CAN STRESS MODULATE THE SEVERITY OF CONVULSIONS AND SUBSEQUENT BEHAVIOURAL OUTCOME?

Maede Ejaredar

B.Sc. in Neuroscience, University of Lethbridge, 2005

A thesis

Submitted to the University of Lethbridge School of Graduate Studies

in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

Department of Neuroscience

University of Lethbridge

LETHBRIDGE, ALBERTA, CANADA

"Human beings are members of a whole,

In creation of one essence and soul.

If one member is afflicted with pain,

Other members uneasy will remain.

If you have no sympathy for human pain,

The name of human you cannot retain"

Sheikh Saadi,

Persian poet of the medieval period

To all the individuals who suffer from epilepsy.

THESIS ABSTRACT

Status epilepticus is prolonged seizure activity lasting for more than 30 minutes that result in a series of morphological alterations in the brain as well as some changes in the behaviour. Experiment 1 examined the relation between status epilepticus, spontaneous seizures, hippocampal volume and the behaviour of rats that had experienced status epilepticus. Experiment 2 examined the relation that stress and status epilepticus had on hippocampal volume, spontaneous convulsions and a subset of the behaviours from Experiment 1.

The results of this thesis confirm that status epilepticus is associated with hippocampal volume loss. Status epilepticus and/or reductions in hippocampal volume may result in behavioural impairments, especially in spatial memory. Unlike some of the other studies, stress did not change the hippocampal volume nor did it interact with status to produce further change. Further investigations are required to confirm whether stress has a compounding effect on status epilepticus and subsequent seizure disorders.

ACKNOWLEDGEMENTS

When I was a little girl I dreamed about becoming a neuroscientist. I am now one step closer for this dream to become true. What a long journey it has been. The writing of this thesis has never been a singular effort. I am indebted to many individuals. First, I would like to thank my supervisor, Dr. Deb Saucier for her guidance throughout this project. She walked me through step by step, allowing me to strengthen my writing skills by editing every paragraph of this work kindly and patiently. Second, I would like to thank Dr. Darren Hannesson my previous supervisor, who introduced me to animal models of epilepsy and raised my awareness of the importance of research in the epilepsy field. I would like to thank my committee members Dr. Gerlinde Metz, Dr. Brent Selinger and Dr. Rob Sutherland for their ongoing intellectual input throughout this project. I would like to thank my external examiner, Dr. Cam Teskey for taking the time to read my thesis and for his valuable insight on this manuscript. I would also like to thank Dr. david Gregory the chair of the examination for accepting to be a member of my examination committee. I would like to thank NSERC for the funding for the projects in this thesis.

I started working in neuroscience laboratory in the first year of my undergraduate degree. During the past six years I have had the privilege of learning different techniques and skills from many individuals, whose names would make an endless list. I would like to thank them all.

I would specially like to appreciate Dr Abdie Kazemipur, who introduced me to the fascinating world of statistics. I would like to thank Jamshid Faraji for teaching me the ziggurat task, answering my endless questions and his invaluable intellectual input. I

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

ACTH	Adreno-corticotropic hormone
ANOVA	Analysis of Variance
CA	Cornu Ammonis
cm	centimeter
CRH	Corticotrophin Releasing Hormone
DAPi	4',6-diamidino-2-phenylindole
EEG	Electro-encephalographic
HPA	Hypothalamic Pituitary Adrenal
i.p.	intra-peritoneal
KA	Kainic Acid
KA+	Kainic Acid treated rats
KA-	Saline treated rats
KA+/+	Rats treated with KA that experienced SE
KA+/-	Rats treated with KA that did not experience SE
KA-/-	Rats treated with Saline that did not experience SE
M	Mean
ml	milliliter
mg	milligram
MRI	Magnetic Resonance Imaging
PBS	Phosphate-Buffer Solution
PET	Positron Emission Tomography
PTSD	Post -Traumatic Stress Disorder
PVN	Para-ventricular Nucleus
TLE	Temporal Lobe Epilepsy
SA	Saline
SD	Standard Deviation
SE	Status Epilepticus
SEM	Standard Error of the Mean

THESIS PURPOSE

Temporal lobe epilepsy (TLE) is the most common type of epilepsy in humans (Hauser et al., 1991; 1993). The causes of TLE are mainly unknown; however, many individuals with TLE have a history of status epilepticus (SE) (Lothman & Bertram, 1993). SE is a condition characterized by seizures that recur for more than thirty minutes (de Lorenzo et al., 1995; de Lorenzo et al., 1996; Shorvon, 1996). SE leads to a variety of morphological changes in a variety of structures in the brain (Bernasconi et al., 2000; Bernasconi et al., 2003; Bernasconi et al., 2005; Du et al., 1993; Pitkänen & Sutula, 2002). However, of particular interest to this thesis is neuronal loss in the hippocampus of individuals who have experienced SE, also known as hippocampal sclerosis (Babb et al., 1984). Commonly, hippocampal sclerosis is manifested as a reduction in volume of the hippocampus (Lee, et al., 1995) and may be observed in individuals with TLE.

Although the hippocampus is known to be involved in numerous behaviours (Eichenbaum, 1999; 2000), there are few studies that have examined the relationship between hippocampal volume decrease resulting from SE and the subsequent behavioural changes (c.f. Pulsipher et al., 2006; Van Paesschen et al., 1997). Thus, the first experiment in this thesis will investigate the potential behavioural impairments that result from SE, by using a rat model of SE-induced epilepsy. This experiment will also examine the relation between hippocampal volume and behaviour, as well as the relation between spontaneous seizures and behaviour.

Similar to SE, exposure to stress has been associated with region-specific neuronal loss, with reductions in hippocampal volume in human and non-human animals

(Czeh, et al., 2001; Gurvits, et al., 1996; Starkman et al., 1992). As well, behavioural impairments are also observed following stress, presumably related to hippocampal volume decreases associated with stress (Starkman et al., 1992; Starkman et al., 1999). Thus, reductions in hippocampal volumes occur as a result of both SE and stress. Therefore, the second experiment of this thesis will examine the potential effects that stress has on SE, by investigating the effect that stress has on SE, with respect changes in hippocampal volume and associated behaviours. As in experiment 1, a rat model of SE-induced epilepsy will be used.

GENERAL INTRODUCTION

Definition of Epilepsy

Epilepsy is a term given to many syndromes, which are all characterized by spontaneous recurrent seizures (Engel, 1996; Engel, 2001). Epilepsy is the second most common neurological disorder in humans (Engel, 1996) with a prevalence rate of approximately 1%, although for most individuals who suffer from epilepsy there is no known cause (Figure 1). However, for some individuals, particularly those who have seizure activity originating from temporal lobe structures, there is a history of either prolonged febrile seizures, trauma, or status epilepticus (SE) (Lothman & Bertram, 1993). As epilepsy develops over a period of time, it is the most commonly acquired chronic neurological disorder in adults (Engel, 1996).
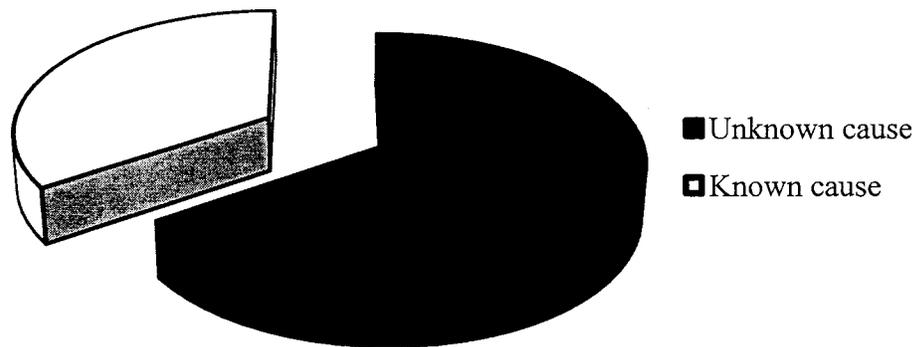
Figure 1. The proportion of individuals with known or unknown causes of epilepsy; in the majority of individuals the cause is unknown (data obtained from Hauser et al., 1993).

The word seizure has its basis in the Latin word saicre, meaning "to take possession of" (Lowenstein, 2005). Seizures are abnormal, excessive, and synchronous neuronal activity that is observed using electro-encephalographic (EEG) recording. Seizures may be accompanied by convulsions (involuntary movements) (Engel, 1996; Engel, 2001), which are involuntary movements. Convulsions can either be manifest sustained contractions of the muscles and /or oscillating contraction and relaxation of muscles. When contractions are sustained, it is referred to as the tonic phase. The oscillation of contraction and relaxation is referred to as the clonic phase (Engel, 1996; Engel, 2001).

Of particular relevance to this thesis are individuals who have experienced SE, as I am studying a rat model of SE-induced epilepsy. SE is characterized by recurrent seizures that last for more than thirty minutes (de Lorenzo et al., 1995; de Lorenzo et al., 1996; Shorvon, 1996). SE has a prevalence rate of approximately 1% (Corey et al., 2004; de Lorenzo et al., 1995; de Lorenzo et al., 1996). There is a high mortality rate (20-60%) associated with SE (Logroscino et al., 2005; Shorvon, 1996). However, of those who survive SE, many will develop TLE after a seizure-free latent period; this latent period may last from few months to as long as a year (French et al., 1993; Lothman & Bertram, 1993; Spencer & Spencer, 1994; Mathern et al., 1995). The progression of SE to TLE is sometimes accompanied by cognitive and anxiety disorders (Bernasconi et al., 2005; Pitkänen & Sutula, 2002).

Classification of Epilepsy

Epilepsy is categorized into a number of subtypes based on the type of seizures and their clinical manifestations (Engel, 2001). Seizures are classified as either partial (also referred to as focal) or generalized (Figure 2). Partial seizures are those in which the seizure activity is restricted to one discrete area of the brain. The symptoms of partial seizures may vary depending on the site of the epileptic focus (i.e. where the seizure activity originates from within the brain). Generalized seizures occur when the seizure activity becomes widespread throughout the brain. Generalized seizures can be more severe, with symptoms ranging from convulsions to loss of consciousness (Engel, 2001).

Epilepsy

|

Spontaneous recurrent seizures

|

Localized ?

Yes   No

Partial   Generalized

Consciousness ?

Yes   No

Simple   Complex

Seizure origin?

Temporal lobe structures

Temporal Lobe Epilepsy
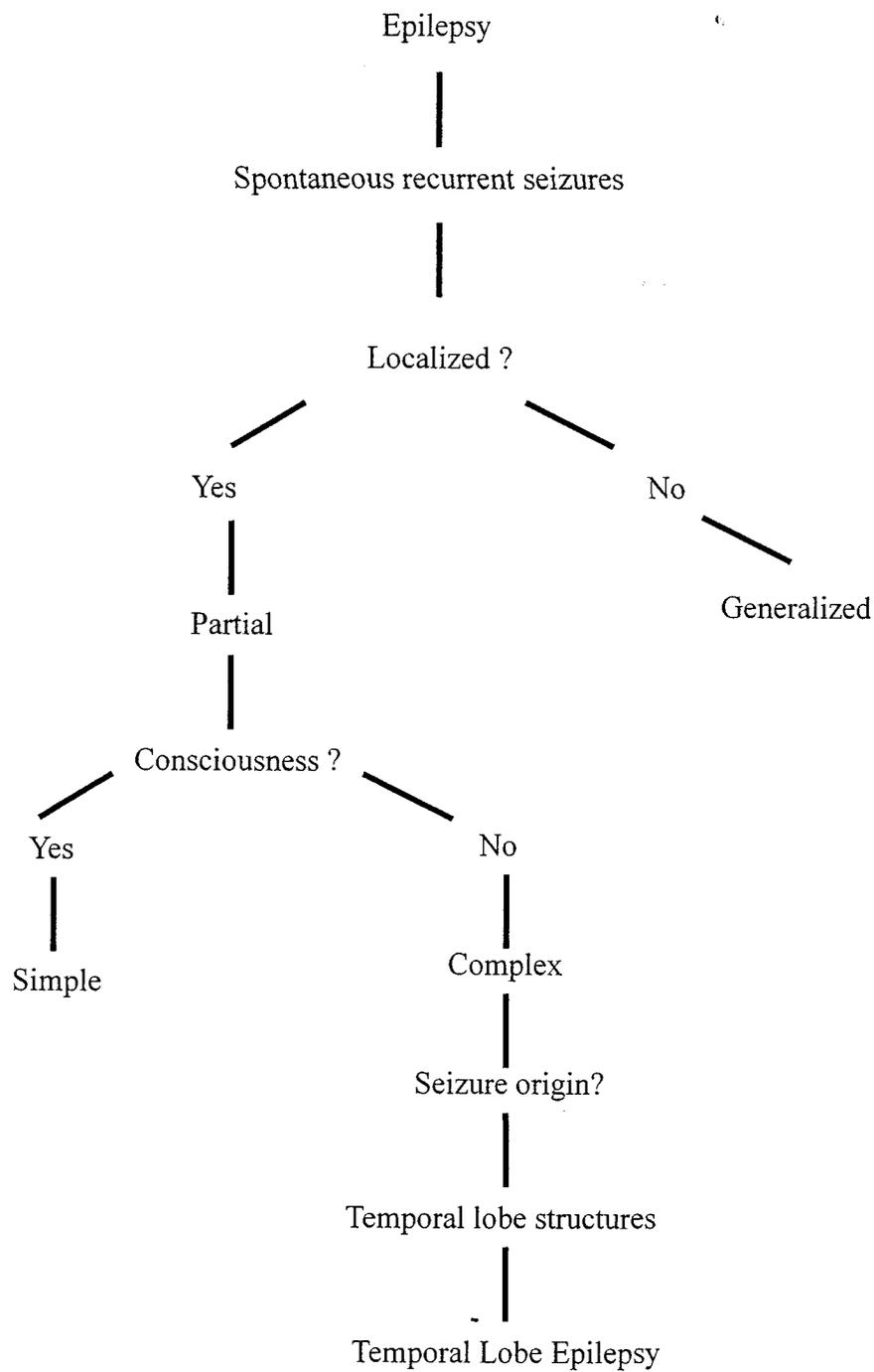
Figure 2. Classification of epilepsy based on the type of seizure activity.

Partial seizures can be further categorized based on the level of consciousness that is maintained during the seizure (Engle, 2001). When consciousness is fully preserved during the seizure, the seizure is considered 'simple'; hence, the seizure is classified as a partial simple seizure. On the other hand, if consciousness is impaired, the seizure is considered more 'complex' and hence it is classified as a partial complex seizure (Engel, 2001; Wieser, 2004).

Temporal Lobe Epilepsy

Partial complex seizures are the most common type of seizures and account for 40- 50% of all forms of epilepsy (Löscher & Schmidt, 1993; 1994). The majority (70-85%) of complex partial seizures originate from the medial temporal lobe structures, including the hippocampus and the amygdala (French, et al., 1993; Engel, 1996). Thus, complex partial seizures are often referred to as Temporal Lobe Epilepsy (TLE) (Löscher, 2002; Wieser, 2004).

Status Epilepticus and Neuronal Loss

As mentioned earlier, many individuals with TLE have a history of prolonged febrile seizures, trauma, or SE. Individuals with TLE tend to exhibit characteristic patterns of neuronal loss, which was first described by Sommer in 1880 (as cited in Gates & Cruz-Rodriguez, 1990). These include reductions in the medial temporal lobe, which occur in about 60 – 70 % of the individuals with TLE (Babb et al., 1984). Specifically, reductions may be due to damage to the hippocampus (Hermann et al., 2005; Lee et al., 1995; Pulsipher et al., 2006; Van Paesschen et al., 1997). The damage may be rather specific to areas within the hippocampus, as Babb et al., (1984) found cell loss of up to

60% of granule cells, 60% of CA3 pyramidal cells, 60% of CA2 pyramidal cells, and 80% of CA1 pyramidal cells in individuals with TLE.

Similarly, SE is associated with morphological change in the mesial temporal lobe (Babb et al., 1991; Sperk, 1994). Specifically, hippocampal sclerosis, or the loss of neurons in Cornu Ammonis (CA) 1, CA3 and dentate gyrus of the hippocampus, is associated with reductions in the volume of the hippocampus (Babb et al., 1991; Pirttila et al., 2001). Individuals who died from SE had 44% fewer pyramidal cells in CA1 and 65% fewer pyramidal cells in CA3, as compared to age-matched controls (deGiorgio et al., 1992). Thus, SE leads to a specific pattern of neuronal loss in the hippocampus that is similar to the pattern observed in individuals with TLE.

The reduction in hippocampal volume may be associated with decreased hippocampal function in individuals with TLE (Bernasconi et al., 1999; Bernasconi et al., 2003; Bortz, 2003; Du, et al., 1993; Hermann et al., 1997). For instance, there is an association between hippocampal atrophy and memory impairments observed in individuals with TLE (Pedersen & Dam, 1986). Notably, since SE is associated with damage to temporal lobe structures similar cognitive impairments may be associated with SE.

Although the hippocampus is the structure that is grossly affected by TLE, extra-hippocampal damage can be detected as well (Bernasconi et al., 1999; Bernasconi et al., 2003; Du, et al., 1993). Reductions in the perirhinal cortex (Bernasconi et al., 1999), entorhinal cortex (Due et al., 1993; Salmenperä et al., 2000), and amygdala (Bernasconi et al., 2003) are also observed. These reductions in volume may lead to different behavioural impairments observed in individuals with TLE (Abrahams et al., 1997;

8

Schwarcz & Witter, 2002; Vannest et al., 2008; Zentner et al., 1999). All of these behavioural changes are reviewed below.

