceptibility in the SwLo rats was not due to a difference in the concentration of pilocarpine present in the brain (i.e., differences in pilocarpine pharmacokinetics), hippocampal tissue samples

were analyzed for pilocarpine levels 5, 10, and 15 min following administration of pilocarpine (380 mg/kg, i.p.). Brain pilocarpine levels increased over time in both SwLo and SwHi rats, but no differences between lines were observed (Figure 2.1B). 2-way ANOVA revealed a significant effect of time ($F_{2,12} = 8.13$, $p < 0.01$) but not line ($F_{1,12} = 0.07$, $p = 0.80$) or time x line interaction ($F_{2,12} = 0.63$, $p = 0.55$).

Increasing current electroshock seizures

To assess whether differences in seizure susceptibility were restricted to chemoconvulsant-induced seizures, we tested SwLo and SwHi rats in an increasing current electroshock seizure paradigm. As shown in Figure 2.2, SwLo rats seized at a lower electroshock threshold than SwHi rats, ($t_{10} = 9.391$, $p < 0.0001$).

Pilocarpine-induced spontaneous seizures

Five weeks following a 1 hr period of status epilepticus induced by pilocarpine (380 mg/kg, i.p.), SwLo and SwHi rats were video recorded and assessed for the appearance of spontaneous limbic seizures during the dark cycle. Approximately 25% (2/9) of the SwLo rats had spontaneous seizures, while none of the SwHi rats did. The next day, rats were subjected to a 15-min swim stress during the light cycle, and again assessed for spontaneous seizures during the following 12-hour dark cycle. Both of the SwLo rats that had spontaneous seizures the previous night, plus two additional SwLo rats, had spontaneous seizures, for a total of 4/9 (44%), while none of the SwHi rats had spontaneous seizures. This difference did not achieve statistical significance ($p = 0.10$) (Tabb, 2008).

Electrical kindling

SwLo and SwHi rats did not differ in initial stimulation threshold for the amygdala or hippocampus (Figure 2.3A & B). While amygdala kindling threshold did not change over time for either line, 4/9 SwHi rats required an increase in threshold to achieve electrographic seizures during hippocampal kindling, whereas none of the SwLo rats did, resulting in a significant difference in final threshold stimulation level between rat lines (Figure 2.3B). A 2-way repeated measures ANOVA revealed a main effect of line ($F_{1,17} = 5.915$, $p = 0.0264$), initial vs. final time point ($F_{1,17} = 6.066$, $p = 0.0248$), and a line x time point interaction ($F_{1,17} = 6.066$, $p = 0.0248$). Bonferroni's post-hoc tests showed a significant difference between the initial and final threshold value in SwHi rats ($t_{17} = 3.395$, $p < 0.01$) and between final threshold values of SwHi and SwLo rats ($t_{17} = 2.959$, $p < 0.05$). Furthermore, SwLo rats showed more wet dog shakes during both amygdala and hippocampal kindling ($t_{281} = 5.526$, $p < 0.0001$ for amygdala, $t_{1214} = 8.004$, $p < 0.0001$ for hippocampus; Figure 2.3C & D). There were no significant differences between SwLo and SwHi rats in the rate of progression to any seizure stage, including the number of stimulations required to reach a fully kindled state, for the amygdala or hippocampus (Figure 2.3E & F).

2.5 Discussion

The results presented here support the validity of SwLo rats as an animal model of epilepsy and depression comorbidity. The depression-like phenotypes of SwLo rats (i.e. low activity in the forced swim test that is reversible by chronic but not acute

antidepressant treatment, decreased response to dopaminergic drugs (Weiss *et al*, 1998b; West *et al*, 1999a; West *et al*, 1999b; West *et al*, 1998)) have been described previously. Here, we show that SwLo rats have increased acute susceptibility to seizures induced by chemoconvulsants and electroshock, and also show exacerbated epileptogenesis in the chronic pilocarpine model and certain parameters of electrical kindling.

Seizure susceptibility and epileptogenesis in the SwLo rat

We reported previously that SwLo rats had a higher incidence of mortality following kainic acid-induced limbic seizures but no differences in generalized flurothyl-induced tonic-clonic seizure susceptibility, suggesting that SwLo rats might be particularly prone to limbic seizures (Tabb *et al*, 2007). This distinction is of interest because there is evidence to suggest that temporal lobe epilepsy has an increased association with depression compared with other forms of epilepsy (Kondziella *et al*, 2007; Piazzini *et al*, 2001). Our present results appear to partially support this idea, as SwLo rats are also more sensitive to acute limbic seizures induced by pilocarpine, as well as spontaneous limbic seizures in the weeks following pilocarpine-induced status epilepticus (Tabb, 2008). However, this association is complex, and this is an area of ongoing study and debate, as reviewed by Hoppe and Elger (Hoppe and Elger, 2011). Rates of depression are also elevated in patients with non-limbic generalized epilepsies as well. This suggests that structures outside of the temporal lobe may also be implicated in the comorbidity, as supported by the SwLo rats' lower threshold for electroshock-induced tonic seizures, which likely also involve brainstem structures (Shehab *et al*, 1995).

The appearance of spontaneous seizures in the weeks following pilocarpine-induced status epilepticus in SwLo rats, although not statistically significant, is an important extension of our previous findings. The presence of spontaneous seizures in nearly half of the SwLo rats, but none of the SwHi rats, suggests increased epileptogenesis in addition to the acute differences in seizure susceptibility. This enhanced epileptogenesis is also more relevant to the clinical evidence supporting a comorbidity between depression and the development of epilepsy.

Given the importance of the amygdala and hippocampus in both limbic seizures and depression, we suspected that electrical kindling would be accelerated in these brain regions in SwLo rats. While we did not find this epileptogenic criterion to be altered in the SwLo rats, we did observe an increased incidence of wet dog shakes during kindling. Furthermore, many of the SwHi rats required an increase in stimulation magnitude to achieve threshold for electrographic seizures during the kindling process, while none of the SwLo rats did. Bragin and colleagues reported a similar threshold increase during kindling in rats that had been previously treated with an intrahippocampal injection of kainic acid (Bragin *et al*, 2002). They interpreted this phenomenon as a compensatory protective response to seizure generation and propagation, consistent with evidence that a variety of compensatory cellular and molecular changes occur in the epileptic brain (McNamara, 1994). If the same processes account for the increased threshold in both the intrahippocampal kainic acid and electrical kindling paradigms, this would suggest that SwLo rats lack this adaptive protective capacity. It is also possible that SwHi rats have an inherently seizure-resistant brain, which is consistent with our finding that none of the SwHi rats developed spontaneous seizures following pilocarpine-induced status

epilepticus, even following a swim stress that increased the incidence of spontaneous seizures in the SwLo line.

Potential mechanisms underlying the SwLo seizure phenotypes

Genetic risk factors contribute significantly to epilepsy, depression, and the interaction between these diseases. For example, over 50% of epileptic patients with depression have a family history of psychiatric illness (Kanner *et al*, 1999; Robertson *et al*, 1987). One advantage of the SwLo rats as a model of epilepsy and depression comorbidity is that these animals have been selectively bred for over 50 generations and are genetically homogenous (Weinshenker *et al*, 2005) (unpublished data). Furthermore, cross-fostering experiments with SwHi rats have revealed that the SwLo swim test phenotype is heritable and controlled by the genotype of the offspring rather than being due to potential differences in rearing (unpublished data and C. West, personal communication). Thus, mapping and identifying genes controlling depression-like behavior and seizure susceptibility differences between SwLo and SwHi rats is possible using quantitative trait loci (QTL) analysis. While QTL and other linkage analyses have been employed to map and identify risk factor genes for epilepsy or depression separately, the SwLo and SwHi rats seem to represent an excellent tool for the discovery of comorbidity genes.

It is not clear *a priori* what types of genes underlie the SwLo phenotypes, but we speculate that noradrenergic dysfunction could be an important contributor. A large body of evidence indicates that norepinephrine has potent anticonvulsant and antiepileptogenic properties (Giorgi *et al*, 2006; Giorgi *et al*, 2004; Kokaia *et al*, 1989; Seo *et al*, 2000;

Stanton, 1992; Szot *et al*, 1999; Weinshenker *et al*, 2002). For example, norepinephrine depletion accelerates the development of hippocampal kindling and increases carbachol-induced wet dog shakes in rats, while central injection of norepinephrine decreases wet dog shakes (Ferencz *et al*, 2001; Kokaia *et al*, 1989; Turski *et al*, 1982) (but see (Bortolotto and Cavalheiro, 1986)). These findings are of particular interest because SwLo rats have a selective decrease of norepinephrine in the hippocampus (Weiss *et al*, 2008), and chronic administration of norepinephrine reuptake inhibitors reverses depression-like phenotypes in SwLo rats in the swim test (Weiss *et al*, 1998b; West *et al*, 1998). Cholinergic dysfunction is another potential culprit. For example, we have shown in this study that SwLo rats are more susceptible to seizures and epileptogenesis induced by the muscarinic acetylcholine receptor agonist, pilocarpine. SwLo rats also have a higher incidence of wet dog shakes during kindling, and disturbances in the muscarinic system have been implicated in wet dog shakes induced by carbachol (Turski *et al*, 1984).

Conclusion

While multiple studies have been conducted assessing depression-like phenotypes in rodent models of epilepsy, the SwLo rats are, to our knowledge, the only example of animals selected and developed specifically for depression-related behavior that also show phenotypes relevant to epilepsy. Thus, the SwLo rats represent a unique model for identifying the genes and mechanisms underlying comorbid depression and epilepsy, as well as a preclinical tool to screen novel therapeutics for safety and efficacy in treating both diseases simultaneously. Although the focus of this study was on the SwLo rats as a model for susceptibility to epilepsy and depression, the SwHi rats are also of interest.

Because the depression- and epilepsy-like phenotypes of "wild-type", non-selected rats tend to fall somewhere in between those of SwLo and SwHi rats (Tabb *et al*, 2007; Weiss *et al*, 1998b; Weiss *et al*, 2008) (unpublished data), the SwHi rats may be a model for comorbidity resilience.

2.6 Acknowledgements

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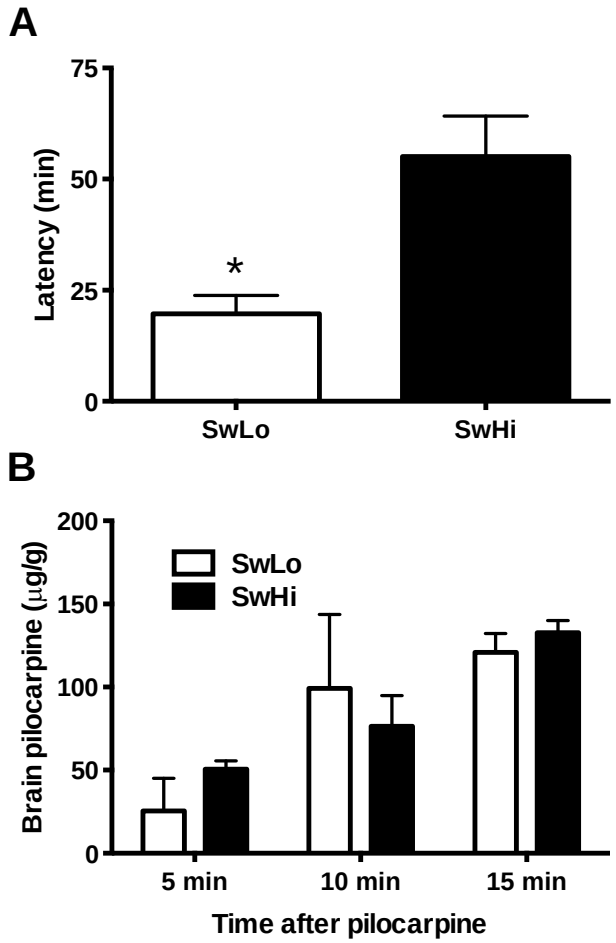
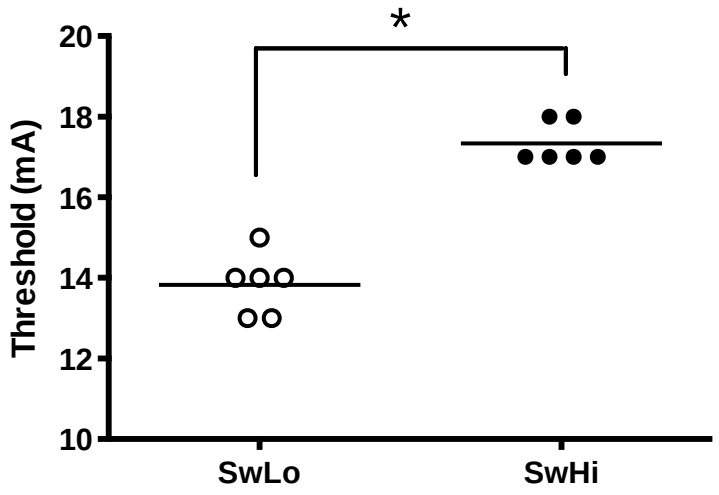
Figure 2.1 SwLo rats have a shorter latency to pilocarpine-induced seizures than SwHi rats that is independent of pilocarpine pharmacokinetics.



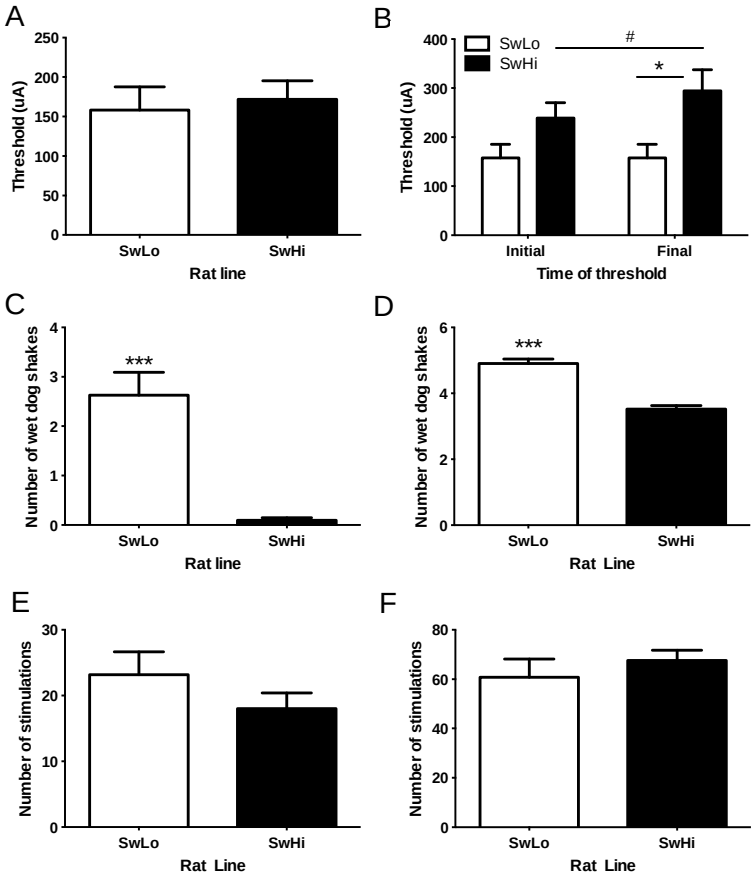
SwLo rats have a shorter latency to pilocarpine-induced seizures than SwHi rats that is independent of pilocarpine pharmacokinetics. (A) SwLo (n = 11) and SwHi (n = 11) rats were injected with atropine methyl bromide (2 mg/kg, i.p.), followed by pilocarpine (380 mg/kg, i.p.) 30 min later, and latency (mean ± SEM) to limbic motor seizures was measured. * $p < 0.01$ compared with SwHi rats (Tabb, 2008). (B) SwLo (n = 3) and SwHi (n = 3) rats were treated with atropine and pilocarpine as above, euthanized 5, 10, or 15 min later, and hippocampal pilocarpine levels (mean ± SEM) were measured by HPLC.

