EXERCISE-INDUCED NEUROPROTECTION AGAINST KAINIC ACID-INDUCED SEIZURES IN THE RAT: ROLE OF GALANIN

by

JENNY REISS

(Under the Direction of Philip Holmes)

ABSTRACT

Studies have demonstrated that exercise decreases the symptoms of a variety of neurological disorders, but the mechanism through which exercise provides this protection is unknown. Excitotoxicity is a common cause of cell death underlying a number of brain disorders. Neuronal hyperexcitability is reduced by the neuropeptide galanin, and galanin mRNA is up-regulated by 3 weeks of activity wheel running. The following studies tested whether activity wheel running can reduce excitability and seizure behaviors induced by kainic acid. The importance of exercise-induced up-regulation of galanin was determined by injecting the galanin antagonist M-40 prior to administration of kainic acid. Seizure-induced behaviors and excitability were decreased in exercising animals following intraperitoneal and intracerebroventricular (ICV) administration of kainic acid. This effect was significantly attenuated when M-40 was injected prior to ICV kainic acid. These findings indicate that exercise induced up-regulation of galanin is a necessary factor in exercise-induced neuroprotection against excitotoxicity.

INDEX WORDS: Physical activity, Kainic Acid, Galanin, Neuroprotection, Seizure

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JENNY REISS

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JENNY REISS

Major Professor: Philip Holmes

Committee: Rodney Dishman
Andrea Hohmann

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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CHAPTER 1

INTRODUCTION

Literature Review

Chronic physical activity has been shown to improve brain functions and protect the brain from a variety of different insults such as epilepsy, stroke, stress, and neurodegenerative disease. Clinical data has shown that a physically active lifestyle can reduce the risk of Alzheimer's disease and dementia of any type as well as improve cognitive function in the elderly (Fratiglioni et al., 2004; McAuley et al., 2004; Churchill et al., 2002; Laurin et al., 2001; Heyn, et al., 2004; Kramer et al., 2006). Studies have also shown that exercise can attenuate behavioral deficits and decrease cell damage induced by neurological insult in experimental animals and genetic models of disease (Gobbo and O'Mara 2005; Carro et al., 2001; Arida et al., 2004; Setkowicz and Mazur 2006; Kiraly and Kiraly 2005; Adlard et al., 2005; Li et al., 2004; Mabandla et al., 2004). Exercise induces many known changes in the brain that likely contribute to improved cognitive functioning and cell survival, but the mechanism through which exercise can protect the brain is not known.

A number of recent studies in animal models demonstrate that physical activity is capable of reducing behavioral and neurotoxic effects induced by excitotoxic (Setkowicz and Mazur 2006; Arida et al., 2004; Gobbo and O'Mara 2005; Carro et al., 2001) and hypoxic insults (Li et al., 2004; Endres et al. 2003; Wang et al., 2001). Excitotoxicity is a common mechanism of cell death and may be induced experimentally by administration of excitotoxins, electrical stimulation (kindling), and stroke (hypoxia). Damage induced by these insults produces excessive

glutamatergic signaling which damages hippocampal regions and produces behavioral impairments in hippocampal dependent tasks such as spatial memory (McEwen 2000; Dawson et al., 1995; Doble 1999). 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). Short term (five days) voluntary activity wheel running also 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).

Temporary occlusion of the middle cerebral artery (MCA) is an experimental model of stroke. Death of tissue after MCA occlusion is caused by glutamate released from dying neurons and glia and a subsequent neurotoxic effect mediated through NMDA receptors (Slevin et al., 2005). Treadmill training for at least 2 weeks decreases infarct volume and behavioral deficits induced by temporary occlusion of the (MCA) (Wang et al., 2001; Li et al., 2004, Endres et al. 2003).

Although neurodegenerative diseases include a number of different pathological symptoms they share excessive glutamatergic signaling and oxidative damage as a common mediator of cell death (Doble 1999). Activity wheel running decreased amyloid load in the hippocampus and cortex and reduced deficits in acquisition of a water maze task in a transgenic model of Alzheimer's disease (Adlard et al., 2005;

Wolf et al., 2006). Voluntary activity wheel running prior to and following injection of 6-Hydroxydopamine (6-OHDA) into the medial forebrain bundle reduces contralateral rotations induced by injection of apomorphine, indicating a protective effect on dopamine neurons (Mabandla et al., 2004, Howells et al., 2005). Although the destruction of dopamine neurons was not significantly different between exercising and sedentary animals median values of cell death were reduced by 14% (Howells et al., 2005). Long term treadmill training significantly attenuated age-related increases in oxidative stress markers, decreased age-related deficits in behavioral tests and increased lifespan in mice (Navarro et al., 2004). Treadmill training also decreased age related degeneration of Purkinje cells in the cerebellum of rats (Larsen, et al., 2000).