Behavioural Impairments Associated with Temporal Lobe Epilepsy

Beyond the seizures themselves, TLE is often associated with behavioural changes (Butterbaugh et al., 2005; Devinsky & Vasquez, 1993; Engelberts et al., 2002) that include cognitive deficits, emotional impairments, and psychosocial problems (Dodrill, 1986; 2002 a; 2002 b). TLE mostly affects mesial temporal structures, such as the hippocampus, amygdala and perirhinal cortex. Thus, the nature of the observed impairment may be dependent on the structure that has been the most affected by TLE.

Cognitively, TLE has been associated with progressive memory loss (Helmstaedter., 2002; Helmstaedter & Kockelmann, 2006; Helmstaedter & Kurthen, 2001; Helmstaedter et al., 2003), including both verbal and non-verbal memory (Aikia et al., 1995; Giovagnoli et al., 2005), and spatial reference memory and short-term memory (Abrahams et al., 1997; Grippo et al., 1996). These types of deficits are thought to reflect hippocampal damage, whereas deficits in face recall or deficits autobiographical memory are thought to reflect damage to the perirhinal cortex (Dupont, et al., 2000 ; Hermann et al.,1997; Lah et al., 2006; O'Brien et al., 2003).

Emotionally, individuals with TLE exhibit approximately 24-72% lifetime prevalence of affective disorders (Blumer et al., 1995; Silberman et al., 1994; Victoroff, 1994); including fear and anxiety disorders (Dodrill & Batzel, 1986; Torta & Keller, 1999). Psychosocially, individuals with TLE have significantly more problems with anxiety, depression and self-esteem when compared to their age-matched controls

(Altshuler et al., 1999; Barry et al., 2000; Cockerell et al., 1996; Hermann et al., 2000; Jacoby et al.,1996). These deficits are thought to reflect amygdala damage.

Finally, individuals with TLE, as well as those who have experienced SE, exhibit motor and attentional speed deficits (Hernandez et al., 2002; Piazzini et al., 2006). This is thought to reflect damage to the motor system of the brain.

A variety of factors are noted to enhance the strength and degree of these impairments, including: earlier age of seizure onset (Alessio et al., 2004; Lespinet et al., 2002; O'Leary et al., 1981; O'Leary et al., 1983), which is related to increased time since diagnosis of TLE (Alessio et al., 2004); and higher seizure frequency (Dodrill, 1986). Individuals with earlier onset of seizures exhibit more severe impairments, particularly in verbal and non-verbal memory tasks (Lespinet et al., 2002; O'Leary et al., 1981; O'Leary et al., 1983). Frequency (Dodrill, 1986) and duration (Jokeit & Ebner, 1999) of seizures was positively correlated with cognitive impairments such as disturbances in spatial memory (Bortz, 2003). As the duration and the number of generalized tonic-clonic seizures that an individual experienced were positively associated with reduction in hippocampal volume (Hermann et al., 1997; Pulsipher et al., 2006), suggesting that hippocampal pathology may lead to cognitive impairments associated with TLE.

Thus, as reviewed nature of the observed deficit(s) depend(s) on factors as varied as including age of onset, seizure frequency, and site of damage. However, many of these factors cannot be manipulated in individuals with TLE. Thus, the use of animal models to identify the extent and the nature of these impairments is essential.

Methods of Studying Temporal Lobe Epilepsy.

Ethical considerations associated with studying humans' result in largely pseudo-experimental or correlational studies predominating in human research on TLE. One way to perform experimental studies of TLE is to use animal models of TLE. There are several advantages associated with the use of animal models. As noted above, factors such as drug history, the age of onset of seizures and focus of seizures can be controlled and manipulated when using animal models. As such, a variety of animal models have been developed to study TLE and its associated behavioural impairments (c.f. Coulter et al., 2002; Löscher, 1997). Among the different animal models, kindling and kainic acid (KA) are the most common. Kindling is a procedure in which repeated electrical stimulation triggers progressively stronger seizure responses (Goddard et al., 1969). In the KA model, the rat experiences a single bout of SE and develops spontaneous convulsions after a latent period. Very little is known about the behavioural effects of KA. Thus, this thesis will focus on only the KA model.

Kainic Acid Model of TLE

KA was isolated in 1950 from the seaweed *Digenea simplex* (Ben-Ari, 1985; Sperk, 1994). KA is a glutamatergic agonist that is neurotoxic and it produces excessive neuronal excitation (Coyle, 1983). Injections of KA to specific sites in the central nervous system result in lesions that spare axons that are passing through the injected site (Coyle et al., 1978; Coyle et al., 1981) but kills the cell bodies that are in the injected site. Olney et al. (1979) found that intra-peritoneal (i.p.) administration of KA in immature mice caused rapid necrotic changes in the soma and dendrites of neurons. However, afferent fibers and axons in the necrotic area appeared normal (Onley et al.,1979). Of

11

relevance to this thesis, injection of KA leads to prolonged SE (Nadler & Cuthbertson, 1980). SE is observed following injections of KA into either cerebrospinal fluid (Nadler & Cuthbertson, 1980), the hippocampus (Cavalheiro et al., 1982; Nadler et al., 1980; Tanaka et al., 1992), amygdala (Ben Ari & Lagowska, 1978) or an intraperitoneal (i.p.) injection of KA (Ben- Ari, 1981; Buckmaster & Dudek, 1997) lead to the development of the KA model of TLE, as injection(s) of KA lead(s) to development of spontaneous seizures in rats.

The morphological changes that take place in the brain following KA-induced SE resemble those observed in the humans with TLE (Cendes et al., 1993; Cendes et al., 1995; Teitelbaum et al., 1990). Similar to TLE, KA-induced SE often results in the occurrence of lesions, especially in the hippocampus, the amygdala and the entorhinal cortex (Ben-Ari et al., 1979; Sperk et al., 1985). The pattern of neuronal loss and morphological alterations resemble those that have been seen in human TLE (Morimoto et al., 2004; Wieser, 2004).

In order to induce TLE, KA can be injected systemically as either a single large dose (usually between 8-15 mg) or as several low doses (usually 3-5 mg) (Buckmaster & Dudek, 1997). Approximately 5 minutes after the injection, rats stop moving and begin to stare. About 9 minutes after the injection, the first epileptic activity is detected by EEG (Tuunanen et al., 1999). About 20 minutes after the injection the epileptic activity spreads to the motor cortex and wet-dog shakes, chewing and head nodding can be observed. As the epileptic activity progresses throughout the motor cortex, rats rear and lose postural control. Within about one hour after the injection the first signs of SE are observed. If there is no intervention, SE usually lasts for about four to six hours. Within about 4-6

12

weeks after cessation of SE, KA-treated rats often exhibit spontaneous convulsions (Cavalheiro et al., 1982; Tanaka et al., 1988). Due to this long latent phase, Löscher (2002) called the model the post-SE model of partial complex epilepsy.

Even though there is not much information available about the nature of the brain's electrical activity during SE, Marthern et al. (1993) measured the time course of hippocampal interictal seizure activity after intra-hippocampal KA injection. Four different stages were identified following injection of KA: (1) the acute phase (first 10 days post KA-induced SE), (2) the active phase (10-30 days), (3) the latent phase (30-90 days), and (4) the chronic phase (after 90 days). They found that the frequency of the seizure activity decreased during the latent phase and increased again during the chronic phase. The increase of seizure activity in the chronic phase was correlated with the development of seizure activity originating from the hippocampus and the neuronal loss in the hippocampus. These findings indicate that there is an association between the different stages of development of the TLE following SE and the morphological change in the hippocampus.

Some of the difficulties associated with the KA-model of TLE is that it is highly variable, as rats from the same litter may respond differently to the same dose of KA (Buckmaster & Dudek, 1997; Morimoto et al., 2004). In addition, various rat strains (Golden et al., 1991; Hort et al., 2000) at different ages (Sperber et al., 1991) respond differently to the drug. For instance, Wistar-Fuzh rats respond at lower doses and with shorter latency to KA relative to Long-Evans or Sprague- Dawley rats (Golden et al., 1991). Further, spontaneous seizure development following KA injection is also variable

(Morimoto et al., 2004; Sperk, 1994), making epileptogenesis difficult to control. Further, unlike many individuals with TLE, KA-induced SE can also result in widespread brain damage that is not always observed in humans with TLE (Wieser, 2004). Finally, the KA-model of TLE can result in high mortality rates (for review see Löscher, 2002; Sperk, 1994). Despite these difficulties, the KA-model of TLE remains very popular.

Behavioural Impairments Associated with SE

In rodents, SE has been associated with numerous behavioural impairments, particularly in the memory domain. Rats and mice that experienced SE were impaired in both short-term and long-term visual-spatial learning and memory (Cilio et al., 2003; Detour et al., 2005; Gröticke et al., 2008). The deficits in spatial memory correlated positively with the severity of hippocampal damage (Cilio et al., 2003; Letty et al., 1995). As well, rats that have experience SE also exhibited reductions in motor function, perhaps due to hyperactivity (Kubova et al., 2000) which is known to be associated with hippocampal damage (Whishaw & Jarrard, 1995). Thus, similar to what was observed with humans with TLE, SE is associated with hippocampal damage and with expected behavioural impairments.

The behavioural impairments associated with SE are not limited to hippocampally dependent tasks, but may extend to disturbances in extra-hippocampal areas such as perirhinal cortex. On a task of conspecific recognition, Lim et al. (2007) found that seizure susceptible mice were impaired in recognizing previously encountered conspecifics. However, this was not observed on a task of object discrimination, as Detour et al. (2005) failed to observe significant deficits in rats that had experienced SE.

However, it must be noted that different species and tasks were used, and that the kind of impairment that may be detected through these tasks may differ.

In summary, KA-induced SE is one of the most commonly used models of TLE. KA-induced SE leads to reductions in hippocampal and extra hippocampal volumes. These pathologies may be associated with memory (Lim et al., 2007) and motor impairments (Kobova et al., 2000; Lai et al., 2002). Thus, careful examination of the effects of SE on motor and memory tasks, as well as the relation between hippocampal pathology and performance on these tasks is warranted.

Temporal Lobe Epilepsy and Stress

Several lines of evidence suggest that stress may exacerbate seizure disorders. Many individuals with epilepsy exhibit an association between seizure frequency and stress (Schmid-Schonbein, 1998). Further, individuals with TLE may experience a substantial amount of stress (Galimberti et al., 2005), and have higher levels of the stress hormone cortisol (Calabrese et al., 1993; Gallagher et al., 1984; Galimberti et al., 2005). Interestingly, common morphological changes take place in the brain following either SE or chronic severe stress (Bernasconi et al., 2003; Gurvits et al., 1996; Lee et al., 1995; McEwen, 1999). When combined with the observation that structures that are commonly involved in TLE also play an essential role in the regulation of the stress response (Baram & Hatalski, 1998; Herman & Cullinan, 1997; Herman et al., 2003), it appears that there may be a relation between stress and TLE.

In animal models of epilepsy, corticosterone administration enhances the development of convulsions (Edwards et al., 1999; Edwards et al., 2000; Karst et al.,

1999; Taher et al., 2005; Talami et al., 1995; Weiss et al., 1993 a; Weiss et al., 1993 b). Similarly, exposure to stressful conditions is also associated with enhanced rates of seizure development. Interestingly, this increased susceptibility to seizures was blocked by spironolactone, which is a glucocorticoid receptor antagonist (Roberts & Keith, 1994 b). Thus, exposure to stress hormones is associated with enhanced seizure vulnerability in non-human animals.

In human and non-human animals, both SE and stress results in neuronal loss in the hippocampus. Moreover, the hippocampus plays a major role in development of seizures and regulation of stress response. Stressful conditions as well as exposure to corticosterone have an impact on the number of seizures in humans (Schmid-Schonbein, 1998) and seizure threshold in animal models (Edwards et al., 1999; Edwards et al., 2000; Karst et al., 1999; Taher et al., 2005; Weiss et al., 1993 a). Furthermore, these effects of stress can be reversed by corticosterone inhibitors (Roberts & Keith, 1994 a; Roberts & Keith, 1994 b; Talami et al., 1994). Thus, stress may intensify the expression of TLE.

Stress Response

Stress is a condition that happens in response to exposure to noxious situations, such as predator smell or cold. The stress response is defined as a series of physiological and behavioural alterations that are designed to lead to re-establishment of homeostasis (Herman et al., 2003). There are several ways to induce stress in animals. For instance, restraining the movement of rats by placing them in a tube, rotation of group membership, excessive exposure to cold or to predator smell, or maternal separation early in life are all common ways to induce stress in lab animals. All lead to excessive secretion of corticosterone into the bloodstream (Herman et al., 2003).

16

Stress responses involve activation of the neuro-endocrine system including the hypothalamus, pituitary gland, and adrenal gland, thus it is referred to as Hypothalamic, Pituitary Adrenal (HPA) axis (Figure 3). The end product of activation of the HPA axis is cortisol in humans and corticosterone in rodents. First, Corticotrophin Releasing Hormone (CRH) is released from Para-Ventricular Nucleus (PVN) of hypothalamus, thereby stimulating the anterior pituitary. The stimulation of anterior pituitary leads to the secretion of adrenocorticotropin hormone (ACTH) into the blood stream. Finally, ACTH reaches the adrenal cortex where it results in the production and secretion of glucocorticoids (Herman et al., 2003).

Hippocampus

↓ (-)

Para-ventricular nucleus (PVN) of hypothalamus

↓ (+)

Corticotrophin releasing hormone (CRH)

↓ (+)

Anterior pituitary

↓ (+)

Adrenocorticotropin hormone (ACTH)

↓ (+)

Adrenal cortex

↓ (+)

(-)   Corticosteroids

(-)

(-)

(-)
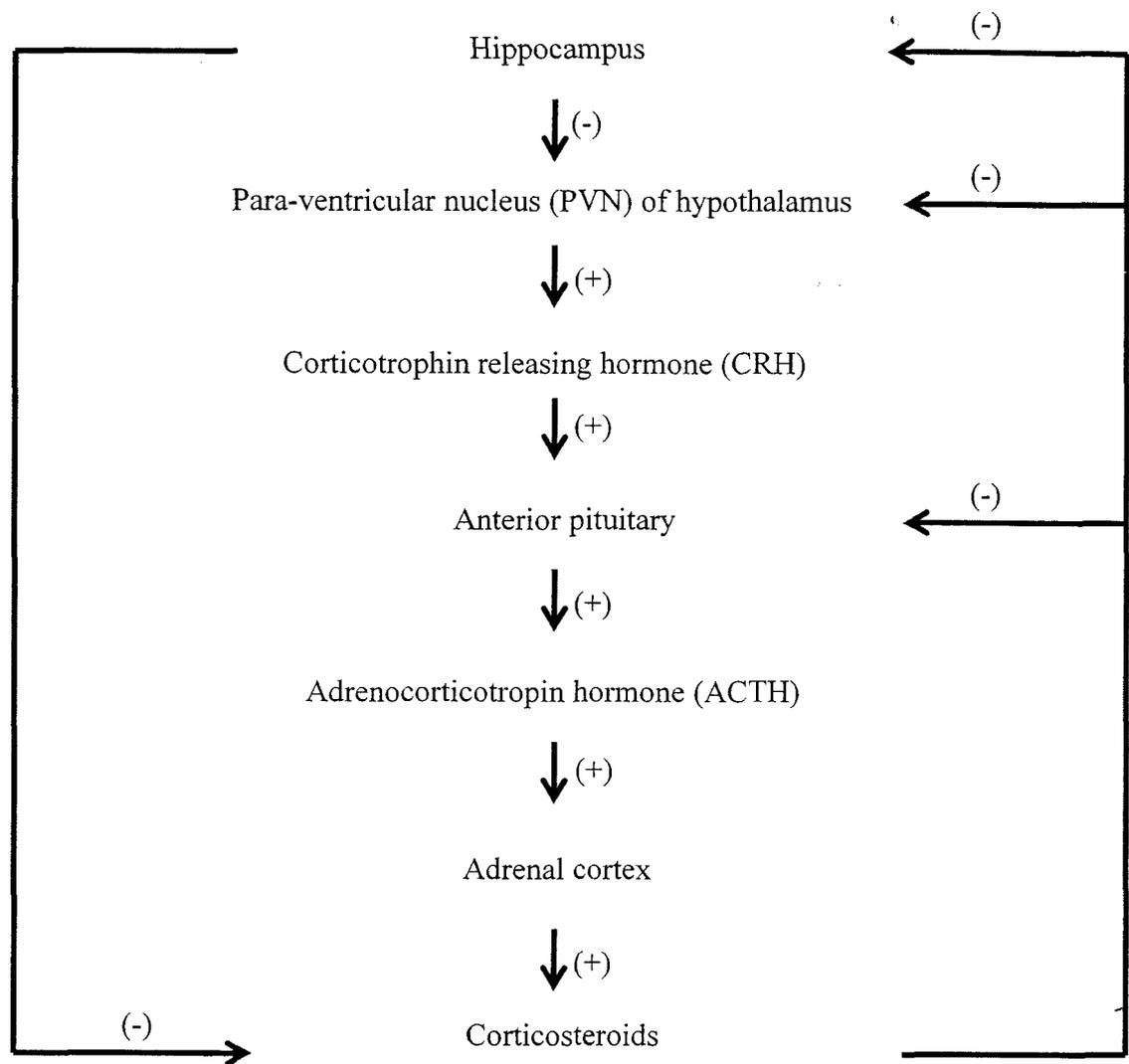
Figure 3. Components of the stress response. CRH is released from PVN, which then stimulates the anterior pituitary. The stimulation of anterior pituitary leads to the secretion of ACTH into the blood stream. Finally, ACTH results in secretion of corticosteroids from the adrenal cortex (positive signs). Secretion of corticosteroids into the blood stream controls the further production in a negative feedback through the suppression of PVN and the anterior pituitary (negative signs).