Figure 2.2 SwLo rats are more susceptible to electrically-induced seizures than SwHi rats.



SwLo rats are more susceptible to electrically-induced seizures than SwHi rats. Increasing current was delivered via earclip electrodes to SwLo and SwHi rats (n = 6 per group), and threshold stimulation required to induce tonic hindlimb flexion was recorded. * $p < 0.0001$.

Figure 2.3 Kindling parameters in SwLo and SwHi rats.



Kindling parameters in SwLo and SwHi rats. SwLo and SwHi rats (n = 6-10 per group) were implanted with electrodes in the amygdala or hippocampus. Initial electrographic seizure threshold was determined, and threshold stimulations were delivered twice per day until rats reached a fully kindled state, defined as 3 consecutive rearing/falling seizures. Shown are the mean ± SEM of initial and final threshold used to induce an electrographic seizure during amygdala (A) and hippocampal (B) kindling, the number of wet dog shakes during amygdala (C) and hippocampal (D) kindling, and number of stimulations required to reach a fully kindled state in the amygdala (E) and hippocampus (F). * $p < 0.05$, *** $p < 0.0001$ compared to SwHi rats, # $p < 0.01$ compared to initial threshold for that rat line.

CHAPTER 3:

ANTIDEPRESSANT AND ANTICONVULSANT EFFECTS OF EXERCISE IN A RODENT MODEL OF EPILEPSY AND DEPRESSION COMORBIDITY

3.1 Abstract

The bi-directional comorbidity between epilepsy and depression is associated with severe challenges for treatment efficacy and safety, often resulting in poor prognosis and outcome for the patient. Improvements to current treatment strategies are needed, and several non-pharmacological therapies, including aerobic exercise, have gained interest for their safety and efficacy in the clinical population. However, many of the mechanisms underlying the beneficial effects of exercise in epilepsy and depression are unknown, and animal models of the comorbidity would be useful for elucidating these mechanisms. We have shown previously that SwLo rats selectively bred for low activity in the forced swim test (FST) also have increased susceptibility to seizures induced by several chemoconvulsants and electrical stimulation compared with their SwHi counterparts that were bred for high activity in the FST, and thus display phenotypes consistent with depression and epilepsy comorbidity. We therefore compared sedentary SwLo and SwHi rats to those given ad libitum access to a running wheel for 3-4 weeks, and then measured struggling duration in the FST to assess depression-like behavior and latency to pilocarpine-induced limbic motor seizures to assess seizure susceptibility. Average distances run per day, in km, were similar between SwLo and SwHi rats. Exercise significantly increased both struggling duration and seizure latency in SwLo rats, but had no effect in SwHi rats. These findings reveal a dual antidepressant and anticonvulsant effect of exercise in a rodent model of comorbid epilepsy and depression.

3.2 Introduction

The bi-directional comorbidity that exists between depression and epilepsy is associated with many challenges for treatment. Patients with this comorbidity are often refractory to many commonly used pharmacotherapies, require more frequent hospitalizations, and have worsened prognosis (Kanner, 2006). Particularly troubling is the association between many anticonvulsant medications and depressed mood, as well as the relationship between antidepressants and increased risk of seizure (Hesdorffer *et al*, 2009; Judge *et al*, 2011). These factors make safe and effective treatment of comorbid epilepsy and depression very difficult, and clearly emphasize the need for novel therapeutic approaches. Of particular interest are non-pharmacological approaches, given their relative lack of negative and potentially dangerous side effects as compared to many pharmacological appproaches. One particularly promising non-pharmacological therapy is aerobic exercise.

Several studies have demonstrated the ability of aerobic exercise to decrease seizure frequency and/or epileptiform discharges (Denio *et al*, 1989; Eriksen *et al*, 1994; Nakken, 1999; Vancini *et al*, 2010) and improve mood in patients with depression (Arida *et al*, 2012; Babyak *et al*, 2000; Mata *et al*, 2012; Villaverde Gutierrez *et al*, 2012). Furthermore, in many epilepsy cases, exercise improves mood even if it does not also affect seizure frequency (McAuley *et al*, 2001). Many of these same studies emphasize the low risk and relative safety of exercise in patients with epilepsy. Unlike the negative side effect profiles associated with many pharmacotherapies, exercise is associated with many positive benefits, particularly in cardiovascular and overall physical health (Arida

et al, 2008; Eriksen *et al*, 1994). Combined, these studies indicate that exercise may be both safe and efficacious in treating comorbid epilepsy and depression.

Although many of the same neurochemicals implicated in depression and epilepsy separately and together are also altered by exercise, including the noradrenergic, serotonergic, GABAergic, dopaminergic, and galaninergic systems (Arida *et al*, 2012; Lundstrom *et al*, 2005; Sciolino *et al*, 2012), a precise understanding of the underlying mechanisms and their interactions remains to be discovered. Animal models are useful for teasing apart the respective contributions of each of these factors, given their controlled and regimented environment, diet, and exercise exposure. Indeed, several animal models have been used to assess the efficacy of exercise as a treatment for epilepsy or depression separately and have contributed to our understanding of a variety of neural substrates to this efficacy. For example, rats allowed to voluntarily exercise on an activity wheel for 3 weeks had less severe seizures following kainic acid administration. Importantly, these effects were blocked when the rats were pre-treated with M-40, a galanin receptor antagonist, suggesting that the galaninergic system is necessary for this effect (Reiss *et al*, 2009). This finding was also supported by additional studies that demonstrated an increase in prepro-galanin mRNA expression in the noradrenergic locus coeruleus (LC) following chronic voluntary exercise (Sciolino *et al*, 2012). Animal models have also been used to demonstrate the antidepressant effects of exercise, and indicate a causal role for increased levels of BDNF (Sartori *et al*, 2011).

However, few, if any, studies have used animal models to assess the effects of exercise on both depression and epilepsy together in a model of comorbidity. It is therefore unknown whether exercise will be an efficacious therapeutic strategy in these

models, or how the underlying mechanisms responsible for any beneficial effects of exercise may be similar to or different from those mechanisms previously implicated in depression or epilepsy singularly. Furthermore, studying the mechanisms underlying antidepressant and/or anticonvulsant effects of exercise in an animal model of this comorbidity may suggest novel targets for pharmacotherapies that may be safer and more efficacious for patients than currently available treatments. To this end, we have used the SwLo rat, a model of depression and epilepsy comorbidity, and the SwHi rat, their depression- and epilepsy-resistant counterparts. By examining the effects of voluntary exercise on depression-related behaviors in the forced swim test (FST) and susceptibility to pilocarpine-induced seizures in both the SwLo and SwHi rats, we provide evidence for the efficacy of exercise in treating epilepsy and depression comorbidity in a rodent model that can then be used for mechanistic studies and testing of novel pharmacotherapies for safety and efficacy.

3.3 Methods

Animals and housing

SwLo and SwHi rats were selectively bred based on FST phenotype, as described previously (Weiss *et al*, 1998b). Briefly, at 3 months of age, rats were fitted with "water wings" made from a plastic bubble and placed in a tank (65 cm high, 30 cm diameter) of 25°C water (14 cm from the top) for 10 min. Duration of struggling (active movement of all 4 paws, forepaws breaking surface of water) and floating (immobility) were measured by a trained researcher blinded to the strain of the rat.

12-14 male rats of each line were randomly assigned to the exercise condition, while 9 male rats of each line were randomly assigned to the sedentary condition. Rats were between 1.5-3.5 months of age at the beginning of the experiments, and were from generations 56-58 of the SwHi and SwLo rat lines. All rats were experimentally naïve to the FST and pilocarpine, and were singly housed. Food and water were available ad libitum, with lights on from 0700 to 1900 hours. All experiments were conducted in accordance with Emory University IACUC approval.

Voluntary exercise

Rats in the exercise condition were given free 24-h access to a stainless steel rodent activity wheel (Mini Mitter, Bend, OR). Each wheel was connected to an electromagnetic counter that measured the number of wheel revolutions, which were recorded daily between approximately 0900 and 1000. The daily distance run was calculated for each rat by multiplying the number of wheel rotations by the wheel circumference (107.75 cm) and converting to km.

Forced swim test

Rats were tested in the FST as described in the Animals and Housing section, with minor modifications. Animals were exposed to a 15 minute swim test, and summary data was calculated for 5 min, 10 min, and 15 min bins. Rats were swum following three weeks of exercise or sedentary conditions. All other conditions of the FST were as described above. Following the FST, rats were returned to their exercise or sedentary conditions for one additional week.

Pilocarpine administration

One week following the FST, rats were assessed for seizure susceptibility by measuring latency to pilocarpine-induced seizures, as described (Epps *et al*, 2012a). Briefly, rats were injected with atropine methyl bromide (2 mg/kg, s.c., Sigma-Aldrich, St Louis, MO) 30 min prior to pilocarpine hydrochloride administration (380 mg/kg, i.p., Sigma-Aldrich, St Louis, MO), and latency to limbic motor seizure was measured during continuous video monitoring. Limbic motor seizures were characterized by rearing and falling behaviors with bilateral forelimb clonus. A booster dose of 190 mg/kg pilopcarine was administered to any rat that did not demonstrate a limbic motor seizure within the first hour following pilocarpine administration. Animals were euthanized 2 h after pilocarpine administration or following 30 min of continuous seizing, whichever came first.

Statistical analysis

Running data were analyzed using a two-way ANOVA. FST and pilocarpine data were analyzed using a Student's t-test. All data analysis was conducted using Prism GraphPad 6.0 and IBM SPSS 19.0.

3.4 Results

Average daily running distances increased over time and were similar between SwLo and SwHi rat lines (Figure 3.1). There was a main effect of time on running distance, ($F_{26, 624}$=19.82, p<0.0001), but no effect of rat line ($F_{1, 624}$=0.2628, p=0.6129) or rat line x time interaction, ($F_{26, 624}$=1.49, p=0.06).

Following 3 weeks of running wheel exposure, struggling and floating duration were assessed in the FST. Exercise modestly, but significantly, increased struggling duration during the first 5 min of the FST in SwLo rats (t_{21}=1.890, $p<0.05$; Figure 3.2, panel A), but not SwHi rats (t_{19}=0.01291, p=0.9898; Figure 3.2, panel B). No changes in struggling were observed at the 10 or 15 min time bins, and no changes were observed in floating behaviors in either rat line at any time point (data not shown).

Following one additional week of running, pilocarpine (380 mg/kg, i.p.) was administered, and latency to limbic motor seizure was measured. Exercise significantly increased seizure latency in SwLo rats (t_{21}=3.942, $p<0.001$; Figure 3.3, panel A), but not SwHi rats (t_{19}=1.050, p=0.3071; Figure 3.3, panel B).

3.5 Discussion

Given the ineffectiveness and negative side effects associated with treatment in many patients with comorbid epilepsy and depression, improved therapeutic strategies are clearly necessary. Indeed, many antidepressants enhance the risk of seizure, particularly at high doses, and many anticonvulsant medications cause depressed mood (Hesdorffer *et al*, 2009; Judge *et al*, 2011), severely limiting the pharmacotherapies available to these patients. While several non-pharmacological strategies, such as aerobic exercise, have demonstrated benefit in the clinical population with depression and/or epilepsy, much remains to be understood about their underlying mechanisms of action. Elucidation of these mechanisms would enhance our understanding of the diseases of interest, as well as suggest new targets for pharmacological therapies that may be safer and more efficacious for treating these patients. While exercise does demonstrate

anticonvulsant and/or antidepressant effects in patients, not all patients are physically able to participate in an exercise program, and novel pharmacotherapies that target the same substrates as aerobic exercise may be an excellent treatment strategy for these patients.

Animal models are an excellent tool for studying the mechanisms underlying the beneficial effects of exercise in depression and epilepsy, and can also be utilized for screening novel pharmacotherapies. To this end, we have characterized the depression- and seizure-susceptible phenotypes of the SwLo rat line following 3-4 weeks of voluntary activity wheel exercise. SwLo rats that exercised showed a significant increase in struggling in the FST compared to sedentary SwLo rats, suggestive of an antidepressant effect. By contrast, no changes in struggling were observed in SwHi rats, indicating that the antidepressant effects of exercise were selective for the depression-susceptible SwLo rat. Interestingly, chronic treatment with canonical tricyclic antidepressants (e.g. imipramine, desipramine) shows the same profile of selectivity for the SwLo rat; that is, chronic antidepressant treatment increases struggling in the SwLo, but not SwHi, rat (West *et al*, 1998). Exercise also had anticonvulsant properties in SwLo rats, as shown by an increased latency to pilocarpine-induced seizure. Again, this effect was restricted to the seizure-susceptible SwLo rats, and was not seen in the SwHi rats.

This study demonstrates that aerobic exercise is antidepressant and anticonvulsant in a rodent model of depression and epilepsy comorbidity. However, many questions remain to be addressed. It would be of interest to understand the duration of these beneficial effects of exercise. This study was limited in its assessment to one time point immediately following exercise. Additional studies which test animals at multiple time

points following the cessation of exercise would be useful to understanding the permanency of these changes in depression- and seizure-sensitivity. One may also speculate that habituation or sensitization to the effects of exercise may occur following long-term exposure, and thus studies utilizing a longer time period of exercise may help to identify whether or not these effects change over the course of prolonged exercise. Furthermore, while chronic exposure has demonstrated antidepressant and anticonvulsant effects, shorter, more acute exposure to exercise may serve as a stressor (Hackney, 2006). Given the association of stressful experiences with pro-convulsive and pro-depressant effects (Haut *et al*, 2003; Sperling *et al*, 2008; Swaab *et al*, 2005), it would be of interest to determine the effects of shorter exposures to exercise as well, and to determine the critical window of exercise exposure required for maximal beneficial effect.

Now that we have identified an antidepressant and anticonvulsant potential of aerobic exercise, the SwLo and SwHi rat models provide an excellent tool for identifying the neurobiological changes resulting from exercise and for screening novel pharmacotherapies based on these mechanisms for their safety and efficacy in treating the comorbidity. One particularly intriguing mechanism is the involvement of the galaninergic system. Galanin appears to play a key role in epilepsy-related phenotypes and anticonvulsant actions, as reviewed by Robertson et al (Robertson *et al*, 2011) and Lerner and Sankar (Lerner *et al*, 2008). For instance, decreases in galanin or its receptors (GalR1, GalR2) have been associated with increased susceptibility to a variety of seizure types (Mazarati and Lu, 2005; Mazarati *et al*, 2004; McColl *et al*, 2006). Conversely, increases in galaninergic transmission suppress seizures (Lu *et al*, 2010; Weinshenker, 2008; White *et al*, 2009). Galanin may also modulate mood. A recent genome wide

association study found a SNP in the galanin gene associated with major depressive disorder (Wray *et al*, 2012). Knockout of GalR2 in mice is associated with depression-like behaviors (Lu *et al*, 2008), providing further support for a relationship between galanin and depression. However, decreases in galanin have also been associated with antidepressant effects (Rovin *et al*, 2012; Weiss *et al*, 1998a; Weiss *et al*, 2005), suggesting that the relationship between galanin and depression may be complex and requires further analysis. Intriguingly, galanin mRNA expression is increased specifically in the LC, the sole source of the neurotransmitter norepinephrine in the forebrain, following voluntary exercise (Holmes *et al*, 2006; Reiss *et al*, 2009; Sciolino *et al*, 2012). Mice overexpressing galanin in the LC are seizure resistant (Kokaia *et al*, 2001), while intracerebral administration of the galanin receptor antagonist, M-40, abolishes the anticonvulsant effects of exercise (Reiss *et al*, 2009). Interestingly, galanin suppresses LC neuron firing, a property shared by most antidepressant drugs (Grant and Weiss, 2001; Picciotto *et al*, 2010). Increases in plasma galanin have also been reported in humans following exercise (Legakis *et al*, 2000). Combined, these data suggest that increased galanin expression in the LC may underlie, at least in part, the antidepressant and anticonvulsant effects of exercise.