There is evidence that a number of exercise-induced changes in cellular signaling systems play an important role in exercise-induced neuroprotection against excitotoxic and hypoxic insults. For example, exercise increases endothelial nitric oxide synthase (eNOS) which increases endothelium dependent vasodilation as well as regional cerebral blood flow. Exercise is not protective against MCA occlusion in transgenic mice deficient in endothelial nitric oxide (eNOS) indicating that eNOS up-regulation is critical to stroke protection by physical activity (Endres et al. 2003). In a more direct manner the exercise induced up-regulation of the growth factor IGF-1 is critical to reducing the behavioral deficits induced by a neurotoxic insult to the hippocampus (Carro et al., 2001). Although these factors are important they do not explain the mechanism through which exercise reduces the behavioral deficits and cell loss induced by excitotoxicity.

Previous research in our laboratory has revealed that physical activity up-regulates prepro-galanin messenger RNA (mRNA) levels in the locus coeruleus after several weeks of activity wheel exposure or treadmill training (O'Neal et al., 2001; Van Hoomisen et al., 2004). Galanin is an amino acid neurotransmitter and trophic factor that regulates neural activity in several brain structures, including the hippocampus. Galanin coexists with norepinephrine in locus coeruleus neurons, and retrograde tracing/double labeling experiments reveal that the hippocampus receives extensive GALergic innervation via projections from the locus coeruleus (Kask et al., 1995; Melander et al., 1986). 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 anti-seizure 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). Previous research thus clearly demonstrates that galanin functions as an endogenous neuroprotective factor for the hippocampus.

Purpose of this Study

Based on observations that exercise decreases damage induced by a number of different insults, we hypothesized that exercise would be protective against cell death and seizures induced by kainic acid. To test this hypothesis rats were allowed access to

an activity wheel for 3 weeks prior to an intraperitoneal (IP) injection of kainic acid.(KA) Seizure-induced locomotor activity and behaviors as well as *c-fos* immunoreactivity, an indirect indicator of enhanced neuronal activity (Sperk 1994) were compared between active and sedentary animals. In a subsequent experiment we aimed to test the hypothesis that exercise-induced up-regulation of galanin is part of the mechanism through which exercise decreases seizure behaviors. The galanin antagonist M-40 was injected prior to intracerebroventricular (ICV) administration of KA. Seizure-induced behaviors were compared between groups.

CHAPTER 2

EXPERIMENTAL PROCEDURE

Subjects

Adult male Sprague-Dawley rats (n = 42; 4-6 per cell for experiment 1 and n=34; 8-9 per cell for experiment 2) 150g 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 versus running conditions and drug versus 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 30X30X30 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 injection of physiological saline or 7, 10 or 14 mg/kg kainic acid. These doses of kainic acid have been shown to produce mild, moderate and severe seizures in rats respectively. In experiment 2 after 18 days all rats had cannula implanted.

Cannulae Implantation and Administration of M-40 and Kainic Acid

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: 1.5mm, ventral: 3 mm according to the rat brain atlas of Paxinos and Watson (1986). They were attached to the scull using 3 stainless steel screws and dental acrylic and protected by small plastic tubing. Rats then received 2mg/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 fast-green dye ICV in a concentration of 2µg/ml following decapitation. The brain was then removed and sectioned coronally through the lateral ventricles (approximately .5mm anterior to bregma) and ventricle IV (approximately 9 mm posterior to bregma) 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) followed by an injection of physiological saline (KA+SAL) or an injection of M-40 (20 µg) followed by an injection of kainic acid (KA+M-40).

Behavioral Measures: Seizure Rating Scale

Experiment 1:

Immediately following injections of kainic acid (or physiological saline) 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 3 hours. Behavioral observations were conducted by an investigator blind to treatment conditions. Observations began immediately following injections and continued for 2 hours. Behavior was also recorded

with a video camera. Two rats 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 1-5 on seizure severity. The rating scale was based on the occurrence of seizure-typical behaviors (Hoffman et al., 2003).

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 {instead of biclonus seizure activity}

5= death.

Experiment 2

Immediately following ICV injections, rats were placed in the Med Associates automated open field activity monitor, used in experiment 1. Procedures previously used were replicated but due to the subconvulsive dose of kainic acid that was injected a 0 was included to differentiate behavior that included only wet dog shakes. Changes are italicized below. Animals were returned to their home cages following the 2 hour observation period and were observed daily for 3 days.

0 = wet dog shakes only

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

2 = minor behaviors, chewing, salivation and rearing without loss of balance; *(rearing with forelimb clonus)*

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

4= tonic clonic seizures {instead of biclonus seizure activity}

5= death.