Glucocorticoids are steroid hormones that are secreted from the adrenal cortex in response to stress. These hormones are essential and adaptive under specific situations. However, prolonged exposure to glucocorticoids may increase the likelihood of pathological conditions such as insulin-resistant diabetes, hypertension and immuno-suppression (Sapolsky et al., 2000). As they are lipophilic, glucocorticoids can pass the blood brain barrier and affect the brain. Glucocorticoids act via membrane receptors and that are located in different regions in the brain, including the temporal lobe (de Kloet et al., 1998; Joels, 2001) and thus may affect temporal lobe functionally (Belanoff et al., 2001).

Hippocampus and Stress

The hippocampus and other temporal lobe structures play a central role in the regulation of stress response (Herman et al., 2003). The hippocampus regulates the secretion of glucocorticoids through negative feedback to the hypothalamus (Herman et al., 1989; Jacobson & Sapolsky, 1991; Sapolsky et al., 1984; Sapolsky et al., 1985; Sapolsky et al., 1991). The hippocampus has the highest level of corticosteroid receptors in the brain (de Kloet et al., 1998), suggesting that the hippocampus is one of the most sensitive structures to corticosteroid concentrations in the brain. Thus, repeated exposure to high levels of glucocorticoids leads to a down-regulation of hippocampal glucocorticoid receptors that in turn reduce the ability of the hippocampus to control glucocorticoid negative feedback.

In humans, MRI has revealed that there is a positive relation between exposure to combat-related stressors and hippocampal atrophy (Bremner et al., 1995; Gurvits et al.,

1996). Similarly, individuals with Cushing's syndrome, a condition that results in excessi2ve secretion of glucocorticoids, exhibit a decrease in hippocampal volume (Starkman et al., 1992; Starkman et al., 1999). Thus, excessive secretion of corticosteroids has been associated with hippocampal volume loss in humans.

Prolonged stress has been associated with cellular atrophy of the hippocampus in monkeys (Uno et al., 1989) and in rats (McEwen, 1999; Sapolskey et al., 1985). Microscopic examination revealed irreversible shortening of the dendrites in the CA3 region of the hippocampus (Magarinos & McEwen, 1995; McEwen, 1999; Woolley et al., 1990). These reductions in hippocampal volume can be as much as approximately 50% (Luine et al., 1994). Thus, there is an association between secretion of corticosteroids and changes in hippocampal morphology. Damage to the hippocampus exhibit enhanced corticosterone secretion (Fendler et al., 1961; Knigge, 1961; Knigge & Hays, 1963). Hence, the hippocampus is very sensitive to changes in the concentration of the stress hormones in the bloodstream.

There are two main types of receptors in the brain for the hormones that are secreted from the adrenal gland (de Kloet et al., 1998). These two receptors are different in their affinity and localization in the brain. Mineralocorticoid receptors have about ten times higher affinity for cortisol when compared to glucocorticoid receptors (de Kloet et al., 1998). Thus, during a stressful situation the mineralocorticoid receptors become saturated first. During prolonged exposure to stress, glucocorticoid receptors play a larger role, as the mineralocorticoid receptors can no longer respond.

20

Cognition and Stress

In humans, experiencing stressful conditions or being exposed to glucocorticoids chronically can lead to impairments in cognitive processes, particularly memory (de Kloet et al., 1999). Chronic treatment with glucocorticoids or those with Cushing's syndrome both exhibit disruptions in declarative memory (Keenan et al., 1996; Starkman et al., 1992; Waber et al., 2000). Sustained stress or prolonged exposure to glucocorticoids also decreases performance in hippocampal dependent tests, such as spatial memory tasks (Keenan et al., 1996; Starkman, 1992; Waber et al., 2000).

In rodents, impairments can be detected in different stages of learning and memory (Oitzl & deKloet, 1992), such as acquisition (Oitzl et al., 2001) or the retrieval of the information (de Quervain et al., 1998; de Quervain et al., 2000; Roozendaal et al., 2003). For instance, de Quevain et al. (1998) observed that rats with higher levels of corticosterone exhibited impaired spatial ability. Impairments in spatial ability has been observed in a number of different spatial tasks following either exposure to stressors or exogenous application of corticosterone (Luine et al., 1994; Luine et al., 1993). Infusion of RU-486 (a glucocorticoid antagonist) into the dorsal hippocampus led to improved spatial ability (Oitzl et al., 2001). All together these findings suggest that chronic exposure to either exogenously administered corticosterone or exposure to stressful conditions lead to disruption of spatial memory.

Epilepsy and Stress

Stress has been shown to affect the expression of epilepsy in animal models. Corticosterone administration enhances the development of seizures both in kindling and

21

KA models (Edwards et al., 1999; Edwards et al., 2002; Karst et al., 1999; Lee et al., 1989; Taher et al., 2005; Weiss et al., 1993 a; Weiss et al., 1993 b). Interestingly, this effect was reversed when the glucocorticoid antagonists were administered (Talmi et al., 1995). For instance, Karst et al. (1999) found that high doses of corticosteroids (100 mg/day, subcutaneous) in rats were associated with lower numbers of stimulations required to reach a stage 5 seizures. However, the dose of corticosterone that was administered in this study was much higher than the physiological level during a stress response. Taher et al. (2005) administered a much lower dose (3 mg/ 100 ml of drinking water in 4.5 mg/kg/day). They found that electrical kindling in amygdala was facilitated in the corticosterone-treated group. These findings indicate that chronic exposure to corticosterone before kindling procedure enhances epileptogenesis by lowering the seizure threshold. Similarly, exposure to stressful conditions lowers the seizure threshold. For instance, Salzberg et al. (2007) used kindling of the amygdala and found that maternal separation early in life is associated with enhanced rates of kindling. Thus, maternal separation early in life is a stressor that facilitates the development of seizures later in life.

Adrenal glands as the source of steroid hormones in the body play an important role in the seizure threshold. The removal of adrenal gland reduces the rate of seizure development. Cottrell et al. (1984) found that removal of adrenal glands reduced the number of stimulations required to reach stage 5 seizures in hippocampus. Edwards et al. (1999) found that higher doses of corticosterone (30 μg/ml in drinking water) are associated with lower seizure threshold in adrenalectomized rats.

The findings in the kindling model are also observed in the KA model of TLE. Roberts & Keith (1994 a) tested adult male mice that were administered with corticosterone (1 mg/kg) one hour prior to intravenous KA administration. They found that corticosterone treated mice had lower seizure threshold. Interestingly, this increased susceptibility was blocked by spironolactone (5 mg/ kg), which is a glucocorticoid receptor antagonist (Roberts & Keith, 1994 a). It is deducible then, that corticosterone reduces the seizure threshold both in kindled and KA animal models; further, inhibition of steroid hormonal pathways reverses this process.

Similarly, Lai et al. (2006) tested the effects of maternal separation on SE. The rats that experienced maternal separation early in life had higher HPA axis activity and thus higher levels of corticosteroid secretion in response to SE. Seizure threshold was assessed by the dosage of pentylenetetrazol (PTZ) that was required to induce the first clonic convulsion. The rats that had experienced separation early in life had reduced seizure threshold in response to PTZ (Lai et al., 2006). Interestingly, metyrapone, a corticosteroid synthesis inhibitor, reversed the effects of maternal separation (Lai et al., 2006).

Both SE and stress results in neuronal loss in the hippocampus. Moreover, hippocampus plays a major role in development of seizures and regulation of stress response. Stressful conditions as well as exposure to corticosterone have an impact on the number of seizures in humans and seizure threshold in animal models. Furthermore, these effects of stress can be reversed by corticosterone inhibitors. Thus, stress may intensify the expression of TLE.

Rationale

SE is associated with morphological alterations in the brain and in behavioural change that is associated with these neural pathologies (Pitkänen & Sutula, 2002). However, the nature of these behavioural alterations is not very well defined. Thus, the first goal of this thesis was to replicate previous findings regarding the hippocampal volume reduction and the development of spontaneous convulsions as a result of experiencing KA-induced SE. In addition, possible behavioural deficits associated with KA-induced SE were investigated using a battery of behavioural tests (including those that measure motor skill, spatial cognition and object discrimination memory). Based on the human and rodent literature, it was hypothesized that rats that experienced SE would be impaired on all of these tests (Figure 4).

As structures such as the hippocampus are strongly affected by SE and stress and they play an essential role in the regulation of the stress response (Herman & Cullinan, 1997), the second goal of this thesis was to investigate how stress affects hippocampal volume, the frequency of spontaneous convulsions, as well as possible behavioural deficits. The second experiment was also designed to examine the compound effects that stress and KA-induced SE had on hippocampal volume, the frequency of spontaneous convulsions, and possible behavioural deficits, using a subset of behavioural tests from Experiment 1. It was hypothesized that there would be an additive effect of SE and stress on hippocampal volume. That is, rats that experienced SE and were exposed to stress would exhibit the lowest hippocampal volumes; rats that experienced SE with no exposure to stress or control rats exposed to stress would exhibit intermediate hippocampal volume; and finally, control rats with no exposure to stress would exhibit

the largest hippocampal volume. Behaviourally, it was hypothesized that rats that experienced SE and exposed to stress would exhibiting the most impairment; intermediate impairments would be observed for either the group of rats that experienced SE with no exposure to stress or the control rats that were exposed to stress; and finally control rats with no exposure to stress would exhibit the least amount of impairment.
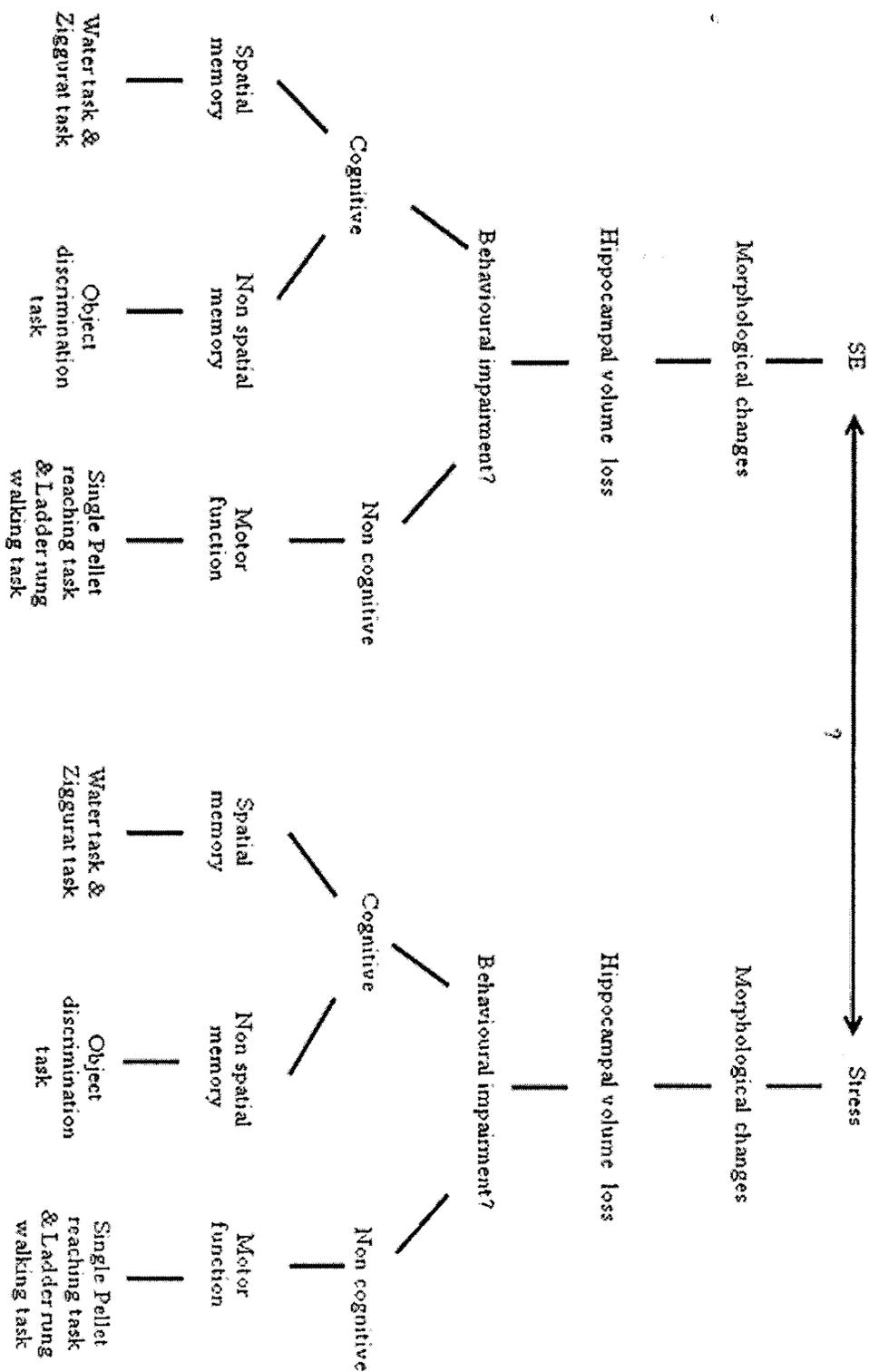
Figure 4. Compound effects of SE and stress on the brain and behaviour.

EXPERIMENT 1. KAINIC ACID INDUCED STATUS EPILEPTICUS AND BEHAVIOUR

Introduction

KA-induced SE is one of the most commonly used animal models of TLE (Sperk, 1994). The morphological changes that take place in the brain following SE, such as hippocampal volume reduction or sclerosis, resemble those detected in the tissue obtained from individuals with TLE (Wieser, 2004). Thus, it was hypothesized that rats with KA-induced SE would have decreased hippocampal volume when compared to saline (SA) treated controls.

It has been shown that the extent of SE-induced damage is positively correlated with both: the duration of SE (Pitkänen & Sutula, 2002); and the number of spontaneous seizures (Kotloski et al., 2002; Sutula et al., 1995). Thus, it was hypothesized that there would be a positive correlation between the duration of SE and the amount of hippocampal volume loss. Further, it was hypothesized that there would be a positive correlation between the number of spontaneous convulsions and the amount of hippocampal volume loss.

SE is associated with deficits in spatial memory, typically as measured by the water task. It was hypothesized that KA-treated rats that experienced SE would have a longer latency in finding the hidden platform in water task when compared to controls. To further investigate the nature of the spatial memory deficit, the rats were tested on another hippocampally dependent spatial task called the ziggurat task. The ziggurat task is a food-motivated task that requires rats to learn the location of the food, which is

hidden on one of the raised platforms (ziggurats), using distal cues (Faraji et al., 2007). It was hypothesized that KA-treated rats that experienced SE would have a longer latency to find the goal ziggurat when compared to controls.

As KA-induced SE has also been associated with impairments in motor tasks (Kubova et al., 2000) and KA-induced SE has been associated with hippocampal damage which may lead to hyperactivity (Whishaw & Jarrard, 1995; Good & Honey, 1997). Thus, more complex motor tasks were incorporated, such as the ladder rung walking task (Metz & Whishaw, 2002) and single pellet reaching task (Whishaw et al., 1993) into this experiment. It was hypothesized that the KA-treated rats would display less dexterity and would fall more often when compared to controls on the motor tasks.

The damage associated with KA-induced SE is not typically limited to the hippocampal region, as it potentially involves damage to areas such as the perirhinal cortex (Du et al., 1993), and the amygdala (Moore & Baker, 2002). As the perirhinal cortex is known to be involved in discrimination memory (Brown & Aggleton, 2001; Ennaceur & Aggleton, 1997; Suzuki et al., 1993), the rats were tested on a task of object discrimination memory (Ennaceur & Delacour, 1988). It was hypothesized that KA-treated rats would show a reduced object discrimination memory when compared to controls.

Methods

Subjects

Adult male Long–Evans hooded rats (Charles River) weighing 350–450 g at the beginning of the study were used as subjects. Rats were maintained in pairs in Plexiglas cages throughout the course of experiment. All experimental procedures were carried out during the light portion of the 12:12 hour light/dark cycle. During testing periods, rats were fed 15- 20 g of laboratory chow after daily behavioral sessions to maintain their weights at 85–90% of free-feeding body weight. Water was available *ad libitum* throughout the experiment.

All rats were handled at least once every 48 hours throughout the experiment. All procedures were in accordance with the guidelines established by the Canadian Council of Animal Care as approved by the University of Lethbridge Animal Care Committee.

Rat Treatment

Each rat was randomly assigned to one of two different groups. The kainic acid (KA+) group ($n=15$) received a single injection of KA (10 mg/kg, dissolved in 1 ml/kg SA, injected i.p.). A second group of rats ($n=15$) received a single dose of SA (SA; 1 ml/kg) and served as control group (KA-). Immediately following the KA or SA injection, rats were monitored for a minimum 6 hours. Monitoring continued until convulsions were not observed for a period of 30 minutes. Epileptic activity (convulsions) was video recorded (Camera model: Canon Zr 500) for further analysis.