Other mechanisms are also of interest. Brain-derived neurotrophic factor (BDNF) and other neurtrophic factors have also been reported to increase following exercise (Sartori *et al*, 2011), and have likewise been implicated in both depression (Kozisek *et al*, 2008; Li *et al*, 2011; Sartori *et al*, 2011; Yu *et al*, 2012) and epilepsy (He *et al*, 2004; Heinrich *et al*, 2011; Kotloski *et al*, 2010; LaFrance *et al*, 2010; Paradiso *et al*, 2011). Additionally, alterations in neurogenesis have been associated with exercise (Mustroph *et*

al, 2012; Vivar *et al*, 2012), antidepressant efficacy (Eisch and Petrik, 2012; Petrik *et al*, 2012), and epilepsy (Danzer, 2012; Marucci *et al*, 2012; Scharfman and Gray, 2007; Scharfman and McCloskey, 2009). Increases in corticotropin releasing hormone (CRH) and/or plasma corticosterone (cortisol in humans) have been associated with depression and epilepsy, both separately and together (Binder and Nemeroff, 2010; Kumar *et al*, 2011; Mazarati *et al*, 2009; Pineda *et al*, 2011; Sawyer and Escayg, 2010; Tolmacheva *et al*, 2012; Wu *et al*, 2012), and decreases in CRH have been demonstrated following chronic exercise (Droste *et al*, 2003; Droste *et al*, 2006) (but, see also (Droste *et al*, 2007)), perhaps explaining the anticonvulsant and antidepressant effects of chronic exercise.

This study has characterized a potentially important non-pharmacological therapy for treatment of epilepsy and depression in a rat model of this comorbidity, and set the stage for further studies to elucidate the underlying mechanisms and provide a tool for screening of novel therapeutic strategies. Pharmacotherapies targeting the galaninergic system or any others found to mediate the benefits of exercise could then be screened for their safety and efficacy in the SwLo rat line, providing important clinical relevance to these studies.

3.6 Acknowledgements

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Figure 3.1 Average daily voluntary exercise in SwLo and SwHi rats.

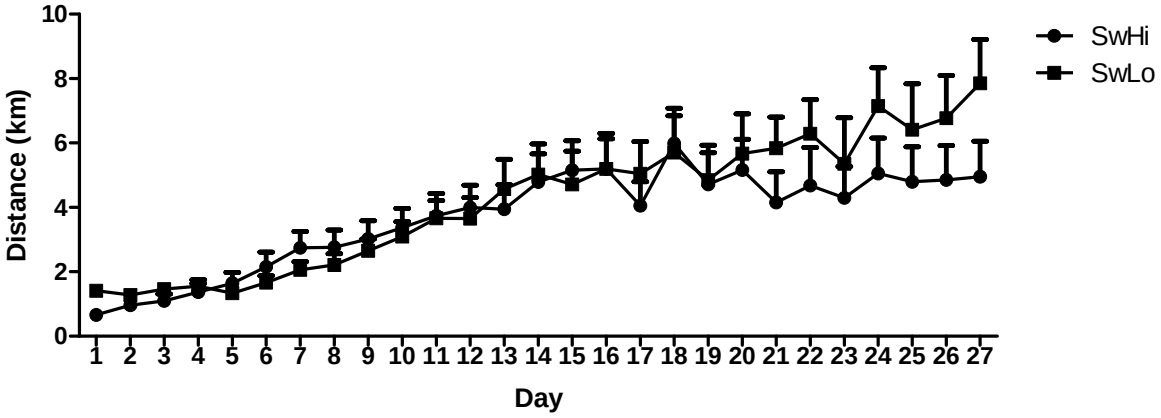

Figure 3.1 Average daily voluntary exercise in SwLo and SwHi rats. SwLo and SwHi rats were allowed 27 days of voluntary exercise on an activity wheel, and distance run (in km) was calculated daily. Shown are mean ± SEM km per day, n=12-14 per line.

Figure 3.2 Antidepressant effects of exercise on FST struggling in SwLo rats.

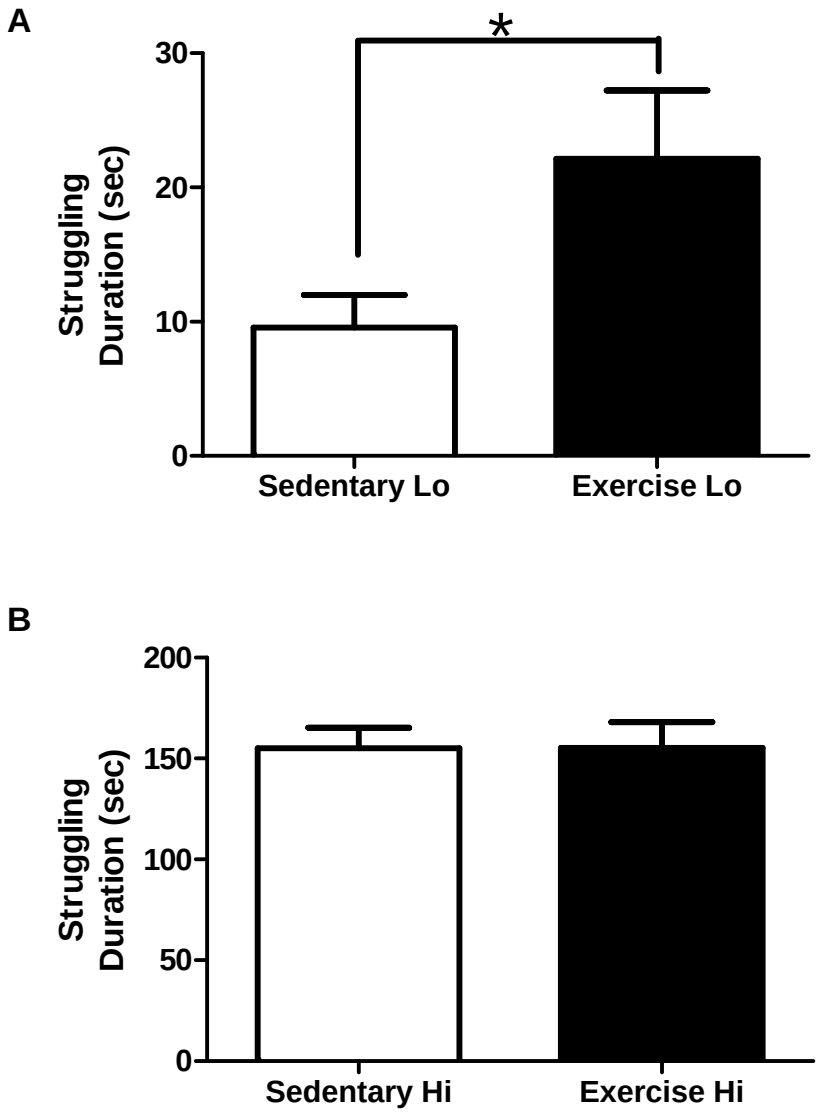

Figure 3.2 Antidepressant effects of exercise on FST struggling in SwLo rats. Rats were given access to running wheels (Exercise) or a regular home cage (Sedentary) for 3 weeks, and behavior was assessed in the FST. Shown is the mean ± SEM struggling duration during the 5-min test. *$p<0.05$, n=9-14 rats per group.

Figure 3.3 Anticonvulsant effects of exercise on latency to pilocarpine-induced seizure in SwLo rats.

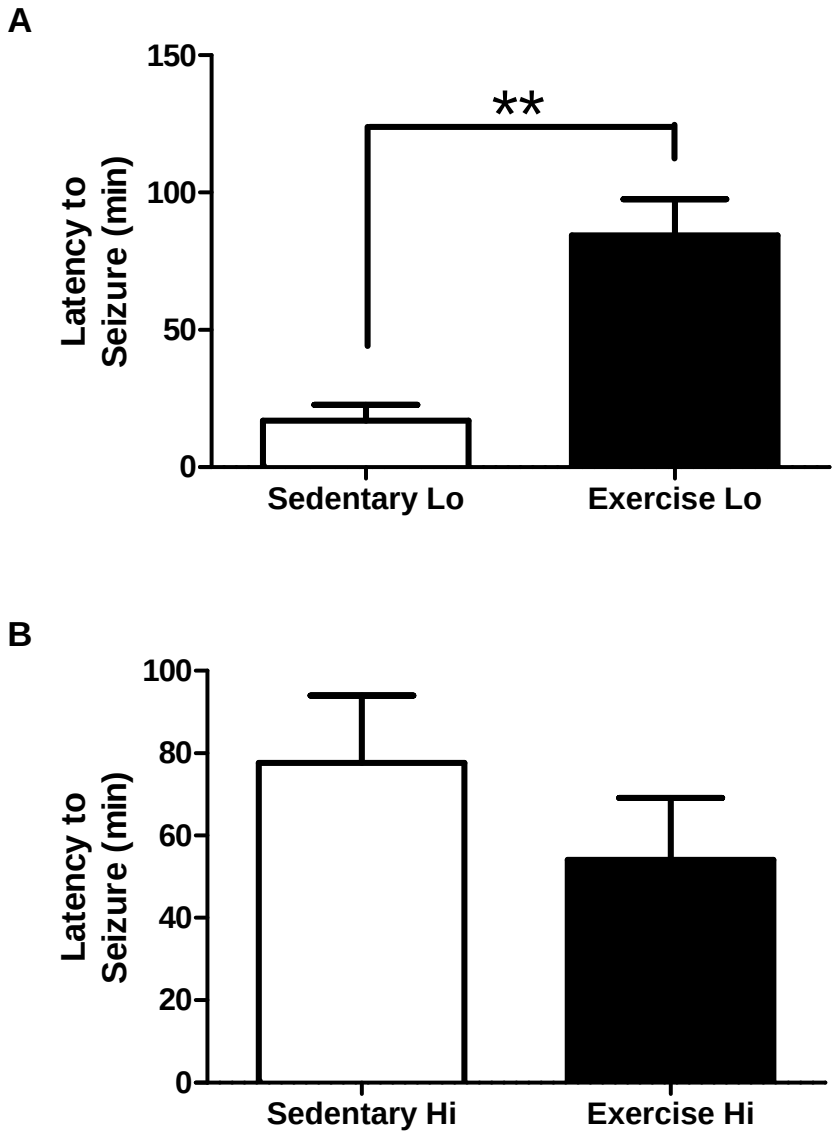

Figure 3.3 Anticonvulsant effects of exercise on latency to pilocarpine-induced seizure in SwLo rats. After the FST, rats were returned to their sedentary or exercise cages. One week later, seizure susceptibility was tested. Shown is the mean ± SEM latency to limbic motor seizure following administration of pilocarpine (380 mg/kg i.p.). **$p<0.001$, n=9-14 rats per group.

CHAPTER 4:

CONCLUSIONS AND FUTURE DIRECTIONS

4.1 Summary

We have demonstrated herein that the SwLo rat line serves as a valid animal model of epilepsy and depression comorbidity. These rats were selectively bred from outbred Sprague-Dawley rats based on a depression-like phenotype of increased immobility on the forced swim test (FST) (Weiss *et al*, 1998b; West *et al*, 1998), and also demonstrate several other depression- and anhedonic-like phenotypes (Lin *et al*, 2012; Weiss *et al*, 2008; West *et al*, 1999a; West *et al*, 1999b). Their counterparts, the SwHi rats, were selectively bred for a depression-resistant phenotype of increased struggling on the FST. Interestingly, the SwLo rats also demonstrated enhanced seizure susceptibility to pilocarpine- and electroshock-induced seizures, as well as enhanced epileptogenesis in particular parameters of hippocampal and amygdala kindling and pilocarpine-induced spontaneous seizures, while the SwHi rats show resistance to these phenotypes (Epps *et al*, 2012a). We have also shown antidepressant and anticonvulsant effects of a non-pharmacological therapy, aerobic exercise, in SwLo, but not SwHi rats, using the FST and latency to pilocarpine-induced seizures. We therefore present the SwLo rats as a useful tool for future studies of the underlying mechanisms of comorbid epilepsy and depression, and for screening novel pharmacotherapies for safety and efficacy in treating these phenotypes.

To our knowledge, these studies are the first to characterize seizure susceptibility and epileptogenesis in a rodent model of depression, making them a unique model of this direction of the comorbidity between depression and epilepsy, as summarized in Table 4.1. This development fills an important gap in the field. The comorbidity between epilepsy and depression has clearly been demonstrated to be bi-directional (Hesdorffer *et*

al, 2006; Kanner, 2008), yet all previous animal models have only examined the development of depression-like phenotypes following epilepsy. By taking the opposite approach, we have generated a new model that can be used to address depression-related epilepsy. Because it is unknown how the two directions of the comorbidity may differ in mechanism, treatment strategy, and/or prognosis, it is vital that research be continued on models representing both epilepsy-related depression and depression-related epilepsy. Furthermore, our exercise experiments allowed us to demonstrate the utility of SwLo rats in screening various treatment strategies for their antidepressant and anticonvulsant properties. Together, these experiments have enabled us to establish the SwLo rat as a rodent model of depression-related epilepsy, the first of its kind, and have supported the use of the SwLo model for future studies of the mechanisms and treatments relevant to this comorbidity.

4.2 Seizure susceptibility and epileptogenesis in the SwLo rat line

Because of the increased risk of epilepsy in patients with depression (Hesdorffer *et al*, 2006; Kanner, 2008) and the need for improved animal models to study this comorbidity, we wished to test the SwLo rat model of depression for seizure- and epilepsy-susceptibility. Our previous studies suggested that the SwLo rats may serve as an animal model of depression and epilepsy comorbidity, with specificity for temporal lobe seizures (Tabb *et al*, 2007). The aim of the current set of experiments was to more fully characterize the SwLo rat line as a model of depression and epilepsy comorbidity using a variety of seizure- and epilepsy-related paradigms. We therefore tested the SwLo

rats for susceptibility to acute pilocarpine-induced seizures, development of pilocarpine-induced spontaneous seizures, increasing current electroshock seizures (ICES), and amygdala and hippocampal kindling.