In Situ Hybridization Histochemistry

Immediately following the final behavioral observations in experiment 1 animals were killed by rapid decapitation and brains were removed and stored at -80ºC. Brains were sliced into 12µm sections at the level of the dorsal (Hippocampal Formation) HF on a Microm cryostat. A detailed procedure of hybridization methods used is reported elsewhere (Van Hoomisen et al., 2004). In brief, sections were thaw-mounted onto microscope slides, fixed, 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. Probes were labeled at the 3' end with [^{35}S]-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 x 8-pixel circles placed randomly throughout the brain region of interest (dentate gyrus [DG], Ammon's horn area 1 [CA1], Ammon's horn area 3 [CA3]), in each section of each rat. The investigator was blind to subject condition at the time of quantification.

Data Analysis

Experiment 1

A 4 X 2 (drug X exercise) factorial design was used in this study. The independent variables were (1) drug (saline versus 7, 10 or 14 mg/kg kainic acid) and (2) exercise (activity wheel running vs. sedentary). The dependent measures were seizure observations (described above) and *c-fos* expression in the dentate gyrus, CA1, CA2 and CA3 regions of the hippocampus. An ANOVA followed by Bonferroni post hoc contrasts was used to compare each of the groups using a p-value of .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

The second experiment is a 2 X 2 design, (1) exercise (activity wheel running vs. sedentary) (2) drug combination (KA+SAL or KA+M-40). Effect sizes for simple effects are reported as Cohen's d and computed using G POWER. Statistical power for exercise was .51. Power for drug was .924.

CHAPTER 3

RESULTS

Experiment 1

Voluntary running distance progressively increased during the 21 days of exposure to activity wheels. Mean running distances were 2467m, 4242m, and 5621m per day for week 1, week 2 and week 3 respectively (Fig. 1).

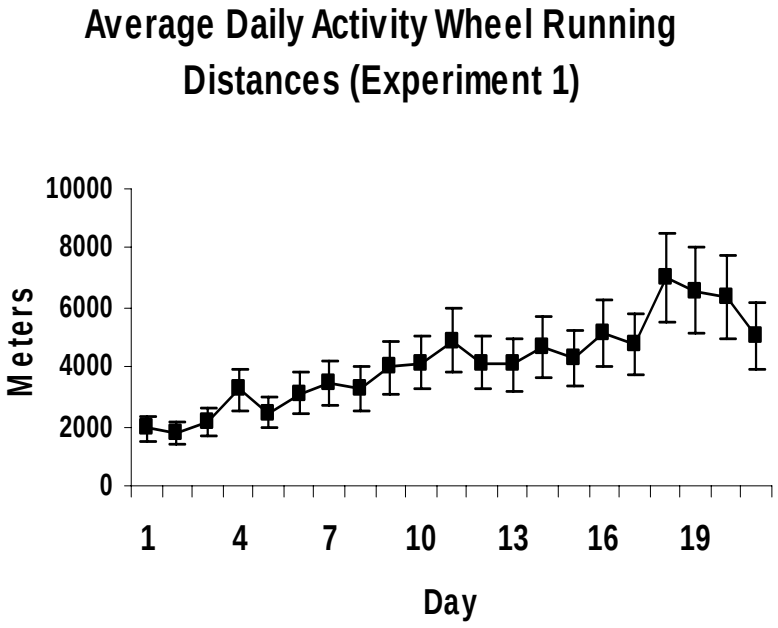

Figure 1: Average running distances for exercising rats (n = 21) expressed as meters per day +/- SEM.

Intraperitoneal KA 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 activity wheel running significantly reduced the behavioral manifestations of seizures induced by KA. ANOVA of seizure ratings for

AW and SED animals at 0, 7, 10 and 14 mg/kg doses of KA 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 tests revealed significant differences for AW10 vs SED10 $t(10) = 4.72$, $p < .01$, $d = 2.7$ and AW14 vs. SED14 $t(8) = 3.89$, $p = .01$, $d = 2.4$.

