THE EFFECT OF EXERCISE ON KAINIC ACID INDUCED SEIZURES

by

JENNY REISS

(Under the Direction of PHILIP HOLMES and RODNEY DISHMAN)

ABSTRACT

Studies have demonstrated that exercise decreases the symptoms of a variety of neurological disorders. Research in our laboratory has revealed that exercise up-regulates prepro-galanin messenger RNA levels in the locus coeruleus after 3 weeks of physical activity in rats. Several other studies have demonstrated that galanin decreases neuronal hyperexcitability both in vivo and in vitro. These findings support the hypothesis that exercise may diminish neural hyperexcitability, possibly through a galaninergic mechanism. The first two experiments (Chapter 3) tested whether voluntary activity wheel running would protect against kainic acid-evoked seizures and whether galaninergic signaling is a necessary factor. The third experiment (chapter 4) used in-vivo voltammetry during kainic acid-induced seizures to compare glutamate release in the hippocampus of rats allowed free access to an activity wheel or sedentary housing. In the first experiment, rats remained sedentary or were given access to running wheels for 3 weeks. After this period, rats received an intraperitoneal (IP) injection of 0, 7 10 or 14 mg/kg kainic acid. Activity wheel running decreased the severity of or eliminated the seizure behaviors and hippocampal c-fos activation induced by kainic acid. In the second experiment rats were injected intracerebroventricularly (ICV) with .2 or .4 µg of kainic acid following either an injection of M-40 (a galanin antagonist) or saline. Activity wheel running decreased the severity

of the behavioral reactions to kainic acid at the .2 µg dose, and M-40 injection decreased this effect. In contrast, there were no detectable differences in behavior or cell loss at the .4 µg dose between exercising and sedentary rats. In the third experiment extracellular hippocampal glutamate was significantly reduced for 30 minutes post injection in exercising compared to sedentary subjects. These findings indicate that exercise induced up-regulation of galanin is a necessary factor in exercise-induced reduction in seizures. Exercise may also reduce excitability by modulating glutamate release.

INDEX WORDS: Physical activity, kainic Acid, galanin, neuroprotection, seizure, glutamate, hippocampus, voltammetry

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CHAPTER ONE

GENERAL INTRODUCTION

Exercise has been shown to be neuroprotective in a number of clinical and animal studies. Clinical research has indicated that exercise can decrease age-related cognitive decline (McAuley et al., 2004; Churchill et al., 2002; Laurin et al., 2001) and reduce the risk of dementia (Fratiglioni et al., 2004; Larson et al., 2006). Aerobic fitness training for 6 months increases brain volume in gray and white matter regions important to cognition while being physically fit also attenuates the loss of gray and white matter in the aged brain (Colcombe et al., 2003; Colcombe et al., 2006)

A number of animal models of Alzheimer's disease (AD) and aging have demonstrated a protective effect of exercise against cognitive decline and AD-like pathology both through behavioral tests and cellular analyses. In one study animals provided with access to an activity wheel for 5 months had significantly decreased Aβ in the frontal cortex and hippocampus and an enhanced rate of learning in the Morris water maze. This experiment also included a group that exercised for 1 month. They had reduced proteolytic cleavage fragments of APP (α-CTFs and β -CTFs) (Adlard et al., 2005). Similar protective effects of exercise on transgenic mice have been found in training studies of aged animals (Nichol et al., 2007) and studies using an enriched environment which includes as activity wheel (Arendash et al., 2004; Jankowsky et al., 2005; Lazarov et al., 2005; Nichol et al., 2009).

Exercise can also increase lifespan and decrease behavioral deficits that accompany the aging process. Mice trained on a treadmill from 28 to 78 weeks extended their lifespan by 9%

and 19% for male and females respectively. Exercise trained mice also showed improved performance on tests of muscular coordination and cognitive ability compared to their sedentary counterparts at 52 weeks. Age-associated increases in oxidative stress markers were also significantly decreased (Navarro et al., 2004). Aged rats trained on a running wheel for 5 weeks show faster acquisition and better retention of the platform location in the Morris water maze compared to age-matched controls (van praag et al., 2005). This demonstrates that even aged animals can benefit from moderate levels of activity.

Treadmill training for at least 3 weeks decreased infarct volume induced by MCA occlusion with reperfusion by 79% and decreased deficits in motor control (Wang et al., 2001; Li et al., 2004). Activity wheel running for 3 weeks prior to MCA occlusion with reperfusion; improved the ability of mice to locate the platform in the Morris water maze, enhanced the survival of new cells in the dentate gyrus, reduced infarct size and sensorimotor deficits upregulated endothelial nitric oxide synthase, and increased cerebral blood flow (Endres et al. 2003; Luo et al., 2007). Endothelial nitric oxide synthase knockout mice do not show any improvements from activity wheel training, demonstrating that it is a crucial factor in exercise induced neuroprotection against ischemic damage (Endres et al., 2003).

Long-term treadmill running delays symptoms and reduces the occurrence and severity of spontaneous recurrent seizures induced by pilocarpine injection in rats (Setkowicz and Mazur 2006, Arida et al., 1999; Arida et al., 2004). Treadmill training also reduces spatial deficits that result from a domoic acid injection in mice (Carro et al., 2001). In addition, treadmill running after pilocarpine injection reduces excitatory postsynaptic potentials evoked by stimulation of hippocampal afferents (Arida et al., 2004). Although these experiments demonstrate a protective effect of aerobic training, the treadmill training and swimming employed in these studies stress

the animals and may confound results (Dishman 1997a; Timofeeva et al., 2003). Short term (five days) of voluntary activity wheel running reduced the learning deficits induced by kainic acid in rats although a reduction of hippocampal cell death was not detected (Gobbo and O'Mara, 2005).

Excitotoxicity through over-activation of glutamatergic synapses is a major mechanism of cell death underlying acute brain insults such as stroke and trauma as well as chronic disorders such as epilepsy and Alzheimer's disease, Parkinson's disease and Huntington's disease (Dawson et al., 1995; Doble 1999; Meldrum 2000; Hynd 2004; Wang 2005; Waxman and Lynch, 2005; Mattson 2007). Stress also damages hippocampal neurons through over-stimulation of glutamatergic synapses. Glutamate receptor blockade has been shown to be neuroprotective and is a treatment modality used to reduce damage in stroke, epilepsy, Alzheimer's disease and stress (Hara and Snyder, 2007; Sonkusare et al., 2005; Scholtzova et al., 2008).

Exercise induces many known changes in the brain that likely contribute to improved cognitive functioning and cell survival. Experiments have consistently demonstrated that exercise increases levels of brain-derived neurotrophic factor (BDN F) and insulin-like growth factor I (IGF-I) (Neeper et al., 1996; Carro et al., 2000). BDNF enhances synaptogenesis and neurogenesis (van Praag et al., 1999; Gómez-Pinilla et al., 2002; van Praag et al., 2002; Redila and Christie, 2005; Stranahan et al., 2007) and promotes long term potentiation (Kiraly and Kiraly, 2005). IGF-I increases BDNF and blood vessel density in the dentate gyrus of the hippocampus and throughout the brain (Lopez-Lopez et al., 2004; Aberg et al., 2006). IGF-I acts on vascular endothelium to activate nitric oxide synthase, thereby promoting vascular health. Nitric oxide produced by the endothelium of cerebral arterioles is an important mediator of endothelium-dependent vasodilation and helps to prevent hypoxic conditions (McCarty 2000). **S**tudies have implicated excessive levels of nitric oxide in excitotoxic cell death following

activation of NMDA receptors and have demonstrated a protective effect of inhibiting its formation during an excitotoxic challenge (Hara and Snyder, 2007). Nitric oxide is an important molecule in the induction of long term potentiation and facilitates excitation (Taqatqeh et al., 2009).

Physical activity may indirectly provide protection from degeneration by modulating the stress response. Studies have demonstrated that physical activity training can attenuate behavioral deficits induced by stress, possibly by moderating monoaminergic responses (Dishman et al., 1996; Dishman et al., 1997; Greenwood et al, 2003; Van Hoomissen et al., 2004; Greenwood et al., 2007). Studies have also shown that exercise training reduced stress induced increases in glucocorticoids (Dishman et al., 1998; Zheng et al., 2006; Sasse et al., 2008). Exercise may reduce excitotoxic cell death by modulating stress induced increases in glucocorticoids (Miller and O'Callaghan, 2003; Kiraly and Kiraly, 2005).

Aside from modulating glucocorticoids, the effects of exercise promote plasticity, neurogenesis and excitability. Activity wheel running rats were reported to have increased AMPA receptors and phosphorylated subunits of the NMDA receptor of (Dietrich et al., 2005). These findings indicate that exercise increases the sensitivity of the hippocampus to glutamatergic input. Upregulation of a factor that modulates the excitability of hippocampal neurons under excitotoxic challenges would be necessary to modulate the increased sensitivity of the hippocampus.

Previous research in our laboratory has identified that physical activity upregulates prepro-galanin messenger RNA levels in the locus coeruleus after 3 weeks of activity wheel exposure (O'Neal et al., 2001; Van Hoomissen et al., 2004). Galanin has been found in a number of studies to act as an anticonvulsant. Galanin administration blocks the release of excitatory

amino acids and protects against cell death in hippocampal cell cultures, while blocking galanin transmission causes increased excitation and cell death (Zini et al., 1993; Elliot-Hunt et al., 2004). Transgenic mice with a disruption in the galanin gene show increased susceptibility to status epilepticus and increased cell death in response to seizure-inducing agents, while mice that over express the galanin gene have decreased seizure susceptibility and cell death (Mazarati et al., 2000; Mazarati et al., 2001; Elliot-Hunt et al., 2004). Galanin or galanin receptor agonists have been demonstrated to decrease excitotoxic effects of models of kindling and status epilepticus in wild type animals and this effect is depleted when followed by galanin receptor antagonists such as M40, M35 or M15 (Mazarati et al., 1992; Mazarati et al., 2001; Saar et al., 2002). Research suggests that galanin acts to suppress glutamate release by hyperpolarizing membranes through the opening of ATP-dependent potassium channels as well as by closing voltage gated calcium channels through g-protein coupled receptors in response to glutamate. Galanin may also inhibit excitatory input postsynaptically by inhibiting long term potentiation, which is mechanistically similar to seizures (Mazarati et al., 2001).

The first two experiments were designed to test whether voluntary activity wheel running can protect against kainic acid-evoked seizures and whether galanin is a critical factor in exercise induced effect. The third experiment tested whether exercise altered extracellular glutamate levels in the hippocampus. In the first experiment Sprague Dawley male rats had access to a running wheel for 3 weeks while another group of rats had no running wheels. At the end of the 3 week period rats were injected intraperitoneally with various doses of kainic acid and behavioral reactions were monitored and scored for 3 hours. After the three hour observation period animals were sacrificed and seizure-induced c-fos activation was quantified in several hippocampal regions. The second experiment tested whether activity wheel running prevents

seizures induced by intracerebroventricular (ICV) injection of kainic acid and whether the galanin antagonist M-40 decreases this protective effect of exercise. The third experiment (chapter 4) used in-vivo voltammetry during kainic acid-induced seizures to compare glutamate release in the hippocampus of rats allowed free access to an activity wheel or sedentary housing.

CHAPTER TWO

LITERATURE REVIEW

Physical inactivity is a modifiable risk factor for a number of conditions that increase an individual's risk for dementia and cognitive decline, including; diabetes, cancer, depression, and obesity (Dishman et al., 2004; Alagiakrishnan et al., 2006). Exercise also decreases the risk of cardiovascular disease and stroke which commonly precede cognitive decline and dementia (Pope et al., 2003; Wendel-Vos 2004; van der Flier and Scheltens. 2005). Physical activity has been found to reduce the risk or symptoms of stress and depression (Lawlor 2001; Salmon 2001; Brosse 2002) which are associated with an increase in the risk of Alzheimer's disease (AD) (Wilson et al 2003; Madrigal et al., 2006). In addition to reducing the occurrence or severity of a number of risk factors for dementia and AD, exercise has been shown to impact age associated declines in cognition.

Some cross-sectional studies support a positive relationship between cardiovascular fitness and cognitive functioning in the elderly (Etnier et al., 1997; Etnier et al., 2006). A meta-analysis of randomized control training studies reported that improvements are most consistently seen for executive control processes, which include higher order functions such as planning, abstract thinking, rule acquisition, and inhibition of inappropriate actions. Greater effect sizes are generally associated with higher levels of activity maintained for longer periods of time (Colcombe and Kramer, 2003).

One study demonstrated that being physically fit can attenuate the loss of gray and white matter in the aged brain. High resolution magnetic resonance imaging scans were acquired from

55 older adults in order to estimate tissue atrophy. Aerobic fitness levels were assessed by maximal oxygen uptake estimates and a walking test. Age related declines in gray matter were observed in the prefrontal, superior parietal and middle inferior cortices and a decline in white matter tracts was also observed. A significant attenuation of age related decline was found in subjects with higher fitness levels (Colcombe et al., 2003). In a randomized control experiment 59 healthy adults 60-79 years old participated in 6 months of aerobic fitness training or a stretching control group. Significant increases in brain volume, in both gray and white matter regions, were found as a function of fitness training for the older adults who participated in the aerobic fitness training but not for the older adults who participated in the stretching and toning (non aerobic) control group (Colcombe et al., 2006). Both of these studies provide biological support for clinical observations of improved cognition in elderly individuals.

The risk of developing AD increases exponentially with age, doubling every 5 years, but being physically active can substantially reduced an individual's risk. Participants with a higher (intensity and frequency) levels of physical activity are less likely to be diagnosed with AD. This relationship has been found in cross-sectional studies including both sexes, monozygotic twins discordant for dementia, and carriers of the apolipoprotein E e4 (APOEe4) allele (Schuit et. al. 2001; Friedland et al, 2001; Pope et al., 2003; Gatz et al., 2006). Longitudinal studies have also demonstrated a protective effect of physical activity begun early and late in life in with greater protective effects for women and carriers of the APOE e4 allele (Laurin et al., 2001; Rovio et al., 2005). An analysis of longitudinal experiments reported that the risk of dementia and AD in the highest physical activity category was reduced by 28% and 45% respectively (Hamer and Chida, 2009). Randomized control trials that include an aerobic activity group have demonstrated that training can improve cognitive functioning in elderly patients (individuals 65 years or older) with

some form of cognitive impairment *(*Palleschi et al., 1996; Heyn et al., 2004; Scherder et al., 2005).

Exercise Protects Against Brain Insults

While the risk of stroke is reduced by exercise in the clinical population, animal models have demonstrated the physical activity can reduce the damage induced by an experimental stroke model. Middle cerebral artery (MCA) occlusion, either permanent (without reperfusion) or temporary (with reperfusion) is a commonly used model of stroke that consistently induces cell loss in a number of brain regions particularly cortical regions and the hippocampus (Butler et al., 2002). Treadmill training for at least 3 weeks decreased infarct volume induced by MCA occlusion with reperfusion by 79% and decreased deficits in motor control (Wang et al., 2001; Li et al., 2004). Activity wheel running for 3 weeks prior to MCA occlusion with reperfusion; improved the ability of mice to locate the platform in the Morris water maze, enhanced the survival of new cells in the dentate gyrus, reduced infarct size and sensorimotor deficits, upregulated endothelial nitric oxide synthase, enhanced arterial vasodialation and increased cerebral blood flow (Endres et al. 2003; Luo et al., 2007). Endothelial nitric oxide synthase knockout mice do not show any improvements from activity wheel training, demonstrating that it is a crucial factor in exercise induced neuroprotection against ischemic damage (Endres et al., 2003).

Exercise can also increase lifespan and decrease behavioral deficits that accompany the aging process. Mice trained on a treadmill from 28 to 78 weeks extended their lifespan by 9 and 19% for male and females respectively. Mice also showed improved performance on tests of muscular coordination and cognitive ability compared to their sedentary counterparts at 52

weeks. Age associated increases in oxidative stress markers were also significantly decreased (Navarro et al., 2004). Aged rats trained on a running wheel for 5 weeks show faster acquisition and better retention of the platform location in the Morris waster maze compared to age-matched controls (van praag et al., 2005). This demonstrates that even aged animals can benefit from moderate levels of activity.

A number of animal models of AD and aging have demonstrated a protective effect of exercise against cognitive decline and AD like pathology both through behavioral tests and cellular analyses. Mouse models depend on expression of human genes with mutations that cause early onset, inherited forms of AD (for example mutant amyloid precursor protein) and lead to overproduction of amyloid plaques in the mouse brain as well as declines in cognition (Cotman and Berchtold, 2007). In one study animals provided access to an activity wheel for 5 months had significantly decreased Aβ in the frontal cortex and hippocampus and an enhanced rate of learning in the Morris water maze. This experiment also included a group that exercised for 1 month. They had reduced proteolytic cleavage fragments of APP (α-CTFs and β -CTFs) (Adlard et al., 2005).