Convulsion Monitoring

Rats were monitored in boxes that were made of corrugated plastic that was 0.4cm thick. The boxes were 42 cm × 42 cm × 41cm (L × W × Ht) (Figure 5).
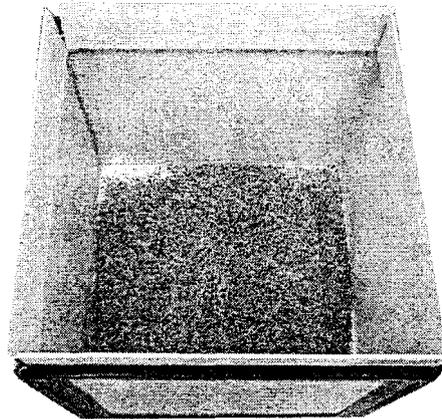
Figure 5. Boxes made out of corrugated plastic to monitor rat's convulsive behavior.

Monitoring lasted for one hour every other day for the first 14 days, and then daily for the remainder of the experiment. The time of the monitoring was selected randomly and was distributed throughout the day. When observed, convulsions were scored according to the following scale: stage 2 convulsions (wet-dog shakes); stage 3 convulsions (unilateral forelimb clonus); and stage 4 (bilateral forelimb clonus); and stage 5 (rearing and falling, interposed with hyperactivity and jumping) (Racine, 1972). The dependent variables were the number of convulsions and the duration of each convulsion.

Behavioural Assessment

Two weeks after the rats had been injected with either KA or SA they were assessed on a battery of tests to detect changes in the sensory and motor functions (see

Figure 6). In the case that there were spontaneous convulsions during the testing the rats were placed back to a holding cage and waited for a period of twenty minutes before it was tested again.

Week I	Week II	Week III	Week IV	Week V	Week VI	Week VII	Week VII

KA Injection

SA Injection

Single Pellet Reaching Task Training

Single Pellet Reaching Task Testing

Ladder Rung Walking Task

Object Discrimination Task Habituation

Object Discrimination Task Testing

Water Task Testing

Ziggurat Task Testing

32
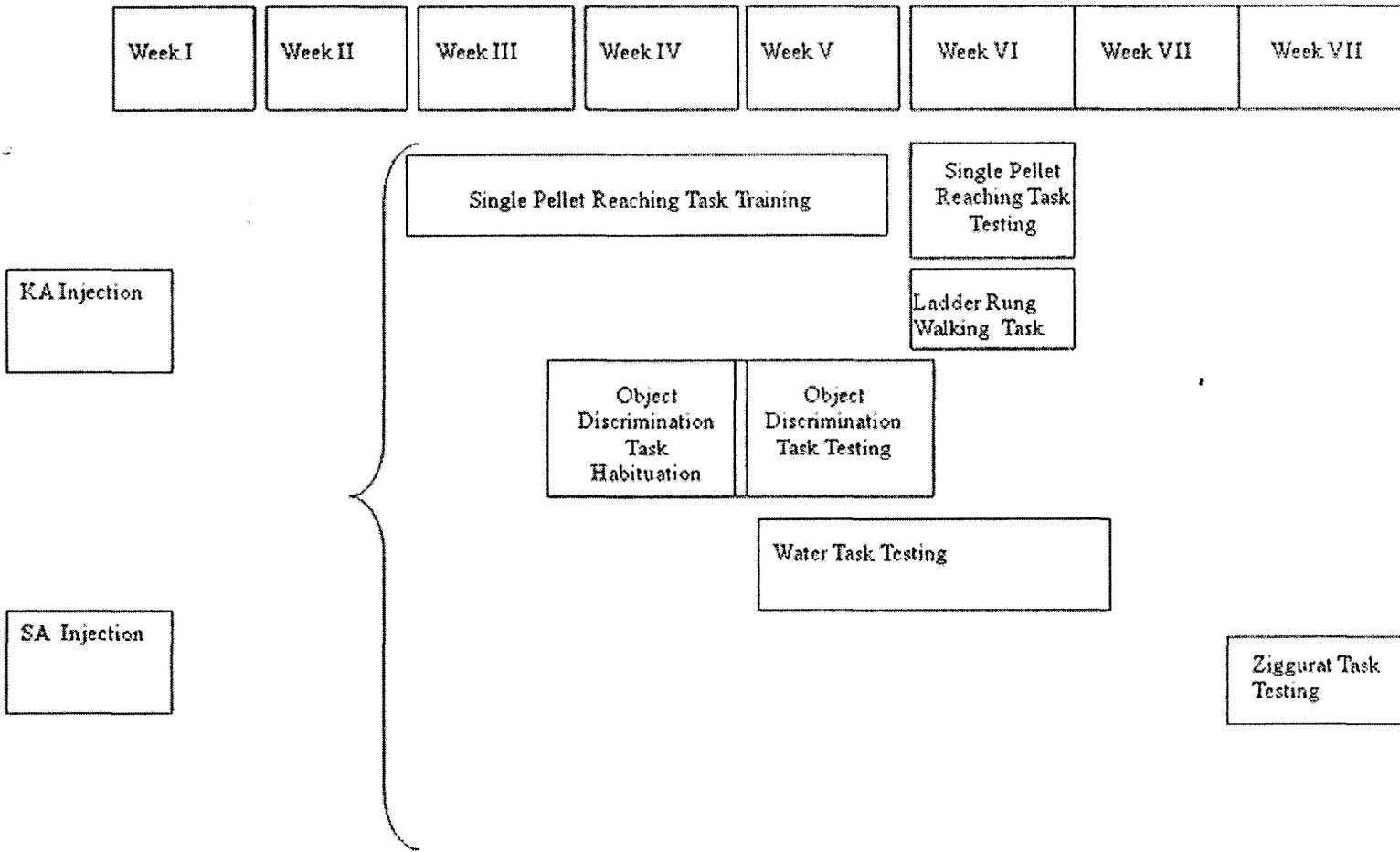
Figure 6. The schedule and order of behavioural testing for Experiment 1.

Single pellet reaching task. This is a task of forelimb function (Whishaw et al., 2003). Rats were placed in a clear Plexiglas box (40 cm × 14 cm × 30 cm high) and filmed from the front (as described by Whishaw et al., 1993). At the centre of the box was a 1 cm wide slit, extending from the floor to a height of 15 cm. On the outside of the box, in front of the slit was a 2 cm wide by 4 cm long shelf (3 cm above the floor) that had 2 indentations that were 2 cm to the right and left of the centre.

Fourteen days after the injection of either SA or KA, rats performed the task for 23 days (21 days of training and 2 days of testing). For the first two days of training, food pellets (50 mg Rodent Chow food pellets, Bioserve Inc., Frenchtown, NJ) were placed in both of the indentations, for 20 trials per day. Following that period, only one pellet was used, which was placed in the indentation contralateral to the limb that the rat had previously preferred to reach with. Training was administered in such a way that when a rat made a successful reach, a short pause (of approximately 10-15 second) preceded presentation of the next food pellet. During the break, the rat ate the pellet and then walked to the back of the box. Finally, the rat repositioned itself at the food aperture for the next trial. All testing took place in the afternoon (between 1 pm and 4:30 pm).

Successful reaches occurred when a rat made more than one reaching movement in which a paw was inserted through the aperture of the cage (Figure 7), resulting in consumption of the food. Single successful reaches occurred when a rat made a single reaching movement in which a paw was inserted through the aperture of the cage, grasped the food with the first attempt and consumed it. Often, however, rats would make more than one attempt before grasping the pellet and consuming it (total attempted

33

reaches). The dependent variables for reaching performance were the total of the
attempted reaches and the number of single successful reaches.
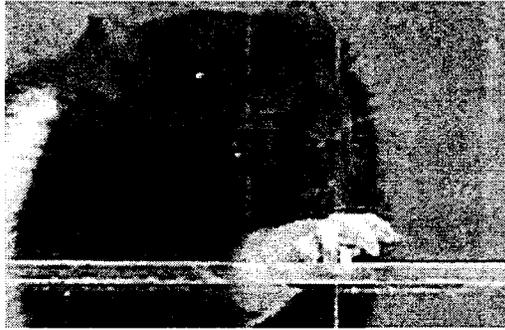
Figure 7. An example of a rat performing a successful reach (modified from Whishaw et
al., 2003).

Ladder rung walking task. This is a task of forelimb and hindlimb function as
well as coordination (Metz & Whishaw, 2002). The rat was placed on a horizontal ladder
rung consisting of side walls made of clear Plexiglas and metal rungs (3 mm diameter) on
the floor that were a variable distance apart (minimum=1 cm apart, maximum=4 cm
apart, see Figure 8). The side walls were 1 m long and 19 cm high measured from the
height of the rungs. The ladder was elevated 30 cm above the ground with a refuge (home
cage) at the end. The width of the alley was adjusted to the size of the rat, so that it was
about 1 cm wider than the rat (Figure 8). This prevented the rat from turning around and
returning to the start position.
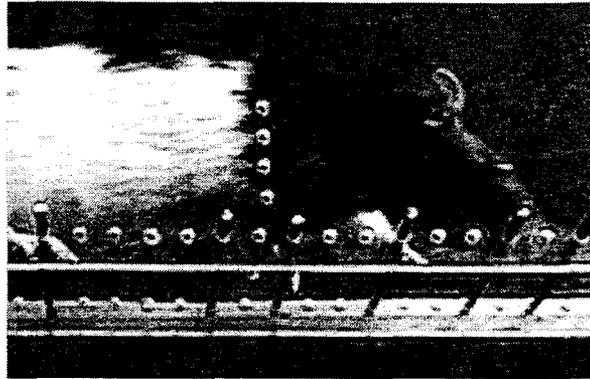
Figure 8. An example of a rat performing the ladder walking task (modified from Metz &
Whishaw, 2002).

Thirty-five days after the injection of either KA or SA, rats performed three

habituation trials within one day. During habituation, the rat was placed in the middle of

the alley and had to walk on the rungs to its home cage, which was located at one end of

the alley. The next day, rats performed 3 test trials of ladder walking that were video-

taped for scoring at a later date (Camera model: Canon Zr 500). The test trials were the

same as the habituation trials, except that the start point was at the opposite end of the

alley from the home cage rather than the middle of the alley. All testing took place

between 10 am and 1pm. The dependent variables for ladder rung walking were the

number of falls and repositions for each limb. A fall occurred when a limb slipped

completely between the rugs. A reposition occurred when an abnormally positioned limb

was corrected, or repositioned, on the rung without losing contact with the rung.

Object discrimination task. This is a task of the rat's ability to discriminate

between a previously encountered object and a novel object, and thus, it is a task of

object discrimination memory (Ennaceur & Delacour, 1988). Testing took place in a

rectangular (2.59 m × 3.96 m) windowless off-white room with one door. Overhead

ventilation fans and a radio produced background noise. The rats were placed in a white

Plexiglas Y-shaped apparatus with equidistant arms (Figure 9). The walls of the Y-

shaped apparatus were 40 cm high and opaque to prevent the rat from examining the

room. The arms in which the objects were placed were short (27 cm long) and narrow (10

cm wide) to minimize locomotor exploration of the testing environment. A video camera

(Camera model: Canon Zr 500) was mounted above the apparatus to record all testing

sessions.

Twenty-eight days after the injection of either KA or SA, rats had 2 days of

habituation sessions. For the habituation sessions, rats were allowed to explore the empty

Y-shaped apparatus for 5 minutes. Rats began the session in the start box (Figure 9).
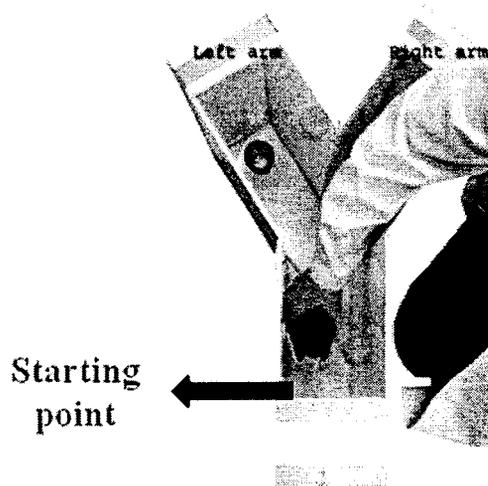
Figure 9. Y-shaped apparatus was used to measure object discrimination memory. The

rat was placed at one end of the box without objects (start box). For habituation the rat

was allowed to explore for 5 minutes (no objects were present), although for the testing

sessions the rats was allowed to explore for 3 minutes.

Twenty-four hours after the second habituation session, the testing sessions began. Each session consisted of two phases: the sample phase and the test phase. In the sample phase, two identical objects were placed in the Y-shaped apparatus, one at the end of each exploration arm. The objects were made of glass, plastic, or metal (Figure 10). The height of the objects ranged from 5 cm to 20 cm, and all objects were affixed to the floor of the apparatus with BluTack © to prevent the rats from moving them. As far as could be determined, the objects had no natural significance for the rats, and they had never been associated with a reinforcer.

Figure 10. An example of objects used in the object discrimination task.

For the sample phase, the rat was placed in the start box and allowed to explore the objects for 3 minutes. Exploration of an object was defined as touching the object with either the nose or forelimb. However, locomoting around or sitting on the object was not considered exploratory behavior. At the end of the sample phase, the rat was removed from the Y-shaped apparatus and was transferred to a holding cage in a room adjacent to the testing room.

After a delay (either 15 or 240 minutes), the rat was placed back in the start box of the Y-shaped apparatus that now contained a third, identical copy of the sample (familiar) object in one arm and a new object in the other. The non-matching objects were typically composed of the same material so that they could not readily be distinguished

37

by olfactory or tactile cues. The rat was allowed to explore the objects for 3 minutes, at the end of which it was removed and returned to its home cage. Exploration was scored similar to the sample phase.

Rats were given two testing sessions. The interval between the habituation day and the first testing day was 24 hours. This was followed by another testing session 48 hours later. The two testing sessions differed in the delay between the sample and test phase (15 versus 240 minutes) and were counterbalanced between groups. For each rat, a different object pair was used, with arm placement of the non-matching objects counterbalanced between groups and across sessions. The floor and walls of the apparatus were wiped with a dry paper towel between rats, but were not cleaned during a session. The experimenter and the person who scored the tapes were blind to the group membership of the rats.

For the choice phase, the time spent exploring the novel and familiar objects was scored from the tapes. The dependent variable was the discrimination index, which was computed as: (the time spent exploring the novel object minus the time spent exploring the familiar objects)/ the total time spent exploring the objects.

Water task. The water task is a task of spatial working memory (Morris, 1981; 1984). Rats were placed in a circular pool that was 150 cm in diameter, with 50 cm high featureless white walls. The pool was filled with water to a height of 26 cm water (with 22±1°C). The water was rendered opaque with 1500–2000 ml of skim milk powder. A clear Plexiglas platform (23 cm high × 10 cm long × 12 cm wide) was located within the pool. The pool was located in a white windowless room (3.1 m × 6.1 m) with a single

door. Varied posters placed on the walls provided visual cues. Background noise was produced by two overhead ventilation fans. A ceiling-mounted camera recorded the movements of the rats. The movements of the rats were recorded and analyzed by a video tracking system (HVS Image 2020 Plus Tracking System, 1998-2002; HVS Image Ltd, UK) and an Acer computer (TravelMate 225X).

Forty-eight days after the injection of either KA or SA, the rats began testing in the water task. Testing occurred daily for 14 days, with 4 trials per day. Rats started each trial at 1 of 4 start locations. The trial was completed when the rat either located the platform or spent 60 seconds swimming without finding the platform. When the rat found the platform, it remained on the platform for 10 seconds and then was placed in a warming chamber to await the next trial. If the rat did not find the platform, it was picked up by the experimenter and placed on the platform where it remained for 10 seconds and then placed in a warming chamber to await the next trial. The minimum inter-trial interval was 20 minutes. Every other day, the location of the platform was moved and randomly assigned. The last two days of testing took place in another room. The data was recorded and analyzed with the same software, however. The dependent variables for water task performance were: latency, or the time it took the rat to find the platform location; path length, or the distance that the rat swam before finding the platform location; and speed or how fast the rat swam in the pool.

Ziggurat task. This is a task of spatial working memory (Faraji et al., 2007). Rats were food-restricted one week prior to the commencement of the ziggurat task, and were maintained at 85% of their initial body weight throughout the experiment. Rats were weighed daily.

Testing was carried out in a white rectangular room (3.1 m ×6.1 m) that had different distal cues (posters) on each wall. A ceiling-mounted camera recorded the movements of the rats. The rat was placed in a box (179 cm × 179 cm × 25 cm in height) constructed from white laminate, which was located on a 50 cm high square table (179 cm wide). The box contained of sixteen pyramidal ziggurats placed 11 cm apart, arranged in a four by four matrix. The ziggurats were identical and made of six levels of square white Styrofoam (21 cm high). The base level of a ziggurat measured 31 cm square with a height of 3.5 cm. Each successive level had an identical height, but was reduced in size by 2 cm. The highest level measured 11 cm square and had a circular hole (1.5 cm in diameter) in the center (Figure 11). The hole was of sufficient size to contain three to five 1 cm long pieces of dry spaghetti (Tradizione brand). The movements of the rats were recorded and analyzed by a video tracking system (HVS Image 2020 Plus Tracking System, 1998-2002; HVS Image Ltd, UK) and an Acer computer (TravelMate 225X).
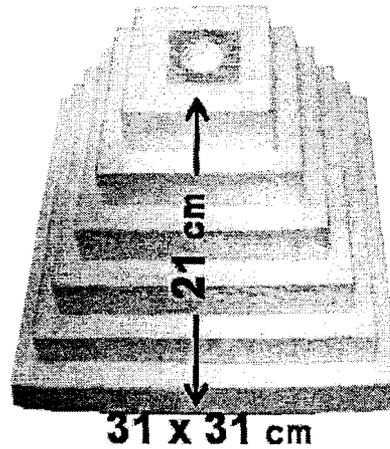
Figure 11. Ziggurats were pyramidal and were made of six levels of square white
styrofoam (21 cm high) with a square base of 31 cm × 31 cm (modified from Faraji et al.,
2007).