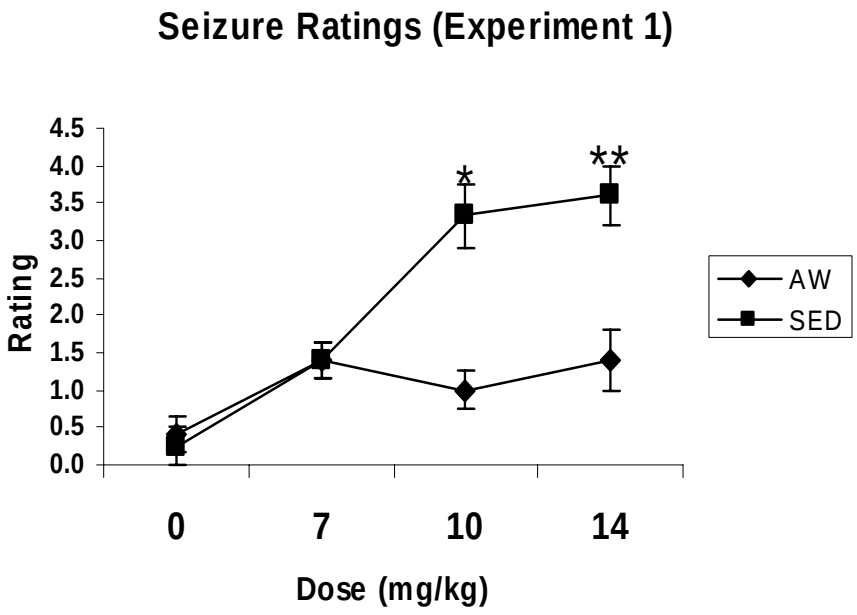

Figure 2: Mean (± SEM) seizure ratings for exercising (AW) and sedentary (SED) rats at 0, 7, 10 and 14 mg/kg doses of kainic acid. ANOVA revealed a significant interaction between condition (AW vs SED) and drug $p < .01$. Seizure ratings increased linearly with dose in SED but plateaued after 7 mg/kg in AW. Post hoc tests revealed significant differences for AW10 vs SED10 $p < .01$* and AW14 vs. SED14 $p = .01$ **.

Consistent with the behavioral data, dose-dependent increases in hippocampal *c-fos* mRNA levels were observed in SED 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.

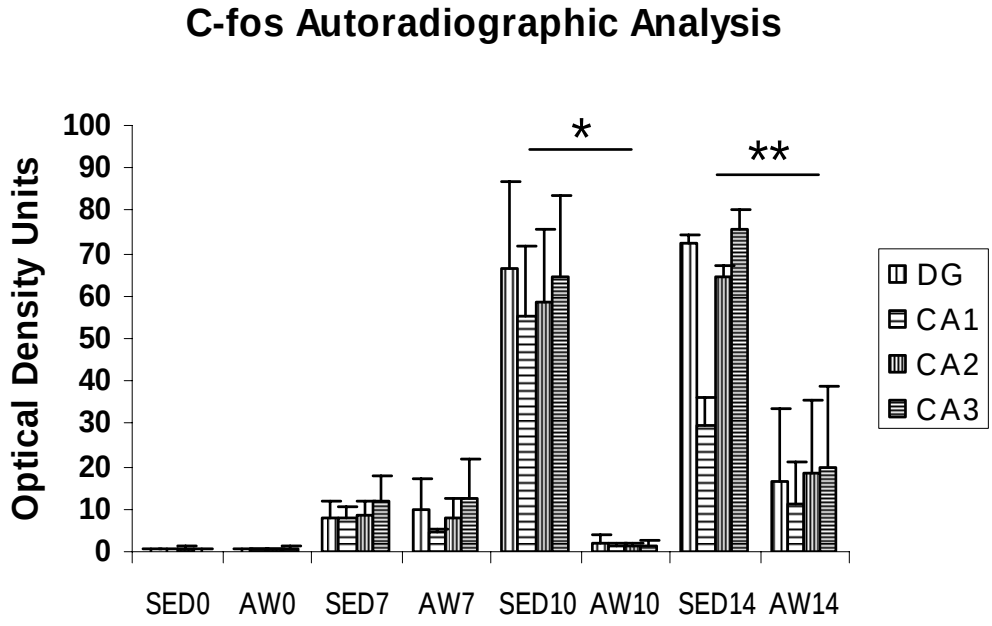

Figure 3: *c-fos* mRNA autoradiographic optical density values in the hippocampal formation for exercising (AW) and sedentary (SED) animals after 0, 7, 10 and 14 mg/kg doses of kainic acid. ANOVA revealed a significant interaction between condition (AW vs SED) and drug of optical density values for CA1, 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) **.