Similar protective effect of exercise on transgenic mice have been found in training studies of aged animals (Nichol et al., 2007) and studies using an enriched environment which includes as activity wheel (Arendash et al., 2004; Jankowsky et al., 2005; Lazarov et al., 2005; Nichol et al., 2009). Canines have also been used in aging studies and like humans they naturally experience amyloid plaques and tangles as well as cognitive decline. An enriched environment that includes exercise has been shown to attenuate age related declines in cognition and oxidative stress markers in beagles (Siwak-Tapp et al., 2008) but studies in mice indicate

that enrichment that does not include an activity wheel may have independent effect on AD-like pathology (Wolf et al., 2006).

Exercise provides protection in other animals models of disease including Parkinson's disease, amyloid lateral sclerosis and Huntington's disease. Exercise training reduces depletion of dopaminergic neurons induced by 6-hydroxydopamine, an animal model of Parkinson's disease (Tillerson et al., 2003; Mabandla et al., 2004; Howells et al., 2005). Mice with a mutation in the Huntington gene develop abnormal motor behaviors and display deficits in motor coordination and cognitive deficits. Mice allowed access to an activity wheel for 10 weeks developed motor abnormalities significantly later than sedentary mice and had normalized levels of rearing behavior in the open field. Activity wheel running also ameliorated cognitive deficits evident in the T maze and Y maze which assess short term and long term spatial memory (Pang et al., 2006). Exercise also enhanced survival in a transgenic mouse model of familial amyloid lateral sclerosis, which is characterized by degeneration of motor neurons. Activity wheel exposure for at least 6 hours a day increased lifespan by about 25 days and delayed the appearance of motor declines by 21 days. Since IGF-I itself was shown to increase lifespan (Kasper et al., 2003) a parallel experiment was run in which each group received IGF-I injections. Exercise had a synergistic effect when combined with injections of IGF-I. IGF-1 levels in the spinal cord were increased 1.37 fold by either exercise or injection, but when the treatments were combined it was increased 15 fold. Survival for the combined treatment was increased by 83 days and motor declines appeared 80 days later than in controls (Kasper et al., 2005). This experiment is an important example of the enhanced effects that a combination of drug exposure and exercise can have. It also suggests that combining different effects of exercise may be more beneficial than individual effects.

A number of studies have demonstrated that exercise can protect the hippocampus from excitotoxic insult. Treadmill training or swimming reduced seizure development following injection of domoic acid, pilocarpine and in kainic acid-induced models of epilepsy (Arida et al., 1998; Arida et al., 1999; Carro et al., 2000; Setkowicz and Mazur, 2006). Although these experiments demonstrate a protective effect of aerobic training, the treadmill training and swimming employed in these studies stress the animals and may confound results (Dishman 1997a; Timofeeva et al., 2003). Short term activity wheel running improved the performance of kainic acid treated rats on spatial learning tasks (Gobbo and O'Mara, 2005).

Excitotoxicity a Common Mechanism of Cell Death

Excitotoxicity through over-activation of glutamatergic synapses is a major mechanism of cell death underlying stroke, epilepsy, a number of neurodegenerative conditions and age related neurodegeneration (Dawson et al., 1995; Wang et al., 2005; Mattson 2007). Glutamate receptor blockade has been shown to be neuroprotective and is a treatment modality used to reduce damage in stroke, epilepsy, Alzheimer's disease and stress (Sonkusare et al., 2005; Hara and Snyder, 2007; Scholtzova et al., 2008). There is an approximately 10,000 fold difference between the intracellular and the extracellular concentration of glutamate, so any disruption of normal ionic gradients, due to excessive depolarization or energy collapse, results in the massive release of glutamate (Dawson et al., 1995; Meldrum 2000). The key process that triggers the excitotoxic cascade is excessive accumulation of glutamate in the synaptic space. This can be achieved by altering the normal cycling of glutamate? to increase release into the extracellular space, a decrease of glutamate uptake from the synaptic space or spillage of glutamate from injured neurons (Mark et al., 2001; Wang et al., 2005) The fast component of cell death from

excitotoxicity occurs within minutes of glutamate excess and is due to the opening of cation channels and depolarization. The entry of sodium into the neuron is accompanied by the passive influx of chloride and water, resulting in the swelling and possible bursting of the cells (Hynd et al., 2004). Glutamate can induce an increase in cytoplasmic calcium by activating N-methyl-D-aspartic acid (NMDA), voltage dependant calcium channels (VDCC) or metabotropic receptors. If calcium is not removed from cytoplasm by activities of the plasma membrane Sodium/Calcium exchanger, Endoplasmic reticulum calcium ATPases and calcium binding proteins, it activates several different cross amplifying cascades which cause delayed neuronal death (Fujikawa 2005; Hara and Snyder, 2007; Greenwood and Connoly, 2007).

Seizure-induced neuronal death is initiated by excessive glutamate release, which activates postsynaptic NMDA receptors and triggers receptor-mediated calcium influx. Increases in glutamate have been observed in the epileptic hemisphere of seizure patients and experimental models of epilepsy (Carlson et al., 1992; Ueda and Tsuru, 1994; Szyndler et al., 2008). During a stroke, permanent or prolonged occlusion of a cerebral artery interrupts blood flow to the brain. The limited availability of glucose and oxygen directly impairs oxidative metabolism in ischemic regions of the affected tissue, depleting ATP and other energy-related metabolites. Ion gradients quickly run down, and glutamate transporters reverse their direction, releasing glutamate into the extracellular space, leading to large increases in neuronal activity which enhances glutamate release (Take et al., 2004; Hazell 2007) The hippocampus is often the focus of epileptic seizures and is a common area of cell death from stroke (During and Spencer, 1993; Marini et al., 2007). When cellular metabolic activity overcomes antioxidant capacity during a stroke or seizures, cells are damaged by reactive oxygen species such as free radicals

and peroxides. When this type of damage occurs it is referred to as oxidative stress (El Kossi and Zakhary, 2001; Freitas 2009).

Stress also damages hippocampal neurons through over-stimulation of glutamatergic synapses (Miller and O'Callaghan, 2000). Stress activates the sympathetic nervous system, the hypothalamic-pituitary-adrenal axis, and increases release of norepinephrine, dopamine, glutamate, epinephrine, corticotrophin-releasing factor and glucocorticoids (Gilad and Gilad, 1995). Increases in glutamate in the hippocampus and cortex from extreme or repeated stress can damage hippocampal neurons and impair spatial memory (Lowy et al., 1995; Bagley and Moghaddam, 1997; Sousa et al., 2000). Adults followed for 4 yrs with the highest cortisol levels had deficits in hippocampal dependent tasks and decreased hippocampal size as revealed by MRI **(**McEwen 2000). The damaging effects of stress are primarily mediated by the NMDA receptor, since blocking it can block stress induced morphological changes in the hippocampus (Madrigal et al., 2006).

Stress induced glucocorticoid secretion can further increase glutamate release, increase the vulnerability of hippocampal neurons to oxidative stress, decrease repair enzymes and suppress neurogenesis (Miller and O'Callaghan 2003; Kiraly and Kiraly, 2005). Stress also increases nitric oxide which contributes to oxidative stress and elevates the pro-inflammatory cytokines; tumor necrosis factor alpha, interleukin I and interleukin 6. Cytokines elevate cyclooxygenase proteins which can produce prostaglandins and generate arachidonic acid. Excessive generation of nitric oxide and upregulated cyclooxygenase-2 (COX-2) and tumor necrosis factor alpha have been observed following seizures, following stroke, and in neurodegenerative conditions (Madrigal et al., 2006).

Aged subjects hyper-secrete cortisol and are vulnerable to the damaging effects of stress (Lowy et al., 1995; McEwen 2000). The life-span of rodents is inversely related to the intensity of their behavioral and neuroendocrine responses to stressful stimuli (Miller and O'Callaghan, 2000). Cognitive processes that depend on proper hippocampal function appear to be particularly age sensitive (Foster and Kumar, 2002) and removal of the adrenal glands can prevent age-related declines and neuronal loss (Lowy et al, 1995). Not all humans or animal subjects experience cognitive decline or hippocampal shrinkage with aging (Gallagher and Nicolle, 1993). Stress increases glutamate and reactive oxygen species, which, according to the free radical theory of aging plays a major role in age associated changes (Goto et al., 2007). The accumulation of reactive oxygen species in the brain has been linked to age related deficits in cognitive function (McEwen 2000; Radak et al., 2001).

Age related changes are associated with a decrease in antioxidants and growth factors which are important to regulating neural survival and reducing vulnerability to injury. Treatment with growth factors (Nerve growth factor and Insulin-like growth factor I) and antioxidants can provide protection against cell loss and behavioral deficits (Gallagher and Nicolle, 1993; McEwen 2000; Dröge and Schipper, 2007). The extent of neuronal damage following glutamate exposure is highly dependent on age related changes (Kannurpatti et al., 2004). Studies have reported reduced glutamate uptake capacity, increased basal extracellular glutamate and loss of glutamate transporters with aging (Segovia et al., 2001; Riederer and Hoyer, 2006). The problem of increased extracellular glutamate is compounded since the aged brain is significantly less responsive to feedback inhibition of phosphate activated glutaminase which generates glutamate and ammonia and can delay glutamate inactivation (Dawson et al., 1995). Furthermore, L-type calcium channel activity increases with aging and is less sensitive to negative feedback. The

delayed negative feedback leads to an increase in long term depression and a decrease in long term potentiation, both of which are likely involved in age related leaning deficits. Glucocorticoids can induce a similar decreased sensitivity to feedback inhibition (McEwen 2000; Foster and Kumar, 2002) Age associated decreases in glucose utilization, energy metabolism, and vascular integrity all contribute to further neurotoxicity (Dröge and Schipper, 2007; Mattson 2007; Dawson et al., 1995). Age related increase in inflammatory proteins combined with decreased repair mechanisms may contribute to the formation of advanced glycation end products (Dröge and Schipper, 2007; Dawson et al, 1995). Advanced glycation end products are formed when glucose and other sugars react with long lived proteins and may result in the buildup of plaques and tangles. Crosslinked proteins can accumulate over time especially in the hippocampus (Riederer and Hoyer, 2006).

Age is the greatest risk factor for AD (Crawford 1996) and crosslinked proteins that accumulate over time combined with impaired glucose utilization are likely important factors in the increased risk with age (Riederer and Hoyer, 2006). Mutations in amyloid precursor protein are associated with early onset, familial AD, while no genetic factor is involved in sporadic, late onset AD (Rego and Oliveira, 2003). Studies show that secreted amyloid precursor protein fulfills synaptotrophic and neuroprotective functions in response to excitotoxicity. Abnormal functioning of secreted amyloid precursor protein may be involved in mechanisms of synaptic damage by failing to maintain normal functioning after an excitotoxic challenge (Maslia et al., 1996). Glutamate dysregulation and excessive activation particularly via the NMDA receptor are proposed to be key players in excitotoxicity, contributing to degeneration of cholinergic neurons in sporadic AD (Riederer and Hoyer, 2006). Neuronal loss is generally restricted to glutamatergic neurons and glutamatergic innervated neurons of the cortex and hippocampus

(Hynd et al., 2004). Glutamate (/aspartate) uptake is 40-50% decreased in the neocortex in Alzheimer's patients (Maslia et al., 1996). Oxidative stress precedes amyloid deposition in the hippocampus of patients with AD and amyloid deposits and neurofibrillary tangles compound oxidative stress. Inflammation promotes neuronal excitability by catalyzing the formation of prostaglandins and by contributing to peroxides activity (Sue and Griffin, 2006; Rego and Oliveira, 2003).

Cognitive and motor deficits positively correlate with oxidative stress levels (Foster et al., 1996) and can be reversed in aged mice by improving antioxidant defenses (Liu et al., 2003). The hippocampus is particularly vulnerable to oxidative stress and, compared to other regions of the brain which are less metabolically active, has lower concentrations of antioxidants (Kiraly and Kiraly, 2005). Kiraly and Kiraly (2005) propose that the process of Aβ deposition in AD can be thought of as a slow ongoing state of injury afflicting patients with AD. Understanding how exercise may ameliorate the effects of excitotoxicity in the hippocampus is extremely important since pathology in this region is common to so many insults and diseases.

Pathogenesis of the majority of neurodegenerative disorders involves a genetic predisposition together with activity of environmental toxins (Rego and Oliviera, 2003; Dawson et al., 1995). Data from studies of patients, animals and cell culture models have established major roles of oxidative stress, impaired energy metabolism and a decrease in calcium homeostasis in the pathogenesis of AD, Parkinson's disease and Huntington's disease (Dröge and Schipper, 2007; Mattson 2007). Specific molecular alterations that result in oxidative stress differ among disorders; amyloid beta in Alzheimer's, mitochondrial alterations in Parkinson's, mutant Huntington in Huntington's disease, ischemia in stroke. However reactive oxygen species and nitrogen species produced, and their consequences for neuronal function, are similar among

disorders. Superoxide, hydrogen peroxide, hydroxyl radical, nitric oxide and peroxynitrite have been implicated in all neurodegenerative diseases (Dawson et al., 1995; Doble 1999; Meldrum 2000; Hynd 2004; Wang 2005; Waxman and Lynch, 2005; Mattson 2007). Understanding how exercise may ameliorate the effects of excitotoxicity in the hippocampus is extremely important since pathology in this region is common to so many insults and diseases.

Exercise Effects That Afford Neuroprotection

A number of effect of exercise are likely involved in neuroprotection, maintaining cognitive functioning during aging and delaying the onset of dementia and AD. Researchers have reported improved cerebral blood flow and maintenance with aging (Swain et al., 2003; Pereira et al., 2007; Ainslie et al., 2008) increased neuronal plasticity (van Praag et al., 1999; Gómez-Pinilla et al., 2002; van Praag et al., 2002; Redila and Christie, 2005; Stranahan et al., 2007) increased synthesis and metabolism of neurotransmitters (Kramer et al., 2006) and increased antioxidant enzymes (Ji 2002; Gomez-Cabrera et al., 2008).

Many researchers attribute improvements to an increase in growth factors. Experiments have consistently demonstrated that exercise increases levels of brain derived neurotrophic factor (BDN F) and insulin like growth factor I (IGF-I) (Neeper et al., 1996; Carro et al., 2000) which reduce excitotoxicity when applied to neurons *in vivo* and *in vitro* (Mattson et al., 1995; Smith 1996; Carro et al., 2001; Escartin et al., 2004). When BDNF is chronically upregulated, as it is with long term activity wheel training, it enhances excitatory transmission in the hippocampus and is considered pro-epileptogenic, (Croll et al., 1999; Binder et al., 2001; Koyama and Ikegaya, 2005) possibly as a result of its enhancement of synaptogenesis and neurogenesis (van Praag et al., 1999; Gómez-Pinilla et al., 2002; van Praag et al., 2002; Redila and Christie, 2005;

Stranahan et al., 2007). BDNF and its receptor Neurotrophic tyrosine kinase, receptor type 2 (TrkB) promote long term potentiation (Kiraly and Kiraly, 2005).

IGF-I stimulates neurogenesis and proliferation in the hippocampus by increasing BDNF. These increases in cell growth do not occur when IGF-I is blocked (Carro et al., 2001; Trejo et al., 2001). IGF-1 can also increase blood vessel density in the dentate gyrus of the hippocampus and throughout the brain (Lopez-Lopez et al., 2004; Aberg et al., 2006). IGF-I acts on vascular endothelium to activate nitric oxide synthase, thereby promoting vascular health. Nitric oxide produced by the endothelium of cerebral arterioles is an important mediator of endothelium-dependent vasodilation and helps to prevent hypoxic conditions (McCarty 2000). **S**tudies have implicated excessive levels of nitric oxide in excitotoxic cell death following activation of NMDA receptors and have demonstrated a protective effect of inhibiting its formation during an excitotoxic challenge (Hara and Snyder, 2007). Nitric oxide is an important molecule in the induction of long term potentiation and facilitates excitation (Taqatqeh et al., 2009).

Physical activity may indirectly provide protection from degeneration by modulating the stress response. Studies have demonstrated that physical activity training can attenuate behavioral deficits induced by stress, possibly by moderating monoaminergic responses (Dishman et al., 1996; Dishman et al., 1997; Greenwood et al, 2003; Van Hoomissen et al., 2004; Greenwood et al., 2007). Studies have also shown that exercise training reduced stress induced increases in glucocorticoids (Dishman et al., 1998; Zheng et al., 2006; Sasse et al., 2008). Exercise may reduce excitotoxic cell death by modulating stress induced increases in glucocorticoids (Miller and O'Callaghan, 2003; Kiraly and Kiraly, 2005).