Fifty-six days following the injection of either KA or SA, ziggurat trials began. In order
to minimize olfactory cues, both the box and ziggurats were cleaned with 5% alcohol after
testing each rat. The rats were habituated for four days, followed by four days of test sessions.
Habituation sessions involved training rats to search the ziggurats for food. On the first and
second day of habituation, the rat was placed in the box containing the 16 baited ziggurats and
allowed to explore the environment for 10 minutes (Figure 12). Each ziggurat was baited with
3-5 small pieces of spaghetti. On the third day the rats were allowed to explore the
environment for 7 minutes, with only 1/2 of the ziggurats baited. On the fourth day, only 1/4 of
the ziggurats were baited and the exploration time was reduced to 5 minutes.
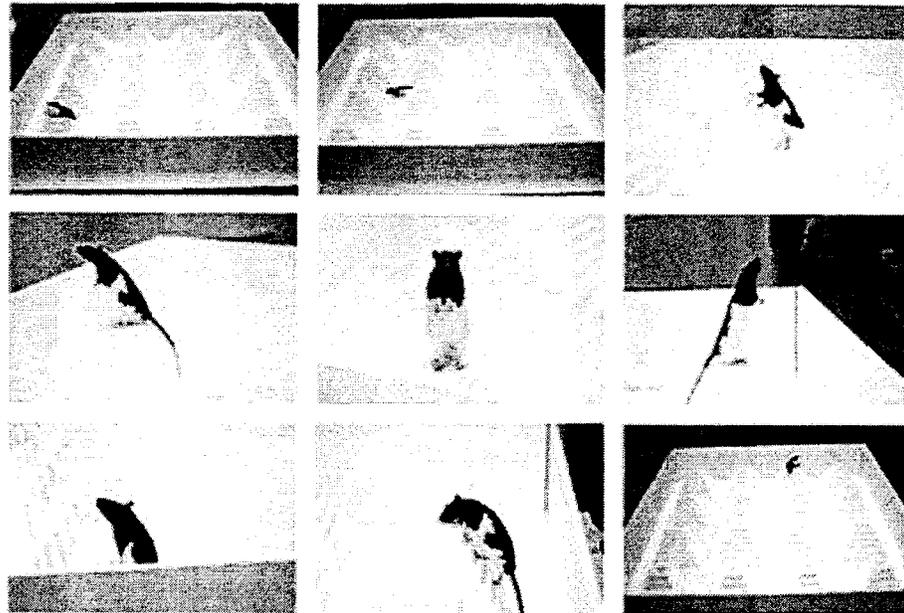
Figure 12. An example of a rat performing the ziggurat task (modified from Faraji et al., 2007).

On the 5th day, the testing sessions began. For testing trials, only 1 of 16 ziggurats was baited (learning day 1). Rats were placed at one of the 4 corner ziggurats facing the outer corner of the box and allowed to explore the environment for a maximum of 3 minutes. Rats completed 10 trials with a minimum inter-trial interval of 30 minutes. Start positions were varied quasi-randomly among the 10 trials. Although timing ceased when the rat found the ziggurat containing the food (the goal ziggurat), the rat was removed from the environment once the food was consumed. On the 6th day, the goal ziggurat remained the same (memory day 1). Rats performed 10 trials as described for day 5. On the 7th day, the goal ziggurat was in a different position, thus rats had to learn a new location (learning day 2). Rats performed 10 learning trials as described above. On the 8th day, the goal ziggurat remained in the same position as on Day 7 (memory day 2). Rats performed 10 trials as described above.

The dependent variables for the ziggurat task were: latency, or the time it took the rat to find the goal ziggurat location; path length, or the distance that it took the rat to find the goal ziggurat location; and speed, or the ratio of distance traveled to the time it took the rat to find the goal ziggurat location. For one of either learning days, the location of the goal ziggurat was in the periphery. For the other learning day, the location of the goal ziggurat was in the centre of the array. The order of the position of the goal ziggurats was counterbalanced among rats and groups.

Euthanasia and Histology

Fifteen days after the completion of the ziggurat task, the rats were deeply anaesthetized with an overdose of sodium pentobarbital (100 mg/kg). When deep anesthesia was achieved, rats were transcardially perfused with phosphate-buffer solution (PBS) containing 0.9% NaCl (pH 7.4; 100-150 ml), followed by 200 ml of PBS containing 4% paraformaldehyde. The brains were then extracted and stored in the fixative solution for 24 hours and then transferred and stored in a cryo-protectant solution (PBS containing 30% sucrose).

The brains were frozen and sectioned in the coronal plane on a sliding microtome. Sections were 40 μm thick. Twelve serial sections were collected and stored in a solution of PBS containing sodium azide (0.02%). Sections from hippocampus, (-1.60 to -6.04 relative to Bregma) were stained with 0.0001% 4',6-diamidino-2-phenylindole (DAPi) (1 mg of DAPi in 10 ml of dH2O) and mounted on 1% gelatin coated slides. Once they were dry, they were cover slipped with fluorescent mounting media containing an anti-fade reagent.

The hippocampal volume was estimated using the Cavalieri method (c.f. Schimitz & Hof, 2005). One twelfth of the sections were chosen for analysis, thus the starting sections from the hippocampus were chosen randomly from each group. Pictures were taken using fluorescent microscope (Zeiss Imager M1) and a camera (Axio Cam). The sections were then analyzed using Image J, a computer program designed for this procedure. A grid, with a size of 0.5 mm², was overlaid on the section randomly and the number of crosses was counted in the hippocampal area. The hippocampal volume was estimated using the following formula:

Volume =Σ Counts × Grid thickness × Section thickness × Sample fraction.

Results

Exclusions

A total of $n=13$ rats had to be excluded from the study. Following the injections of KA, 4 rats did not develop SE. Thus, they ($n=4$) and their KA- cage mates ($n=4$) were excluded. Also, 2 rats in the KA+ group died due to the excessive convulsive activity after injection of KA. Thus, they ($n=2$) and their KA- cage mates ($n=2$) were excluded from KA- group. Due to problems during sectioning during histological analysis, 1 of the KA- group had to be excluded from all analyses. Consequently, the final KA- group consisted of 8 rats and the KA+ group consisted of 9 rats.

Hippocampal Volume

The total number of sections was analyzed with independent sample t-test using group (KA+, KA-) as the independent variable. For the number of sections, t-test

analysis failed to reveal a significant difference between the KA+ group (M=10.556; SD=2.351) and the for KA- group (M=11.625; SD=2.825), t(15)=0.852, p=.598. As predicted, the average hippocampal volume for the KA+ group (M=80.240 mm³; SD=24.270) was significantly lower than the average hippocampal volume for the KA- group (M=112.050 mm³; SD= 24.270), t(15)=2.591, p=.020 (Figure 13).
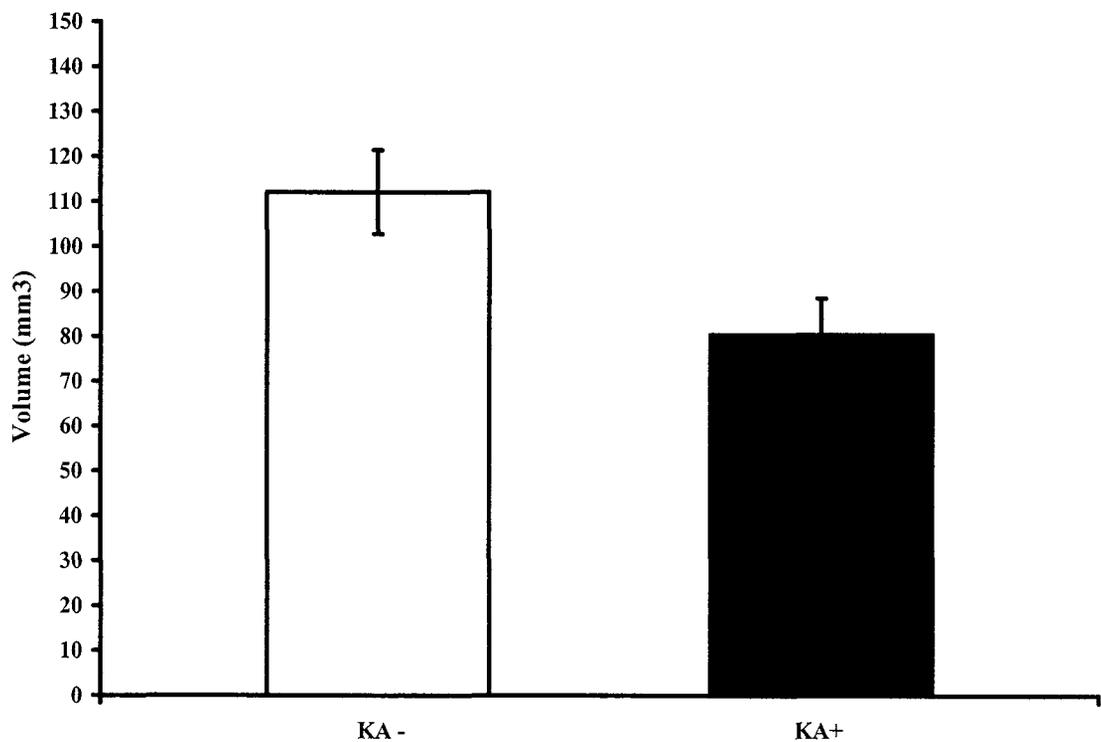
Figure 13. The significant main effect of group for hippocampal volume (mm³). The Bars represent the mean volume ± the standard error of the mean (SEM).

Convulsions

Following the injection of KA or SA, all of the KA+ group exhibited SE and exhibited at least one stage 5 convulsion, with the duration of convulsions lasting (M=18000 seconds; SD=3086.357). During the convulsion monitoring period, all of the

KA+ group exhibited spontaneous convulsions (M=9.222 convulsions; SD=5.357), whereas none of the KA- group did (M=0.000 convulsions; SD=0.000), $t(15)$=-4.852, p<.001. The duration of the spontaneous convulsions were summed, with the result that the KA+ group (M=432.778 seconds; SD= 237.830) had significantly longer convulsions than the KA- group (M=0.000 seconds; SD=0.000), $t(15)$=-5.128, p<.001.

Pearson product-moment correlations were performed between the hippocampal volume and: the duration of SE; the number of spontaneous convulsions; and the total duration of the spontaneous convulsions. For the entire group of rats, there was a significant correlation between hippocampal volume and duration of SE, $r(15)$=-0.572, p<.001. Also, there was a significant correlation between hippocampal volume and the duration of spontaneous convulsions, $r(15)$=-.556, p=.010. However, for the entire group of rats, the analysis failed to reveal a significant correlation between hippocampal volume and the number of spontaneous convulsions, $r(15)$=-.302, p=.120. However, as none of the KA- rats had convulsions, this correlation may be spurious.

As such, separate correlations were performed for the KA+ rats alone. The analysis failed to reveal a significant correlation between hippocampal volume and the number of spontaneous convulsions, $r(7)$=.365, p=.167. Also, the analysis failed to reveal a significant correlation between hippocampal volume and the duration of spontaneous convulsions, $r(7)$=-.319, p=.202 or between hippocampal volume and duration of SE, $r(7)$=-.229, p=.277.

Behavioural Assessment

Single pellet reaching task. The total number of attempts and single successful reaches were summed across the two testing days and a mean value was calculated. The mean values were analyzed with separate independent sample t-tests using group (KA+, KA-) as the independent variable. For the total attempted reaches, t-test analysis failed to reveal a significant difference between KA+ group (M=8.944; SD=1.168) and KA- group (M=11.625; SD=0.632), $t(15)$=1.946, p=.071. For the single successful reaches, t-test analysis failed to reveal a significant difference between KA+ group (M=5.167; SD=3.092) and KA- group (M=7.625; SD=1.788), $t(15)$=1.971, p=.068.

Pearson product-moment correlations were performed among the hippocampal volume and: the total attempted reaches; and the single successful reaches. For the entire group of rats, the analysis failed to reveal a significant correlation between the hippocampal volume and the total attempted reaches, $r(15)$=.309, p=.227 or with the single successful reaches, $r(15)$=.303, p=.236. As before, separate correlations were performed for the KA+ rats alone. This analysis failed to reveal a significant correlation between the hippocampal volume and the total attempted reaches, $r(7)$=.009, p=.982 or with the single successful reaches, $r(7)$=-.022, p=.955.

Ladder rung walking task. The number of repositions and falls were summed across the three trials and were analyzed with separate independent sample t-tests using group (KA+, KA-) as the independent variable. For the total number of repositions, t-test analysis failed to reveal a significant difference between the KA+ group (M=3.444; SD=2.743) and the KA- group (M=5.125; SD=5.986), $t(15)$=0.759, p=.459. For the total

47

number of falls, t-test analysis failed to reveal a significant difference between the KA+ group (M=9.888; SD=3.018) and the KA- group (M=8.750; SD=3.991), t(15)=0.668, p=.514.

As it was possible to investigate differences between the hindlimbs and the forelimbs, separate t-tests were performed for the total number of repositions and the total number of falls. For the total number of repositions of forelimb, t-test failed to reveal a significant difference between the KA+ group (M=2.556; SD=1.424) and the KA- group (M=3.000; SD=3.817), t(15)=0.326, p=.749. For the total number of falls of forelimb, t-test failed to reveal a significant difference between the KA+ group (M=4.444; SD=1.509) and the KA- group (M=3.625; SD=1.768), t(15) =-1.031, p=.319. For the number of repositions of hindlimb, t-test failed to reveal a significant difference between the KA+ group (M=0.889; SD=1.691) and the KA- group (M=2.125; SD=2.474), t(15)=1.215, p=.243. For the total number of falls of hindlimb, t-test failed to reveal a significant difference between the KA+ group (M=5.444; SD=1.740) and the KA- group (M=5.125; SD=2.356), t(15)=-0.321, p=.753.

Pearson product-moment correlations were performed among the hippocampal volume and: the total number of repositions; and the total number of falls. For the entire group of rats, there was a significant positive correlation between the hippocampal volume and the total number of repositions, r(15)=.495, p=.043. However, for the entire group of rats, the analysis failed to reveal a significant correlation between the hippocampal volume and the total number of falls, r(15)=.260, p=.314. As before, separate correlations were performed for the KA+ rats alone. Unlike the results for the entire group, the analysis failed to reveal a significant correlation between the

48

hippocampal volume and the total number of repositions, $r(7)=.345$, $p=.363$. There was

no significant correlation between the hippocampal volume and the total number of falls,

$r(7)=.284$, $p=.460$.

Object discrimination task. Using the discrimination index as dependent variable,

separate one sample t-tests with group (KA+, KA-) as the independent variable were

performed for the trials that had a 15-minute delay and those that had a 240-minute delay.

For 15-minute delay, t-test failed to reveal a significant difference when compared to

baseline zero, $t(15)=0.655$, $p=.522$. Also, for 240-minute delay, t-test failed to reveal a

significant difference when compared to baseline zero, $t(15)=0.634$, $p=.535$.

Using the discrimination index as dependent variable, separate independent

sample t-tests with group (KA+, KA-) as the independent variable were performed for the

trials that had a 15-minute delay and those that had a 240-minute delay. For 15-minute

delay, t-test failed to reveal a significant difference between the KA+ group ($M=0.179$;

$SD=0.585$) and the KA- group ($M=-0.025$; $SD=0.457$), $t(15)=-0.794$, $p=.440$. Also, for

240-minute delay, t-test failed to reveal a significant difference between the KA+ group

($M=0.137$; $SD=0.535$) and the KA- group ($M=0.012$; $SD=0.504$), $t(15)=-0.496$, $p=.627$.

Pearson product-moment correlations were performed among the hippocampal

volume and: the discrimination index for the 15-minute delay; the discrimination index

for the 240-minute delay. For the entire group of rats, the analysis failed to reveal a

significant correlation between the hippocampal volume and the discrimination index for

objects for either the 15-minute delay, $r(15)=-.103$, $p=.695$ or for the 240-minute delay,

$r(15)=.025$, $p=.925$. As before, separate correlations were performed for the KA+ rats

alone. The analysis failed to reveal significant correlation between the hippocampal volume and the discrimination index for objects for either the 15-minute delay, $r(7)=-.398, p=.289$, or the 240-minute delay $r(7)=-.535, p=.137$.

Water task. For latency to find the platform, a repeated measures Analysis of Variance (ANOVA) was performed with day (days 1-14) and trial (trials 1-4) as within-subject measures and group (KA+, KA-) as a between-subject measure. ANOVA failed to reveal a significant difference between the KA+ group ($M=15.572$ seconds; $SD=5.449$) and the KA- group ($M=12.458$ seconds; $SD=2.427$), $F(1,14)=2.346, p=.148$. ANOVA revealed a main effect of day, $F(13,182)=25.294, p<.001$, and a main effect of trial, $F(3,42)=16.772, p<.001$. However, these main effects were mediated by a significant interaction between day and trial, $F(39,546)=2.286, p<.001$ (Figure 14). Notably, the interactions: between day and group $F(13,182)=1.453, p=.139$; between trial and group, $F(3,42)=0.866, p=.466$; and day, trial, and group, $F(39,546)=1.051, p=.390$; all failed to reach significance.

The latency was averaged across the four trials and planned comparisons (Bonferroni) were performed on the learning days and the memory days. For the learning days, planned comparisons were conducted between the two consecutive days that had the same platform location. The analysis failed to reveal a significant difference between: day 1 and day 2, $p=1.000$; day 3 and day 4, $p=.121$; day 5 and day 6, $p=1.000$; day 7 and day 8, $p=1.000$; day 9 and day 10, $p=1.000$; day 11 and day 12, $p=1.000$; and day 13 and day 14, $p=1.000$.