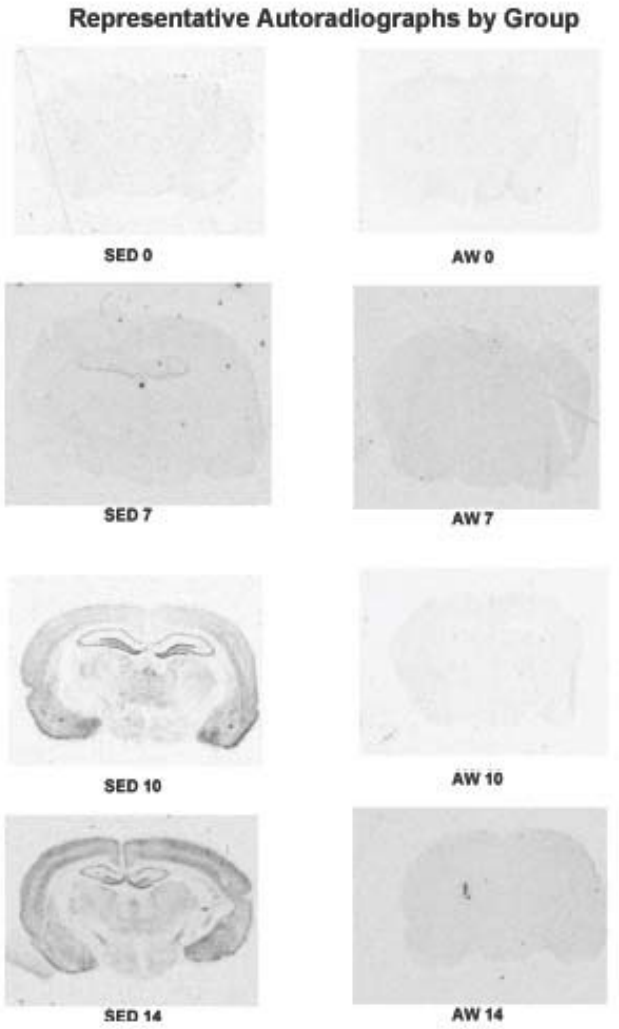

Figure 4: Representative autoradiographs of brain sections hybridized for *c-fos* mRNA. Exercising (AW) or sedentary (SED) rats received 0, 7, 10, or 14 mg/kg of kainic acid. Rats were killed three hours later after kainic acid injection and brains were frozen and sectioned at the level of the hippocampal formation.

Experiment 2

Voluntary running distance progressively increased over the first through 18th day (when surgery took place) of exposure. Between surgery and seizures (day 19-21) average running distance dropped to 2121 and continued to drop to 1172 (figure 5).

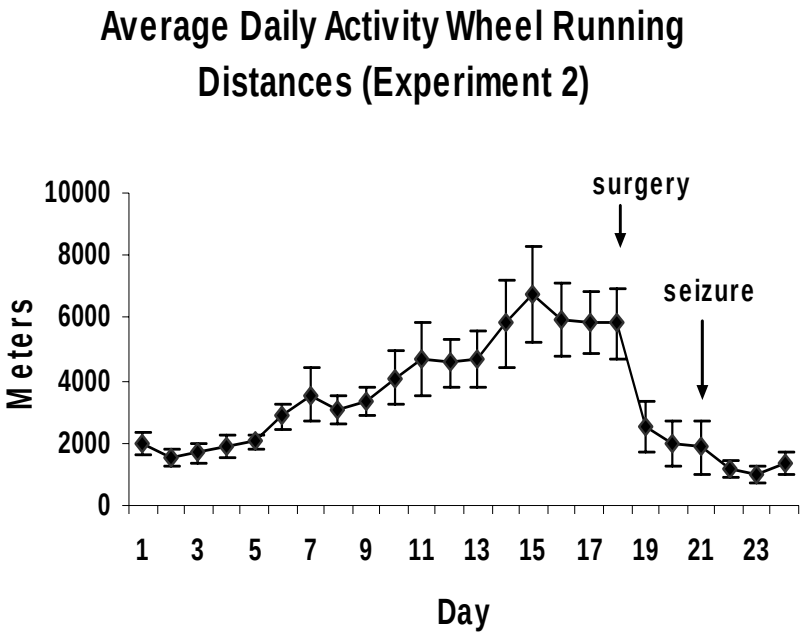

Figure 5: Average running distances for AW rats (n = 21) expressed as meters per day +/- SEM. All surgeries were performed on day 18 of running. KA+SAL and KA+M-40 were injected on day 21 of running.

AW animals showed decreased behavioral ratings to KA+SAL injections and KA+M40 in comparison to SED animals $F(1, 32) = 4.28$, $p = .049$. M-40 injections prior to KA increased seizure scores of AW animals and SED animals $F(1, 32) = 12.31$, $p = .01$ (fig 6). Post hoc t tests reveal a significant difference between AW KA+SAL and SED KA+SAL $t(11) = 2.2$, $p = .02$.