Aside from modulating glucocorticoids, the effects of exercise promote plasticity, neurogenesis and excitability. Activity wheel running rats were reported to have increased

AMPA receptors and phosphorylated subunits of the NMDA receptor (Dietrich et al., 2005). These findings indicate that exercise increases the sensitivity of the hippocampus to glutamatergic input. Upregulation of a factor that modulates the excitability of hippocampal neurons under excitotoxic challenges would be necessary to modulate the increased sensitivity of the hippocampus.

Physical Activity Up-regulates Galanin

Previous research in our laboratory has revealed that physical activity up-regulates prepro-galanin messenger RNA levels in a dose-dependent manner in the locus coeruleus after several weeks of activity wheel exposure or treadmill training (O'Neal et al., 2001; van Hoomisen et al., 2004; Eisenstein and Holmes, 2007; Reiss et al., 2009). Galanin coexists with norepinephrine in at least 80% of locus coeruleus neurons (Holmes and Crawley. 1995) and retrograde tracing/double labeling experiments reveal that the hippocampus receives extensive galaninergic innervation via projections from the locus coeruleus (Melander et al., 1986a; Kask et al., 1995). Galanin is primarily an inhibitory neurotransmitter, and previous studies indicate that galanin functions within the hippocampus to regulate neuronal excitability. Both in vivo and in vitro studies have demonstrated anti-seizure effects of galanin to seizures induced by both kindling and excitotoxins (Mazarati et al., 1998; Mazarati et al., 2000; Elliot-Hunt et al., 2004).

Galanin has been found in a number of studies to act as an anticonvulsant. Galanin administration blocks the release of excitatory amino acids and protects against cell death in hippocampal cell cultures. Conversely blocking galanin transmission causes increased excitation and cell death (Zini et al., 1993; Elliot-Hunt et al., 2004). Transgenic mice with a disruption in the galanin gene show increased susceptibility to status epilepticus and increased cell death in

response to seizure-inducing agents, while mice that overexpress the galanin gene have decreased seizure susceptibility and cell death (Mazarati et al., 2000; Mazarati et al., 2001; Elliot-Hunt et al., 2004). Galanin or galanin receptor agonists have been demonstrated to decrease excitotoxic effects of models of kindling and status epilepticus in wild type animals and this effect is depleted when followed by galanin receptor antagonists such as M40, M35 or M15 (Mazarati et al., 1992; Mazarati et al., 2001; Saar et al., 2002).

While galanin influences seizure activity, seizure activity also influences galanin in a time dependent manner. Status epilepticus is accompanied by a rapid depletion of galanin from hilar and CA3 fibers of the hippocampus which persists for at least one week. Status epilepticus also induces the expression of galanin-immunoreactivity by neurons in the hilar region of the dentate gyrus, which is absent in control animals (Mazarati et al., 1998). Research suggests that galanin acts to suppress glutamate release by hyperpolarizing membranes through the opening of ATP-dependent potassium channels as well as closing voltage-gated calcium channels through g-protein coupled receptors in response to glutamate. Galanin may also inhibit excitatory input postsynaptically by inhibiting long term potentiation, which is mechanistically similar to seizures (Mazarati et al., 2001).

The hippocampus receives extensive connections from the locus coeruleus and septum which are sites of high galanin-immunoreactivity (Mazarati et al., 1998). Dentate gyrus cell layers contain the highest density of galanin-immunoreactive fibers and also receive excitatory glutamatergic input from the entorhinal cortex. Stimulation of the perforant path of the entorhinal–dentate gyrus projection by brief electrical impulses will reliably produce seizures. Mazarati (2004) has proposed that seizures result when glutamatergic excitation exceeds galanin inhibition of the hippocampus.

Both GALR1 and GALR2 receptors are involved in the anti-seizure effects of galanin (Lu et al., 2005; Kanter-Schlifke et al., 2007). GALR1 appears to function presynaptically to inhibit the release of glutamate by opening ATP dependent potassium channels or closing voltage gated calcium channels (Mazarati 2004). GALR2 is most commonly reported to inhibit excitation postsynaptically by mediating the release of calcium from intracellular stores and opening calcium dependent chloride channels (Mazarati et al., 2001; Lang et al., 2007). In addition GALR2 phosphorylates the serine/theonin kinase Akt and extracellular signal-related kinase (ERK) which reduces caspase 3 and caspase 9 activity, therefore promoting neuronal survival (Elliot-Hunt et al., 2007; Lang et al., 2007). Previous research thus clearly demonstrates that galanin functions as an endogenous neuroprotective factor for the hippocampus. If galanin up-regulation through exercise is capable of exerting this same type of response to excitotoxic insult, it would indicate an additional mechanism through which exercise is neuroprotective.

The studies performed assess the effects that physical activity training has on kainic acid induced seizures. The first two studies were performed to determine whether 3 weeks of activity wheel running reduces behavioral and cellular responses to an excitotoxic insult and whether a galaninergic mechanism modulates exercise induced changes. The third study determines whether physical training alters extracellular glutamate levels in the hippocampus following kainic acid injection. Physical activity reduced seizure ratings induced by intraperitoneal and intracerebroventricular kainic acid injection. Training also reduced c-fos mRNA levels in the hippocampus, which is an indirect measure of excitability. When galanin preceded intracerebroventricular kainic acid injection seizure ratings did not differ between active and sedentary rats indicating that galanin is a necessary factor in the effects of exercise on seizures. Training also modulated kainic acid induced extracellular glutamate during the first 30 minutes

following injection. Physical activity may also be neuroprotective by modulating the effects of glutamate release induced by an excitotoxic challenge.

CHAPTER THREE

CHRONIC ACTIVITY WHEEL RUNNING REDUCES KAINIC ACID-INDUCED SEIZURES IN THE RAT: POSSIBLE ROLE OF GALANIN

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Abstract

Studies in both humans and rodents suggest that exercise can be neuroprotective, but the mechanisms by which this occurs are still poorly understood. Three weeks of voluntary, physical activity in rats upregulates prepro-galanin messenger RNA levels in the locus coeruleus. Galanin is a neuropeptide extensively coexisting with norepinephrine that decreases neuronal hyperexcitability both in vivo and in vitro. Thus, exercise may diminish neural hyperexcitability through a galaninergic mechanism. The current experiments tested whether voluntary activity wheel running would protect against kainic acid-evoked seizures and whether galaninergic signaling is a necessary factor in this protection. In experiment 1, rats were given access to running wheels or remained sedentary for three weeks. After this period, rats received an intraperitoneal (IP) injection of 0, 7, 10 or 14 mg/kg kainic acid. Exercise decreased the severity of or eliminated seizure behaviors and hippocampal c-fos expression induced by kainic acid. In experiment 2, exercising or sedentary rats were injected intracerebroventricularly (ICV) with 0.2 or 0.4 μg of kainic acid following either an injection of M-40 (a galanin receptor antagonist) or saline. Exercise decreased kainic acid-induced seizures at the 0.2 μg dose, and M-40 (6 nmol) decreased this effect. In contrast, there were no detectable differences between exercising and sedentary rats in behavior at the 0.4 μg dose. The results suggest that the protective effects of exercise against seizures are at least partially mediated by regulation of neural excitability through a process involving galanin.

Introduction

Several lines of evidence suggest that exercise protects against a variety of psychological and neurological disorders. Clinical trials and population-based observational studies have

indicated that exercise can ameliorate symptoms of stress and depression, (Brosse et al., 2002; Callaghan, 2004; Salmon, 2001) decrease age-related cognitive decline (McAuley et al., 2004; Colcombe et al., 2004; Laurin et al., 2001), and reduce the risk of dementia (Fratiglioni et al., 2004; Larson et al., 2006). Animal studies have also shown that exercise can modulate stress responses (Dishman et al., 1997a, 1998; Soares et al., 1999; Greenwood et al., 2007) and reduce brain cell loss in aging animals (Larsen et al., 2000). Exercising rats exhibit less neuronal damage when compared to sedentary controls in several animal models of brain insults. For example, decreases in measures of hippocampal cell death were evident in physically active animals following treatments that model stroke and epilepsy (Ang et al., 2003; Arida et al., 1999, 2004; Wang et al., 2001). In another study, rats allowed access to an activity wheel and then injected with 6-hydroxydopamine displayed behavior consistent with sparing of dopaminergic neurons (Mabandla et al., 2004). In addition, voluntary exercise decreases amyloid-beta plaques in a transgenic model of Alzheimer's disease in both cortical and hippocampal regions (Adlard et al., 2005) and improved cognitive abilities and motor competence in transgenic mouse models of Huntington's disease (Pang et al., 2006). However, the mechanisms underlying these apparent protective effects are poorly understood.

Previous research in our laboratory has revealed that physical activity upregulates prepro-galanin messenger RNA levels in a dose-dependent manner in the locus coeuruleus after several weeks of activity wheel exposure or treadmill training (Eisenstein and Holmes, 2007; O'Neal et al., 2001; Van Hoomisen et al., 2004). Galanin is a 29 amino acid neurotransmitter and trophic factor that regulates neural activity in several brain structures, including the hippocampus. Galanin coexists with norepinephrine in at least 80% of locus coeruleus neurons (Holmes and Crawley 1995; Melander et al., 1986a) and retrograde tracing/double labeling experiments reveal

that the hippocampus receives extensive galaninergic innervation via projections from the locus coeruleus (Kask et al., 1995; Melander et al., 1986a).

Galanin is primarily an inhibitory neurotransmitter, and previous studies indicate that galanin functions within the hippocampus to regulate neuronal excitability. Infusion of galanin into the hippocampus inhibits seizures, and the galanin receptor antagonists M-35 and M-40 block the antiseizure effects of galanin (Mazarati et al., 1998). Transgenic galanin "knock-out" mice show increased susceptibility to kainic acid induced seizures (Mazarati et al., 2000). In corroboration of these results, in vitro studies in hippocampal cultures from transgenic mice have confirmed that endogenous galanin reduces excitotoxicity and apoptosis (Elliot-Hunt et al., 2004). The current experiments were designed to test whether voluntary activity wheel running can protect against kainic acid-evoked seizures. In the first experiment, male Sprague-Dawley rats had access to a running wheel for three weeks while another group of rats had no running wheels. At the end of the three week period rats were injected with various doses of kainic acid and behavioral reactions were monitored and scored for 2 h. After the observation period, the animals were killed and seizure-induced c-fos activation was quantified in several hippocampal regions. It was hypothesized that exercised rats would show diminished seizure activity and less c-fos activation. The second experiment examined the role of galanin in modulating this response by testing whether the galanin receptor antagonist M-40 would decrease the protective effect of exercise on seizure severity.

Methods

Subjects

Adult male Sprague-Dawley rats (n=42; 4–6 per cell for experiment 1 and n=80; 5–9 per cell for experiment 2) weighing 150 g–200 g were purchased from Harlan Inc. (Indianapolis, IN) and allowed to adapt to the animal facility for 1 week before behavioral manipulations began. Rats were housed in a humidity and temperature controlled vivarium with lighting maintained on a reverse 12-hour light/dark schedule (lights on 1900–0700). Food and water were available ad libitum and animals were weighed weekly throughout the duration of the study. All procedures were conducted in accordance with NIH Guide for the Care and Use of Laboratory Animals. All animals were randomly assigned to exercise vs. running conditions and drug vs. control groups.

Exercise conditions and drug administration

Rats were randomly assigned to either the activity wheel (AW) or sedentary (SED) condition and all animals were singly housed. Activity wheels (MiniMitter) with a circumference of 105 cm were placed in 30×30×30 cm polycarbonate cages and attached to a magnetic revolution counter. Sedentary rats remained in home cages without running wheels throughout the study. In experiment 1, after 21 days, all rats received an intraperitoneal (IP) injection of physiological saline or 7, 10 or 14 mg/kg kainic acid (Sigma-Aldrich). These doses of kainic acid have been shown to produce mild, moderate and severe seizures in rats respectively. In experiment 2 all rats had cannulae implanted 18 days into the exercise/sedentary condition and were tested on day 21 as in experiment 1. Injections for experiments 1 and 2 were done between 0700 and 1900 h (dark phase).

Cannulae Implantation and Administration of M-40 and Kainic Acid

Since M40 is a peptide that does not cross the blood-brain barrier, experiment 2 employed an ICV route of administration for all compounds rather than the more standard i.p. route. Rats were between 270 and 380 g at the time of cannula implantation and all procedures were performed under aseptic conditions. Rats were anesthetized with a halothane/ oxygen mixture delivered through a vaporizer and nose cone and mounted in the stereotax. The head was shaved and scrubbed with Betadine solution and a longitudinal incision was made along the scalp. Overlying connective tissue and periosteum was scraped away from the scalp. Cannulae (1 cm) were implanted into the following coordinates (measured stereotactically from bregma): posterior: 1.0 mm, lateral: 1.5 mm, ventral: 3 mm according to the rat brain atlas of Paxinos and Watson (1986). They were attached to the skull using 3 stainless steel screws and dental acrylic and protected by small plastic tubing. Rats then received 2 mg/kg banamine subcutaneously and were allowed to recover under a heat lamp before being returned to their cages. Cannula placement was verified at the end of the study by injecting 10 µl fastgreen dye ICV in a concentration of 2 mg/ml following decapitation. The brain was then removed and sectioned coronally through the lateral ventricles (approximately .5 mm anterior to bregma) and ventricle IV (approximately 9 mm posterior to bregma). The presence of dye in the ventricles was then confirmed by visual inspection. Evidence of dye in both ventricles was required for inclusion in the data analysis. Four days after cannula implantation rats received either an injection of kainic acid (.2 µg or .4 µg) followed by an injection of physiological saline (KA+SAL) or an injection of M-40 (6 nmol dissolved in deionized water; Bachem) followed by an injection of kainic acid (KA+M-40). ICV injection volumes for all drugs were 10 µl.

Behavioral Measures: Seizure Rating Scale

Immediately following injections of kainic acid, M-40 or saline (the appropriate combination) rats were placed in a Med Associates automated open field activity monitor, which consists of a 43.3 cm long×43.3 cm wide×30.5 cm high clear plastic observation chamber with infra-red photobeams. Locomotor behavior was measured for 2 h. An investigator blind to treatment conditions conducted the behavioral observations. Observations began immediately following injections and continued for 2 h. Behavior was also recorded with a video camera. Two rats, randomly paired, were observed simultaneously and measures taken included latency and incidences of forelimb clonus, wet dog shakes, as well as tonic clonic seizures. Each rat was also rated on a scale of 0–5 on seizure severity. The rating scale was based on the occurrence of seizure-typical behaviors (Racine, 1972, Hoffman et al., 2003). For ICV kainic acid administration, the scale was modified such that wet dog shakes were scored as a "0" (i.e. were not included in the scoring of seizure behaviors) since the injection procedure itself produced occasional, head shaking that could be confused with seizure activity. Wet dog shakes alone were therefore only scored as a "1" in the IP experiments.

0 = no seizure behaviors (or wet dog shakes only after ICV administration)

1 = minor behaviors including catatonia, wet dog shakes, scratching, sniffing and head bobbing

2 = salivation and rearing in combination with seizure behaviors without loss of balance/ control

3 = minor behaviors, chewing salivation and rearing with loss of balance/ control

4 = tonic/clonic seizures

5 = death

In Situ Hybridization Histochemistry

Rats were killed by rapid decapitation 3 h following kainic acid administration in experiment 1, and brains were removed, frozen, and stored at −80 °C. Brains were cut in 12 μm sections at the level of the dorsal hippocampus and locus coeuruleus using a Microm cryostat. A detailed procedure of hybridization methods used is reported elsewhere (Kaplan et al., 1998; Van Hoomisen et al., 2004). In brief, sections were thaw-mounted onto microscope slides, fixed in formaldehyde, rinsed in PBS, and placed in 0.25% acetic anhydride. Sections were dehydrated with a series of ethanol washes, delipidated in chloroform, rinsed in ethanol, and allowed to dry. Oligonucleotide probes were purchased from Oligos Etc. (Wilsonville, OR). The c-fos probe sequence was complementary to bases 270–319 of rat c-fos mRNA. Galanin oligonucleotide probe sequences were complementary to rat galanin cDNA bases 228–271. Probes were labeled at the 3′ end with [35S]-dATP, terminal deoxynucleotidyl transferase, and tailing buffer. Column separation was used to separate unincorporated nucleotides from the probes. Sections were hybridized with radiolabeled probes in solution containing formamide, NaCl, Tris–HCl, EDTA, sodium pyrophosphate, sodium dodecyl sulfate, heparin sulfate, and dextran sulfate. Brain sections were incubated with the hybridization solution and then subjected to a series of washes to reduce nonspecific binding. Slides were rinsed in deionized water and ethanol and allowed to dry. Sections were exposed to autoradiographic film and then developed. Autoradiographic films were analyzed with a computerized image analysis system to determine optical density (OD) within the HF. The HF was analyzed by taking the average OD of ten 8×8-pixel circles placed randomly throughout the brain region of interest (dentate gyrus [DG], Ammon's horn area 1 [CA1], Ammon's horn area 2 [CA2], Ammon's horn area 3 [CA3]), in each section of each rat.