For the memory days, planned comparisons (Bonferroni) were conducted on two consecutive days with the new platform location. For example, comparisons were made between: day 1 and day 3; day 3 and day 5; and so on. The analysis revealed a significant difference between day 3 and day 5, $p=.041$. However, the analysis failed to reveal a significant difference: between day 1 and day 3, $p=1.000$; between day 5 and day 7, $p=.121$; between day 7 and day 9, $p=.055$; day 9 and day 11, $p=1.000$; and between day 11 and day 13, $p=1.000$.

To examine the simple main effect of trial, the latency was averaged across the fourteen days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed trial 1 was significantly longer than trial 2, $p=.009$. Also, trial 1 was significantly longer than trial 3, $p=.005$; and trial 1 was significantly longer than trial 4, $p<.001$. Trial 2 was significantly longer than trial 4, $p=.050$. However, the analysis failed to reveal a significant difference between trial 3 and trial 4, $p=.590$.
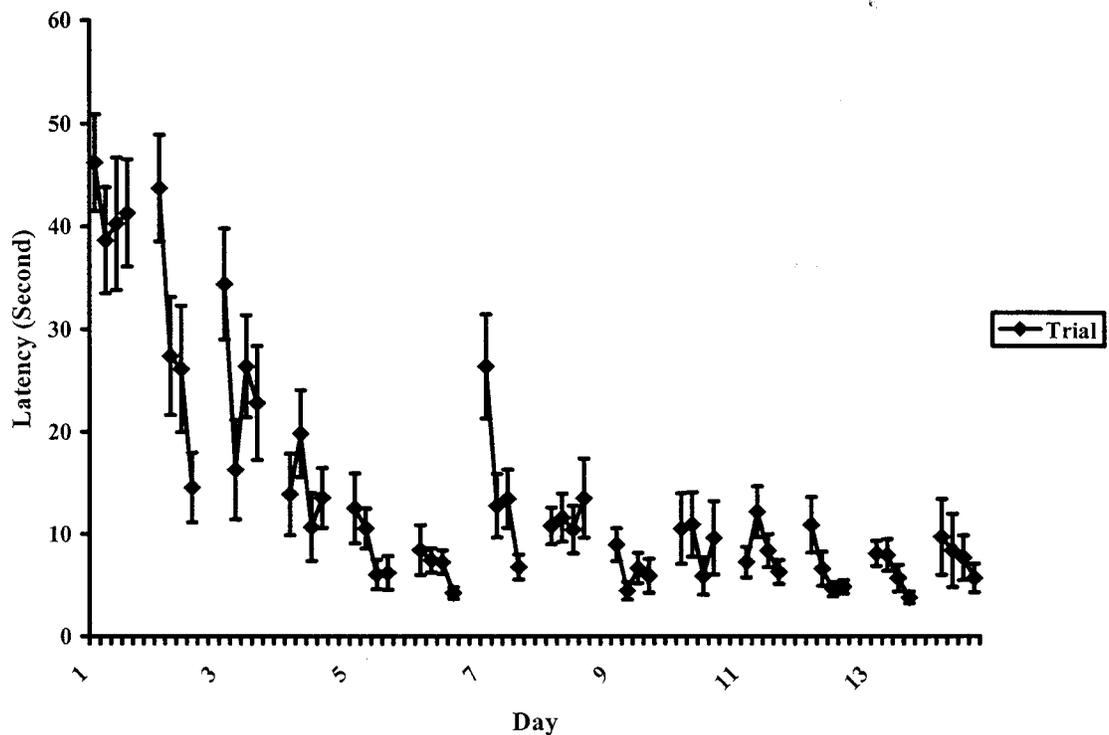
Figure 14. The significant interaction between day and trial for latency in which the rats found the platform in water task. The symbols represent mean latency (seconds) ± the SEM.

For the entire group of rats, Pearson product-moment correlations were performed between the hippocampal volume and the latency with which the rats found the platform for each day (averaged across the trials; Table 1). There was a significant correlation between the hippocampal volume and the latency with which the rats found the platform on day 5, however, the analysis failed to reveal any other significant correlations for the entire group of rats. As before, separate correlations were performed for the KA+ rats alone (averaged across trials; Table 1). There was a significant correlation between the hippocampal volume and the latency with which the rats found the platform on day 8,

however, the analysis failed to reveal any other significant correlations for the KA+ group.

Table 1.

Pearson product-moment correlations for latency in which the rats found the platform in the water task.

	Entire group of rats		KA+ Group	
	Pearson Correlation	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Day1	0.209	0.438	0.522	0.149
Day2	-0.408	0.116	0.069	0.860
Day3	-0.292	0.272	-0.201	0.604
Day4	-0.448	0.082	0.106	0.786
Day5	**-0.546**	**0.029**	-0.483	0.188
Day6	0.098	0.719	0.504	0.166
Day7	-0.198	0.462	0.465	0.208
Day8	-0.006	0.983	**0.708**	**0.033**
Day9	-0.187	0.489	0.601	0.087
Day10	-0.376	0.151	-0.419	0.301
Day11	0.410	0.114	0.079	0.853
Day12	-0.097	0.719	0.365	0.374
Day13	-0.088	0.746	-0.085	0.842
Day14	0.074	0.786	0.588	0.126

Note. The correlation values for the entire group of rats (column 2) and the KA+ group alone (column 4). The significant values are identified in bold.

For the distance traveled, a repeated measures ANOVA was performed with day (days 1-14) and trial (trials 1-4) as within-subject measures and group (KA+, KA-) as a between-subject measure. ANOVA failed to reveal a significant difference between the KA+ group (M=4.621 meters; SD=1.698) and the KA- group (M=3.561 meters; SD=0.723), $F(1,14)$=3.393, p=.087. ANOVA revealed a main effect of day, $F(13,182)$=29.248, $p<.001$; and a main effect of trial, $F(3,42)$=16.752, $p<.001$. However,

these main effects were mediated by a significant interaction between day and trial, $F(39,546)=2.582$, $p<.001$ (Figure 15). Notably, the interactions between: day and group $F(13,182)=1.708$, $p=.062$; trial and group, $F(3,42)=0.503$, $p=.683$; and the day, trial, and group, $F(39,546)=0.896$, $p=.652$; all failed to reach significance.
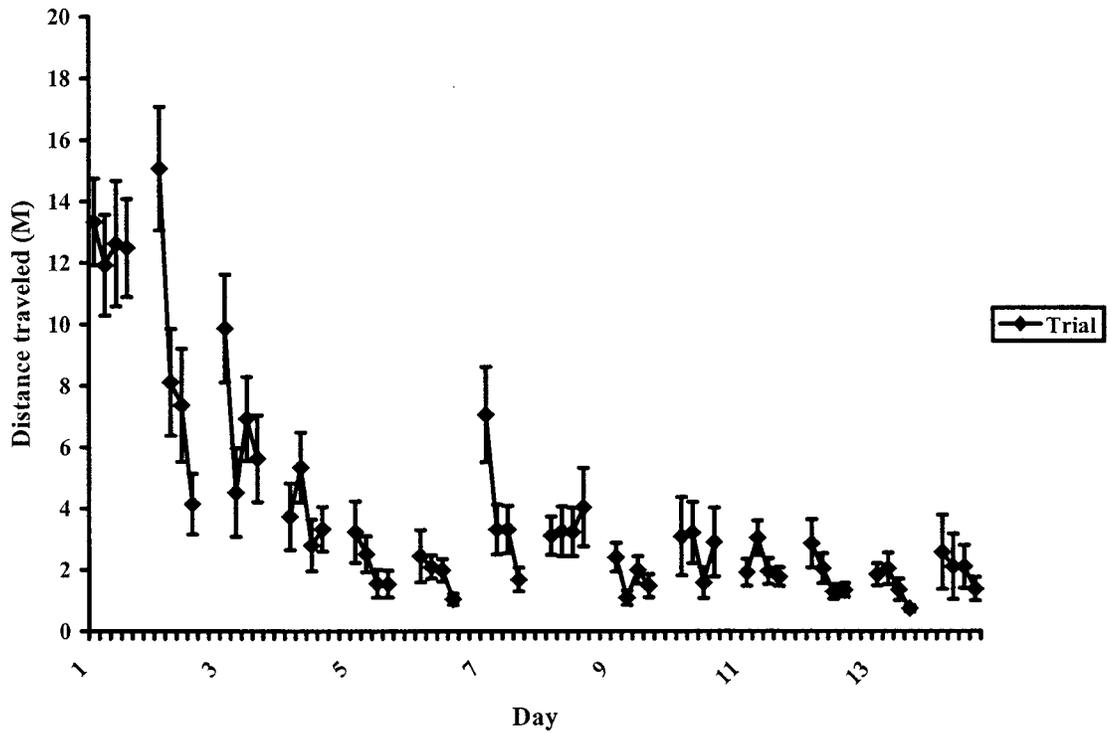
Figure 15. The significant interaction between day and trial for distance which the rats traveled to find the platform in water task. The symbols represent mean distance traveled (meters) ± the SEM.

The distance traveled was averaged across the four trials and planned comparisons (Bonferroni) were performed on the learning days and the memory days. For the learning days, planned comparisons were conducted between the two consecutive days that had the same platform location. The analysis failed to reveal a significant

difference: between day 1 and day 2, $p=.557$; between day 3 and day 4, $p=.346$; between day 5 and day 6, $p=1.000$; between day 7 and day 8, $p=1.000$; between day 9 and day 10, $p=1.000$; between day 11 and day 12, $p=1.000$; and between day 13 and day 14, $p=1.000$.

For the memory days, planned comparisons (Bonferroni) were conducted on two consecutive days with the new platform location. For example, comparisons were made between: day 1 and day 3; day 3 and day 5; and so on. The analysis failed to reveal a significant difference: between day 1 and day 3, $p=.107$; day 3 and day 5, $p=.057$; day 5 and day 7, $p=.082$; day 7 and day 9, $p=.280$, day 9 and day 11, $p=1.000$; and day 11 and day 13, $p=1.000$.

To examine the simple main effect of trial, the distance traveled was averaged across the fourteen days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed trial 1 was significantly longer than trial 2, $p=.010$. Also, trial 1 was significantly longer than trial 3, $p=.008$; and trial 1 was significantly longer than trial 4, $p<.001$. Trial 2 was significantly longer than trial 4, $p=.039$. However, the analysis failed to reveal a significant difference: between trial 2 and trial 3, $p=1.000$; and between trial 3 and trial 4, $p=.449$.

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the distance that the rats traveled to find the platform for each day (averaged across the trials; Table 2). There was a significant correlation between the hippocampal volume and the distance that the rats traveled to find the platform on day 5, however, the analysis failed to reveal any other significant correlations

for the entire group of rats. As before, separate correlations were performed for the KA+ rats alone (averaged across the trials; Table 2). The analysis failed to reveal any significant correlations between the hippocampal volume and the distance that the rats traveled to find the platform for the KA+ group.

Table 2.

Pearson product-moment correlations for distance traveled in which the rats found the platform in the water task.

	Entire group of rats		KA+ Group	
	Pearson Correlation	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Day1	0.324	0.221	0.628	0.070
Day2	-0.307	0.247	0.039	0.921
Day3	-0.280	0.294	-0.053	0.892
Day4	-0.451	0.080	0.180	0.642
Day5	**-0.497**	**0.050**	-0.432	0.246
Day6	0.114	0.674	0.540	0.133
Day7	-0.187	0.489	0.271	0.480
Day8	0.003	0.990	0.509	0.162
Day9	-0.199	0.459	0.245	0.526
Day10	-0.378	0.149	-0.409	0.314
Day11	0.389	0.136	0.104	0.806
Day12	-0.132	0.627	0.254	0.545
Day13	-0.205	0.446	-0.144	0.733
Day14	0.046	0.866	0.585	0.128

Note. The correlation values for the entire group of rats (column 2) and the KA+ group alone (column 4). The significant values are identified in bold.

For speed, a repeated measures ANOVA was performed with day (days 1-14) and trial (trials 1-4) as within-subject measures and group (KA+, KA-) as a between-subject measure. ANOVA failed to reveal a significant difference between the KA+ group (M=0.265 meters/second; SD=0.018) and the KA- group (M=0.254 meters/second;

SD=0.028), *F*(1,14)=1.856, *p*=.195. ANOVA revealed a main effect of day,

F(13,182)=7.884, *p*<.001; and a main effect of trial, *F*(3,42)=3.086, *p*=.037. However,

these main effects were mediated by a significant interaction between day and trial,

F(39,546)=3.582, *p*<.001 (Figure 16). Notably, the interaction between day and group,

F(13,182)=0.702, *p*=.760; trial and group, *F*(3,42)=0.049, *p*=.985; and the day, trial, and

group, *F*(39,546)=1.398, *p*=.059; all failed to reach significance.
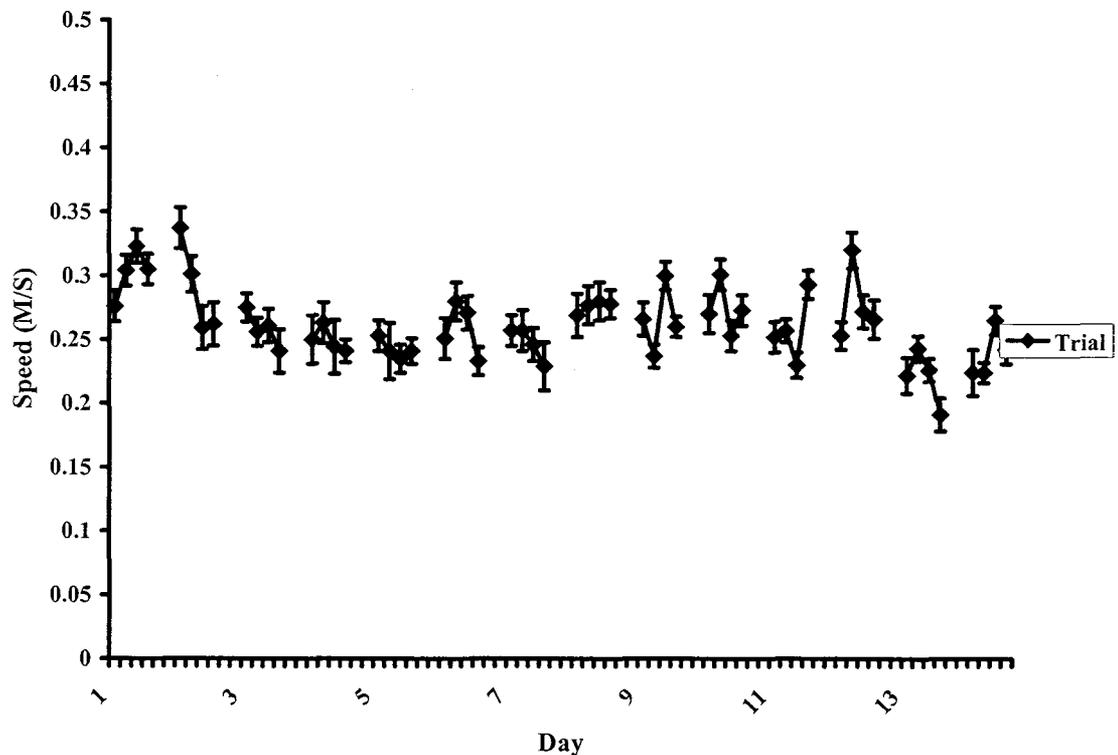
Figure 16. The significant interaction between day and trial for the speed with which the

rats found the platform in water task. The symbols represent the mean speed

(meters/second) ± the SEM.

The speed was averaged across the four trials and planned comparisons

(Bonferroni) were performed on the learning days and the memory days. For the learning

days, planned comparisons were conducted between the two consecutive days that had the same platform location. The analysis revealed a significant difference between day 7 and day 8, $p=.021$. However, there was no significant difference: between day 1 and day 2, $p=1.000$; between day 3 and day 4, $p=1.000$; between day 5 and day 6, $p=1.000$; between day 9 and day 10, $p=1.000$; between day 11 and day 12, $p=1.000$ and; between day 13 and day 14, $p=1.000$.

For the memory days, planned comparisons (Bonferroni) were conducted on two consecutive days with the new platform location. For example, comparisons were made between: day 1 and day 3; day 3 and day 5; and so on. The analysis revealed a significant difference between day 11 and day 13, $p=.016$. However, the analysis failed to reveal a significant difference between: day 1 and day 3, $p=.195$; between day 3 and day 5, $p=1.000$; between day 5 and day 7, $p=1.000$; between day 7 and day 9, $p=1.000$; and between day 9 and day 11, $p=1.000$.

To examine the simple main effect of trial, the speed was averaged across the fourteen days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed trial 2 was significantly slower than trial 4, $p=.043$. However, the analysis failed to reveal significant difference: between trial 1 and trial 2, trial 3, trial 4, $p=1.000$; between trial 2 and trial 3, $p=.460$; and between trial 3 and trial 4, $p=.166$.

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the speed with which the rats swam to find the platform for each day (averaged across the trials; Table 3). The analysis failed to reveal any significant correlation between the hippocampal volume and the speed with which

58

the rats found the platform, for the entire group of rats. As before, separate correlations were performed for the KA+ rats alone (averaged across the trials; Table 3). There was a significant correlation between the hippocampal volume and the speed with which the rats found the platform on day 3, however, the analysis failed to reveal any other significant correlations for the KA+ group.