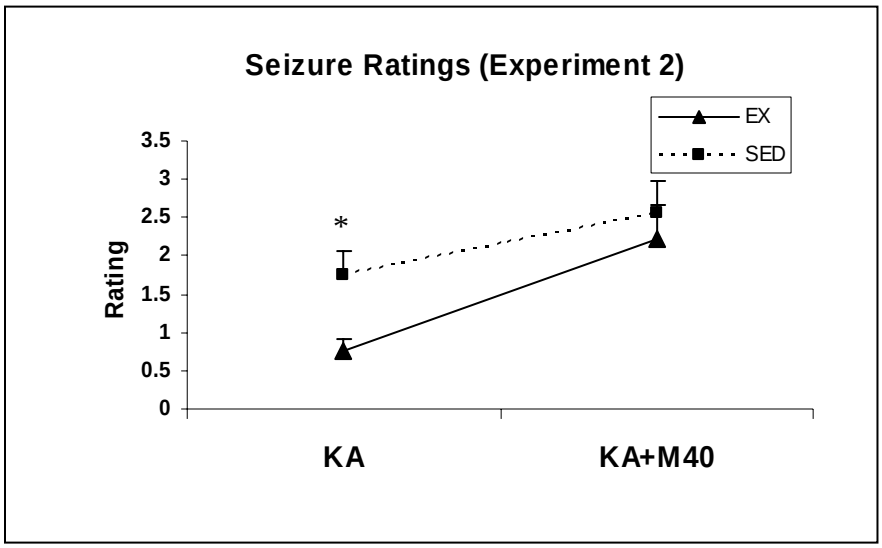

Figure 6: Mean (± SEM) seizure ratings for exercising (AW) and sedentary (SED) rats injected with KA and KA + M40 $p < .05$. ANOVA revealed a significant main effect of condition AW vs SED and drug (KA vs M40 +KA). Post hoc tests revealed significant differences for AW vs SED $p < .05$.

CHAPTER 4

Discussion

Activity wheel running protected against the development and progression of kainic acid-evoked seizures following IP injection. This protection was evident at (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 SED and AW conditions at the 7 mg/kg dose. This subconvulsive dose of KA induced primarily petit mal seizures and not all rats showed detectable reactions. Replication of this experiment with a higher number of animals at the 7 mg/kg dose may be useful since seizure responses at this dose are highly variable and may be better differentiated by a different rating scale that focuses on subconvulsive responses and specific behavioral tests. A 6mg/kg dose of i.p. kainic acid was demonstrated to be too low to induce motor, convulsive seizures but differences in response to novel objects in an open field can be detected in rats treated with this dose in comparison to saline controls (Mikulecká et al., 1999). Although there was no statistically significant difference in *c-fos* optical density values, again this effect may be evident with a higher number of subjects per group given the variability in responses to this dose.

The results of experiment 1 support the hypothesis that exercise decreases kainic acid-induced seizure behavior and excitability in hippocampal regions, but the effect of exercise on the pharmacokinetics of KA are not known. Exercise has been shown to influence the rate of absorbtion, distribution, metabolism and excretion of some drugs (Khazaeinia, Ramsey, and Tam, 2000). If, for example, exercise increases the excretion of KA that is systemically injected, smaller amounts of the drug may cross the blood

brain barrier which would subject AW animals to a smaller dose of the drug. To rule out this possible confound experiment 2 used an ICV route of administration which bypasses any possible difference in systemic pharmacokinetics.

Experiment 2 demonstrated a decreased behavioral response to ICV KA in exercising animals compared to sedentary animals. The protective effect of exercise was significantly decreased when M-40 was injected prior to KA. Increased seizure severity in AW animals injected with M-40 prior to KA supports the hypothesis that increased GAlergic activity is crucial to decreased excitability induced by KA in AW animals

Results of experiment 1 demonstrated that the protective effect of exercise is either decreased or more difficult to detect at low or subconvulsive dose (7mg/kg i.p.) in comparison to higher doses (10 and 14mg/kg). The doses typically used in ICV KA injection are higher (.4 µg - 1.5µg) than the .2 µg dose currently used (Sperk 1994). This low dose was chosen in order to promote survival of sedentary animals receiving KA+M-40. Experiments demonstrate that rats injected with M-35 (a galanin antagonist) prior to perforant path stimulation (PPS) developed sustained status epilepticus while control animals subjected to the same PPS procedure never went into status epilepticus (Mazarati et al., 2001). Due to the effects of M-35 and the pro-seizure effects of decreased GAlergic signaling we hypothesized that in order for KA+M-40 animals to survive for three days after seizures a low dose would be necessary. If neuroprotection is more highly evident at higher doses of ICV injection as it is with IP. injection, replication of experiment 2 with a higher dose of KA may find even greater differences between AW and SED animals.

Animals in experiment 2 showed decreased running following cannula surgery which may affect results. It is not known how long galanin signaling remains following an excessive decrease in physical activity. It has been shown that BDNF signaling begins to decline after 3 days of inactivity, and is diminished after 14 days of inactivity (Berchtold et al., 2005). Physical activity must be above a threshold level of 500 m/day in order to elicit up-regulation of functions such as an increase in the hippocampal level of BDNF mRNA and activated CREB (Tong et al., 2001). Activity levels did remain above this threshold level therefore possibly maintaining exercise induced changes. An analysis of GAL mRNA in control groups (not injected with KA+SAL or KA+M-40) will be necessary to delineate the possible effects of decreased activity of GAL in the locus coeruleus.