Four different adjacent sections were analyzed per rat and OD values for all sections were averaged. The investigator was blind to subject condition at the time of quantification.

Data Analysis

Experiment 1: A 2×4 factorial design was used where there were two exercise (activity wheel running vs. sedentary) by four drug (saline vs. 7, 10 or 14 mg/kg kainic acid) conditions. The dependent measures were seizure observations (described above) and cfos expression in the dentate gyrus, CA1, CA2 and CA3 regions of the hippocampus. A factorial ANOVA followed by Bonferroni post hoc contrasts was used to compare each of the groups using a p-value of 0.05. Effect sizes for simple effects are reported as Cohen's d and computed using G POWER. Statistical power for expected large effects (d>1.5) of drug and exercise exceeded .80.

Experiment 2: A 2×3×2 factorial design was used in which there were two exercise conditions (activity wheel running vs. sedentary) by three drug (saline, SAL, vs. .2 μg vs. .4 μg kainic acid, KA) by two drug (M-40 vs. saline) conditions. Based on results of experiment 1, planned contrasts (one-tailed independent samples) on seizure observations were made. Comparisons tested for seizure observations were EX.2KASAL vs. SED.2KASAL; EX.2KAM40 vs. SED.2KAM40; EX.4KASAL vs. SED.4KASAL; EX.4KAM40 vs. SED.4KAM40. Because of the lack of variance and the number of ties in some groups, seizure ratings were analyzed using Mann Whitney U tests corrected for ties. Galanin expression in the locus coeruleus (for control animals in both experiments 1 and 2) was analyzed using a one-tailed independent samples t-test. Animals from both experiments were combined, as 10 subjects per group provided adequate power in previous experiments for detecting a large effect (d>.80) of exercise on gene expression (Van Hoomisen et al., 2004). Statistical power of the exercise effect was .95. Galanin expression for experimental groups receiving kainic acid was not

included in the analysis. The effect of seizures on galanin expression greatly exceeds an exercise effect, and it is thus not possible to separate the effects of exercise from the effects of seizures.

Results

Experiment 1: Exercise reduces seizures and c-fos mRNA induced by IP kainic acid

Voluntary running distance progressively increased during the 21 days of exposure to activity wheels. Mean running distances were 2467 m, 4242 m, and 5621 m per day for week 1, week 2 and week 3 respectively (Fig. 1). Kainic acid administration caused dose-dependent increases in seizure behavior scores in sedentary rats, with maximal seizure activity observed at the 10 and 14 mg/kg doses (Fig. 2). Voluntary wheel running significantly reduced the behavioral manifestations of seizures induced by kainic acid. ANOVA of seizure ratings for (exercising) AW and SED (sedentary) animals at 0, 7, 10 and 14 mg/kg doses of kainic acid revealed a significant interaction between condition (AW vs. SED) and drug (0, 7, 10 and 14 mg/kg) $F(3,36)=8.52$, $p<.01$. Post hoc t-tests revealed significant differences for AW10 vs. SED10 $t(10)=4.72$, $p<.01$, $d=2.7$ and AW14 vs. SED14 $t(8)=3.89$, $p=.01$, $d=2.4$. Consistent with the behavioral data, dose-dependent increases in hippocampal c-fos mRNA levels were observed in sedentary rats treated with kainic acid. No significant increases were observed in exercising rats (Fig. 3). ANOVA of c-fos autoradiographic optical density values for AW and SED animals at 0, 7, 10 and 14 mg/kg doses of kainic acid revealed a significant interaction between exercise condition and drug for CA1 $F(3,36)=5.51$, $p=.001$; CA2 $F(3,36)=4.59$, $p=.01$; CA3 $F(3,36)=4.63$, $p=.01$ and dentate gyrus (DG) $F(3,36)=5.12$, $p=.01$ regions of the hippocampus (Figs. 3 and 4). Post hoc t-tests revealed a significant difference between AW and SED at the 10 mg/kg dose in CA1 $t(10)=3.25$, $p=.02$; CA2 $t(10)=3.29$, $p=.02$; CA3 $t(10)=3.34$, $p=.02$ and DG

t(10)=3.13, p=.03 and 14 mg/kg dose in CA1 t(8)=3.22, p=.015; CA3 t(8)=2.87, p=.045 and DG t(8)=3.26, p=.03. Effect size (d) ranged from 1.8 to 1.9. Inspection of the representative autoradiographs (Fig. 4) reveals that kainic acid-induced c-fos gene expression was not restricted to the hippocampal formation. Though not quantified in the present experiment, increases in c-fos mRNA are evident in cortical areas as well.

Experiment 2: Exercise reduces seizures induced by ICV kainic acid, reversal by galanin antagonist. Voluntary running distance progressively increased over the first through 18th day (when surgery took place) of the experiment (Fig. 5). AW animals exhibited significantly lower behavioral responses to kainic acid than did SED animals at the 0.2 μg dose (Mann–Whitney U=16.0, p=.013), but that difference between AW and SED animals was not observed when M-40 preceded the kainic acid injection (Mann–Whitney U=32.5, p=.220). AW and SED animals did not differ at the .4 μg dose (Mann–Whitney U=32, p=.332) or when M- 40 preceded the kainic acid injection at that dose (Mann–Whitney U=37.5, p=.376) (see Fig. 6). Levels of galanin mRNA in the locus coeruleus were significantly elevated in AW control animals (SAL in experiment 1 and SAL+SAL in experiment 2) compared to SED control animals t(17)=2.33, p=.016, d=1.07 (Fig. 7).

Discussion

Both studies presented herein provide evidence that activity wheel running protected against the development and progression of kainic acid-evoked seizures. In experiment 1, this protection was evident at the 10 and 14 mg/kg doses for both seizure-related behaviors and c-fos gene expression. There were no detectable differences between the behavioral reactions of the sedentary and activity wheel conditions at the 7 mg/kg dose. This low dose of kainic acid

induced primarily sub-convulsive seizure-related behaviors that are difficult to detect using the Racine seizure rating scale (Mikulecká et al., 1999).

The results of experiment 1 support the theory that exercise decreases kainic acid-induced seizure behavior and neural activation in hippocampal regions. However, the effect of chronic exercise on the resting pharmacokinetics of kainic acid is not known. Changes in fitness may alter drug absorption, distribution or elimination through a number of mechanisms such as increased collateral blood flow, increased plasma volume or altered hepatic enzymes (Persky et al., 2003). Eight and sixteen weeks of activity wheel running has been shown to influence measures of fitness such as skeletal muscle mitochondrial enzymes and maximal oxygen uptake (Davidson et al., 2006; Yano et al., 1997) but three to six weeks of activity running has not (Yiamouyiannis et al., 1992; Yamouyiannis et al., 1993; Dishman et al., 1998, 1996, 1997a, 1995; Dunn et al., 1996), suggesting that such factors were unlikely to be involved in the present experiments.

The ICV route of administration employed in experiment 2 rules out possible differences in systemic pharmacokinetics or body composition. The results of experiment 2 demonstrated a decreased behavioral response to ICV kainic acid at 0.2 μg in exercising animals compared to sedentary animals, and this protective effect of exercise was significantly attenuated when the galanin antagonist M-40 was injected prior to kainic acid. The increased seizure severity in exercising animals injected with M-40 prior to kainic acid supports the hypothesis that enhanced galaninergic transmission dampens kainic acid-induced excitability in exercising animals. Consistent with previous studies showing the pro-convulsant effects of manipulations that interfere with galanin transmission, sedentary rats treated with M-40 and 0.2 μg kainic acid trended toward higher seizure ratings than sedentary rats treated with saline. The absence of any

such differences at the 0.4 μg dose may have been due to a ceiling effect. Though it should be noted that M-40 may exert partial agonist activity at doses exceeding 10 nmol (Lu et al., 2005), the dose of 6 nmol used in the present experiments should not have yielded such effects.

Galanin is primarily an inhibitory neurotransmitter, and both GALR1 and GALR2 receptors mediate the antiseizure effects of galanin (Lu et al., 2005; Kanter-Schlifke et al., 2007). GALR1 appears to function presynaptically to inhibit the release of glutamate by opening ATP dependent potassium channels or closing voltage gated calcium channels (Mazarati, 2004). GALR2 is most commonly reported to inhibit excitation postsynaptically by mediating the release of calcium from intracellular stores and opening calcium dependent chloride channels (Mazarati et al., 2001; Lang et al., 2007). In addition GALR2 phosphorylates the serine/theonin kinase Akt and extracellular signal-related kinase (ERK), which reduces caspase 3 and caspase 9 activity, therefore promoting neuronal survival (Elliot-Hunt et al., 2007; Lang et al., 2007).

Previous research thus clearly demonstrates that galanin functions as an endogenous neuroprotective factor for the hippocampus, which supports the hypothesis that exercise-induced neuroprotection may involve a galaninergic mechanism. Results of in situ hybridization analyses revealed that exercise increased galanin mRNA levels in the locus coeruleus, as previously reported by this laboratory (Eisenstein and Holmes, 2007; O'Neal et al., 2001; Van Hoomisen et al., 2004). This finding reveals a potential circuit through which exercise may prevent hyperexcitability. The anatomy of the locus coeruleus system supports the hypothesis that galanin exerts its inhibitory control over hippocampal circuits via projections from the locus coeruleus.

Nearly all locus coeruleus neurons projecting to the hippocampus contain galanin (Melander et al., 1986b). Both galanin receptors GALR1 and GALR2 have been identified in the

hippocampus, with relatively high densities observed in dentate gyrus and CA3 (Lu et al., 2005; Mazarati, 2004; Kanter-Schlifke et al., 2007). Anatomical tracing studies have revealed the locus coeruleus projections to CA3 are particularly dense (Melander et al., 1986b; Merchenthaler et al., 1993; Xu et al., 1998). This anatomy suggests the locus coeruleus neurons may modulate hippocampal activity via this innervation. Present and previous results reveal that CA3 pyramidal cells are most vulnerable to kainic acid treatment (Ben-Ari and Cossart, 2000; Sperk, 1994). Exercise-induced protection from kainic acid seizures may therefore occur primarily at the level of CA3. The kainic acid model thus provides the opportunity to selectively test the neural circuits that protect CA3 neurons from hyperexcitability. Since the exercise-induced protection against seizures was only observed at low to moderate doses, the hyperexcitability induced by the high dose of kainic acid may overwhelm any protection afforded by an upregulation in endogenous galanin.

In addition to upregulating endogenous neuromodulators such as galanin that may protect against hyperexcitability, exercise also increases the expression of factors that render the hippocampus more vulnerable to excitatory insults. Various modes of exercise cause increases in levels of the neurotrophin brain-derived neurotrophic factor (BDNF) in the dentate gyrus and CA 1–3 cell layers (Berchtold et al., 2005; Neeper et al., 1995; Neeper et al., 1996; Oliff et al., 1998). BDNF enhances excitatory transmission in the hippocampus and is considered pro-epileptogenic (Binder et al., 2001; Croll et al., 1999; Koyama and Ikegaya 2005), possibly as a result of its enhancement of synaptogenesis and neurogenesis (Gómez-Pinilla et al., 2002; Redila and Christie, 2005; Stranahan et al., 2007; van Praag et al., 1999, 2002). A previous experiment has demonstrated that intra-hippocampal injections of kainic acid after long term activity wheel running increased cell death in anesthetized female rats (Ramsden et al., 2003).

The increased cell death reported in this previous experiment may have been mediated by the hippocampal hypertrophy caused by exercise- induced increases in BDNF. It is also important to recognize potential differences in vulnerability to excitotoxicity between awake and anesthetized rats when comparing these previous results to the present experiments. Presumably, anesthesia would eliminate endogenous mechanisms activated by hyperexcitability that exert inhibitory control over the hippocampus, particularly if those mechanisms involve the locus coeruleus system. The difference in responses to the low and high doses of kainic acid in the current experiment implies that exercise-induced neuroprotection is limited to low to moderate levels of excitability.

One potential confound in experiment 2 is a significant decrease in activity wheel running following implantation of cannula, which can be seen in Fig. 5. Previous experiments conducted in this laboratory have shown that rats that undergo surgical manipulations tend to exhibit decreased running. Implantation of cannulae three days before injection allows for a high level of running for most of the three week experimental running period, and it allows for any effect of anesthesia to dissipate before injection on day 21. It is not known whether galanin upregulation would be affected by this decrease in running. It is possible that exercise effects on galanin are graded, in which case a decline in galanin expression would be expected. Although the drop in running was significant, averages for all three of these days were above 1500 m and the time period was short, making it unlikely that significant changes in a chronically upregulated gene occurred. The results of the experiment suggest that galanin levels were high enough to decrease seizure responses, as this effect was attenuated by the antagonist M-40, although the possibility that this effect may have been more substantial remains. A replication of

experiment 2 with cannulae implantation occurring prior to the start of activity wheel running would help resolve this issue.

The reduction in severity of kainic acid-induced seizures has been detected in paradigms similar to those currently employed in which exercise training precedes seizure induction. Exercise attenuates kindling and CA1 hyper responsiveness in kainic acid-induced models of epilepsy (Arida et al., 1998, 1999). Two weeks of treadmill running attenuated seizure development after an injection of domoic acid (Carro et al., 2001) and 45 days of swimming and treadmill training reduced pilocarpine-induced seizure susceptibility (Setkowitz and Mazur, 2006). The treadmill training employed in these studies should be interpreted with caution since treadmill exercise is forced and therefore a significant stressor (Dishman, 1997b; Timofeeva et al., 2003). A study by Gobbo and O'Mara (2005) demonstrated that 5 days of exercise improves spatial learning in kainic acid treated animals while 6 weeks of housing in an enriched environment had no effect. In that study kainic acid-treated rats in the exercise condition showed improved performance in both the Morris maze and object exploration tasks over rats in the sedentary and enriched environment conditions following kainic acid injection.

Studying the neural mechanisms for exercise-induced neuroprotection requires a paradigm that models a general process common to several neuropathologies. Neurotoxicity mediated by glutamatergic transmission is a common mechanism of cell death underlying a variety of neurological insults and disorders such as epilepsy, stroke, trauma and Alzheimer's disease (Doble 1999; Mattson, 2007; Waxman and Lynch, 2005). Evidence for a role of an endogenous factor that is induced by exercise and that regulates hyperexcitability would increase our understanding of the mechanism for exercise induced neuroprotection.

The results of the current experiment provide further evidence that exercise protects against neurological insults. In particular, long term activity wheel running reduces seizure-related behaviors induced by kainic acid administered either centrally or systemically, and this effect was reversed by central administration of the galanin antagonist, M-40. Increases in endogenous galanin may therefore be necessary for decreased seizure severity following exercise. Exercise-induced upregulation of galanin may therefore represent a major mechanism through which exercise protects the brain. Further examination of the involvement of galanin in exercise-induced neuroprotection will aid in the development of behavioral interventions for the prevention and treatment of neurological insults. It will also improve our understanding of the brain's naturalistic, endogenous protective mechanisms.