We demonstrated that SwLo rats exhibited decreased latency to pilocarpine-induced limbic motor seizures when injected with a single dose of 380 mg/kg pilocarpine. Additionally, SwLo rats developed more spontaneous seizures five weeks after pilocarpine-induced status epilepticus (i.e., one hour of continuous limbic motor seizure) (Tabb, 2008). These findings could not be explained by differences in pharmacokinetics, as brain tissue levels of pilocarpine were similar between SwLo and SwHi rats at each of three different time points following pilocarpine administration. Thus, these findings are likely the result of true differences in neuronal excitability, resulting in enhanced seizure susceptibility and epileptogenesis, rather than differences in drug metabolism. Additionally, when administered an increasing current electroshock, SwLo rats had a lower threshold to seizure than did the SwHi rats, providing further support for increased seizure sensitivity to a variety of insults in the SwLo rats.

We were also interested in identifying particular brain regions of importance to the epilepsy-related phenotypes in the SwLo rats. To this end, we conducted electrical kindling studies on the amygdala and hippocampus, two regions known to play a role in depression and epilepsy separately (Epps and Weinshenker, 2012b; Gilliam *et al*, 2007; Kanner, 2004; Kondziella *et al*, 2007; Trimble and Van Elst, 2003). Although we did not find a difference in the overall kindling rate of either brain region, we did note that SwLo rats had a significantly lower threshold stimulation. They also exhibited more wet dog

shake behaviors (Epps *et al*, 2012a), suggestive of possible muscarinic dysfunction (Turski *et al*, 1982; Turski *et al*, 1984).

These findings support the validity of the SwLo rat line as a rodent model of depression-related epilepsy comorbidity, the only known model of its kind. This characterization of the SwLo model is thus an important step forward for the field, as it allows for the study of epilepsy comorbidity in an animal model selectively bred for depression-related phenotypes. Use of such a model will enhance our understanding of the mechanisms underlying depression-related epilepsy, potentially identifying novel targets for pharmacotherapies specific for this patient population. Additionally, it provides a screening mechanism for testing the safety and efficacy of these pharmacotherapies, potentially addressing the current treatment difficulties experienced by these patients and thereby improving their quality of life.

4.3 Antidepressant and anticonvulsant effects of exercise in SwLo rats

Because of the known difficulties in treating patients with comorbid epilepsy and depression due to the high incidence of refractoriness to treatment and potentially dangerous side effects (Hesdorffer *et al*, 2009; Judge *et al*, 2011; Kanner, 2006), we wished to test a non-pharmacological therapy for safety and efficacy using the SwLo rodent model of the comorbidity. We therefore chose to assess depression-like phenotypes on the FST and susceptibility to pilocarpine-induced seizures following chronic exercise or sedentary conditions, given the known improvement of both of these phenotypes following exercise (Arida *et al*, 2009; Sartori *et al*, 2011). The aim of the

current study was to demonstrate the utility of the SwLo rats in screening treatments for the comorbidity and establish a model for future studies assessing the mechanisms underlying any beneficial effects of exercise.

Following three weeks of voluntary exercise on a rodent activity wheel, SwLo rats showed a significant increase in struggling behaviors on the FST as compared to sedentary SwLo rats that were not exposed to an activity wheel, suggestive of an antidepressant effect of exercise. This finding was not demonstrated in the SwHi rats. Following one additional week of activity wheel exercise, the exercise SwLo rats demonstrated an increased latency to pilocarpine-induced seizure (380 mg/kg) as compared to sedentary SwLo rats. Again, this finding was not demonstrated in SwHi rats, suggesting that the antidepressant and anticonvulsant effects of exercise are specific to the SwLo rat.

This study demonstrated the anticonvulsant and antidepressant effects of chronic aerobic exercise in a rodent model of epilepsy and depression comorbidity. To our knowledge, this study is the first to address the benefits of exercise in both depression and epilepsy behaviors in a genetic model of the comorbidity. As such, this provides important evidence that exercise can be a beneficial treatment strategy for depression and epilepsy comorbidity, and suggests that treatment strategies targeting the same neural systems as exercise may be both safe and efficacious for this patient group that tends to be refractory to standard treatments. Future studies into the mechanisms underlying the antidepressant and anticonvulsant effects of exercise may therefore suggest targets for novel pharmacotherapies, which could then be tested for safety and efficacy in SwLo rats. Additionally, exercise is the first therapy that has been tested for both

anticonvulsant and antidepressant activity in the SwLo rat model. The results of this study concur with the general clinical perspective of the beneficial effects of exercise for both epilepsy and depression (Arida *et al*, 2012). These findings, therefore, are an important demonstration of the utility of SwLo rats for screening therapeutic strategies for both antidepressant and anticonvulsant effects, and also indicate the selectivity of these effects to the comorbidity, given the lack of effect seen by the SwHi rats. Thus, this study represents important progress in the development of a rodent model to screen medications for safety and efficacy in treating comorbid epilepsy and depression.

4.4 Future directions

As the first animal model to assess epilepsy-related phenotypes in a depression model, the SwLo rats represent an important advance in the understanding of this bi-directional comorbidity. Nonetheless, many important studies remain to be conducted on this topic utilizing this model. Our first study characterized multiple seizure susceptibility and epileptogenic behaviors in the SwLo rats; however, additional studies to discover the mechanisms underlying these phenotypes are critical to the advancement of our understanding of these disorders and the identification of new therapeutic targets. Our second study evaluated the beneficial effect of aerobic exercise on these phenotypes, but once again, additional studies are required to elucidate the mechanisms underlying the anticonvulsant and antidepressant actions of this therapy.

Seizure susceptibility and epileptogenesis in the SwLo rat line

Our behavioral characterization of seizure- and epilepsy-related phenotypes in the SwLo rats allows for their use in further studies of the mechanisms underlying comorbid epilepsy and depression. Although several other animal models have been used to identify various mechanisms in this comorbidity (Epps *et al*, 2012b), these other models have all taken the approach of assessing depression-related phenotypes in a rodent model of epilepsy. It is unknown how the mechanisms underlying this direction of the comorbidity may differ from those underlying depression-related epilepsy, and thus the SwLo rats may be a useful means to shed light upon these differences or similarities. Better understanding of the mechanisms underlying this direction of the comorbidity may also have important clinical implications for patients with depression who develop comorbid epilepsy, as this may identify new targets for pharmacotherapies, which could then be tested for safety and efficacy using the SwLo rat model.

While much remains to be learned about the mechanisms underlying the SwLo phenotype, speculation can be made regarding these mechanisms based on the current studies to date. For instance, SwLo rats show enhanced susceptibility to pilocarpine-induced seizures and epileptogenesis, as well as increased wet dog shaking in both amygdala and hippocampal kindling (Epps *et al*, 2012a). Pilocarpine is a known muscarinic agonist, and wet dog shakes have also been associated with the muscarinic system (Turski *et al*, 1982; Turski *et al*, 1984), and thus it appears likely that the muscarinic acetylcholinergic system may be implicated in the seizure- and epilepsy-related phenotypes of SwLo rats. Additionally, tissue levels of dopamine and

norepinephrine are reduced in the prefrontal cortex and dorsal hippocampus of SwLo rats (Weiss *et al*, 2008; West *et al*, 1999b), respectively, suggesting a possible role for these neurotransmitters as well. Further characterization of the role of each of these neurotransmitters in both epilepsy and depression phenotypes in the SwLo rats would be informative. For example, one could conduct quantitative real-time PCR (qRT-PCR) or in situ hybridization assays to compare the mRNA expression of cholinergic, noradrenergic, and dopaminergic biosynthetic enzymes, metabolizing enzymes, transporters, and receptors between SwLo and SwHi rats. Electrophysiological studies of the firing rates of cholinergic, noradrenergic, and dopaminergic cells in the brain regions of interest could also provide information about the mechanisms underlying these phenotypes. A variety of experiments could then be conducted to reverse these phenotypes using viral vector or pharmacological means. For example, chronic administration of tricyclic antidepressants, which inhibit the reuptake of norepinephrine, has an antidepressant effect in SwLo rats (West *et al*, 1998), but the effect of these drugs on seizure phenotypes has never been tested. Similarly, it would also be of interest to alter dopamine or acetylcholine transmission using agonists or antagonists, respectively, and assess their effects on depression and seizure phenotypes. Likewise, viral vectors and RNA interference could be used to site specifically alter neurotransmission in SwLo rats in an effort to provide a therapeutic effect. Optogenetic approaches could be used in a similar fashion to stimulate or inhibit specific cell populations (i.e., inhibitory interneurons, etc) to identify their role in epilepsy and depression comorbidity. These approaches could also be used in the SwHi rat line in an effort to create a "SwLo-like" phenotype in these rats.

Genetic analysis of the SwLo and SwHi rat lines would also be informative. Quantitative trait loci (QTL) analysis could be conducted to identify chromosomal regions that are associated with each of the phenotypes of interest. Combining this information with microarray analysis of differentially expressed genes between the two rat lines may provide a list of primary candidate regions of interest that likely associate with the comorbidity, and thus may be useful in providing information about the underlying genetic changes contributing to comorbid epilepsy and depression. The products of these genes may then serve as targets for pharmacotherapies or future study. Preliminary studies of this nature are described further in Appendix A. Additionally, while we have assessed expression levels of only a few candidate transcripts using qRT-PCR (Appendix A), the entire transcriptome could be sequenced and analyzed using RNAseq (whole transcriptome shotgun sequencing) (Wang *et al*, 2009). This would identify many additional candidate genes with differential expression that could be characterized via the pharmacological or viral vector techniques described previously. Knock-out rats for these candidate genes could then be generated, and their phenotypes compared to those of the SwLo and SwHi rats. Analysis of epigenetic differences between the SwLo and SwHi rats could also be used to explore the role of any histone modifications, DNA methylation, or other epigenetic changes in the behaviors of interest. These protein-DNA interactions could be assayed using ChIP-seq, ChIP-on- chip technologies, methyl-specific bisulfite PCR, or Western blotting for various histone modifications, and any identified epigenetic differences between the two rat lines could be characterized via inhibitors of these processes (i.e., DNA methylation could be blocked by administration of a DNMT inhibitor) (Lubin, 2011). Together, these genetic

and epigenetic analyses of the SwLo rats could shed light on the mechanisms underlying comorbid epilepsy and depression and suggest new targets for the development of future pharmacotherapies.

Antidepressant and anticonvulsant effects of exercise in SwLo rats

Our demonstration of antidepressant and anticonvulsant effects of exercise in SwLo rats supports the utility of these rats as a screening tool for novel therapeutics. Additionally, it also suggests the possibility of several new options for new pharmacotherapies. While a growing body of clinical evidence supports the benefits of exercise in depression and epilepsy comorbidity (Arida *et al*, 2012), little is definitively known about the mechanisms underlying these effects. The SwLo rats provide an excellent means with which to identify these mechanisms, and then screen novel pharmacotherapies targeting these mechanisms for their safety and efficacy in treating the comorbidity. We can now speculate on various factors that may underlie antidepressant and anticonvulsant effects in the SwLo rats, based on current understanding of the neural effects of exercise, although further research is clearly warranted before this question can be addressed definitively and in full.

One potential mechanism of interest is galanin, a neuropeptide that is frequently co-released along with norepinephrine. Upregulation of the galaninergic system has been demonstrated following chronic exercise (Holmes *et al*, 2006; Reiss *et al*, 2009; Sciolino *et al*, 2012), and this upregulation may be responsible for the anticonvulsant and/or antidepressant effects of exercise. Future studies could compare the expression level of

galanin and its receptors in both SwLo and SwHi rats under exercise and sedentary conditions using qRT-PCR or in situ hybridization. Additionally, protein levels of galanin and its receptors could be assayed via Western blot for a semi-quantitative measure of their expression, or via immunohistochemistry to determine the distribution of their expression throughout the brain. Galanin receptor agonists, such as galnon, could be chronically administered to SwLo rats via osmotic minipump in an effort to mimic the effects of exercise, thus suggesting new therapeutics that may be safe and efficacious for patients with the comorbidity who are physically unable to exercise. Similar studies could be conducted using site-specific viral vectors to overexpress galanin in the SwLo brain, thereby identifying the brain regions responsible for these effects. Likewise, administration of a galanin antagonist, such as M-40, or use of a viral vector to decrease the expression of galanin in the SwLo rat could perhaps be used to block the beneficial effects of exercise, thereby helping to clarify the necessity or sufficiency of galanin for these phenotypes. The effects of exercise and galaninergic manipulations could also be assessed in other epilepsy- and depression-related phenotypes, such as intracranial self-stimulation (ICSS), electroshock-induced seizures, etc.

Similar studies could also be conducted on other targets of interest, such as BDNF and other neurotrophic factors or neurogenesis. Additionally, a "dose-response curve" could be calculated to determine the duration or time scale of running required for antidepressant and anticonvulsant effects, as well as the duration of these effects after cessation of exercise. Knowing the time scale of the development of these effects may enlighten us to the types of neural changes that are required for the effects (i.e., a slower time scale may reflect genetic changes, neurogenesis and migration of newly born

neurons, etc). Importantly, future studies should also assess any antiepileptogenic effects of exercise. Current treatment strategies are primarily anticonvulsant; that is, they prevent seizures from occurring. However, they do not serve as antiepileptogenics; they are not able to prevent the progression of epilepsy or the neuronal damage that it frequently brings. Here, we have assessed the anticonvulsant effects of exercise by measuring the latency to acute pilocarpine-induced seizure. However, future studies should also address the antiepileptogenic effects of exercise by measuring the number of spontaneous seizures occurring following pilocarpine-induced status epilepticus or various parameters in the electrical kindling paradigm. Identification of a therapeutic target that can serve as an antiepileptic as well as an anticonvulsant and antidepressant would be of extreme importance and benefit to patients with this condition.

Table 4.1 Epilepsy- and depression-related phenotypes of the SwLo rat.

Depression-Related Behaviors	References
Increased floating in forced swim test Reversed by chronic antidepressants	(Weiss *et al*, 1998b) (West *et al*, 1998)
Decreased response to rewarding drugs of abuse	(Lin *et al*, 2012; Weiss *et al*, 2008; West *et al*, 1999a; West *et al*, 1999b)
Increased threshold for intracranial self-stimulation	(C. West, personal communication)
Seizure Susceptibility & Epileptogenesis	**References**
Increased mortality after kainic acid-induced seizure	(Tabb *et al*, 2007)
Decreased latency to pilocarpine-induced seizure	(Epps *et al*, 2012a; Tabb, 2008)
Decreased threshold for electroshock-induced seizures	(Epps *et al*, 2012a)
Increased development of spontaneous seizures in pilocarpine epileptogenesis	(Epps *et al*, 2012a; Tabb, 2008)
Lower final threshold in hippocampal kindling, increased wet dog shaking in amygdala & hippocampal kindling	(Epps *et al*, 2012a)

Table 4.1 Epilepsy- and depression-related phenotypes of the SwLo rat. SwLo rats were selectively bred based on a depression-like phenotype of increased floating and decreased struggling on the forced swim test (FST). They show several anhedonia-related phenotypes, as well as increased susceptibility to kainic acid-, pilocarpine-, and electroshock-induced seizures. Additionally, SwLo rats exhibit increased epileptogenesis following both pilocarpine and kindling insults. Together, this characterization of the depression-, seizure-, and epilepsy-related phenotypes of the SwLo rats validates their use as a rodent model of comorbid depression and epilepsy.