As previously mentioned, the reduction in severity of kainic acid-induced seizures has been detected in similar paradigms to those currently employed. In contrast to the above results, a study using intra-hippocampal injections of KA after activity wheel running found that exercise increased cell death in anesthetized female rats (Ramsden et al., 2003). The effects of intrahippocampal KA treatment in anesthetized rats would be expected to differ markedly from the effects observed in awake, freely behaving animals. Anesthesia would presumably eliminate the influence of extrahippocampal neural circuits, such as the locus coeruleus projections, in regulating hyperexcitability. Increased cell death in this paradigm may have resulted from higher levels of the neurotrophin BDNF which shows increased levels of expression in the hippocampus following exercise (Berchtold et al., 2005). BDNF enhances excitatory transmission in the

hippocampus and is considered pro-epileptogenic (Binder et al., 2001; Koyama and Ikegaya 2005).

The results of the current experiment provide further evidence that exercise is capable of protecting against neurological insults. In particular, long term activity wheel running reduces excitability and behaviors induced by IP kainic acid. Activity wheel running also decreased seizure behaviors induced by ICV kainic acid, and this affect was decreased when the galanin antagonist, M-40, preceded injection. Since galanin up-regulation is necessary for decreased seizure severity, it supports the hypothesis that exercise-induced up-regulation of galanin underlies a major mechanism through which exercise protects the brain. Further study and analysis of cell loss in hippocampal regions for AW and SED animals injected with KA+SAL and KA+M-40 will be necessary to determine whether M-40 affects cell survival in AW animals. Determining 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 how the brain can naturally protect itself from hyperexcitability.

REFERENCES

Adlard PA, Perreau M, Pop V, Cotman CW (2005) Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. J Neurosci 25:4217-21.

Arida RM, Scorza FA, dos Santo NF, Peres CA , Cavalheiro EA (1999) Effect of physical exercise on seizure occurrence in a model of temporal lobe epilepsy in rats. Epilepsy Res 37: 45-52.

Arida, RM, Sanabria ER, da Silva AC, Faria LC, Scorza FA, Cavalheiro EA (2004) Physical training reverts hippocampal electrophysiological changes in rats submitted to the pilocarpine model of epilepsy. Physiol Behav 83:165-171.

Berchtold NC, Chinn G, Chou M, Kesslak JP, Cotman CW (2005) Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. Neuroscience 133:853-861.

Binder DK, Croll SD, Gall CM, Scharfman HE. (2001) BDNF and epilepsy: too much of a good thing? Trends Neurosci.24:47-53.

Carro E, Nunez A, Busiguina S, Torres-Aleman I (2000). Circulating insulin-like growth factor I mediates effects of exercise on the brain. J Neurosci 20:2926-33.

Churchill JD, Galvez R, Colcombe S, Swain RA, Kramer AF, Greenough WT (2002) Exercise, experience and the aging brain. Neurobiol Aging 23:941-955.

Dawson R, Beal MF, Bondy SC, Di Monte DA, Isom GE (1995) Excitotoxins, aging and environmental neurotoxins: Implications for understanding human neurodegenerative diseases. Toxicol Appl Pharmacol 134:1-17.

Doble A (1999) The role of excitotoxicity in neurodegenerative disease: Implications for therapy. Pharmacol Ther 81:162-221.

Elliot-Hunt CR, Marsh B, Bacon A, Pope R, Vanderplank P, Wynick D, (2004) Galanin acts as a neuroprotective factor to the hippocampus. PNAS 101:5105-510.

Endres M, Gertz K, Lindauer U, Katchanov J, Schultze J, Schröck H, Nickenig G, Kuschinsky W, Dirnagl U, Laufs U (2003) Mechanisms of stoke protection by physical activity. Ann Neurol 54:582-590.

Fratiglioni L, Paillard-Borg S, Winblad B (2004) An active and socially integrated lifestyle in late life might protect against dementia. Lancet Neurol 6:343-53.

Gobbo OL, O'Mara SM (2005) Exercise, but not environmental enrichment, improves learning after kainic acid-induced hippocampal neurodegeneration in association with an increase in brain-derived neurotrophic factor. Behav Brain Res 159: 21-26

Heyn P, Abreu C, Ottenbacher KJ (2004) The effects of exercise training on elderly persons with cognitive impairment and dementia: A mete-analysis. Arch Phys Med Rehabil 85:1694-1704.

Hoffman GE, Moore N, Fiskum G, Murphy AZ (2003) Ovarian steroid modulation of seizure severity and hippocampal cell death after kainic acid treatment. Exp Neurol 182: 124-134.

Howells FM, Russel VA, Mabandlaa MV, Kellaway LA (2005) Stress reduces the neuroprotective effect of exercise in a rat model for Parkinson's disease. Behav Brain Res 165:210-220.

Kask K, Langel U, Bartfai T, (1995) Galanin--a neuropeptide with inhibitory actions. Cell Mol Neurobiol 15:653-73.

Khazaeinia T, Ramsey AA, Tam YK (2000) The effects of exercise in the pharmacokinetics of drugs. J Pharm Pharm Sci 3:292-302.