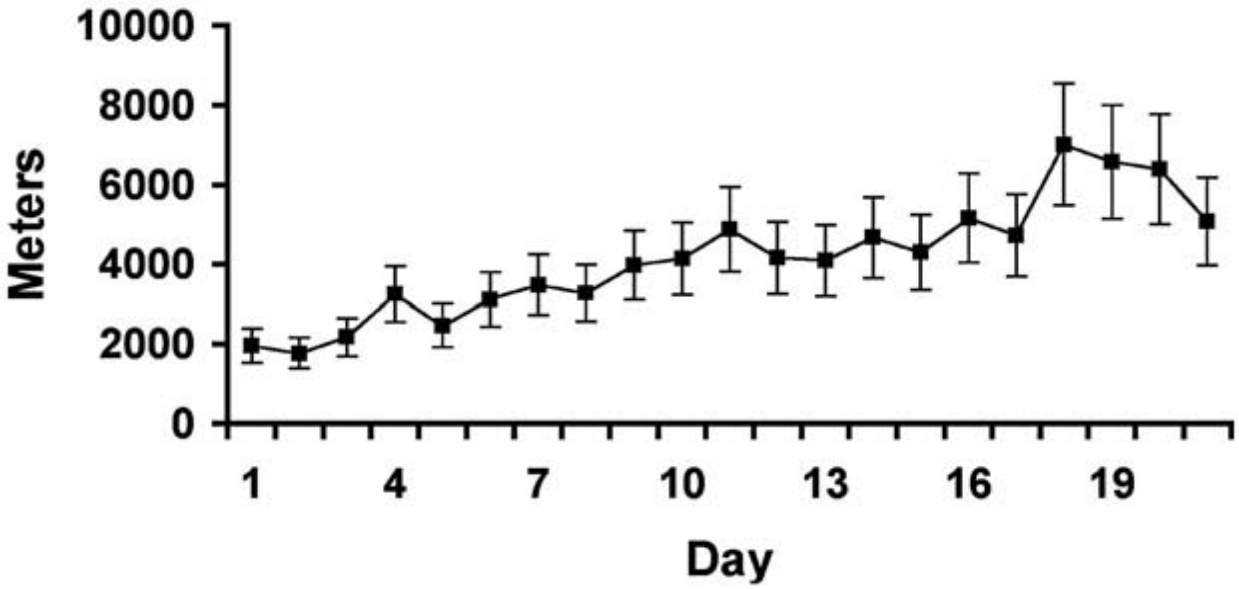

Fig. 3.1: Average daily activity wheel running distances experiment 1. Average running distances for exercising rats (n=21) expressed as meters per day+/−SEM.

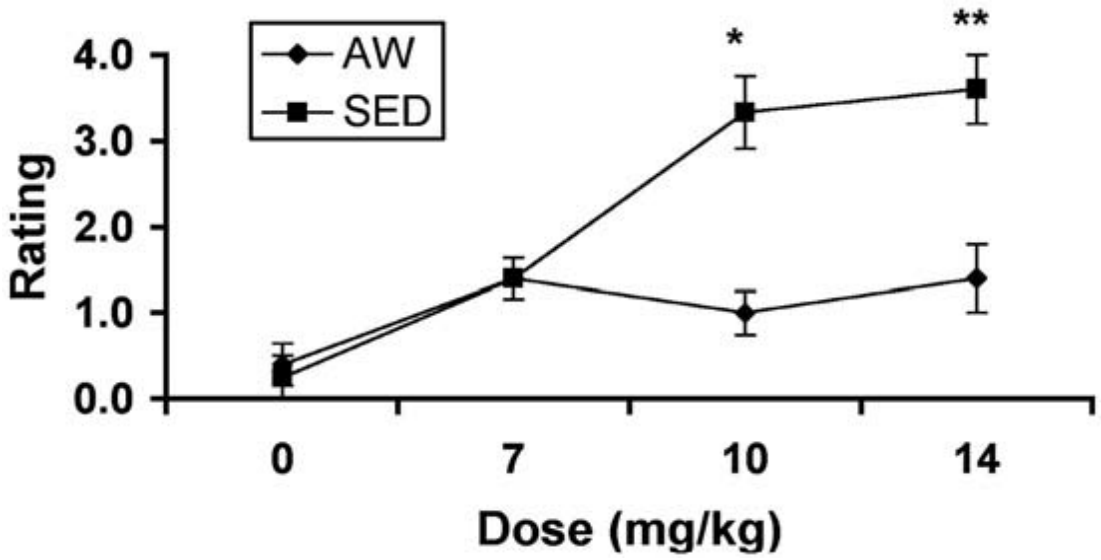

Fig. 3.2: Average seizure ratings following intraperitoneal kainic acid. Mean (±SEM) seizure ratings for exercising (AW) and sedentary (SED) rats at 0, 7, 10 and 14 mg/kg doses of kainic acid. ANOVA revealed a significant interaction between condition (AW vs. SED) and drug $p<.01$. Seizure ratings increased linearly with dose in SED but plateaued after 7 mg/kg in AW. Post hoc t-tests revealed significant differences for AW10 vs. SED10 $p<.01$* and AW14 vs. SED14 $p=.01$ **.

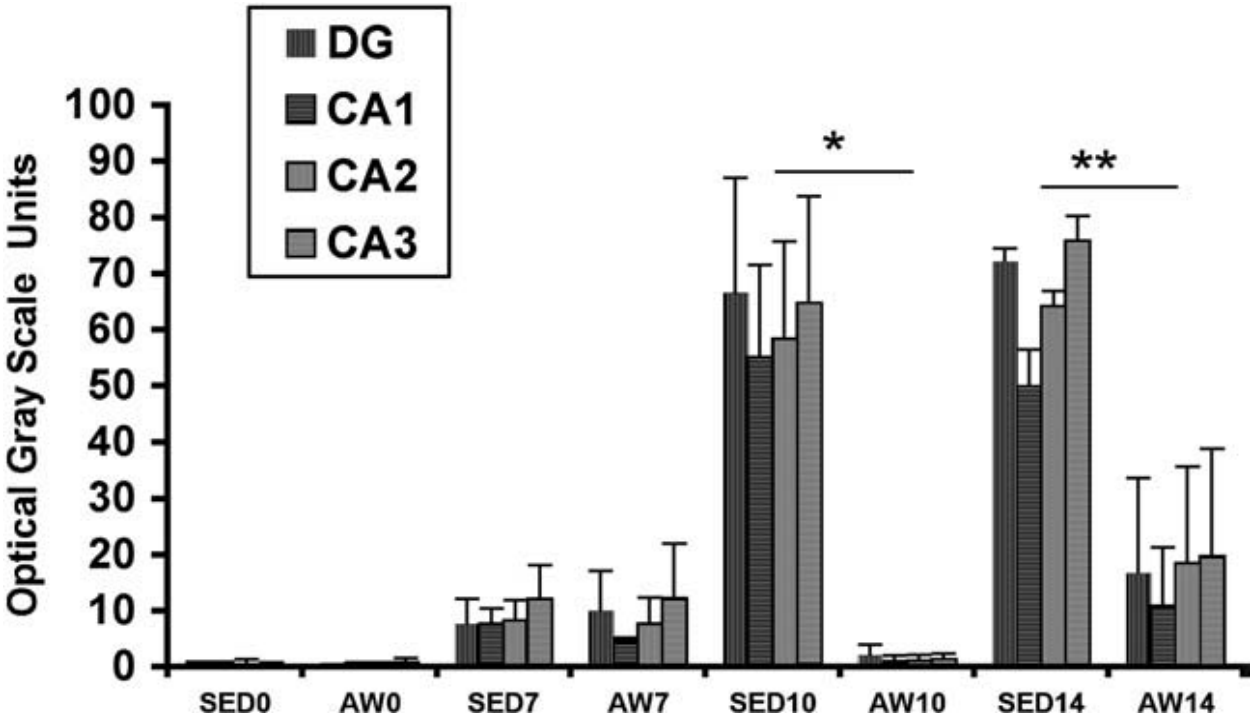

Fig. 3.3: c-fos autoradiographic analysis of the hippocampal formation following intraperitoneal kainic acid. c-fos mRNA autoradiographic optical density values in the hippocampal formation for exercising (AW) and sedentary (SED) animals after 0, 7, 10 and 14 mg/kg doses of kainic acid. ANOVA revealed a significant interaction between condition (AW vs. SED) and drug of optical density values for CA1, CA2, CA3 and DG (p=.001–.01). Post hoc t-tests revealed a significant difference between AW and SED at the 10 mg/kg dose in CA1, CA2, CA3 and DG (p=.02–.03)* and 14 mg/kg dose in CA1, CA3 and DG (p=.015–.045)**

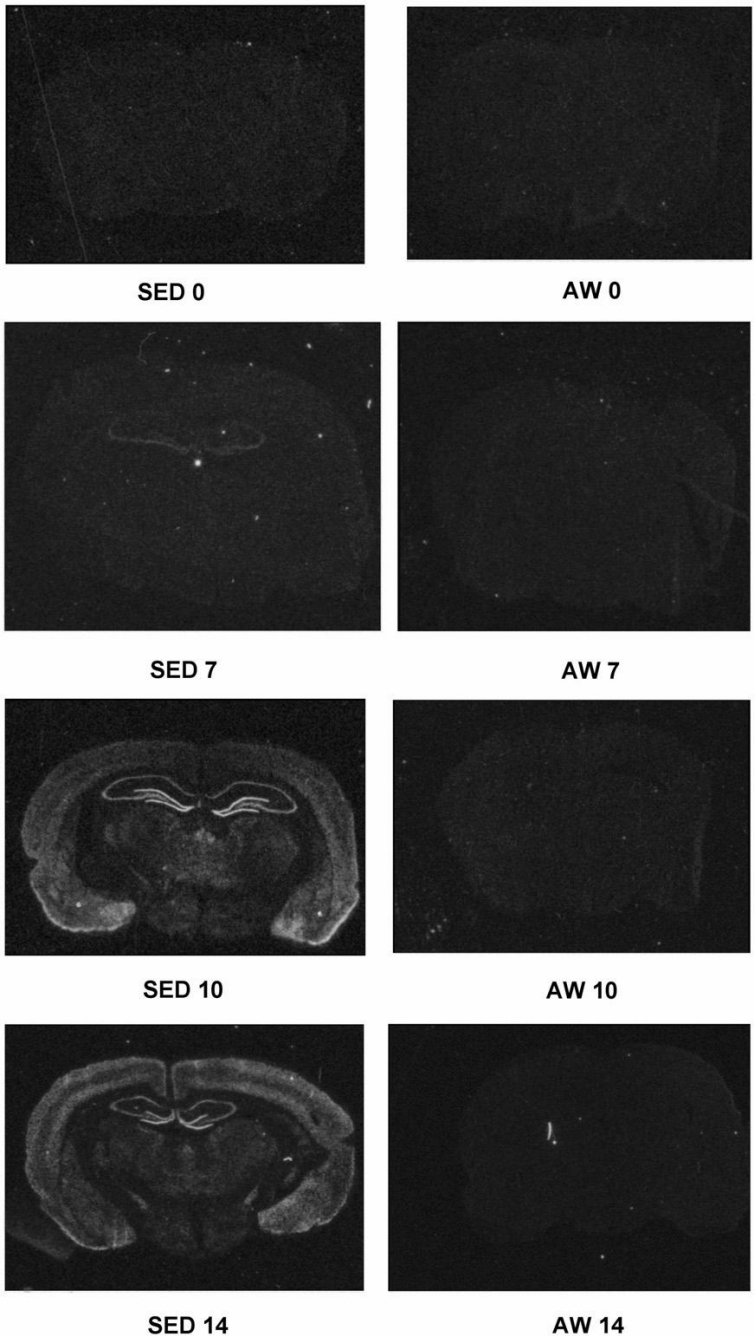

Fig. 3.4: Representative autoradiographs of c-fos mRNA following IP kainic acid.

Representative autoradiographs of brain sections hybridized for c-fos mRNA. Exercising (AW) or sedentary (SED) rats received 0, 7, 10, or 14 mg/kg of kainic acid. Rats were killed 3 h later after kainic acid injection and brains were frozen and sectioned at the level of the hippocampal formation.

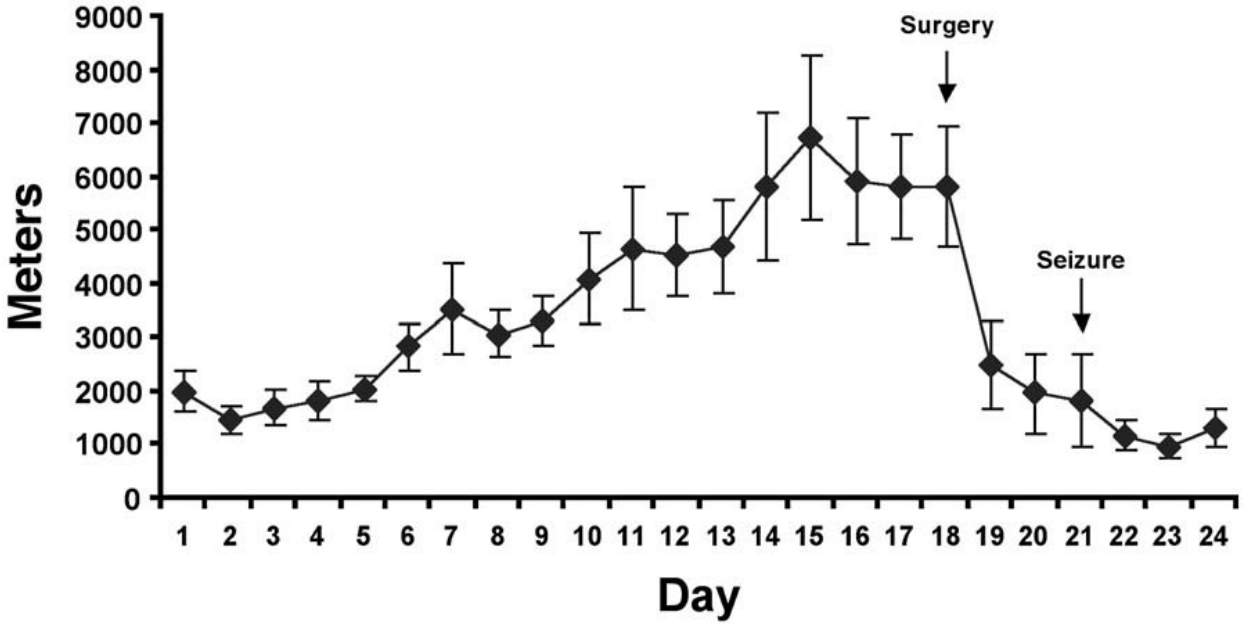

Fig. 3.5: Average daily activity wheel running distances and experiment 2 timeline. Average running distances for AW rats (n=41) expressed as meters per day+/−SEM. All surgeries were performed on day 18 of running. SAL+SAL, KA+SAL or KA+M-40 were injected on day 21 of running.

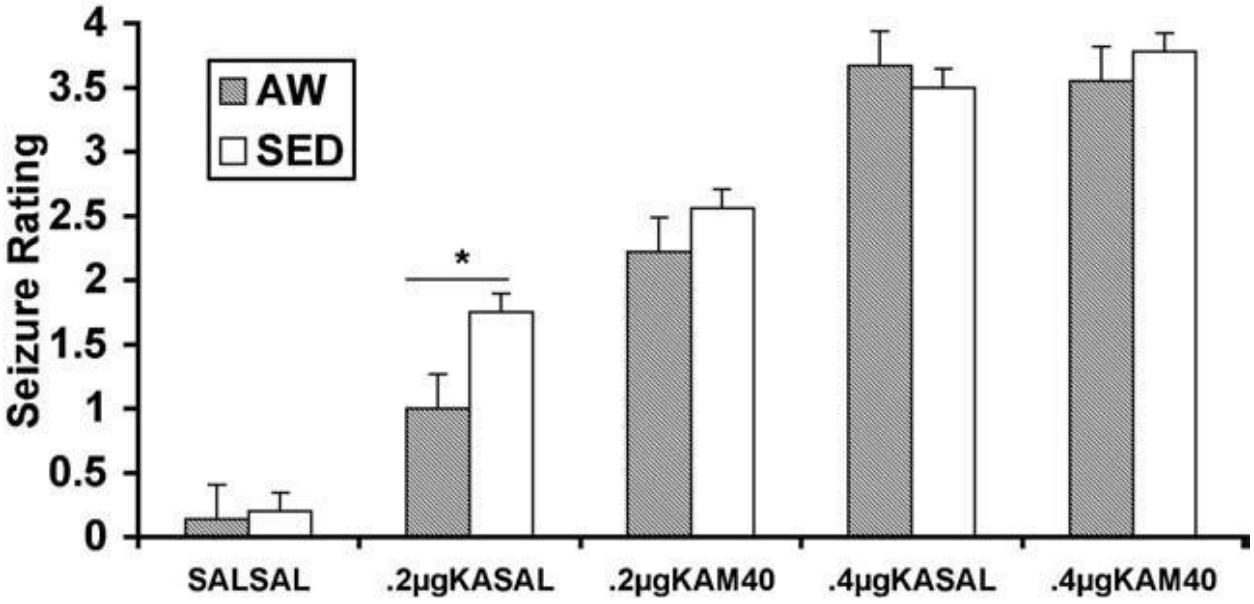

Fig. 3.6: Average seizure rating following ICV M-40 or saline followed by ICV kainic acid. Average seizure ratings for exercising (AW) and sedentary (SED) animals injected with M-40 (6 nmol) or saline (SAL) followed by a 0, .2 or .4 µg dose of kainic acid (n=6, 5, 8, 8, 9, 9, 9, 8, 9, 9 respectively). Planned contrasts indicated that seizure ratings were lower for .2 µg AWKASAL compared to .2 µg SEDKASAL ($p<.01$)*. No significant differences were found between (AW) or sedentary (SED) animals when M-40 preceded a .2 µg or .4 µg dose of kainic acid or when saline preceded a .4 µg dose of kainic acid ($p>.05$).