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APPENDIX 1:

mRNA EXPRESSION ANALYSIS OF A RODENT MODEL OF EPILEPSY AND DEPRESSION COMORBIDITY

A1.1 Abstract

The bi-directional comorbidity between epilepsy and depression is likely explained, at least in part, by genetic influences. Identification of candidate genes that underlie this comorbidity will provide essential information about the mechanisms behind the comorbidity and suggest novel pharmacotherapeutic targets. As a selectively bred rodent model of depression and epilepsy comorbidity, the SwLo rat line is uniquely positioned to provide such information. To this end, we conducted an expression microarray analysis of the SwLo and SwHi rat lines to identify differentially expressed genes that may underlie their phenotypic differences.

We found 210 genes with at least 2-fold differential expression between SwLo and SwHi rats, with 121 of these genes exhibiting higher expression in the SwHi rats, and 89 genes showing higher expression in the SwLo rats. These included a variety of genes known to play a role in depression or epilepsy separately, such as Gabbr1, Ntrk1, Hcn1, and C3. However, we were unable to confirm the expression differences for a subset of the most promising candidate genes using quantitative reverse-transcriptase PCR (qRT-PCR). Despite this lack of positive confirmation for the genes identified by the expression microarray, better understanding of the genetic underpinnings of comorbid depression and epilepsy remains a priority for the field, and future studies using animal models of this comorbidity, like the SwLo rat line, are warranted due to the potential mechanistic insights and clinically relevant drug targets they may provide.

A1.2 Introduction

A strong bi-directional comorbidity exists between major depressive disorder (MDD) and temporal lobe epilepsy (TLE). MDD is characterized by depressed or irritable mood, loss of pleasure in previously enjoyed activities, and a host of additional symptoms for a period of at least two weeks. Patients with TLE exhibit two or more unprovoked complex partial seizures that originate in the temporal lobe of the brain and are characterized by impaired consciousness. These seizures also frequently feature motor automatisms. People with TLE are at an increased risk for developing MDD, and MDD is also associated with an elevated incidence of epilepsy, particularly TLE (Hesdorffer *et al*, 2006; Kanner, 2003; Kanner *et al*, 1999; Kanner *et al*, 2012).

Although many hypotheses have been suggested to explain the association between MDD and TLE, it is clear that genetic factors play a large role. Nearly 50% of patients with comorbid epilepsy and depression have family members with psychiatric diseases, with mood disorders being the most commonly reported psychiatric disease in family history (Kanner *et al*, 1999). Additionally, family history was a significant contributor in mood disorders in children and adolescents with epilepsy (Thome-Souza *et al*, 2004), emphasizing the likely importance of genetic contributions to comorbid epilepsy and depression. Furthermore, expression microarray studies in rodents and humans have identified a number of differentially expressed genes for depression and epilepsy when analyzed separately (Bergstrom *et al*, 2007; Gorter *et al*, 2006; Kang *et al*, 2007; Nakatani *et al*, 2004; Noebels, 2003; Pearson *et al*, 2006). Animal models of depression have revealed decreased expression of genes responsible for signal transduction (Nakatani *et al*, 2004), an upregulation of apoptotic pathways (Bergstrom *et*

al, 2007), and a downregulation of genes encoding potassium channels (Andrus *et al*, 2012; Pearson *et al*, 2006). Similarly, postmortem microarray analysis of human patients showed significant expression changes in genes involved in extracellular signal transduction, regulation of cell cycle and cell death, and inflammation (Aronica and Crino, 2011). Rodent models of epilepsy demonstrate alterations in the expression of genes regulating immune activation, ion channel subunits, synaptic transmission, and GABA receptors (Gorter *et al*, 2006). Comparably, human studies have identified channelopathies as a likely cause of several forms of epilepsy, and also provide evidence for an improper balance of GABAergic and glutamatergic transmission (de Moura *et al*, 2012).

However, little is currently known about the genetic underpinnings of depression and epilepsy comorbidity, and how these may be similar to, or different from, genes involved in epilepsy or depression singularly. It is possible that some of the differences in expression seen in previous studies of depression or epilepsy alone may also have a role in the comorbidity. However, it is also likely that genetic analysis of comorbid epilepsy and depression will identify differences in several other genes that are unique to the comorbidity of these disorders. For example, the HTR2A gene has been associated with panic disorder (Maron *et al*, 2005) and with anxiety disorder alone (Serretti *et al*, 2007), but not with co-morbid panic and anxiety disorders (Maron *et al*, 2005), indicating that at least some genes involved in comorbidity cannot be identified based on a comparison of the genes involved in either disorder alone. Therefore, it is expected that genetic analysis of comorbid epilepsy and depression will provide a more accurate group of candidate genes involved in the comorbidity of depression-like and seizure susceptible

behaviors than would simply comparing genes implicated in either disorder alone. Identification of these genes will help us to better understand the mechanisms and pathways that may be involved in producing co-existent depression and epilepsy, and provide potential therapeutic targets for more efficacious and safe medications.

Human studies of genetic associations with epilepsy or depression are often limited in power and generalizability due to confounding factors such as environment, age of disease onset, and medication history. Animal models thus provide a useful tool because they have controlled environmental exposures and known history in which to study the genetic underpinnings of comorbid epilepsy and depression. Advances in the field of rodent genetics have offered a variety of techniques with which to address these questions. One way to identify genes that contribute to phenotypic differences is by examining differences in gene expression, such as with expression microarrays. In this technique, mRNA is isolated from the two strains of interest from a given brain region (e.g. hippocampus) and hybridized to a microarray containing probes for all known expressed genes. The expression profiles from the two strains are compared, and a list of genes with differential expression is generated. These genes may then serve as candidates for future analysis of their role in explaining the different seizure and/or depression-related phenotypes seen in the animal model.

While several studies have assessed the role of various genes in rodent models of epilepsy that also show depression-like phenotypes, these analyses have not been conducted on a model of depression that also shows epilepsy-related phenotypes. To this end, we conducted a microarray analysis to identify genes with differential expression between SwLo and SwHi rats. The SwLo rat is the only animal model to date in which

pre-existing genetic vulnerability to depression-like phenotypes has conferred seizure susceptibility. The SwLo rats are selectively bred for decreased struggling in the forced swim test (FST) (Weiss *et al*, 1998), and also display depression-like anhedonic phenotypes and enhanced sensitivity to pilocarpine-, kainic acid-, electroshock-, and kindling-induced seizures and epileptogenesis (Epps *et al*, 2012; Tabb *et al*, 2007; West *et al*, 1999). Conversely, their SwHi counterparts show a depression-resistant phenotype of increased struggling in the FST and decreased anhedonia, and do not show increased sensitivity to these seizure-induction paradigms.

Our primary region of interest for this analysis was the hippocampus, which is known to be involved in both depression (Kronmuller *et al*, 2008; Maletic *et al*, 2007) and epilepsy (particularly TLE) (Majores *et al*, 2007) in humans and animal models, and has been specifically implicated in the comorbidity of these diseases (Gilliam *et al*, 2007; Kondziella *et al*, 2007). Furthermore, the SwHi/SwLo rats show the largest neurochemical differences in this region. SwLo rats have a significant reduction in norepinephrine levels in the dorsal hippocampus as compared to SwHi rats (Weiss *et al*, 2008). Interestingly, decreases in noradrenergic tone have been linked to both depression and seizure susceptibility phenotypes (Jobe *et al*, 1999; Kanner, 2008; Ressler and Nemeroff, 1999; Weinshenker *et al*, 2002). Chronic administration of norepinephrine reuptake inhibitors increases struggling in the SwLo, but not SwHi, rats (West *et al*, 1998), suggesting that changes in norepinephrine in the dorsal hippocampus may contribute significantly to the SwLo depression-related phenotypes. Identifying genes that play an important role in comorbid epilepsy and depression in a rodent model by expression microarray analysis of the dorsal hippocampus will enhance current

understanding of the mechanisms underlying this comorbidity and identify potential targets for drug development.

A1.3 Methods

Animals and housing

SwLo and SwHi rats were selectively bred based on FST phenotype, as described previously (Weiss *et al*, 1998). Briefly, at 3 months of age, rats were fitted with "water wings" made from a plastic bubble and placed in a tank (65 cm high, 30 cm diameter) of 25°C water (14 cm from the top) for 10 min. Duration of struggling (active movement of all 4 paws, forepaws breaking surface of water) and floating (immobility) were measured by a trained researcher blinded to the strain of the rat. Five male rats of each line between 5 and 7 months of age from generation 44 were used for the expression microarray analysis. All rats except one had prior exposure to the FST at 3 months of age. An additional 5-6 animals of each line from generation 55 were anesthetized and decapitated for the qRT-PCR experiment. These rats were approximately 3 months of age and experimentally naive to the FST. All rats were housed in groups of 2-3 per cage with food and water available ad libitum and lights on from 0700 to 1900 hours. All experiments were conducted in accordance with Emory University IACUC approval.

Tissue extraction & RNA isolation

Five animals of each line (SwLo and SwHi) were anesthetized with Isofluorane and decapitated. The dorsal hippocampus was isolated on ice, flash-frozen, and stored at

-80°C until further use. Tissue samples were homogenized in TRIzol solution (Invitrogen) using a motorized Kontes Pellet Pestle, and RNA was isolated using the TRIzol Plus RNA Isolation kit (Invitrogen). Briefly, the aqueous phase containing RNA was separated using chloroform. Ethanol was added to precipitate the RNA, and the RNA was allowed to bind to a spin column. After a wash step, the RNA was eluted using RNAse-free water. RNA purity and concentration were verified using a Nanodrop spectrophotometer, and the RNA was stored at -80°C.

Microarray

All microarray procedures were performed by the Yerkes Microarray Core using an Affymetrix GeneChip® Rat Genome 230 2.0 Array. This chip contains 31,099 probes and represents transcripts and variants from approximately 28,700 rat genes, with sequences chosen from GenBank, dbEST, and RefSeq. Each sample was run on an individual array. Quality control of the RNA samples was assessed using an Agilent 2100 Bioanalyzer. Samples were then amplified and labeled using biotin. After hybridization to the GeneChip, chips were washed and stained using an Affymetrix fluidics station. Chips were scanned using an Affymetrix 3000 scanner, and data was collected and processed using the GeneChip Operating Software (GCOS).

Analysis

Microarray analysis was performed using GeneSpring GX software (Agilent). The expression in SwHi samples was compared to the SwLo samples. Data was filtered based on the present or marginal criterion, such that only genes that were expressed in at

least one of the 10 samples under analysis were considered in further analyses. Next, a parametric test was run, with variances assumed equal, $p<0.05$, for a fold change of at least 2. To correct for multiple testing, a Benjamini and Hochberg False Discovery Rate was applied. This generated a list of genes that had a difference of at least 2-fold in expression level between SwHi and SwLo rats at $p<0.05$.

Genes passing the aforementioned criteria were then grouped into functional classes and pathways using Ingenuity Pathways Analysis (Ingenuity Systems, www.ingenuity.com), and the genes were overlaid onto a global molecular network based on their identifiers in the Ingenuity Pathways Knowledge Base. The functional analysis was then used to identify the functions and diseases that were significantly represented in the data set, i.e., those functions or diseases that have a known association with numerous genes in the data set. A p-value was calculated using the Fischer's exact test to determine the statistical significance of the enrichment of these functions or diseases in the data set; this value represented the probability that the biological function or disease was represented in the data set by chance alone. Genes that show greater than two-fold expression difference between lines and have been previously implicated in either epilepsy or mood disorders were primary candidates for follow-up via qRT-PCR confirmation.

Real-time polymerase chain reaction

qRT-PCR was used to confirm expression results for the primary candidate genes. RNA samples from 5-6 male rats of each line from generation 55 were used to synthesize cDNA for qRT-PCR using the SuperScript III First-Strand Synthesis System (Invitrogen)

for biological replication. These rats were all approximately 3 months of age and FST-naive. A subset of two RNA samples from each line that was previously used for microarray analysis was also tested for technical replication.

Intron-spanning primers were designed using Primer3 Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and were manufactured by Invitrogen. Sequences for all primers are shown in table A1.1. All primers were tested on an agarose gel to verify single band products of expected size.

A Bio-Rad CFX96 was programmed to perform qRT-PCR amplification and melting curve analysis using SYBR® Green SuperMix for iQ™ (Quanta). A β actin standard curve of 10-fold serial dilutions of cDNA from a non-selectively bred rat line was used, and a threshold cycle (C(t)) value was calculated. SwLo and SwHi samples were normalized to this standard curve, and differences between the normalized C(t) values for SwLo and SwHi rats were compared using a two-way ANOVA with Sidak's multiple comparisons posthoc test.

A1.4 Results

Microarray data was filtered based on the present or marginal criterion, such that only genes expressed in at least one of the 10 samples were considered further. Of the 31,099 probe sets on the array, 23,358 passed this filter; these probes were then filtered based on fold change of at least 2, generating a list of 222 genes (Figure A1.1).

Following a parametric test, with variances assumed equal ($p<0.05$) and application of a Benjamini and Hochberg False Discovery Rate to correct for multiple testing, 210 genes with significantly different expression between the two lines were

identified. Of these 210 genes, 121 had at least two-fold higher expression in the SwHi rats than the SwLo. Of these, 20 were deemed of particular interest based on the results of the Ingenuity Pathways analyses (Table A1.2). In the SwLo rats, 89 genes showed higher expression than in SwHi rats, and 16 were of particular interest (Table A1.3). Key genes with differential expression included the Gabbr1 and Gabrb2 genes (SwHi > SwLo), as GABA receptor pathologies have been implicated in epilepsy and several affective disorders (Kauffman *et al,* 2008; Sequeira *et al*, 2007; Sperk, 2007). Also of interest were the K+ channel genes Kcnab2 (SwHi > SwLo), Hcn1 (SwLo > SwHi), and Kcnj10 (SwLo > SwHi), given the role of potassium channels in cell excitability and epilepsy (Bender *et al*, 2003; Heilstedt *et al*, 2001; Shang *et al*, 2005). Ntrk1 (SwHi > SwLo) was of interest given its known role in kindling epileptogenesis (Li *et al*, 2005); this neurotrophic receptor is decreased in postmortem human brains of suicide victims as well, suggesting a possible involvement in depression and suicidal ideation (Dwivedi *et al*, 2009). Various components of the complement pathway (C3 and C5r1, SwLo > SwHi) have been implicated in Rasmussen's encephalitis, a neurodegenerative disease characterized by seizures (Whitney and McNamara, 2000). COMT (SwHi > SwLo) is important in catecholamine metabolism, and evidence supports its involvement in seizure susceptibility and affective response (Baune *et al*, 2008; Schlesinger *et al*, 1975).

qRT-PCR analysis was used to confirm and validate the microarray results for Gabbr1 (SwHi > SwLo), Ntrk1 (SwHi > SwLo), Hcn1 (SwLo > SwHi), and C3 (SwLo > SwHi) candidate genes in the biological replicates from generation 55 (n=5-6 per line). Contrary to the microarray results, normalized C(t) values of these genes did not differ between SwLo and SwHi rats using this method of quantification, $F(1, 31)=0.03682$,

p=0.8491 (Figure A1.2A). Additionally, qRT-PCR analysis was used to assess the validity of the microarray results for Gabbr1 (SwHi > SwLo) and Hcn1 (SwLo > SwHi) for a subset of the original RNA samples from the microarray experiment (n=2 per line). Results of these technical replicates were similar to those for the biological replicates, with similar expression levels of these genes between SwLo and SwHi rats, $F(1,4)=2.247$, $p=0.2082$ (Figure A1.2B). Combining these data also revealed no significant differences between SwLo and SwHi rats for any of the genes of interest, $F(1,39)=0.02370$, $p=0.8784$ (Figure A1.2C).