Table 3.

Pearson product-moment correlations for speed in which the rats found the platform in the water task.

	Entire group of rats		KA+ Group	
	Pearson Correlation	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Day1	-0.055	0.835	-0.321	0.399
Day2	0.063	0.810	0.055	0.888
Day3	0.112	0.669	**0.699**	**0.036**
Day4	0.299	0.244	0.487	0.183
Day5	-0.026	0.921	0.204	0.598
Day6	0.210	0.420	0.617	0.077
Day7	0.248	0.336	-0.082	0.834
Day8	0.006	0.982	-0.261	0.497
Day9	-0.217	0.403	-0.569	0.110
Day10	0.021	0.940	0.259	0.535
Day11	-0.009	0.974	0.056	0.896
Day12	-0.017	0.950	-0.061	0.886
Day13	-0.245	0.361	-0.074	0.862
Day14	-0.250	0.350	0.224	0.595

Note. The correlation values for the entire group of rats (column 2) and the KA+ group alone (column 4). The significant values are identified in bold.

Ziggurat task. For latency to find the goal ziggurat, a repeated measures ANOVA was performed with day (days 1-4) and trial (trials 1-10) as within-subject measures and treatment (KA+, KA-) as a between-subject measure. ANOVA revealed that the KA+

rats took longer to find the goal ziggurat than the KA- rats, $F(1,14)=8.325$, $p=.012$

(Figure 17). ANOVA also revealed a main effect of day, $F(3,42)=10.565$, $p<.001$

(Figure 18) ; and a main effect of trial, $F(9,126)=5.488$, $p<.001$ (Figure 19). However, the

analysis failed to revealed a significant interaction of day and trial, $F(27,378)=1.150$,

$p=.279$. Notably, the interactions between day and group, $F(3,42)=1.967$, $p=.134$; trial

and group, $F(9,126)=1.900$, $p=.058$; and day, trial, and group, $F(27,378)=0.828$, $p=.715$,

all failed to reach significance.
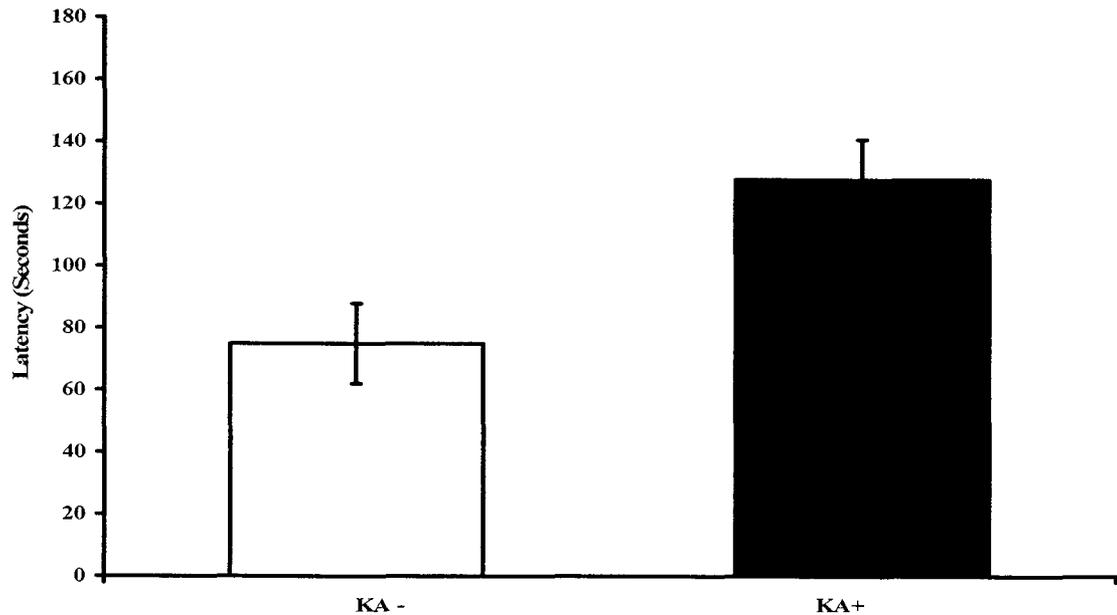
Figure 17. The significant main effect of group for the latency with which the rats found

the goal ziggurat. The bars represent mean latency (seconds) ± the SEM.
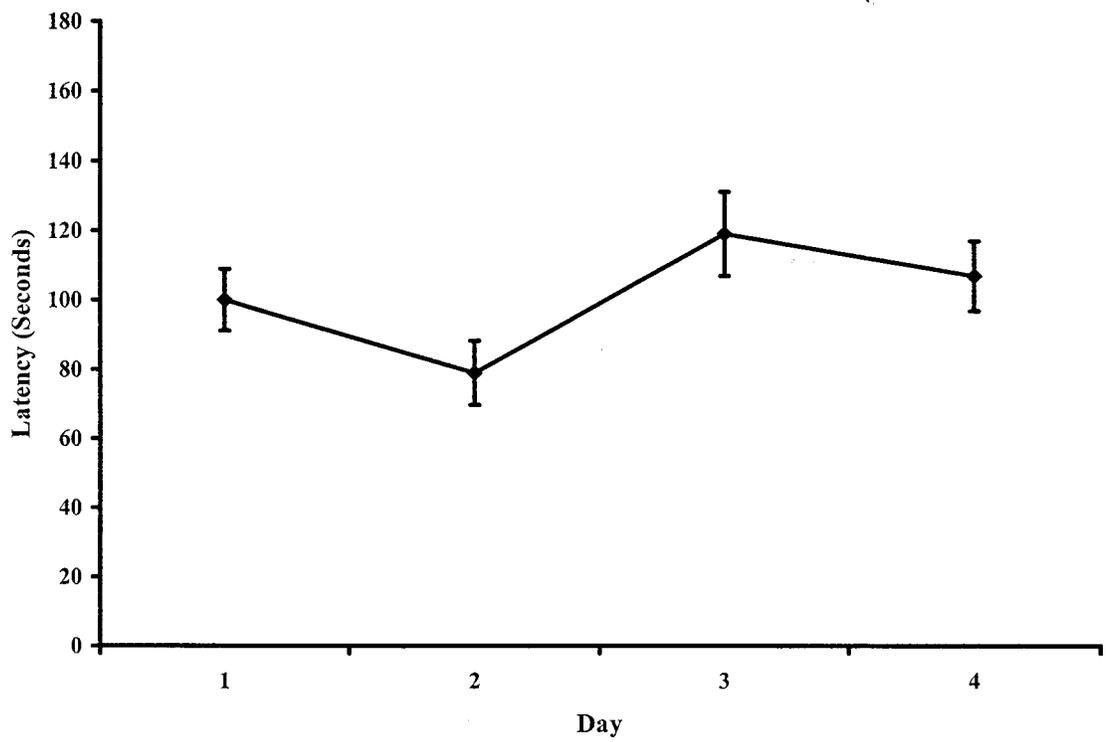
Figure 18. The significant main effect of day for the latency with which the rats found

the goal ziggurat. The symbols represent mean latency (seconds) ± the SEM.
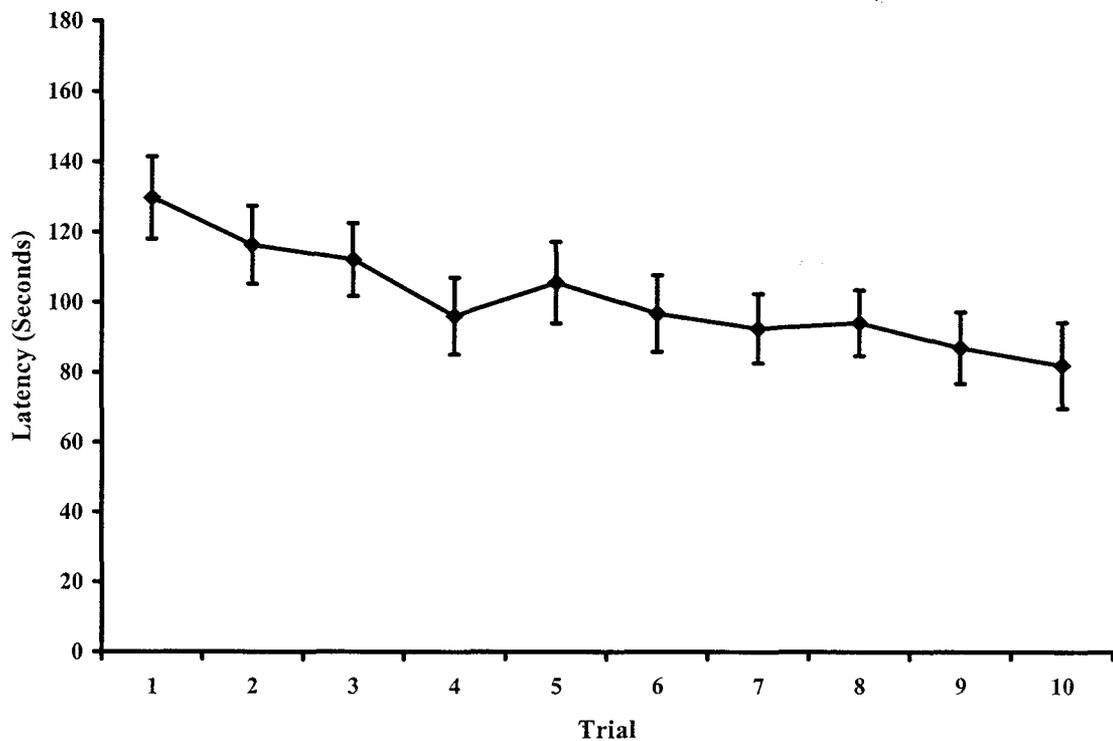
Figure 19. The significant main effect of trial for the latency with which the rats found

the goal ziggurat. The symbols represent mean latency (seconds) ± the SEM.

The latency was averaged across the ten trials and planned comparisons

(Bonferroni) were performed on the learning days and the memory days. For the learning

days, planned comparisons were conducted between the two consecutive days that had

the same goal ziggurat location. The analysis revealed day 1 was significantly longer than

day 2, $p=.006$. However, the analysis failed to reveal a significant difference between day

3 and day 4, $p=.485$.

For the memory days, planned comparisons (Bonferroni) were conducted on two

consecutive days with the new goal ziggurat location. For example, comparisons were

made between: day 1 and day 3. The analysis failed to reveal a significant difference between day 1 and day 3, $p=.241$.

To examine the simple main effect of trial, the latency was averaged across the four days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed trial 1 was significantly longer than trial 9 and trial 10, $p=.014$. Also, trial 2 was significantly longer than trial 9, $p=.044$; and trial 2 was significantly longer than trial 10, $p=.049$. However, the analysis failed to reveal any other significant differences between the trials.

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the latency with which the rats found the goal ziggurat for each day (averaged across trial, Table 4). There was a significant correlation between the hippocampal volume and the latency with which the rats found the goal ziggurat on day 1, however, the analysis failed to reveal any other significant correlation for the entire group of rats. As before, separate correlations were performed for the KA+ rats alone. The analysis failed to reveal any other significant correlation between the hippocampal volume and the latency with which the rats found the goal ziggurat for the KA+ group.

Table 4.

Pearson product-moment correlations for latency in which the rats found the goal

ziggurat in the ziggurat task.

	Entire group of rats		KA+ Group	
	Pearson Correlation	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Day 1	**-0.513**	**0.042**	-0.166	0.695
Day 2	-0.285	0.284	0.134	0.752
Day 3	-0.378	0.149	0.116	0.784
Day 4	-0.413	0.112	0.188	0.655

Note. The correlation values for the entire group of rats (column 2) and the KA+ group

alone (column 4). The significant values are identified in bold.

For the distance traveled, a repeated measures ANOVA was performed with day

(days 1-4) and trial (trials 1-10) as within-subject measures and group (KA+, KA-) as a

between-subject measure. ANOVA failed to reveal a significant difference between the

KA+ group (M=8.198 meters; SD=2.447) and the KA- group (M=7.487 meters;

SD=2.469), $F(1,14)$=0.335, p=.572. However, ANOVA revealed a main effect of day,

$F(3, 42)$=7.413, p<.001 (Figure 20) and a main effect of trial $F(9, 126)$=7.205, p<.001

(Figure 21). Unlike the other measures, the interaction between day and trial failed to

reach significance $F(27,378)$=1.116, p=.316. Notably, the interaction between: day and

group, $F(3,42)$=0.482, p=.067; trial and group, $F(9,126)$=0.806, p=.611; and day, trial,

and group, $F(27,378)$=0.897, p=.617, all failed to reach significance.
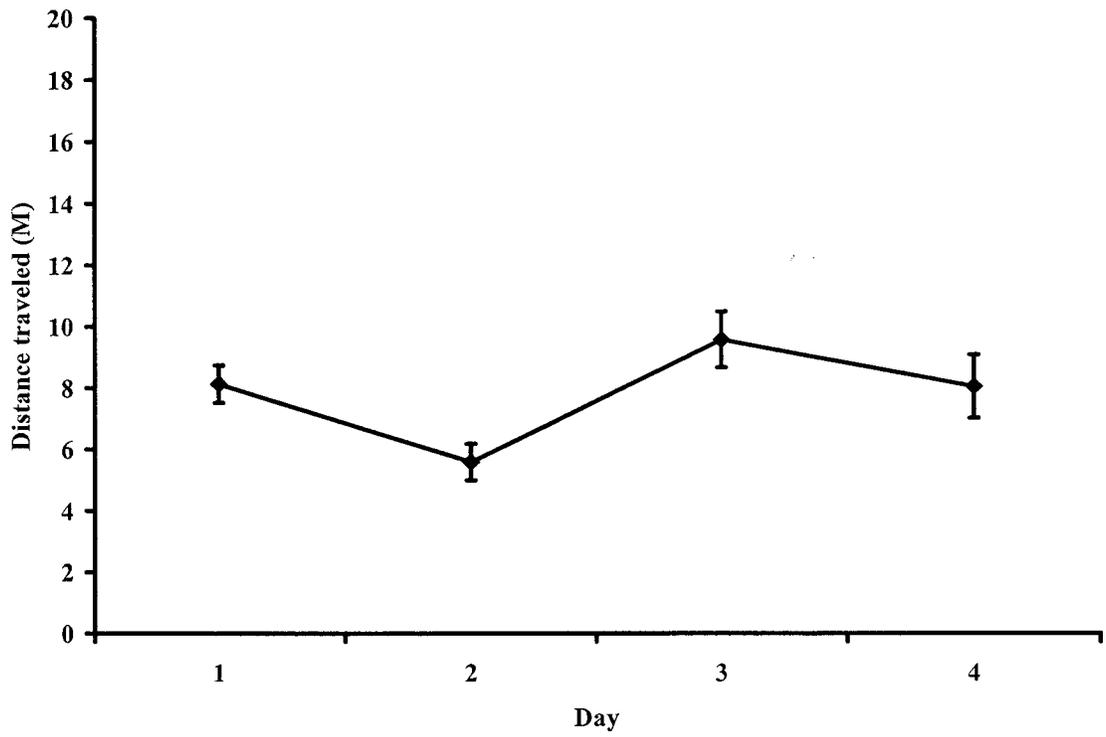
Figure 20. The significant main effect of day for the distance that the rats traveled to find

the goal ziggurat. The symbols represent mean distance traveled (meters) ± the SEM.

Figure 21. The significant main effect of trial for the distance that the rats traveled to find

the goal ziggurat. The symbols represent mean distance traveled (meters) ± the SEM.

The distance traveled was averaged across the ten trials and planned comparisons

(Bonferroni) were performed on the learning days and the memory days. For the learning

days, planned comparisons were conducted between the two consecutive days that had

the same goal ziggurat location. The analysis revealed the distance traveled on day 1 is

significantly longer than day 2, *p*=.004. However, the analysis failed to reveal significant

difference between day 3 and day 4, *p*=.696.

For the memory days, planned comparisons (Bonferroni) were conducted on two

consecutive days with the new goal ziggurat location. For example, comparisons were

made between: day 1 and day 3. The analysis failed to reveal a significant difference between day 1 and day 3, $p=.605$.

To examine the simple main effect of trial, the distance traveled was averaged across the four days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed the distance traveled in trial 1 was significantly longer than trial 9, $p=.038$; and trial 1 was significantly longer than trial 10, $p=.026$. Also, the distance traveled in trial 2 was significantly longer than trial 10, $p=.022$; and trial 5 was significantly longer than trial 10, $p=.042$. However, the analysis failed to reveal there any other significant differences among the trials.

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the distance that the rats traveled to find the goal ziggurat, on a given day (Table 5). The analysis failed to reveal any significant correlation between the hippocampal volume and the distance that the rats traveled to find the goal ziggurat for the entire group of rats. As before, separate correlations were performed for the KA+ rats alone among the hippocampal volume and the distance which the rats traveled to find the goal ziggurat, on a given day (Table 5). The analysis failed to reveal any significant correlation between the hippocampal volume and the distance that the rats traveled to find the goal ziggurat for the KA+ group.

Table 5.

Pearson product-moment correlations for distance traveled with which the rats found the

goal ziggurat.

	Entire group of rats		KA+ Group	
	Pearson Correlation	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Day 1	-0.334	0.206	0.149	0.724
Day 2	0.304	0.252	0.587	0.126
Day 3	-0.119	0.661	0.179	0.672
Day 4	-0.068	0.803	0.183	0.664

Note. The correlation values for the entire group of rats (column 2) and the KA+ group

alone (column 4). There are no significant values identified.