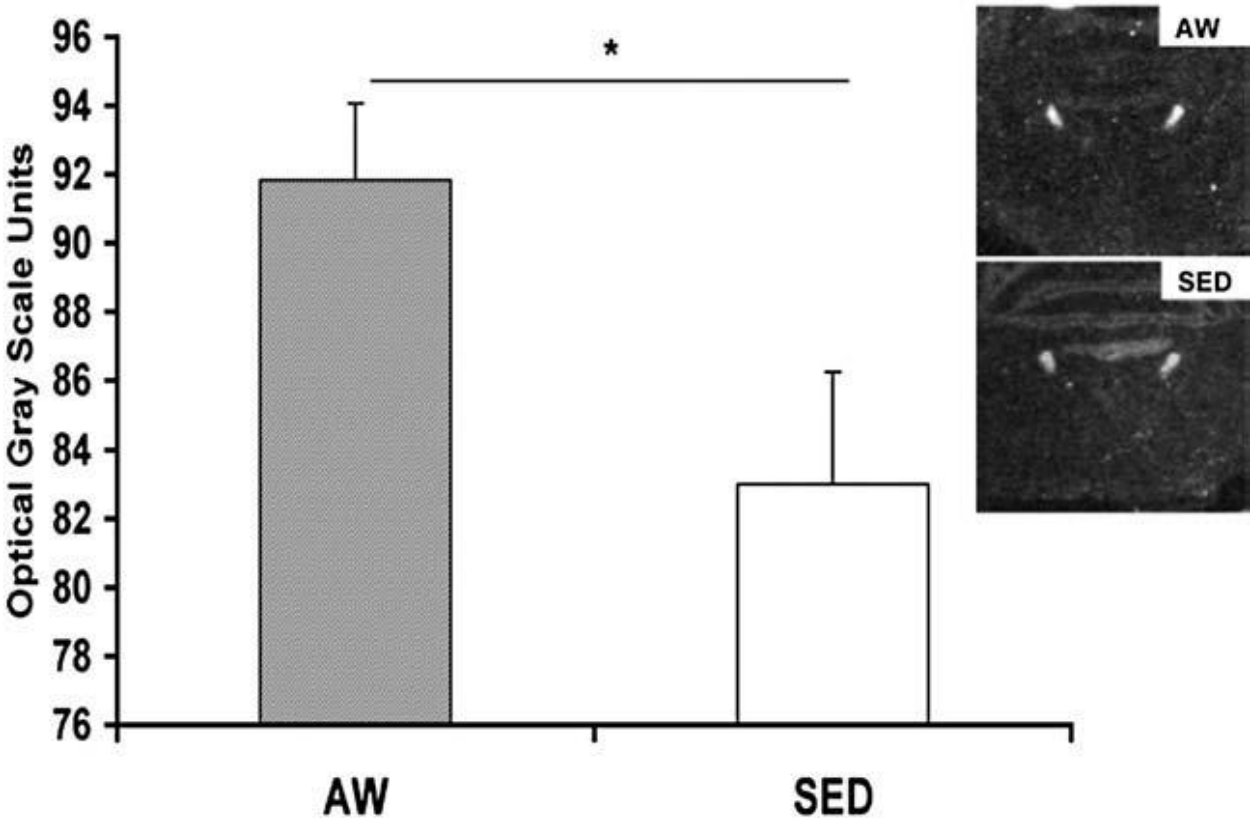

Fig. 3.7: Galanin autoradiographic analysis. Mean (±SEM) galanin (GAL) mRNA optical density in the locus coeruleus. Exercising (AW) control subjects had significantly increased GAL mRNA levels compared to sedentary (SED) control subjects p<.025*. Insets show representative autoradiographs (dark field) of the locus coeruleus from an AW and a SED subject.

CHAPTER FOUR

IN VIVO VOLTAMMETRY OF GLUTAMATE RELEASE FOLLOWING KAINIC ACID-INDUCED SEIZURES IN THE HIPPOCAMPUS OF CHRONICALLY ACTIVE VS. SEDENTARY RATS: AN OBSERATIONAL STUDY

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Abstract

Studies in this laboratory demonstrated that long term activity wheel running protects against the development and progression of kainic acid-induced (KA) seizures. Three weeks of voluntary physical activity in rats also upregulates prepro-galanin messenger RNA levels in the locus coeruleus. Galanin acts to suppress glutamate release by hyperpolarizing membranes through the opening of ATP-dependent potassium channels as well as closing voltage gated calcium channels through g-protein coupled receptors in response to glutamate. When the galanin antagonist M-40 was injected prior to KA the effect of exercise on seizure reduction was significantly attenuated indicating that enhanced galaninergic transmission is a critical factor in reducing kainic acid-induced excitability in exercising animals. Studies on extracellular glutamate release following kainic acid injection report increases in glutamate levels during seizures. The current experiment is designed to test the hypothesis that exercise regulates the release of glutamate into the hippocampus under excitotoxic conditions. We used in-vivo voltammetry following kainic acid-induced seizures to compare extracellular glutamate in the hippocampus of rats allowed free access to an activity wheel or sedentary housing. Glutamate levels were lower among wheel runners during the first 30 minutes after kainic acid injection. Observation of individual seizure graphs also revealed differences in response patterns between groups.

Introduction

Excitotoxicity through over-activation of glutamatergic synapses is a major mechanism of cell death underlying acute brain insults such as stroke and trauma as well as chronic disorders such as epilepsy, Alzheimer's disease, Parkinson's disease and Huntington's disease (Dawson et al., 1995; Doble 1999; Hynd et al., 2004; Mattson 2007, Wang 2005; Waxman and Lynch, 2005;

Meldrum 2000). Stress also damages hippocampal neurons through over-stimulation of glutamatergic synapses. The aged brain is more vulnerable to these insults (Lowy et al., 1995; McEwen 2000; Miller and O'Callaghan, 2000). Glutamate receptor blockade has been shown to be neuroprotective and is a treatment modality used to reduce damage in stroke, epilepsy, Alzheimer's disease and stress (Hara and Snyder, 2007; Scholtzova et al., 2008; Sonkusare et al., 2005).

Studies demonstrate that exercise can reduce symptoms of stress and depression (Brosse et al., 2002; Callaghan 2004; Salmon 2001), decrease age-related cognitive decline (McAuley et al., 2004; Colcombe et al., 2004; Laurin et al., 2001), and reduce the risk of dementia (Fratiglioni et al., 2004; Larson et al., 2006) in clinical studies. Animal models of Parkinson's disease, Alzheimer's disease, Huntington's disease and stroke have shown that exercising rats exhibit less neuronal damage when compared to sedentary controls (Adlard et al., 2005; Mabandla et al., 2004; Pang et al., 2006; Wang et al., 2001). Animal studies have also shown that exercise can decrease stress induced impairments (Dishman et al., 1997; Dishman et al., 1998; Soares et al., 1999; Greenwood et al., 2007) and reduce brain cell loss in aging animals (Larsen et al., 2000).

A number of studies have demonstrated that exercise can protect the hippocampus from excitotoxic insult. Treadmill training or swimming reduced seizure development following injection of domoic acid and pilocarpine and in kainic acid-induced models of epilepsy (Arida et al., 1998; Arida et al., 1999; Carro et al., 2000; Setkowicz and Mazur, 2006). Although these experiments demonstrate a protective effect of aerobic training, the treadmill training and swimming employed in these studies stress the animals and may confound results (Dishman 1997a; Timofeeva et al., 2003). Short term activity wheel running improved the performance of kainic acid (KA) treated rats on spatial learning tasks (Gobbo and O'Mara, 2005). Studies in this

laboratory demonstrated that long term activity wheel running protects against the development and progression of intraperitoneally injected kainic acid and reduced seizure-induced c-fos gene expression. Activity wheel running also reduced seizure behaviors induced by a low dose of intracerebroventricular kainic acid (Reiss et al., 2009). This effect was significantly decreased when the galanin antagonist M-40 was injected prior to KA (Reiss et al., 2009). Results of in-situ hybridization analyses revealed that exercise increased galanin mRNA levels in the LC, as previously reported by this laboratory (Eisenstein and Holmes, 2007; O'Neal et al., 2001; Reiss et al., 2008; Van Hoomisen et al., 2004). The increased seizure severity in exercising animals injected with M-40 prior to KA provides evidence that enhanced galaninergic transmission is a critical factor in reducing kainic acid-induced excitability in exercising animals. Galanin coexists with norepinephrine in at least 80% of locus coeruleus neurons (Holmes and Crawley, 1995) and retrograde tracing/double labeling experiments reveal that the hippocampus receives extensive galaninergic innervation via projections from the locus coeruleus (Kask et al., 1995; Melander et al., 1986).

Results from previous experiments in this laboratory demonstrate that exercise prevents the initiation of seizures, as it significantly decreased seizure ratings, a measure of seizure initiation (Reiss et al., 2009). Previous research therefore suggests that exercise may regulate release of glutamate into the hippocampal region. The current experiment is designed to test the hypothesis that exercise regulates the release of glutamate into the hippocampus under hyperexcitable conditions. Some studies on extracellular glutamate release following kainic acid injection report increases in glutamate levels during seizures (Ding et al., 1998; Rigaud-Monnet et al. 1995; Stein-Behrens et al., 1994; Ueda et al., 2001; Ueda et al., 2002). The current experiment will use in-vivo voltammetry to compare glutamate release in the hippocampus of

rats allowed free access to an activity wheel or sedentary housing following kainic acid-induced seizures. Due to the novel nature of this type of experiment, characteristic differences from each group will be reported in order to determine areas of focus for future experiments.

Methods

Subjects

Adult male Sprague-Dawley rats weighing 150g - 200g were purchased from Harlan Inc. (Indianapolis, IN) and allowed to adapt to the animal facility for 1 week before behavioral manipulations begin. Rats were housed in a humidity and temperature controlled vivarium with lighting maintained on a reverse 12-hour light/ dark schedule (lights on 1900 - 0700). Food and water were available ad libitum and animals were weighed weekly throughout the duration of the study. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All animals were randomly assigned to exercise versus sedentary conditions and were singly housed.

Exercise protocol

For the exercise condition activity wheels (MiniMitter) with a circumference of 105 cm were placed in 30X30X30 cm polycarbonate cages and attached to a magnetic revolution counter (Med Associates). Daily running distances were determined by multiplying the wheel circumference by the number of revolutions. Sedentary rats remained in home cages without running wheels. Animals were assigned to exercise or sedentary conditions following the one week adaptation period to the vivarium. Rats were housed in sedentary or exercise conditions for 21 days. On day 21 guide cannulae and sensors were implanted.

Guide cannulae implantation

All procedures were performed under aseptic conditions. Rats were anesthetized with a halothane/oxygen mixture delivered through a vaporizer and nose cone and mounted in the stereotax. The head was shaved and scrubbed with Betadine solution and a longitudinal incision was made along the scalp. Overlying connective tissue and periosteum were scraped away from the scalp. The guide cannula was implanted into the following coordinates measured stereotactically from bregma: posterior -3.8mm, lateral 2.0 mm, ventral 1mm; according to the rat brain atlas of Paxinos and Watson (1986). These coordinates allow for the probe, which extends 3mm beyond the guide cannula to terminate in the hilar region of the dentate gyrus of the dorsal hippocampal formation. The housing for the wireless potentiostat was affixed with the cannula to the skull with screws and dental acrylic. The calibrated glutamate sensor probe was then inserted through the guide cannula and connected to the potentiostat. Rats received 2mg/kg banamine subcutaneously and were allowed to recover under a heat lamp before being returned to their cages. When the rats were returned to their home cages the telemetry signal recorded by the receiver was verified.

Glutamate Baseline and Control Injection

Glutamate levels were measured continuously and stored automatically by the Pinnacle software on the interfaced computer. Voltammetry recordings occurred two days prior to kainic acid treatment in order to establish a stable baseline and eliminate any influence of anesthesia. A control injection of subcutaneous banamine (2mg/kg) was given during lights off (0700-1900) one day prior to kainic acid injection in order to measure glutamate responses to an injection and serve as a control for each subject.

Kainic acid treatment and measurement of seizure behaviors

Kainic acid (Sigma) was dissolved in physiological saline and injected IP in volumes of 1ml per kg in a dose of 10 mg/kg. Immediately following injection, rats were placed in an open field chamber (50 X 50 X 45 cm). Behavioral observations were conducted by an investigator blind to treatment conditions. The sessions was also recorded by video camera. The incidences and time of occurrence of the following were recorded: staring, sprawling, opthalmoptosis, chewing, wet dog shakes, salivation, head bobbing, forelimb clonus, hindlimb clonus, fecal boli and tonic clonic seizures (with loss of postural control). The rating scale was based on that employed by Racine (1972) and modified by Hoffman (2003): 1 = minor behaviors including catatonia (staring), wet dog shakes, scratching, sniffing and head bobbing; 2 = minor behaviors, chewing, salivation, forelimb clonus and rearing without loss of balance/ postural control; 3= minor behaviors, chewing, salivation, forelimb and hindlimb clonus and rearing with brief loss of balance/ postural control; 4 = tonic clonic seizures; 5 = death.

Behavioral observations took place for 2 hours after injection. After two hours rats were monitored every 20 minutes for another hour and then returned to their home cages. Data recording continued for 24 hours after the testing session in order to determine a maximum value of glutamate as well as the time it takes for glutamate levels to return to baseline (if this occurs within 24 hours). Data points remained reliable for most animals for up to 6 hours so this time point was used in addition to the original 3 hours to look at broader changes in the data. After the 6 hour time point many animals were either missing larger portions of data or data recordings looked to be hidden by a lot of artifact or unexplained increases and decreases. Twenty one hours after the 3 hour session, rats were euthanized by rapid decapitation and brains were extracted and frozen.

Quantitative measures of hippocampal glutamate

The time course of changes in glutamate and the peak level of glutamate over the course of the three hour session in which seizures were scored served as the main measures of kainic acid-evoked glutamate release. Data recorded in PAL were recorded in nanoamps. Nanoamp levels from PAL were converted to micromolar glutamate using a linear conversion obtained from the calibration procedure for each individual probe. Baseline values for 3 hour data were calculated by averaging all micromolar glutamate levels that were graphed in PAL (using the filter function) over 15 minutes prior to injection. Difference from baseline for each average or each individual data point for 3 hour data is therefore equal to that data point minus the baseline. Difference from baseline was then used in all data comparisons. This conversion allows comparisons between animals since the actual value of the baseline is unknown and therefore arbitrary. The same procedure was used for observing the time course of change in glutamate following banamine injection, except with a smaller time scale. A ten minute pre-injection baseline was calculated for banamine data and 1 minute averages of post injection differences from baseline were used to express subject responses to banamine.

The Pinnacle data acquisition software permits "time stamping" which allows annotation of the real-time record of glutamate levels with identification of seizure behaviors (e.g. wet dog shakes, forelimb clonus). Inspection of the seizure behavior and corresponding glutamate record were used to determine whether there is a relationship between these behaviors and glutamate levels, and whether these relationships differ between sedentary and exercising rats.

Verification of cannula placement

Brains were removed, frozen on dry ice and stored at -80 ˚C. Twelve micron sections of the forebrain at the level of the dorsal hippocampal formation (region corresponding to plate 29

in the rat brain atlas of Paxinos and Watson) were cut using a Microm cryostat and sections were mounted on gelatin/chromium potassium sulfate-coated microscope slides. Approximately every fifth section was stained with 0.1% thionin in order to visualize anatomical landmarks. Cannula placement was verified by histological examination of brains as they were sectioned. Rats with probe placements outside of the dentate gyrus were excluded from the analysis.

Data Analysis

A chi square was performed to determine whether the number of deaths resulting from kainic acid injection differed between groups. Measures of the time course of glutamate changes (in five minute intervals after kainic acid treatment, in one minute intervals after banamine injection) were analyzed by mixed model 2-group (wheel runners vs. sedentary) x time (5, 10, 15, 20, 25, 30 min after injection) ANOVA with time of glutamate recording as the repeated measure. Significance was set at $p < .05$. Based on our prior findings that wheel running is protective against seizures induced by kainic acid (Reiss et al., 2009), one-tailed tests were used. A scatterplot was made for the following variables to detect possible relationships; seizure score and maximum glutamate; seizure score and average daily running distance; average daily running distance and maximum glutamate. A correlation coefficient was obtained for each graph.