Kiraly MA, Kiraly SJ (2005) The effect of exercise on hippocampal integrity: review of recent research. Int'l J Psychiatry in Medicine 35:75-89.

Koyama R**,** Ikegaya Y (2005) To BDNF or not to BDNF: that is the epileptic hippocampus. Neuroscientist 11:282-7.

Kramer AF, Erickson KI, Colcombe SJ (2006) Exercise, cognition, and the aging brain. J Appl Physiol 101:1237-1242.

Larsen JO, Skalicky M, Viidik A (2000) Does long-term physical exercise counteract age-related Purkinje cell loss? A stereological study of rat cerebellum. J Comp Neurol 428:213-22.

Laurin D, Verreault R, Lindsay J, MacPherson K, Rockwood K (2001) Physical activity and risk of cognitive impairment and dementia in elderly persons. Arch Neurol 58:498-504.

Li J, Luan X, Clark JC, Rafols JA, Ding Y (2004) Neuroprotection against transient cerebral ischemia by exercise pre-conditioning in rats. Neurological Research 26:404-408.

Mabandla M, Kellaway L, St.Clair Gibson A, Russell VA (2004) Voluntary running provides neuroprotection in rats after 6-hydroxydopamine injection into the medial forebrain bundle. Metab Brain Dis 19:43-50.

Mazarati, AM, Hantao L, Soomets U, Sankar R, Shin D, Katsumori H, Langel U, Wasterlain CG (1998) Galanin modulation of seizures and seizure modulation of hippocampal galanin in animal models of status epilepticus. J Neurosci 18:10070-10077.

Mazarati AM, Hohmann JG, Bacon A, Liu H, Sankar R, Steiner RA, Wynick D, Wasterlain CG (2000) Modulation of hippocampal excitability and seizures by galanin. J Neurosci 20:6276-6281.

Mazaratii A, Langel U, Bartfai T (2001) Galanin: an endogenous anticonvulsant. Neuroscientist 7:506-517.

McAuley E, Kramer AF, Colcombe SJ (2004) Cardiovascular fitness and neurocognitive function in older adults: a brief review. Brain Behav Immun 18:214-220.

McEwen BS (2000) Allostasis, allostatic load and the aging nervous system: Role of excitatory amino acids and excitotoxicity. Neurochem Res 25:1219-31.

Melander T, Hokfelt T, Rokaeus A, Cuello AC, Oertel WH, Verhofstad A, Goldstein M (1986) Coexistence of galanin-like immunoreactivity with catecholamines, 5-hydroxytryptamine, GABA and neuropeptides in the rat CNS J Neurosci 6:3640-54.

Navarro A, Gomez C, López-Cepero JM, Boveris A (2004) Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. Am J Physiol Regul Integr Comp Physiol 285:505-11.

Mikulecká A, Hlinák Z, Mares P (1999) Behavioural effects of a subconvulsive dose of kainic acid in rats. Behav Brain Res 101:21-28.

O'Neal HA, Van Hoomissen JD, Holmes PV, Dishman RK (2001) Prepro-galanin messenger RNA levels are increased in rat locus coeuruleus after treadmill exercise training. Neurosci Lett 299:69-72.

Ramsden M, Berchtold NC, Kesslak P, Cotman CW, Pike CS (2003) Exercise increases the vulnerability of rat hippocampal neurons to kainate lesion. Brain Res 971:239-244.

Setkowicz Z, Mazur A (2006) Physical training decreases susceptibility to subsequent pilocarpine-induced seizures in the rat. Epilepsy Res 71:142-148.

Slevin M, Krupinski J, Kumar P, Gaffney J, Kumar S (2005) Gene activation and protein expression following ischemic stroke: strategies toward neuroprotection. J Cell Mol Med 9:85-102.

Sperk G (1994) Kainic acid seizures in the rat. Prog Neurobiol 42:1-32.

Tong L, Shen H, Perreau VM, Balazs R, Cotman CW (2001) Effects of exercise on gene-expression profile in the rat hippocampus. Neurobiol Dis 8:1046-1056.

Van Hoomisen JD, Holmes PV, Zellner AS, Poudevigne A, Dishman RK (2004) Effects of beta-adrenoreceptor blockade during chronic exercise on contextual fear conditioning and mRNA for galanin and brain-derived neurotrophic factor. Behav Neurosci 118:1378-90.

Wang, RY, Yang YR, Yu SM (2001) Protective effect of treadmill training on infarction in rats. Brain Res 922:140-143.

Wolf SA, Kronenberg G, Lehmann K, Blankenship A, Overall R, Staufenbiel M, Kempermann G (2006) Cognitive and physical activity differently modulate disease progression in the amyloid precursor protein (APP)-23 model of Alzheimer's disease. Biol. Psychiatry 60:1314-23.