A1.5 Discussion

In order to elucidate the genetic underpinnings of comorbid epilepsy and depression, we conducted hippocampal gene expression analysis on the SwLo rat model of depression and epilepsy. The goal of this study was to identify genes that may provide insight into the relationship between these diseases in humans. Because many antidepressants are pro-convulsant and some anticonvulsants can have a depressive effect (Hesdorffer *et al*, 2009; Judge *et al*, 2011), understanding the mechanisms underlying this comorbidity is important for creating safe and effective medications, and could provide targets for diagnostic screening tests.

Our microarray results identified over 200 genes that were differentially expressed in the hippocampus between the SwLo and SwHi rat lines, and thus may be promising candidates for explaining the epilepsy- and depression-susceptible phenotypes of the SwLo rats. Unfortunately, these results were not confirmed via qRT-PCR analysis. One possibility for the lack of confirmation by qRT-PCR is the potential presence of

genetic drift in the SwLo and SwHi breeding colony over the course of successive generations. However, the absence of significant differences in both biological and technical replicates from both lines makes this a less likely explanation. It is also possible that β actin is a poor choice of housekeeping gene to normalize SwLo and SwHi C(t) values; however, expression levels of β actin were similar between SwLo and SwHi rats, suggesting that this is an appropriate choice of housekeeping gene. An additional possibility relates to the choice of primers for qRT-PCR. It is possible that the microarray assayed a different portion of the transcript than the qRT-PCR primers did (i.e., the microarray may have measured the 3' region of the transcript, while the qRT-PCR primer may target the transcript mid-region). If different areas of the transcript are expressed differently, perhaps via alternative splicing, this may lead to lack of correlation between the microarray and the qRT-PCR (Ding *et al*, 2007). Finally, it is also possible that this subset of genes were "false positives" on the microarray. Because of the inherent multiple testing that occurs in a microarray analysis of 31,000 probes, the risk of Type 1 error is high (Verducci *et al*, 2006). We made every attempt to reduce this risk by using a Benjamini and Hochberg's False Discovery Rate to correct for Type 1 error, but it is possible that false positives may have appeared nonetheless. Additionally, a positive control was not included on any of the qRT-PCR assays. Thus, it cannot be definitively stated that our assays were able to detect a difference in expression between lines even if one was present. Future experiments of this nature should include positive controls to demonstrate differences in gene expression in samples with well-defined known differential expression in order to address this issue.

Analysis of mRNA expression was restricted to the dorsal hippocampus in this study. The hippocampus appears to be an excellent target region for multiple reasons: the hippocampus is known to play a key role in both TLE and MDD (Gilliam *et al*, 2007; Kanner *et al*, 2012; Kondziella *et al*, 2007; Maletic *et al*, 2007), and it is also the site of the largest neurochemical differences measured in the SwLo vs SwHi rats (Weiss *et al*, 2008). Although gene expression differences in one brain regions are often reflected in others, it is possible that specific alterations in other brain regions (e.g. amygdala, cortex, etc) also contribute to the phenotypes of interest, and these regions would be of interest for future study as well.

An additional limitation of this study is that expression microarrays provide no information about which of these genes actually contributes to the phenotypic differences between the lines. Thus, even if the genes identified by the microarray were determined not to be false positives, further studies would still be necessary to determine which of the 220 identified genes actually *cause* the epilepsy- and depression-susceptible phenotypes of the SwLo rat. Future studies could utilize quantitative trait loci (QTL) analysis, combined with the expression microarray results, to identify a small subset of genes that show differential expression *and* map to a genomic region that underlies, at least in part, both the depression-like and seizure susceptibility phenotypes. Additionally, further characterization of any identified candidate genes would be useful towards understanding their role in this comorbidity. For example, viral vectors could be used to regionally overexpress a gene that is decreased in the SwLo rats in an effort to "rescue" the depression-like and seizure susceptible phenotypes. By contrast, RNA interference could be used to knock-down expression in a SwHi rat to create a "SwLo-like"

phenotype. Additionally, transgenic or knockout mice for primary candidate genes may be available or could be created for analysis. Lastly, pharmacological approaches could be utilized to characterize the role of these genes in the phenotypes of interest. Administration of a selective agonist for a gene product with decreased expression in SwLo rats may increase activation of the channel or receptor of interest, and may therefore exhibit an antidepressant and/or anticonvulsant effect in these animals. Likewise, use of an antagonist to decrease activation of these same channels or receptors of interest that are overexpressed in the SwHi may create a "SwLo-like" phenotype in these animals as well, thereby shedding light on its role in these phenotypes.

Despite the lack of positive confirmation for the microarray results in these experiments, more studies of this sort are warranted. Enhanced understanding of the genetic mechanisms underlying comorbid epilepsy and depression phenotypes may provide new therapeutic targets that will be safe and efficacious for a patient population that faces many treatment challenges. Additionally, candidate genes may serve as potential targets for diagnostic screening, identifying patients who are at greater risk for developing the comorbidity and assisting doctors in targeting therapeutic strategies for these patients.

A1.6 Acknowledgements

We thank Yuhua Li and Nick Wagar of the Yerkes Microarray Core for their assistance with the expression microarray and data analysis, and Joshua Suhl and the Steve Warren lab (Department of Human Genetics, Emory University) for assistance with qRT-PCR and use of the Bio-Rad CFX96 machine. These experiments were supported by funding for David Weinshenker (NIH Grant 5 RO1 NS053444) and Alisha Epps (Epilepsy Foundation Fred Annegers Fellowship 121961, NINDS Grant 1F31NS065663-01, and NIDA T32 Institutional Training Grant DA015040).

Table A1.1 Primer sequences used for qRT-PCR.

β Actin	Forward 5'- CTA AGG CCA ACC GTG AAA AG -3'
	Reverse 5'- ATC ACA ATG CCA GTG GTA CG -3'
C3	Forward 5'- TCT TCT CTC TGG CTG CCA AC -3'
	Reverse 5'- CAC ATC TGC CTC CTT GGT G -3'
GABBR1	Forward 5'- GTT CCT CAT CGG GTG GTA TG -3'
	Reverse 5'- CTT TTC AGC CGC TTG GTT AG -3'
Hcn1	Forward 5'- CAA CTT CAA CTG CCG GAA AC -3'
	Reverse 5'- CCT TGG TCA GCA GGC ATA TT -3'
Ntrk1	Forward 5'- CTG GGG CTA ACT CTG GTC AA -3'
	Reverse 5'- CAC CAG TGA TGC TGT TCC AC -3'

Table A1.1 Primer sequences used for qRT-PCR. Intron-spanning primers were designed using Primer3 Plus software (http://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and were manufactured by Invitrogen from the listed sequences.

Table A1.2 Differentially expressed genes with higher expression in SwHi vs. SwLo.

Genbank Access. ID	Gene Symb.	Gene Name	Fold Change	Cell Signaling	Cell Growth & Proliferation	Development	Metabolism	Transport	Regulation of Gene Expression	Immune Function	Seizure Implications	Psychiatry
AF220102	Ilf3	Interleukin enhancer binding factor 3	11.83		X				X			
AM084708	Zfhx1B	Zinc Finger Homeobox 1b	5.88			X			X		X	
Y10369	Gabbr1	gamma-aminobutyric acid (GABA) B receptor 1	5.566	X		X	X				X	
M14400	Ckb	Creatine Kinase, Brain	4.346				X					
X52140	Itga1	Integrin Alpha 1	4.188	X	X	X				X		
NM_019310	Il8ra	Interleukin 8 receptor, alpha	3.961	X	X		X	X		X		
BC085858	Rnmt	RNA (guanine-7) methyltransferase	3.802		X				X			
X15512	Apoc1	Apolipoprotein C-1	3.099	X			X	X				X
AY574277	Nrcam	Neuron-glia-CAM related cell adhesion molecule	2.981	X	X	X						X
M85301	Slc9a4 (Nhe4)	Solute carrier family 9, member 4 (Sodium-Hydrogen Exchange Protein, Isoform 4)	2.877					X				
AB027155	Pde10a	Phosphodiesteraase 10A	2.794	X								X
AY574251	Gabrb2	Gaba-A Receptor Beta 2 subunit	2.747	X		X	X	X			X	X
M85214	Ntrk1	Neurotrophic tyrosine kinase receptor, type 1	2.663	X	X	X	X			X	X	X
Y09164	Scn7a	Sodium Channel, Voltage-gated, type VII, alpha	2.649					X				
NM_017304	Kcnab2	Potassium Voltage-gated channel, shaker related subfamily, beta member 2	2.5					X			X	
BC087076	LOC 498564	similar to integrin, beta-like 1	2.384	X								
BC081850	Comt	Catechol-O-methyltransferase	2.331	X			X	X			X	X
BC061985	Ndufa10	NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 10	2.07				X					X
NM_001106840	Gsta4	Glutathione S-transferase, alpha 4	2.055	X			X					
L05489	Hbegf	Heparin-binding EGF-like growth factor	2.01	X	X	X	X		X	X		

Table A1.2 Differentially expressed genes with higher expression in SwHi vs. SwLo.

Of 121 genes with higher expression in SwHi than SwLo, 20 were deemed to be of particular interest based on their known involvement in either epilepsy or depression separately, and/or their classification in the Ingenuity Pathways Analysis.

Table A1.3 Differentially expressed genes with higher expression in SwLo vs. SwHi.

Genbank Access. ID	Gene Symb.	Gene Name	Fold Change	Cell Signaling	Cell Growth & Proliferation	Development	Metabolism	Transport	Regulation of Gene Expression	Immune Function	Seizure Implications	Psychiatry
BC085359	Alb	Albumin	5.75	X	X		X	X	X			X
AY208182	Abca1	ATP-binding cassette, sub-family A (ABC1), member 1	5.03	X			X	X		X		
M85300	Slc9a3 (Nhe3)	Solute carrier family 9, (sodium/hydrogen exchanger) member 3	4.27					X				X
AB011531	Slit3 (Megf5)	Slit homolog 3	3.97	X		X						
M84156	Mapt	Microtubule-associated protein tau	3.23	X		X	X	X				X
AJ781499	Pofut1	Protein O-fucosyltransferase 1	3.16	X		X	X		X			
AF247450	Hcn1	Hyperpolarization-activated cyclic nucleotide-gated potassium channel 1	2.87					X			X	
BC059152	CD74	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	2.67	X	X		X	X	X	X		X
BC083750	Dcn	Decorin	2.67	X	X	X	X	X		X		
U72497	Faah	Fatty acid amide hydrolase	2.39				X				X	
U27558	Kcnj10	Potassium inwardly-rectifying channel, subfamily J, member 10	2.38			X		X			X	
BC078756	Ccr5	Chemokine (C-C motif) receptor 5	2.24	X	X		X	X		X		
L33894	Dck	Deoxycytidine kinase	2.15		X		X		X	X		
AB003042	C5r1	Complement Component 5, receptor 1	2.09	X			X	X		X	X	
X52477	C3	Complement Component 3	2.07	X	X		X	X		X	X	
BC078880	Pkib	Protein kinase inhibitor beta, cAMP dependent, catalytic	2.01	X							X	

Table A1.3 Differentially expressed genes with higher expression in SwLo vs. SwHi.

Of 89 genes with higher expression in SwHi than SwLo, 16 were deemed to be of particular interest based on their known involvement in either epilepsy or depression separately, and/or their classification in the Ingenuity Pathways Analysis.

Figure A1.1 Differential genetic expression of SwHi and SwLo rats.

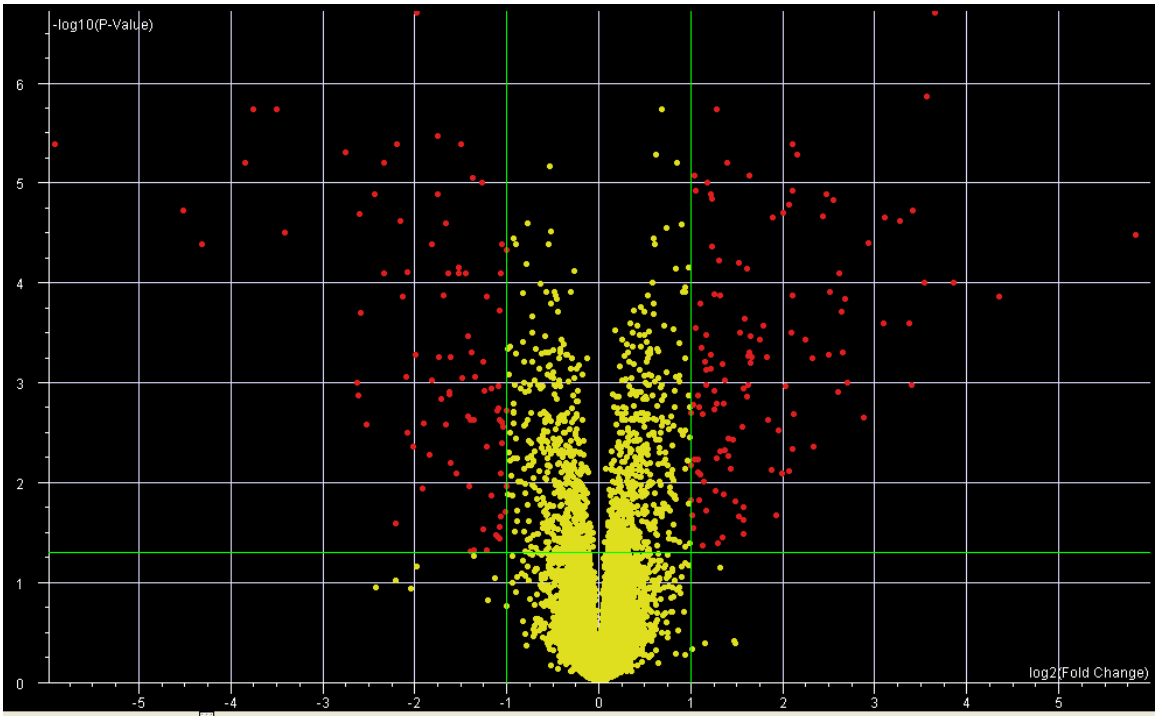

Figure A1.1 Differential genetic expression of SwHi and SwLo rats. This volcano plot shows 210 genes with significantly different expression between SwHi and SwLo (in red), based on a parametric test assuming equal variances. Benjamini & Hochberg False Discovery Rate was applied, and differential expression was determined by a fold difference >2 and $p<0.05$. X-axis shows log2(fold change) and y-axis shows log10(p value).

Figure A1.2 Normalized C(t) analysis of candidate gene expression in SwLo and SwHi rats.