Results

Calibration Procedure

Each probe was calibrated in order to convert current measured in nano amps by PAL into micromoles of glutamate. The calibration was performed by adding 10 µL of a 5 mM glutamate stock solution at a time to phosphate buffered saline. After the addition of each 10 µL (resulting in increments of 10 µM glutamate) amount the corresponding nano amp value was

recorded. Figure 1 shows a typical calibration procedure with its corresponding linear equation. An R^2 value of .9999 demonstrates the accuracy of this procedure. The R^2 values for all probes used in the experiment had a range of .992 to .999. Previously published research using concurrent dialysis and the Pinnacle voltammetry system has confirmed that the system selectively and sensitively measures extracellular glutamate. Voltammetry readings have been shown to correlate with dialysis measures in the amygdala (Song et al., 2004). The methodology of the sensor is discussed in Hu et al 1994.

Probe Placement

Histological analysis was performed to verify placement of each glutamate probe. Analysis of adjacent sections revealed that evidence of damage was limited to a range of less than 48 microns.

Responses to banamine injection in AW vs. SED rats

Averages of responses to (control) banamine injections revealed no significant differences between groups. Observation of the time course of glutamate averaged over 1 minute shows a trend for a change from baseline in AW animals that is 40uM higher than the change for SED which is only 1uM. The two minute time point also shows the same trend, with an increased response of about 30 uM. At the three minute time point and after the glutamate recordings decrease in AW animals compared to baseline from -5 up to -20uM. Four of six sedentary subjects showed a detectable increase from baseline while just one of the 5 sedentary subjects showed an increase that could be differentiated from baseline. Furthermore the maximum increases of the AW subjects were approximately 3, 14, 100 and 300 μM compared to a 33 μM increase in the SED subject that responded.

Seizure scores in response to kainic acid in AW vs. SED rats

Average seizure score for AW and SED rats are shown in figure 3. A t - test revealed that the scores were not significantly different. Although scores were not significantly different a dose of 10mg/kg was fatal to 4 of 7 SED subjects (a score of 5 = death) while only 1 of 7 AW subjects died from the injection. Furthermore 3 of the 4 SED subjects died before 40 minutes post injection, the fourth died 120 minutes post injection. The death of the AW subject was 115 minutes post injection. Kainic acid at the dose used is more fatal to SED subjects and also more quickly fatal than it is to AW subjects.

Response to kainic acid in AW vs. SED rats

Measures of the time course of glut release over three hours presented as 5 minute averages of μM difference from baseline are shown in Fig 4. There was a group effect $F(1,8) = 3.56$, $p = .046$, revealing that glutamate levels were lower among wheel runners during the first 30 minutes after kainic acid injection. Averages of μM difference for glutamate between AW and SED reveal that during the first 30 minutes SED subjects show a greater than 100 μM increase from baseline compared to AW animals. Three of the initial 5 sedentary subjects depicted in this graph died by the 40 minute time point so averages for SED subjects after the 40 minute time point are not an accurate representation of the SED group. This graph does illustrate that for half of the SED group increases in glutamate compared to baseline values are immediate and highly fatal. Observation of the AW group averages shows the greatest increases at much later time points, specifically 90, 110 and 115 minutes. Because of the high amount of variability obtained when averaging all animals, other measures of seizure responses are helpful in making comparisons between groups.

Selected time stamped graphs for AW and SED rats

Time stamping which allows annotation of the real-time record of glutamate levels with identification of seizure behaviors is shown in fig 5 and 6. Inspection of seizure behaviors and corresponding glutamate record did not reveal a clear relationship although one pattern was seen in a few. The sedentary subject in figure 5 shows that the first seizure behavior (forelimb clonus) corresponds with a peak in glutamate while the subsequent behaviors occur randomly. This subject experienced three separate bouts of tonic clonic seizures followed by death shortly after. Of particular interest is that all 3 bouts of tonic clonic seizures occur when glutamate is closer to baseline instead of during the 300 μM increases above baseline. Figure 6 shows seizure behaviors of an AW subject time stamped onto its seizure graph. All of the seizure behaviors for this subject correspond to the rising phase or peaks of glutamate. Unlike behavior during the first half hour for this subject, seizures during the second half hour occur at random and do not correspond to peaks. Although some subjects only show a correspondence between the first behavior and peak in glutamate like the SED subject in figures 5, the correspondence can be seen for longer periods like the AW subject in Fig 6. Other subjects do show a similar pattern in which early seizure behaviors correspond to peaks in glutamate and later behaviors do not (5 subjects). Some subjects show no correspondence to glutamate peaks and seizure behaviors (7 subjects).

Observations of individual subject graphs for AW and SED seizures

Table 1a and b show subject ratings, the maximum increase in glutamate compared to baseline and the latency in minutes post injection to reach the maximum increase for SED and AW subjects respectively. Some observations can be made from this table. The time to reach maximum increase is higher for many of the AW subjects than it is for the SED subjects. Two

SED subjects, 8 and 11, show latencies lower than the minimum latency of 29 minutes for AW subjects. These two subjects also show the highest increases, of 347 µM and 396 µM above baseline, for the SED subjects. The SED group has 3 of 5 subjects with max Glut increases above 50 µM, while the AW group only has 1 of 7 subjects. A scattertplot of seizure rating and maximum glutamate did not reveal a relationship between variables.

Additional observations of Kainic Acid injection graphs-latency to glut increase

The decrease in glutamate preceding an increase in AW subjects is common to most animals. Four of 6 of the AW subjects showed a decrease in glutamate that was 2 SD less than the baseline (a fifth showed a similar pattern of decrease but it was only 1 SD below baseline). Only one of the 6 SED subjects showed a decrease in glutamate that precedes an increase.

Discussion

This experiment is to our knowledge the first attempt to identify differences in kainic acid-induced glutamate release this glutamate sensor probe marketed by Pinnacle technology. Seizures induced by kainic acid show high variability both in response to an initial injection and also in the temporal occurrence of spontaneous seizures. This report demonstrates that it is possible to identify differences between seizure groups using a glutamate sensor probe. Maximum increase in glutamate as well and the latency to glutamate changes are two factors that can be helpful in comparing seizure data. We also identified that the pattern of glutamate response (for example does a decrease precede an increase) may help determine different mechanisms that regulate seizure severity. Although a large amount of data is generated using the glutamate sensor probe the identification of areas of focus provide an important example for future studies that aim to identify the effects of drugs or behavioral manipulations on seizures.

The results of this experiment reveal that a glutamate sensor probe implanted in the dorsal hippocampus is a useful tool in identifying differences in responses to kainic acid injection between AW and SED subjects. Group averages of μM difference from baseline and observation of the latency to reach the maximum increase in this difference demonstrate that SED subjects show increases in glutamate more quickly than AW subjects. Observation of individual seizure graphs also reveals that most AW subjects show a decrease from baseline glutamate prior to any increase while only one of the SED subjects shows this pattern. Observations of latency to a significant increase in glutamate, latency to maximum increase in glutamate and maximum glutamate increase reveal some interesting trends for differences between groups.

In order to determine that measured glutamate responses were specific to kainic acid and not just the stress of receiving an injection a control (banamine) injection was performed for each subject. Average responses to this injection higher for AW than SED subjects for 2 minutes post injection. An increase for first the 2 minutes could represent that injection is a greater stressor to AW animals than SED since an increase in hippocampal extracellular glutamate typically occurs following a stressor (Lowy et al., 1995; Bagley and Moghaddam 1997). In our laboratory AW animals typically scratch, bite and attempt to escape when being handled while SED animals tend to explore and respond more passively to being handled. Increased defensive behavior of physically active rats has been observed in the open field, elevated plus maze and during handling (Burghardt et al., 2004) so future studies should address differences for active and sedentary animals in extracellular glutamate following both individual and repeated stressors of different durations.

Inspection of group means from seizure graphs indicates that kainic acid acts more quickly and is more fatal to SED subjects than AW subjects. The most striking difference between the AW and SED seizure graphs is that a majority of AW subjects showed a decrease in glutamate relative to baseline prior to any increase in glutamate while only 1 of the SED subjects showed this pattern of response. Possible interpretation is that glutamate release is decreased following injection of kainic acid in AW but not SED subjects by the neuropeptide galanin.

Previous research in our laboratory has revealed that physical activity up-regulates prepro-galanin messenger RNA levels in a dose dependent manner in the locus coeruleus after several weeks of activity wheel exposure or treadmill training (Eisenstein and Holmes, 2007; O'Neal et al., 2001; Van Hoomisen et al., 2004; Reiss et al., 2009). Galanin coexists with norepinephrine in at least 80% of locus coeruleus neurons (Holmes and Crawley, 1995) and retrograde tracing/double labeling experiments reveal that the hippocampus receives extensive galaninergic innervation via projections from the locus coeruleus (Kask et al., 1995; Melander et al., 1986a). Galanin is primarily an inhibitory neurotransmitter, and previous studies indicate that galanin functions within the hippocampus to regulate neuronal excitability. Both in vivo and in vitro studies have demonstrated anti-seizure effects of galanin to seizures induced by both kindling and excitotoxins (Mazarati et al., 1998; Mazarati et al., 2000; Elliot-Hunt et al., 2004). When the galanin antagonist M-40 is injected intracerebroventricularly prior to kainic acid the reduction in seizure severity afforded to exercising animals is attenuated indicating that galanin is an important part of the mechanism through which exercise reduces seizures.

Both galanin receptors GALR1 and GALR2 have been identified in the hippocampus, with relatively high densities observed in dentate gyrus and CA3 (Lu et al., 2005; Mazarati 2004; Kanter-Schlifke et al., 2007). Both GALR1 and GALR2 receptors are involved in the antiseizure

effects of galanin (Lu et al., 2005; Kanter-Schlifke et al., 2007). GALR1 appears to function presynaptically to inhibit the release of glutamate by opening ATP dependent potassium channels or closing voltage gated calcium channels (Mazarati 2004). GALR2 is most commonly reported to inhibit excitation postsynaptically by mediating the release of calcium from intracellular stores and opening calcium dependent chloride channels (Mazarati et al., 2001, Lang et al., 2007).

Although both receptor subtypes are involved in reducing seizure severity, GALR2 functions to shorten the duration or maintenance phase of seizures, while GALR1 is involved in the early initiation phase of seizures. Results from the current and previous experiments in this laboratory (Reiss et al., 2009) demonstrate that exercise prevents the initiation of seizures as it significantly decreased seizure ratings, a measure of seizure initiation. If GALR1 receptors are activated immediately by injection of kainic acid they may decrease the release of glutamate into the hippocampus and result in a decrease in extracellular glutamate. This early activation of GALR1 receptors may also result in a delay in glutamate increase that was also observed in AW subjects. Activation of GALR2 may also function in the maintenance phase of AW seizures to reduce fatalities when glutamate levels do increase at later time points.

No relationship was found between seizure scores and maximum glutamate levels in the hippocampus. The rating scale used in this experiment was based on the occurrence of seizure-typical behaviors and their progressive increase in severity has been used in many experiments (Racine 1972, Hashimoto et al., 1998; Hoffman et al., 2003; Riba-Bosch and Pérez-Clausell 2004; Veiga et al., 2005). One reason scores may not match maximum glutamate levels is the rise in glutamate in the hippocampus may only represent a small portion of the glutamate increase as a whole since it spreads through other regions of the brain such as the motor cortex

during intense convulsions. This interpretation may also explain why the tonic clonic seizures occurred during lower levels of glutamate measured by the probe in Fig 5. It may also be an explanation for why many animals showed a correspondence with seizure behaviors and glutamate peaks early on during seizures such as the example in Fig 6. Future studies should assess sub convulsive doses of excitotoxins as well as behavioral indices to determine whether a relationship exists.

A number of recent studies in animal models demonstrate that physical activity is capable of reducing behavioral and neurotoxic effects induced by excitotoxic insult (Setkowicz and Mazur 2006; Arida et al., 2004; Gobbo and O'Mara 2005; Carro et al., 2001; Reiss et al., 2009) but it also paradoxically increases long term potentiation (van Praag et al., 1999), a process with similar mechanisms underlying epileptogenesis induced by kindling (Meador 2006). Various modes of exercise cause increases in levels of the neurotrophin brain-derived neurotrophic factor (BDNF) in the dentate gyrus and CA 1-3 cell layers (Berchtold et al., 2005; Neeper et al., 1995; Neeper et al., 1996; Oliff et al., 1998). BDNF enhances excitatory transmission in the hippocampus and is considered pro-epileptogenic (Binder et al., 2001; Croll et al., 1999; Koyama and Ikegaya, 2005; Koyoma et al., 2004), possibly as a result of its enhancement of synaptogenesis and neurogenesis (Gómez-Pinilla et al., 2002; Redila et al., 2005; Stranahan et al., 2007; van Praag et al., 1999; van Praag et al., 2002). Exercise has also been shown to increase AMPA receptors, phosphorylated subunits of the NMDA receptor and binding of MK-801 which binds to open NMDA receptors in the cortical post synaptic density of activity wheel running rats (Dietrich et al., 2005). These findings indicate that exercise increases the sensitivity of the hippocampus to glutamatergic input. A regulation of glutamate release would be a

necessary mechanism induced by exercise in order to compensate for the increased excitability and synaptogenesis it induces via BDNF.

Experiments have demonstrated that exercise increases levels of a number of different growth factors but brain derived neurotrophic factor is most consistently reported and has been shown to play important roles in supporting neuronal growth and survival. The mechanism underlying how exercise can protect against a variety of neurological insults is poorly understood. The current experiment provides evidence that exercise alters levels of hippocampal extracellular glutamate induced by kainic acid injection. Future experiments are needed to determine the mechanism responsible for this change. Injecting a galanin antagonist such as M-40 prior to seizure induction would help determine whether a galaninergic mechanism is responsible for the differences observed between active and sedentary rats. Delineating the anatomy and physiology through which exercise protects the brain from overstimulation of glutamate will aid in the prevention and treatment of stress related disorders as well as neurological insults.

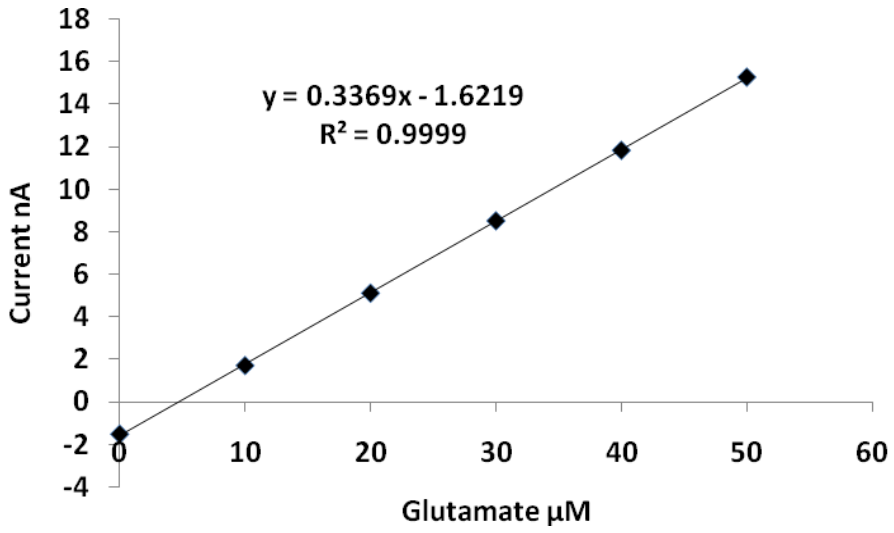

Fig 4.1: Individual calibration procedure for each probe allows the calculation of a linear function which is used to converts nano amps to uM glutamate.

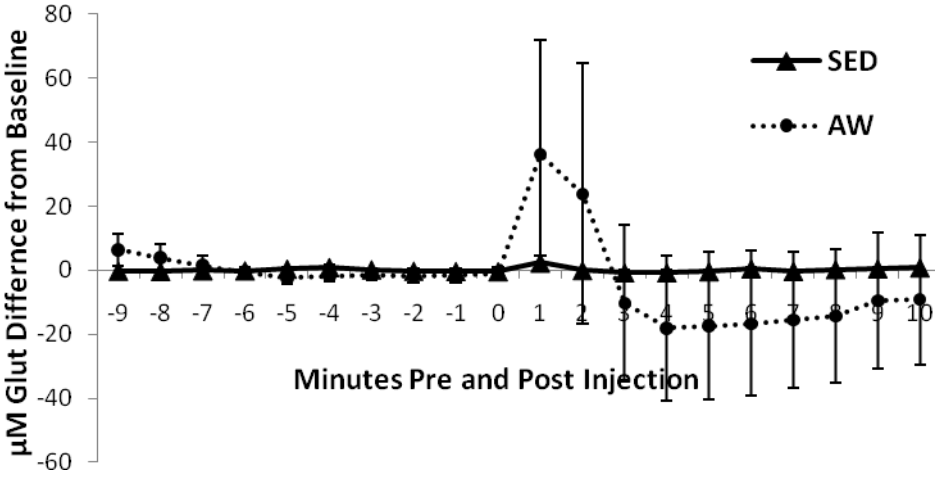

Fig 4.2: SED (n=5) and AW (n=6) Banamine (control) Injection: 1 minute averages ± SEM of micromolar glutamate difference from 10 minute pre-injection baseline.