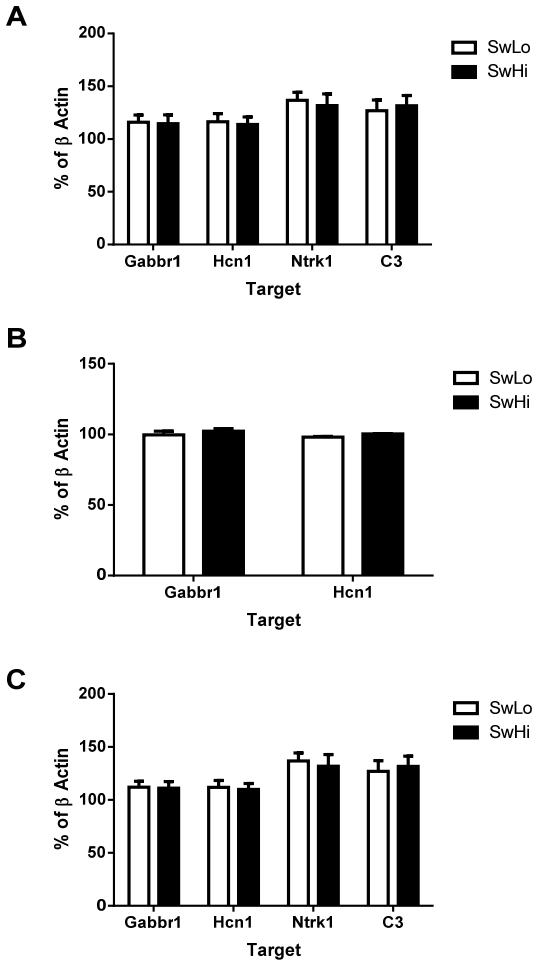

Figure A1.2 Normalized C(t) analysis of candidate gene expression in SwLo and SwHi rats. Threshold cycle values were calculated for Gabbr1, Ntrk1, Hcn1, and C3 in SwLo and SwHi rats and normalized to a β actin standard curve. SwLo and SwHi rats do not differ in their expression levels of the tested genes in biological replicates from generation 55 (panel A, n=5-6 per line), technical replicates from generation 44 (panel B, n=2 per line) or biological and technical replicates combined (panel C, n=7-8 per line). Shown are mean $\pm$ SEM normalized to β Actin (% of β Actin expression).

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APPENDIX 2:

OPERANT PSYCHOSTIMULANT SELF-ADMINISTRATION IN A RAT MODEL OF DEPRESSION

Adapted from:

Lin SJ, Epps SA, West CH, Boss-Williams KA, Weiss JM, Weinshenker D (2012). Operant psychostimulant self-administration in a rat model of depression. Pharmacology, Biochemistry, and Behavior **103**(2): 380-385.

A2.1 Abstract

Depression and psychostimulant addiction are comorbid conditions; depression is a significant risk factor for psychostimulant abuse, and the rate of depression in drug addicts is higher than in the general population. Despite the prevalence of this comorbidity, there are few animal models examining psychostimulant abuse behaviors in depression. We have shown previously that while rats selectively bred for depression-like phenotypes (SwLo) have blunted mesolimbic dopamine (DA) signaling and locomotor responses to dopaminergic drugs, they voluntarily administer excessive amounts of psychostimulants compared to normal or depression-resistant (SwHi) rats in oral consumption paradigms. To determine whether this increased drug intake by depression-sensitive rats extends to operant self-administration, we assessed fixed ratio-1, progressive ratio, extinction, and reinstatement responding for cocaine and amphetamine in SwLo and SwHi rats. Contrary to the oral consumption results, we found that the SwHi rats generally responded more for both cocaine and amphetamine than the SwLo rats in several instances, most notably in the progressive ratio and reinstatement tests. Food-primed reinstatement of food seeking was also elevated in SwHi rats. These results provide further insight into the neurobiology of depression and addiction comorbidity and caution that oral and operant psychostimulant self-administration paradigms can yield different, and in this case, opposite results.

A2.2 Introduction

Addiction and depression comorbidity

High rates of depression in cocaine abusers were first reported over 20 years ago (Weiss *et al*, 1986), and experience in drug abuse clinics has continued to support an association between psychiatric disorders and substance abuse (Kilbey *et al*, 1992). In general, cocaine abuse and dependence are associated with increased risk for depression, with lifetime rates of major depression ranging from 25%-61% in cocaine abusers versus ~10% for community control populations (Rounsaville, 2004). The likelihood of substance abuse/dependence disorders and depression to occur together in the same individuals is ~ 5 times greater than would be expected by the prevalence of each disorder alone, though the complex factors contributing to this comorbidity remain a matter for debate (Grant, 1995; Regier *et al*, 1990; Rounsaville, 2004; Volkow, 2004).

The self-medication hypothesis

One of the leading theories to explain depression and drug addiction comorbidity is the "self-medication hypothesis," stemming from common risk factors and similarities in the neurobiology of depression and drug dependence. This theory posits that individuals with underlying depression have deficits in brain reward systems and may turn to drugs that create euphoric feelings to compensate for their intrinsic anhedonia and motivational deficiencies (Markou *et al*, 1998). The mesolimbic dopamine (DA) system, particularly the DA neurons in the ventral tegmental area (VTA) that project to the nucleus accumbens (NAc), is a critical neural substrate for the rewarding and reinforcing

effects of psychostimulant drugs of abuse (Bardo, 1998; Feltenstein and See, 2008; Leshner and Koob, 1999; Leyton, 2007). Furthermore, there appears to be an association between reduced DA function and depression because key symptoms associated with major depression, such as anhedonia, are consistent with a hypofunctioning DA reward system (Markou *et al*, 1998; Nestler and Carlezon, 2006; Weiss *et al*, 1986).

SwLo rats as a model of addiction and depression comorbidity

We have shown previously that SwLo rats selectively bred for low activity in the forced swim test model of depression have impaired mesolimbic DA signaling and self-administer excessive amounts of psychostimulants in an oral consumption paradigm compared to SwHi rats bred for high activity in the forced swim test (Weiss *et al*, 1998; Weiss *et al*, 2008; West *et al*, 1999a; West *et al*, 1999b). This constellation of phenotypes—a hypofunctional reward system characterized by depression-like behavior, combined with an increased propensity to self-administer psychostimulants—is consistent with the self-medication hypothesis and provides an intriguing model for studying depression and addiction comorbidity. However, oral drug self-administration paradigms have some inherent limitations, as they are subject to potential differences in taste and involve an inborn, "automatic" behavior (drinking) rather than a learned, operant behavior that is more reminiscent of human drug addiction. To confirm the persistence of DA system dysfunction in the depression-sensitive line, we first tested amphetamine-induced locomotion in more recent generations of SwLo and SwHi rats. Next, to further explore motivation for drug-seeking behavior, we assessed several

aspects of operant i.v. amphetamine and cocaine self-administration in SwLo and SwHi rats.

A2.3 Methods

Animals

Male SwHi and SwLo rats were bred in-house, as described (Weiss *et al*, 1998), and were 2-4 months of age at the time of testing. All subjects were singly housed and received *ad libitum* access to food and water unless otherwise noted. Rats were maintained in a temperature-controlled environment on a 12 h reverse light/dark cycle with the lights on from 1900 to 0700 hours for self-administration experiments. For the amphetamine locomotion experiments, rats were maintained on a conventional 12 h light/dark cycle with the lights on from 0700 to 1900 hours.

Rats used for the self-administration experiments were acclimated to the vivarium for 1 week prior to food training. All animals were treated in accordance with NIH policy, and experiments were approved by the Emory Institutional Animal Care and Use Committee.

Amphetamine-induced locomotor activity

Rats were individually housed in clear acrylic cages in an activity-monitor room with *ad libitum* access to food and water. Movement was tracked using eight parallel infrared beams positioned at 5-cm intervals along the length of the cage. To exclude repetitive movements in a small area, each beam break that was different from the

previous four breaks was recorded by a computer as a unit of "ambulatory activity." Rats were allowed to habituate to the room for 3 days and were handled for several minutes each day. All animals received a vehicle injection (0.9% saline) and, 2 days later, an injection with amphetamine (0.5 or 1.0 mg/kg in a volume of 5 ml/kg; Sigma-Aldrich, St. Louis, MO). Ambulatory activity was measured for 1 h immediately following injection. Each animal was tested with a single dose of amphetamine.

Food training

Prior to catheterization surgery, rats were trained to lever-press on a fixed ratio-1 (FR1) schedule for food (45 mg pellets; Fisher Scientific, Pittsburgh, PA) in standard rat operant chambers (Med Associates, St Albans, VT) as we have described (Schroeder *et al*, 2010). Each chamber was equipped with a house light, two retractable levers (active and inactive), stimulus lights above both levers, and a food pellet dispenser. Inactive lever presses had no consequence. A computer with MED-PC software (MED Associates) controlled the program and recorded data. Food training sessions lasted for 8 h, or until the animal obtained at least 100 food pellets with a 70% selection for the active lever. Most rats achieved these criteria in a single session, although some required a few sessions.

Surgery

Rats were anesthetized with isoflurane and implanted with intravenous jugular catheters using standard methods, as we have described (Schroeder *et al*, 2010). Catheters were flushed twice daily with 0.05 ml gentamicin (4 mg/ml) and 0.1 ml heparin solution

(30 U/ml in sterile saline) for three days following surgery, then once daily. Catheter patency was verified by infusing methohexital sodium (20 mg/ml, IV), which results in rapid muscle tone loss when administered intravenously. Rats were allowed at least 5 days of recovery time before commencing self-administration experiments.

Amphetamine self-administration

Daily self-administration sessions on a FR1 schedule lasted for a maximum of 2 h or until rats received 40 drug infusions. At the beginning of each session, rats received a non-contingent infusion of amphetamine (0.1 mg/kg, i.v.; Sigma-Aldrich). During the sessions, each active lever press resulted in an amphetamine infusion (0.1 mg/kg in a volume of 167 µl/kg) and illumination of a stimulus light above the lever. A timeout period of 20 s followed the infusion, in which active lever presses had no programmed consequences. After the timeout period, the stimulus light was extinguished. Inactive lever presses were recorded, but had no programmed consequences. Daily FR1 sessions continued until the rats met the criteria for stable responding (number of drug infusions varied by <20% of the mean, active lever response was at least 20, and preference for the active lever was at least 75% for 3 consecutive days, with a minimum of 5 total days of cocaine self-administration). The day after reaching maintenance criteria for FR1 responding at the 0.1 mg/kg/infusion dose, rats were trained on a FR1 schedule with 0.25 mg/kg amphetamine. After reaching criteria for the second FR1 paradigm, the progressive ratio (PR) schedule commenced, in which each subsequent infusion of amphetamine (0.1 mg/kg) required a greater number of active lever presses than the last,

as described (Richardson and Roberts, 1996). The equation used for the number of active presses for each infusion was:

$$\textit{Response Requirement (rounded to nearest integer)} = (5e^{(\textit{injection number}*0.2)}) - 5,$$

such that the response requirements for the first 25 infusions were 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, and 737. Amphetamine infusions were accompanied by the same programmed consequences as described for the FR-1 schedule. Sessions lasted until the rats reached breakpoint, defined as the number of infusions received prior to a 1 h period in which no rewards were obtained, or for a maximum of 6 h. Rats were considered to have reached a stable response level using the same criteria as the FR1 schedule, but for a minimum of 3 total days on the PR schedule. A subset of the rats were then tested for PR responding for a higher dose of amphetamine (0.25 mg/kg/infusion).

Cocaine self-administration and reinstatement

Separate groups of rats were used for cocaine self-administration experiments, and conditions were identical to those used for amphetamine self-administration, except that the reinforcer for the FR1 and PR sessions was cocaine (0.5 mg/kg/infusion; National Institute of Drug Abuse Drug Supply Program, Bethesda, MD).

The day following meeting criteria for stable PR responding, lever pressing was extinguished in daily 2 h sessions that had the same contingencies as the FR1 schedule, except responding on the active lever had no programmed consequences and did not result in a cocaine infusion. Behavior was considered extinguished when active lever

presses over 3 consecutive days were <25% of the average number of active lever presses during the last 3 days of progressive ratio.

The day following meeting extinction criteria, rats were pretreated with cocaine (10 mg/kg, i.p.) immediately before a 2 h reinstatement session under extinction conditions. A subset of the rats were also tested for reinstatement following administration of a lower dose of cocaine (1 mg/kg, i.p.).

Food self-administration and reinstatement

Separate groups of rats were used in the food self-administration experiments, and FR1, PR, extinction, and reinstatement conditions were identical to those used for drug self-administration, with the following exceptions. Rats were placed on restricted diets of 16 g of normal rat chow per day, which was given at least 1 h after the end of food self-administration sessions. Active lever presses were reinforced with a 45 mg food pellet (Fisher Scientific), and sessions lasted for 1 h or when 60 pellets were obtained. Following extinction, rats were reinstated for food in a 2 h session as we have described previously (Schroeder *et al*, 2010). At the beginning of the session, 3 food pellets were non-contingently delivered in the first 10 s, and a food pellet was delivered non-contingently every 3 min. Active lever responses had no programmed consequences during reinstatement.

Statistical analysis

Data were analyzed by Student's t-test when comparing 2 groups, and by ANOVA followed by Bonferroni post hoc tests when comparing more than 2 groups, using Prism 5.0 for Macintosh.

A2.4 Results

SwLo rats have decreased amphetamine-induced locomotion

We reported previously that amphetamine-induced locomotion is attenuated in SwLo rats compared to SwHi rats (West *et al*, 1999a). Because those data were obtained more than a decade and dozens of generations ago, we determined whether that phenotype has continued to be co-inherited with the phenotype being selected for (low activity in the forced swim test). We found that amphetamine-induced locomotion was still reduced in recent generations of SwLo rats (Figure A2.1). A two-way ANOVA showed a main effect of line ($F_{1,26}$=20, $p<0.001$), dose ($F_{2,26}$=23.07, $p<0.0001$), and a line x dose interaction ($F_{2,26}$=4.08, $p<0.05$). Posthoc tests revealed that amphetamine-induced locomotion was significantly lower in SwLo rats compared with SwHi rats at both the 0.5 mg/kg ($t=3.937$, $p<0.01$) and 1 mg/kg ($t=2.57$, $p<0.05$) doses. These results indicate that a dysfunctional DA system has persisted in SwLo rats.

Amphetamine- and cocaine-seeking behavior during progressive ratio and reinstatement responding is attenuated in SwLo rats

To determine whether the differences between SwLo and SwHi rats in stimulant-induced locomotion and oral consumption extended to operant drug-seeking behavior, we assessed several aspects of i.v. amphetamine and cocaine self-administration. While no differences were detected during FR1 responding for amphetamine at two different doses (0.1 and 0.25 mg/kg/infusion), SwLo rats had a significantly lower breakpoint during PR self-administration of both doses compared to SwHi rats (Figure A2.2). A two-way ANOVA showed a main effect of line ($F_{1,11}$=16.64, $p<0.01$), and posthoc tests revealed that breakpoint was significantly lower in SwLo rats at the 0.1 mg/kg ($t=3.21$, $p<0.05$) and the 0.25 mg/kg ($t=2.67$, $p<0.05$) doses. Inactive lever presses were low during all phases and were not significantly different between lines.

Results were similar for cocaine-self administration (Figure A2.3). No differences were detected during FR1 responding for cocaine, but SwLo rats had a significantly lower breakpoint during PR self-administration ($t=2.37$, $p<0.05$). While the low priming dose of cocaine (1 mg/kg, i.p.) did not induce reinstatement behavior in rats of either line, the high priming dose of cocaine (10 mg/kg, i.p.) significantly reinstated active lever pressing compared to extinction responding in SwHi rats, but not SwLo rats. A two-way ANOVA showed a main effect of self-administration phase ($F_{2,30}$=20.77, $p<0.0001$) and line ($F_{1,30}$=5.16, $p<0.0001$), and a phase x line interaction was just at the significance cut-off ($F_{2,30}$=3.31, $p=0.05$). Posthoc analysis revealed that active lever responding at the high dose was significantly higher in SwHi rats compared to extinction ($t=5.25$, $p<0.001$)

and compared to SwLo rats ($t=3.41$, $p<0.05$). Inactive lever presses were low during all phases and were not significantly different between lines.

Food-primed reinstatement of food seeking is reduced in SwLo rats

To determine whether the differences in amphetamine and cocaine responding between SwLo and SwHi rats were specific to stimulant drugs or generalized to natural rewards, we assessed several aspects of operant food self-administration (Figure A2.4). No significant differences were observed between lines during FR1 or PR food self-administration, but food-primed reinstatement responding was reduced in SwLo rats. A two-way ANOVA showed a main effect of phase ($F_{1,14}=6.15$, $p < 0.05$) and line ($F_{1,14}=6.61$, $p<0.05$). Posthoc analysis revealed that active lever presses were significantly lower in SwLo rats compared with SwHi rats during reinstatement ($t=2.53$, $p<0.05$). Active lever presses also tended to be lower in SwLo rats during extinction, but the difference did not reach statistical significance. Inactive lever presses were low during all phases and were not significantly different between lines.

A2.5 Discussion

SwLo rats as a model of depression and addiction comorbidity

As summarized in the Introduction, depression and drug addiction are comorbid disorders, potentially due to an attempt by depressed individuals to compensate for intrinsic anhedonia and motivational deficiencies by taking drugs (i.e. the "self-medication" hypothesis). Because animal models have been particularly useful for

understanding the neurobiological substrates underlying depression and addiction individually, an animal model displaying features of both disorders might be equally informative for unraveling the underpinnings of comorbidity. We examined operant psychostimulant self-administration in SwLo and SwHi rats for 3 reasons. First, SwLo animals have low activity in the forced swim test (the trait they were selected for) and other anhedonic-like behaviors (Weiss *et al*, 1998) (our unpublished data). Second, they display decreased amphetamine-induced locomotor activity, altered behavioral responses to apomorphine, and impaired DA signaling in the nucleus accumbens (West *et al*, 1999a; West *et al*, 1999b). Third, SwLo rats voluntarily consume more cocaine and amphetamine in oral self-administration paradigms (Weiss *et al*, 2008). This constellation of phenotypes—a hypofunctional reward system characterized by depression-like behavior and anhedonia, combined with an increased propensity to self-administer psychostimulants—is consistent with the self-medication hypothesis and provides an intriguing model to study depression and addiction comorbidity. The SwLo rats are also appealing because their behavioral phenotypes are heritable, and both depression and addiction have substantial genetic components in humans (Levinson, 2006; Nestler, 2000). While we did not test non-selected "wild-type" rats in this study, our previous results have shown that the relevant phenotypes of non-selected rats (e.g., forced swim test activity, oral amphetamine consumption) typically fall somewhere in between those of SwLo and SwHi rats (Weiss *et al*, 1998; Weiss *et al*, 2008).

Operant psychostimulant self-administration

Contrary to our predictions and previous oral consumption results, SwLo rats actually displayed decreased amphetamine- and cocaine-seeking behavior compared with SwHi rats. These differences were particularly evident when contingencies were high (PR schedule) or non-reinforced (reinstatement). These results suggest that the self-medication hypothesis may not be applicable to the SwLo rats. These animals have hypofunctional DA systems, which may suppress multiple stimulant-induced behaviors, including locomotor activity and operant self-administration. The differences between SwHi and SwLo rats were particularly evident during drug-primed reinstatement, a behavior known to require DA transmission in the NAc (Schmidt *et al*, 2005). Thus, it is possible that a hypofunctional accumbal DA system in the SwLo rats can account for the decrease in drug-seeking behavior observed in these animals. Interestingly, the levels of DA and its metabolites in the striatum of SwLo rats are similar to that of SwHi rats, but are low in the prefrontal cortex, while postsynaptic DA receptor signaling is altered in SwLo rats (Weiss *et al*, 2008; West *et al*, 1999b). We did not measure DA release or transmission in this study, and it is possible that other neurotransmitter systems that are impacted by psychostimulants, such as norepinephrine, are altered in SwLo rats and contribute to the phenotypes.

A trivial explanation for the increased oral drug intake observed in the SwLo rats could be altered taste preferences. However, this seems unlikely because SwLo rats consumed more amphetamine solution regardless of whether sucrose was added to it or not (Weiss *et al*, 2008). Thus, we offer several alternative explanations for the discrepancy between oral and operant psychostimulant-seeking behavior. Because

operant behaviors require learning, while drinking is innate, differences in cognitive performance could contribute to differences in operant behavior. However, SwLo and SwHi rats acquired self-administration at similar rates and learn spatial memory tasks with equal efficiency (our unpublished data). The explanation we currently favor is that the low exploratory and drug-induced motor activity may be masking an increased propensity for psychostimulant intake. Compared to SwHi rats, SwLo animals have reduced activity under circumstances that tend to elicit active, assertive motor behavior, such as a novel environment (Weiss *et al*, 1998), which could affect motivation to execute operant drug-seeking responses. In addition, amphetamine-induced locomotor activity, which could help drive operant responding for the drug during the self-administration sessions, is also impaired in SwLo rats. Consistent with this idea, individual and selected differences in self-administration are often (although not always) positively correlated with novelty-seeking and stimulant-induced locomotor activity (Belin *et al*, 2011; Mandt *et al*, 2008; Meyer *et al*, 2010; Schramm-Sapyta *et al*, 2011). Food-primed reinstatement of food seeking is also impaired in SwLo rats, consistent with a decreased motivation to perform operant tasks. Although we tested 2 different doses of amphetamine and 1 dose of cocaine, it is important to note that we did not examine full dose-response relationships in this study. Thus, it is possible that different results would be obtained if additional doses were tested.

Conclusion

We undertook this study to determine whether the increase in oral psychostimulant self-administration observed in a rodent model of depression with

impaired DA transmission extended to operant self-administration. Contrary to our hypothesis and the oral self-administration data, SwLo rats displayed reduced drug-seeking behavior during PR and reinstatement responding for both cocaine and amphetamine. Thus, at the doses tested, these results do not support the use of SwLo rats to study the self-medication hypothesis of drug addiction, and highlight the different outcomes that can be obtained using oral vs. operant self-administration paradigms. Future studies examining these rats in other addiction-related paradigms that measure drug seeking but do not require operant responses, such as conditioned place preference, are warranted. It may also be informative to test the self-administration behavior of SwHi and SwLo rats using additional cocaine and amphetamine doses, as well as other classes of drugs such as opiates and nicotine.

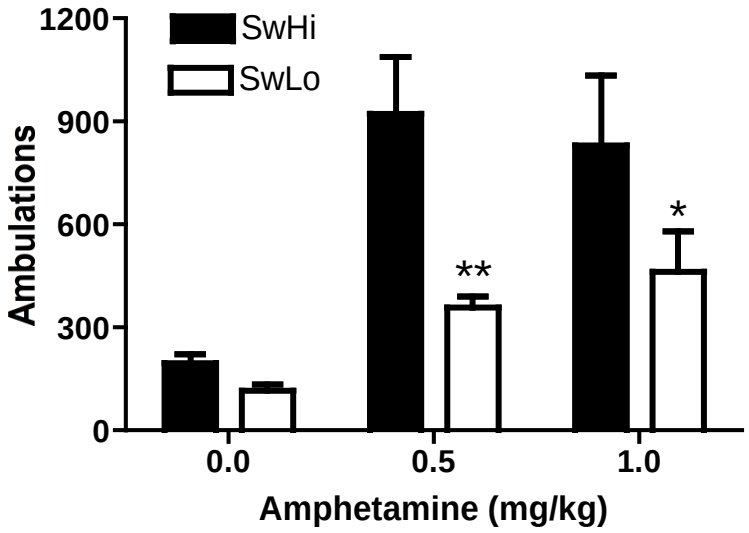
A2.6 Acknowledgements

This work was supported by an Emory SURE Fellowship (to SJL), the National Institute of Neurological Disease and Stroke (NS065663 to SAE), and the National Institute of Drug Abuse (DA027535 to DW).

A2.7 Conflict of Interest

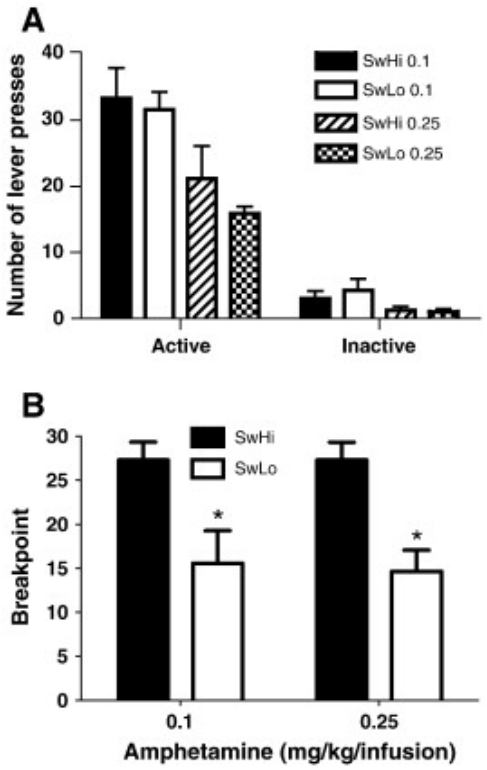
DW is co-inventor on a patent concerning the use of selective DBH inhibitors for the treatment of cocaine dependence (US-2010-015748-A1; “Methods and Compositions for Treatment of Drug Addiction”). The other authors declare no conflicts of interest.

Figure A2.1 Amphetamine-induced locomotion is reduced in SwLo rats.



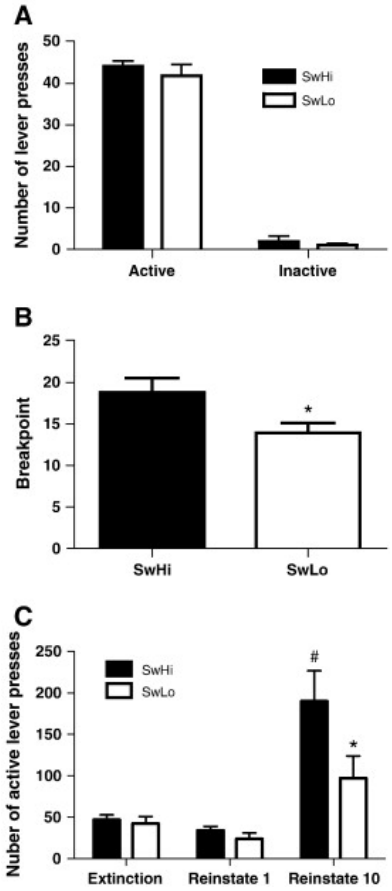
Amphetamine-induced locomotion is reduced in SwLo rats. SwHi and SwLo rats were injected with vehicle (n=8 per group) or amphetamine (0.5 or 1 mg/kg, i.p.; n=4 per group), and locomotor activity was measured for 1 h. Shown are mean ± SEM ambulations (consecutive beam breaks). *$p<0.05$, **$p<0.01$ compared with SwHi rats at that dose.

Figure A2.2 Amphetamine self-administration in SwHi and SwLo rats.



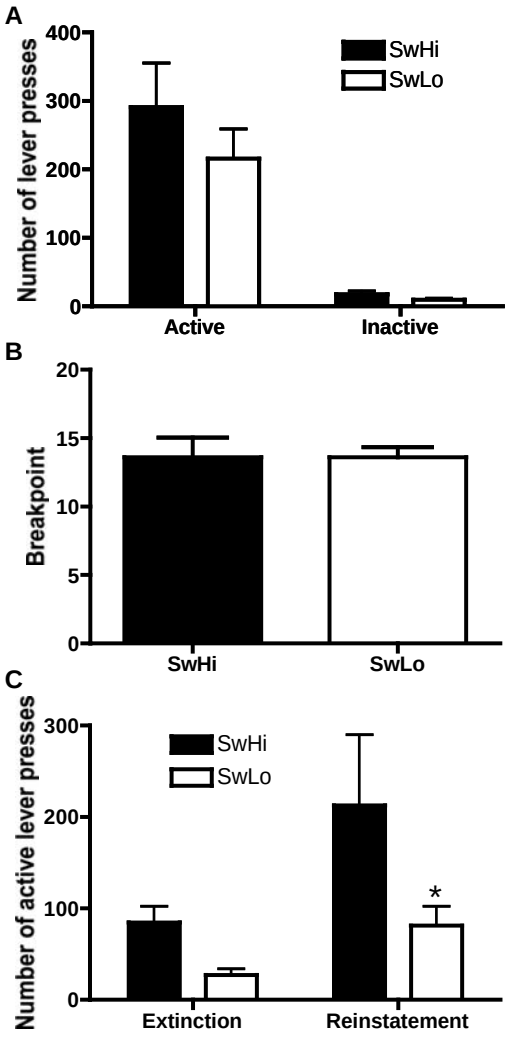
Amphetamine self-administration in SwHi and SwLo rats. SwHi and SwLo rats were trained to self-administer amphetamine (0.1 mg/kg/infusion) on an FR1 schedule. After reaching maintenance criteria, rats were trained to self-administer a higher dose of amphetamine (0.25 mg/kg/infusion) on an FR1 schedule. After reaching maintenance criteria for the higher dose, rats were tested for PR responding for the lower dose (0.1 mg/kg). Shown is the mean ± SEM of (A) active and inactive lever presses during stable FR1 responding at 0.1 mg/kg/infusion (SwHi, n=7; SwLo, n=5) and 0.25 mg/kg/infusion (SwHi, n=5; SwLo, n=5) and (B) number of rewards earned at breakpoint during stable PR responding at 0.1 mg/kg/infusion (SwHi, n=5; SwLo, n=4) and 0.25 mg/kg/infusion (SwHi, n=2; SwLo, n=4). *$p<0.05$ compared to SwHi rats.

Figure A2.3 Cocaine self-administration and reinstatement in SwHi and SwLo rats.



Cocaine self-administration and reinstatement in SwHi and SwLo rats. Rats were trained to self-administer cocaine (0.5 mg/kg/infusion) on an FR1 schedule. After reaching maintenance criteria, rats were tested for PR responding at the same dose of cocaine. Rats were then extinguished and tested for cocaine-primed (1 or 10 mg/kg i.p.) reinstatement ("Reinstate 1" and "Reinstate 10", respectively) the day after meeting extinction criteria. Shown is mean ± SEM of (A) active and inactive lever presses during stable FR1 responding (SwHi, n=7; SwLo, n=8), (B) number of rewards earned at breakpoint during stable PR responding (SwHi, n=7; SwLo, n=8), and (C) active lever presses during stable extinction and reinstatement (n=6 per group). *$p<0.05$ compared to reinstatement for SwHi rats, #$p<0.001$ compared to extinction for SwHi rats.

Figure A2.4 Food self-administration and reinstatement in SwHi and SwLo rats.



Food self-administration and reinstatement in SwHi and SwLo rats. Rats were trained to self-administer 45 mg food pellets on an FR1 schedule. After reaching maintenance criteria, rats were tested for PR responding for food pellets. Rats were then extinguished and tested for food-primed reinstatement the day after meeting extinction criteria. Shown is mean ± SEM of (A) active and inactive lever presses during stable FR1 responding, (B) number of rewards earned at breakpoint during stable PR responding, and (C) active lever presses during stable extinction and reinstatement. *$p<0.05$ compared to reinstatement for SwHi rats. N=4-5 per group.

A2.8 References

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