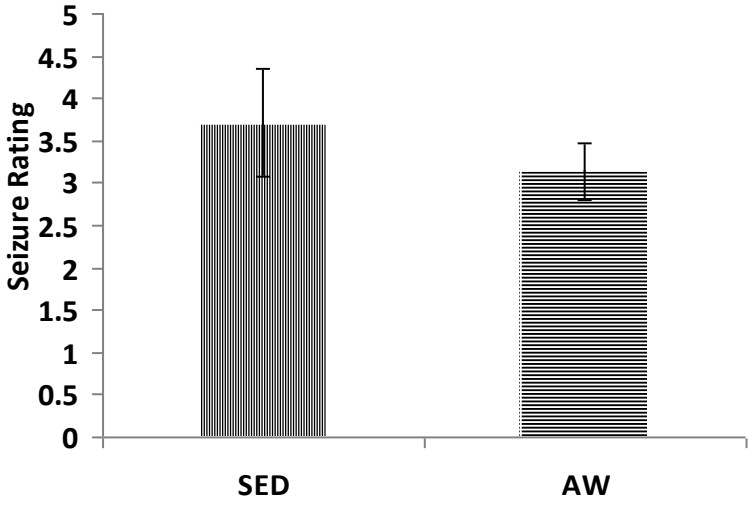

Fig 4.3: Average seizure score ± SEM for AW and SED (n=7, 7) animals.

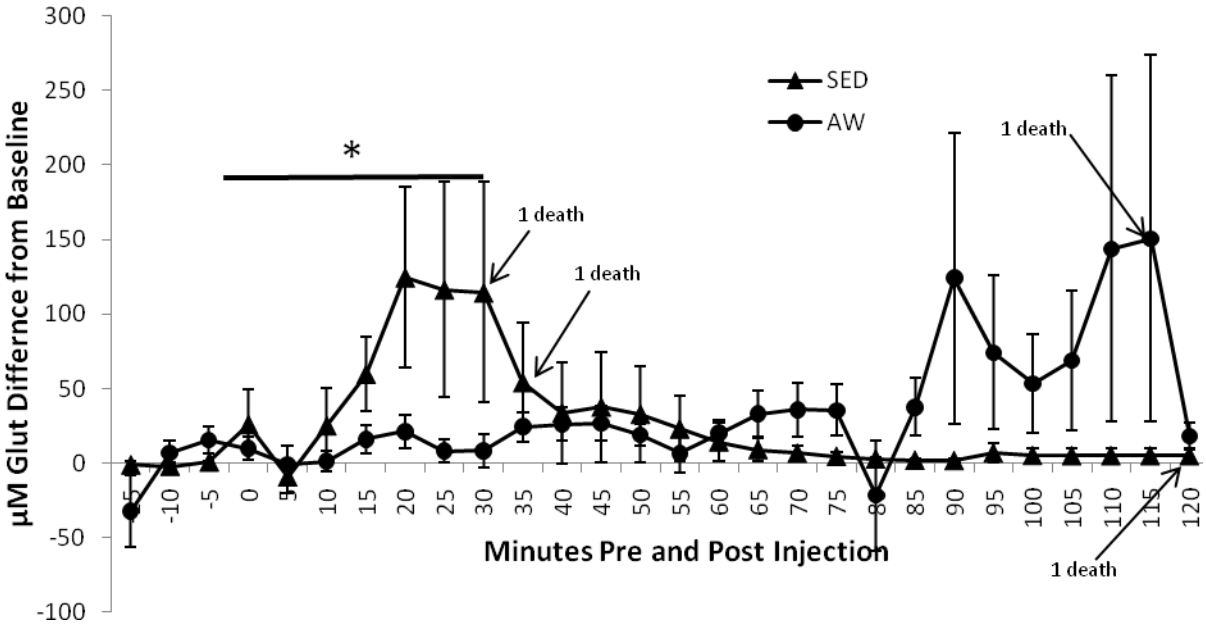

Fig 4.4: SED (n=5) and AW (n=6) KA Injection: 5 minute averages ± SEM of micromolar glutamate difference from 20 minute pre-injection baseline

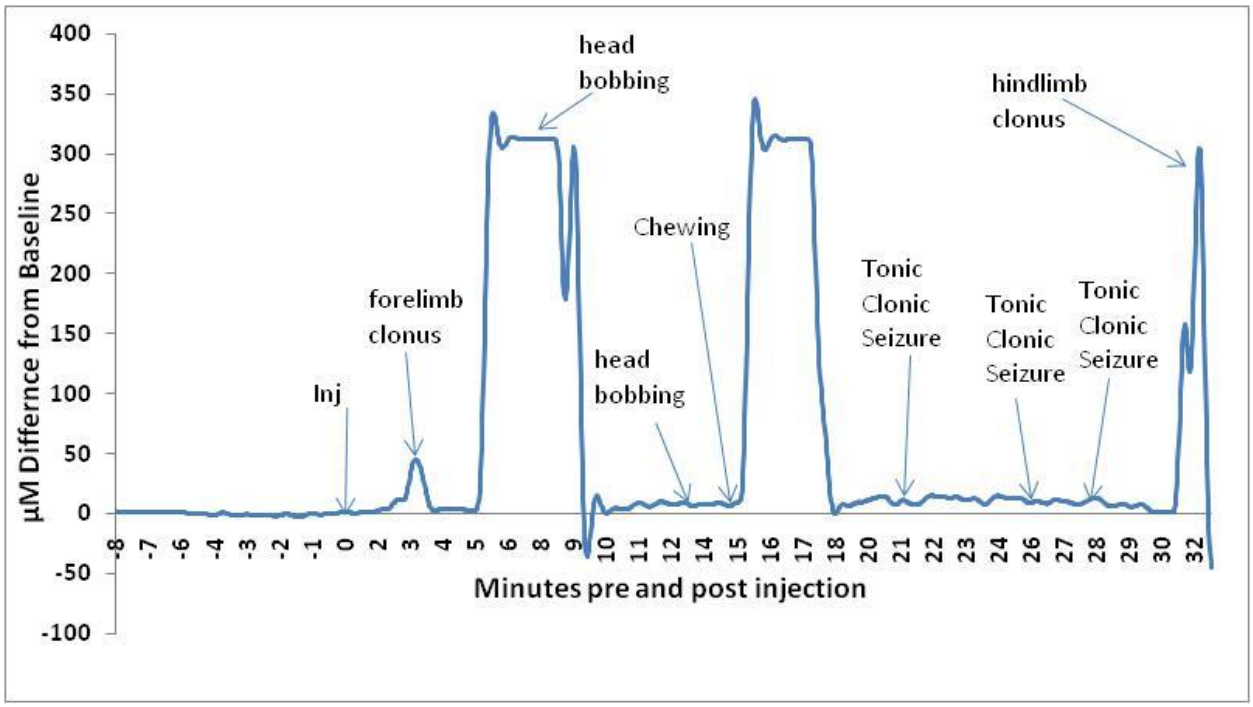

Fig 4.5: Seizure behaviors time stamped onto the first 32 minutes of a sedentary subject's seizure graph. This subject died at 33 minutes post injection.

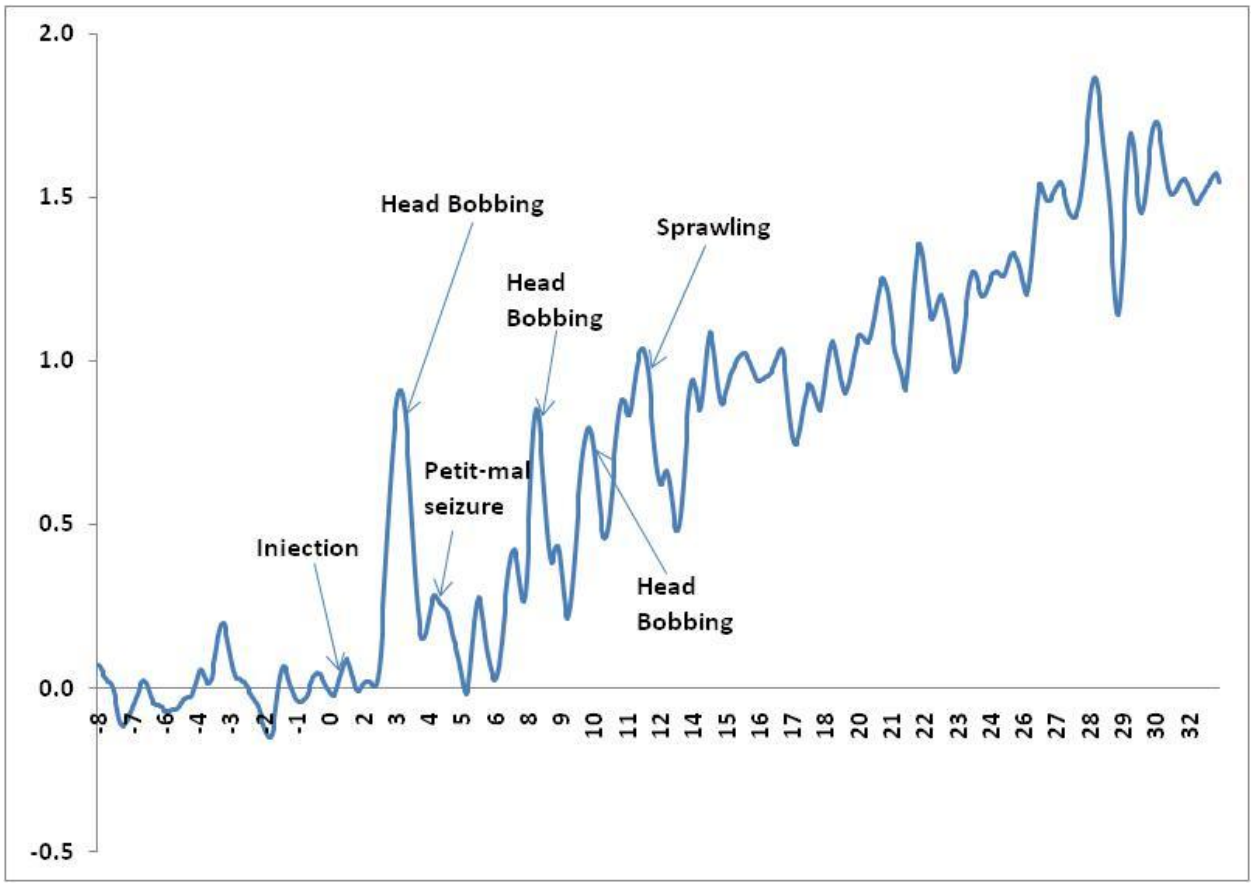

Fig 4.6 Seizure behaviors time stamped onto the first 32 minutes of an AW subject's seizure graph.

Table 1a and 1b: Subject rating, maximum increase and the latency in minutes to reach the maximum increase in glutamate following kainic acid injection

Table 1a

SED

Subject	**Rating**	**Max Increase (µM)**	**Time to Max (min)**
5	1	182	30 min
1	3	.6	67
8	5	347	8
11	5	396	16
15	5	36.4	92

Table 1b

AW

Subject	**Rating**	**Max Increase (µM) and type**	**Time to Max (min)**
12	2	47	67
2	3	10	50
3	3	17	29
6	3	4	106
14	3	18	59
9	5	825	53

CHAPTER FIVE

GENERAL DISCUSSION

Experiment 1 and 2 (ch3) provide evidence that activity wheel running protected against the development and progression of kainic acid-evoked seizures. In experiment 1, this protection was evident at the 10 and 14 mg/kg doses for both seizure-related behaviors and c-fos gene expression. There were no detectable differences between the behavioral reactions of the sedentary and activity wheel conditions at the 7 mg/kg dose. This low dose of kainic acid induced primarily sub-convulsive seizure-related behaviors that are difficult to detect using the Racine seizure rating scale (Mikulecká et al., 1999).

The results of experiment 2 (ch3) demonstrated a decreased behavioral response to ICV kainic acid at 0.2 μg in exercising animals compared to sedentary animals. This protective effect of exercise was significantly attenuated when the galanin antagonist M-40 was injected prior to kainic acid. The increased seizure severity in exercising animals injected with M-40 prior to kainic acid supports the hypothesis that enhanced galaninergic transmission dampens kainic acid-induced excitability in exercising animals. Consistent with previous studies showing the pro-convulsant effects of manipulations that interfere with galanin transmission, sedentary rats treated with M-40 and 0.2 μg kainic acid trended toward higher seizure ratings than sedentary rats treated with saline. The absence of any such differences at the 0.4 μg dose may have been due to a ceiling effect. Though it should be noted that M-40 may exert partial agonist activity at doses exceeding 10 nmol (Lu et al., 2005), the dose of 6 nmol used in the present experiments should not have yielded such effects.

Experiment 3 (ch4) is to our knowledge the first attempt to identify differences in kainic acid-induced glutamate release using the glutamate sensor probe marketed by Pinnacle technology. The results of this experiment reveal that a glutamate sensor probe implanted in the dorsal hippocampus is a useful tool in identifying differences in responses to kainic acid injection between AW and SED subjects. Inspection of group means from seizure graphs indicates that glutamate levels were significantly lower among wheel runners during the first 30 minutes after kainic acid injection. Group averages of μM difference from baseline and observation of the latency to reach the maximum increase in this difference, demonstrate that SED subjects show increases in glutamate more quickly than AW subjects. Observation of individual seizure graphs also reveals that most AW subjects show a decrease from baseline glutamate prior to any increase while only one of the SED subjects shows this pattern. Observations of latency to a significant increase in glutamate, latency to maximum increase in glutamate and maximum glutamate increase reveal some interesting trends for differences between groups.

The most striking difference between the AW and SED seizure graphs is that a majority of AW subjects showed a decrease in glutamate relative to baseline prior to any increase in glutamate while only 1 of the SED subjects showed this pattern of response. A possible interpretation is that glutamate release is decreased by the neuropeptide galanin following injection of kainic acid in AW subjects, but not in SED subjects. Future experiments are needed to determine the mechanism responsible for this change. Injecting a galanin antagonist such as M-40 prior to seizure induction, as was done in experiment 2, would help determine whether a galaninergic mechanism is responsible for the differences observed between active and sedentary rats.

A number of recent studies in animal models, in addition to the current results, demonstrate that physical activity is capable of reducing behavioral and neurotoxic effects induced by excitotoxic insult (Setkowicz and Mazur 2006; Arida et al., 2004; Gobbo and O'Mara 2005; Carro et al., 2001) but it also, paradoxically, increases long term potentiation (van Praag et al., 1999), a process with similar mechanisms underlying epileptogenesis induced by kindling (Meador 2006). Various modes of exercise cause increases in levels of the neurotrophin brain-derived neurotrophic factor (BDNF) in the dentate gyrus and CA 1-3 cell layers (Berchtold et al., 2005, Neeper et al., 1995, Neeper et al., 1996, Oliff et al., 1998). BDNF enhances excitatory transmission in the hippocampus and is considered pro-epileptogenic (Binder et al., 2001, Croll et al., 1999, Koyama and Ikegaya 2005, Koyoma et al., 2004), possibly as a result of its enhancement of synaptogenesis and neurogenesis (Gómez-Pinilla et al., 2002, Redila et al., 2005, Stranahan et al., 2007, van Praag et al., 1999, van Praag et al., 2002). Exercise has also been shown to increase AMPA receptors, phosphorylated subunits of the NMDA receptor and binding of MK-801 which binds to open NMDA receptors in the cortical post synaptic density of activity wheel running rats (Dietrich et al., 2005). These findings indicate that exercise increases the sensitivity of the hippocampus to glutamatergic input, in contrast to the findings of experiment 1 and 2. A regulation of glutamate release would be a necessary mechanism induced by exercise in order to compensate for the increased excitability and synaptogenesis it induces via BDNF. Although BDNF plays an important role in supporting exercise induced neuronal growth and survival, the mechanism underlying how exercise can protect against a variety of neurological insults is poorly understood. The current experiments provide evidence that exercise decreases kainic acid induced seizures and that exercise induced upregulation of galanin is a critical factor in decreasing excitability. We have also demonstrated that exercise alters levels of hippocampal

extracellular glutamate induced by kainic acid injection. Delineating the anatomy and physiology through which exercise protects the brain from overstimulation of glutamate will aid in the prevention and treatment of stress related disorders as well as neurological insults.

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