For the speed, a repeated measures ANOVA was performed with day (days 1-4)

and trial (trials 1-10) as within-subject measures and group (KA+, KA-) as a between-

subject measure. ANOVA failed to reveal a significant difference between the KA+

group (M=0.132 meters/second; SD=0.087) and the KA- group (M=0.148 meters/

second; SD=0.052), $F(1,14)$=0.186, p=.673. ANOVA revealed a main effect of day,

$F(3,42)$=9.464, p<.001, and a main effect of trial, $F(9,126)$=9.712, p<.001. However, this

was mediated by a significant interaction between day and trial, $F(27,378)$=10.085,

p<.001 (Figure 22). Notably, the interactions: between day and group, $F(3,42)$=0.765,

p=.520; trial and group, $F(9,126)$=0.713, p=.696; and day, trial, and group,

$F(27,378)$=0.530, p=.976, all failed to reach significance.
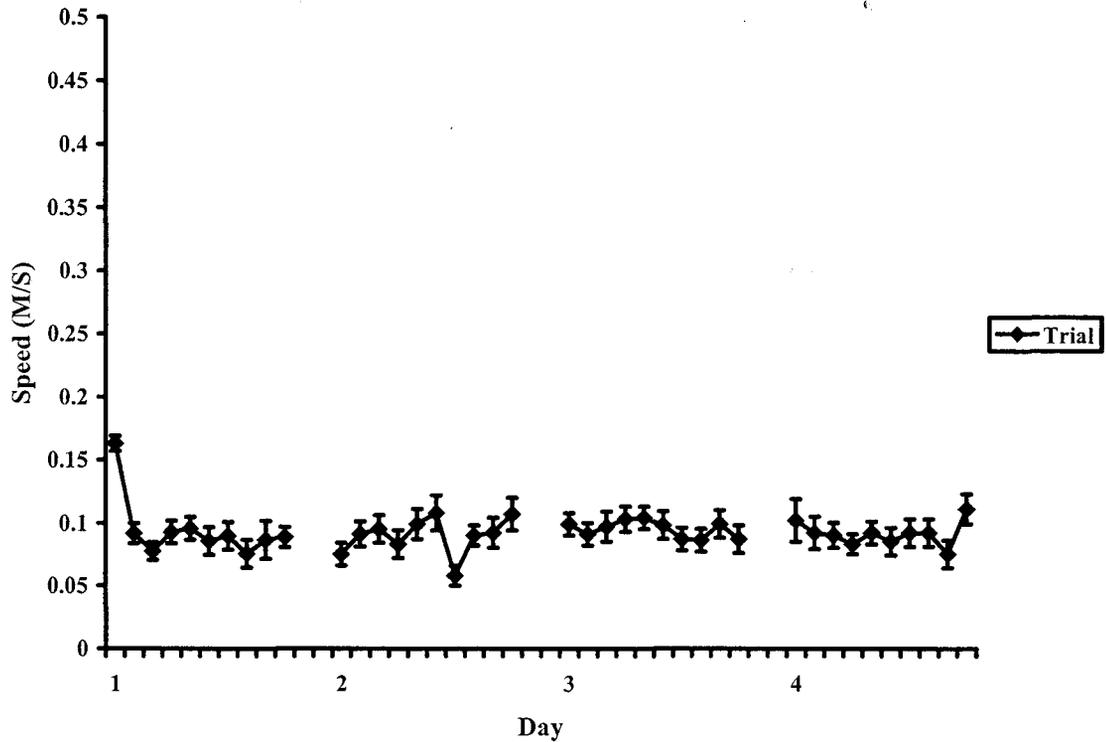
Figure 22. The significant interaction between day and trial for speed in which the rats

swam to find the goal in ziggurat task. The symbols represent mean speed

(meters/seconds) ± the SEM.

The speed was averaged across the ten trials and planned comparisons

(Bonferroni) were performed on the learning days and the memory days. For the learning

days, planned comparisons were conducted between the two consecutive days that had

the same goal ziggurat location. The analysis revealed rats found the goal significantly

faster during day 1 than day 2, $p=.049$. However, the analysis failed to reveal a

significant difference between day 3 and day 4, $p=1.000$.

For the memory days, planned comparisons (Bonferroni) were conducted on two

consecutive days with the new goal ziggurat location. For example, comparisons were

69

made between: day 1 and day 3. The analysis failed to reveal a significant difference between day 1 and day 3, $p=.055$.

To examine the simple main effect of trial, the speed was averaged across the four days and planned comparisons (Bonferroni) were performed among all trials. The analysis failed to reveal any significant difference.

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the speed with which the rats found the goal ziggurat, on a given day (Table 6). The analysis failed to reveal any significant correlation between the hippocampal volume and the speed with which the rats found the goal ziggurat for the entire group of rats. As before, separate correlations were performed for the KA+ group alone among the hippocampal volume and the speed in which the rats found the goal ziggurat, on a given day (Table 6). The analysis failed to reveal any significant correlation between the hippocampal volume and the speed with which the rats found the goal ziggurat for the KA+ group.

Table 6.

Pearson product-moment correlations, for speed in which the rats found the goal

ziggurat.

	Entire group of rats		KA+ Group	
	Pearson Correlation	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Day 1	0.286	0.282	0.673	0.067
Day 2	0.347	0.188	0.060	0.888
Day 3	0.397	0.128	0.038	0.928
Day 4	0.450	0.080	0.013	0.976

Note. The correlation values for the entire group of rats (column 2) and the KA+ group alone (column 4). There are no significant values identified.

The number of errors was calculated as the number of ziggurats that were explored prior to finding the goal ziggurat. For the number of errors, a repeated measures ANOVA was performed with day (days 1-4) and trial (trials 1-10) as within-subject measures and group (KA+, KA-) as a between-subject measure. ANOVA failed to reveal a significant difference between the KA+ group (M=4.203 errors; SD=0.687) and the KA- group (M=2.875 errors; SD=0.687), $F(1,14)$=1.921, p=.187. However, ANOVA revealed a main effect of day, $F(3, 42)$=8.089, p<.001 (Figure 20) and a main effect of trial $F(9, 126)$=4.784, p<.001 (Figure 21). Unlike the other measures, the interaction between day and trial failed to reach significance $F(27,378)$=0.990, p=.482. Notably, the interaction between: day and group, $F(3,42)$=0.133, p=.940; trial and group, $F(9,126)$=1.035, p=.416; and day, trial, and group, $F(27,378)$=1.231, p=.201, all failed to reach significance.
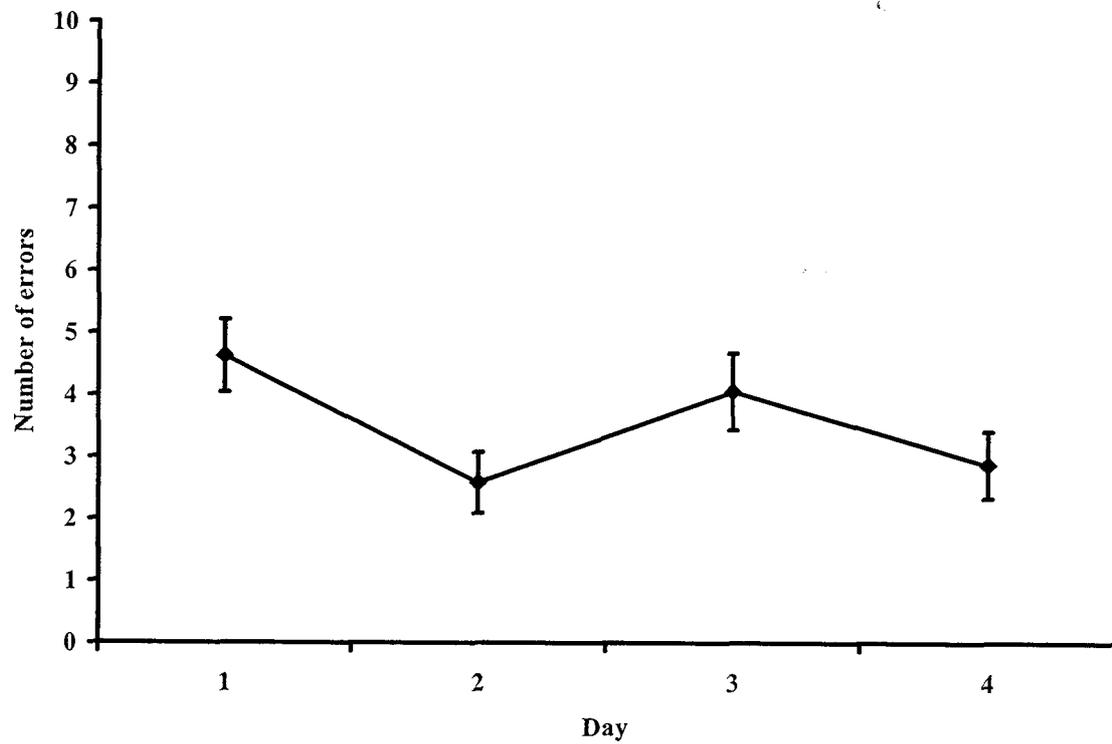
Figure 23. The significant main effect of day for the number of errors that the rats made

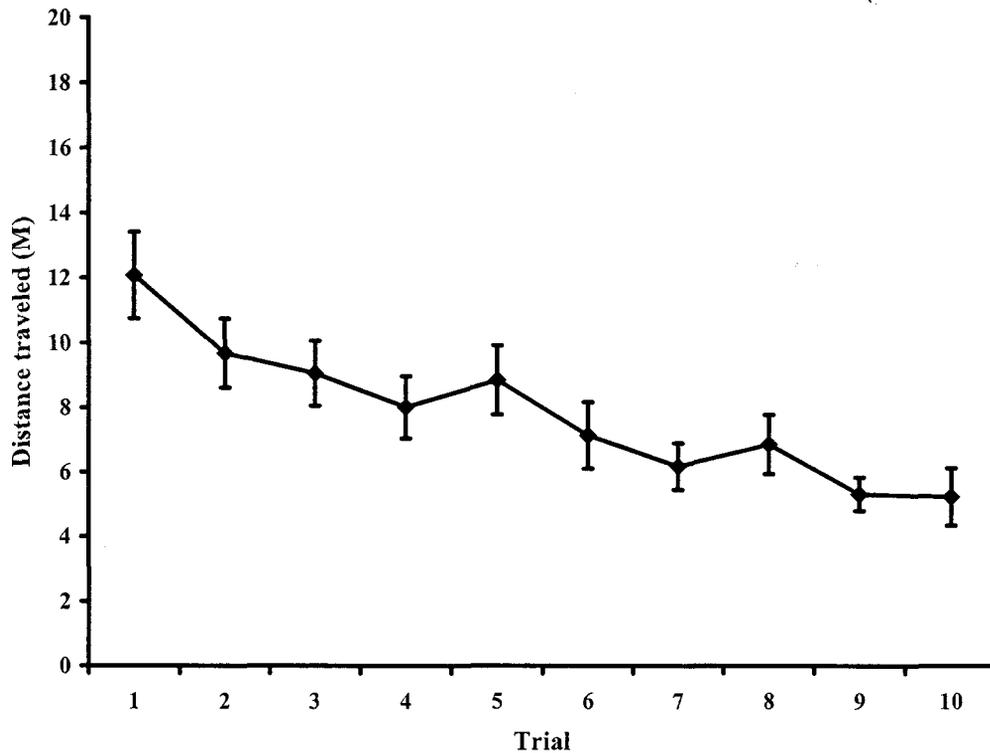to find the goal ziggurat. The symbols represent mean number of errors ± the SEM.
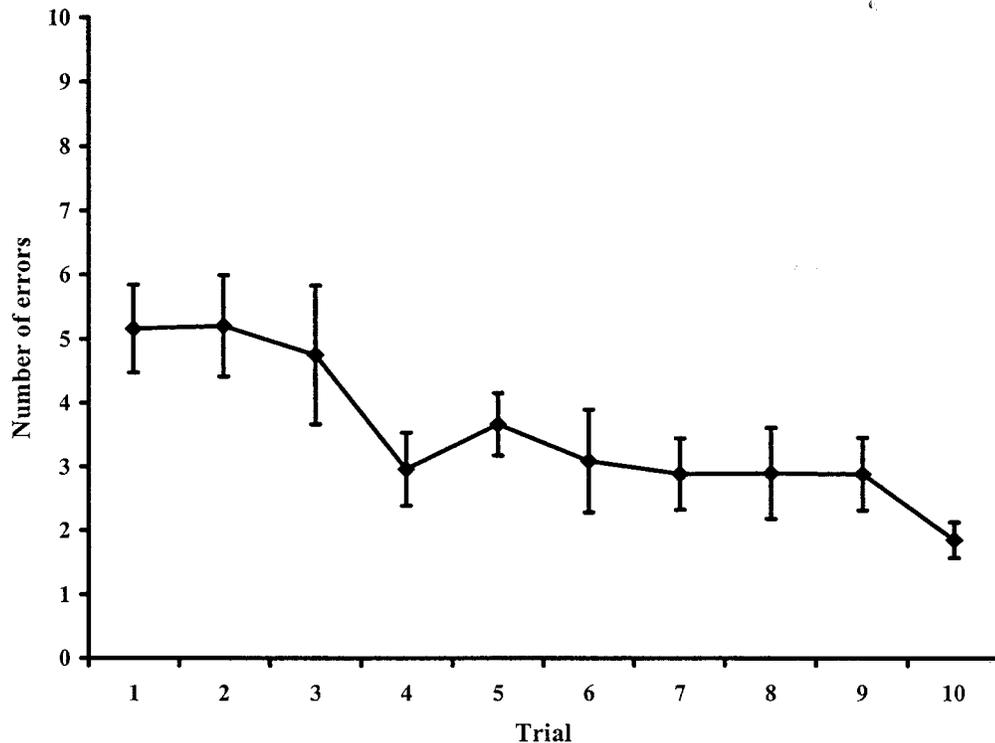
Figure 24. The significant main effect of trial for the number of errors that the rats made to find the goal ziggurat. The symbols represent mean number of errors ± the SEM.

The number of errors was averaged across the ten trials and planned comparisons (Bonferroni) were performed on the learning days and the memory days. For the learning days, planned comparisons were conducted between the two consecutive days that had the same goal ziggurat location. The analysis revealed the number of errors in day 1 was significantly more than day 2, $p=.024$. Also, the analysis revealed the number of errors in day 3 was significantly more than day 4, $p=.005$.

For the memory days, planned comparisons (Bonferroni) were conducted on two consecutive days with the new goal ziggurat location. For example, comparisons were

made between: day 1 and day 3. The analysis failed to reveal a significant difference between day 1 and day 3, $p=1.000$.

To examine the simple main effect of trial, the number of errors was averaged across the four days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed that: the number of errors in trial 1 was significantly more than trial 10, $p=.016$; the number of errors in trial 2 was significantly more than trial 10, $p=.010$; and that the number of errors in trial 5 was significantly more than trial 10, $p=.044$. However, the analysis failed to reveal any other significant differences among the trials.

Discussion

The aim of this study was twofold. Firstly, the experiment intended to replicate the effects of SE on hippocampal volumes and to demonstrate that rats treated with KA would develop spontaneous convulsions. Secondly, the study looked for behavioural impairments associated with KA-induced SE in rats.

Consistent with other studies and as hypothesized, there was a reduction in hippocampal volume in rats that experienced SE when compared with SA-treated controls. Furthermore, there was a negative correlation between the duration of SE and hippocampal volume (Table 7), suggesting that rats that had a longer duration of SE had more hippocampal volume loss.

74

Table 7.

Summary of the correlations between hippocampal volume and the convulsion analysis

for Experiment 1.

Convulsion Analysis	Correlation with Hippocampal Volume
Duration of status epilepticus	Negative correlation
Number of spontaneous convulsions	No correlation
Duration of spontaneous convulsions	No correlation

Unlike what was predicted, there was no significant correlation between the number of spontaneous convulsions and the hippocampal volume loss. Several studies have indicated that the number of spontaneous seizures and hippocampal volume were negatively correlated (de Rogalski Landrot et al., 2001; Kotloski et al., 2002; Sutula et al., 1995). For instance Kotloski et al., (2002) found that experiencing 35 generalized seizures results in deficits in the performance of hippocampally-dependent tasks, such as radial-arm-maze (Olton & Samuelson, 1976). Further, there is a direct relationship between the extent of damage and the number of siezures. There are two possible reasons for the lack of relation observed: 1. Time of day that the rats were monitored; and 2. Lack of statistical power.

Seizure activity is associated with circadian rhythms (Roberts & Keith, 1994 b), with maximum seizure activity occurring at 6:30 p.m..Thus, it may be that my procedure that sampled convulsive behavior at random times across the day, thereby eliminating possible effects that could have been observed only at one time of the day. However,

75

consistent with the majority of the literature, it was decided that a procedure that randomized observation periods provided the best opportunity to observe spontaneous convulsions.

A second possibility and perhaps a more probable one is that the sample size was too small in order to detect an existing correlation. That is, there is not enough statistical power ($\beta=0.833$). In fact, Cohen (as cited in Tabachnick & Fidell, 2001) suggests that correlations of larger than 0.25 are considered large and the obtained correlation value was certainly larger than that. Thus, it is likely that there was a negative strong relationship, which is consistent with the predictions, between what and what that failed to reach significance due to insufficient statistical power.

Contrary to the hypothesis, there was no correlation between the duration of spontaneous convulsions and the hippocampal volume loss. Similar to the seizure frequency, the correlation may not have been detected, as there was not enough statistical power, since there was a correlation value was also in the range considered large by Cohen (as cited in Tabachnick & Fidell, 2001). Thus, it is likely that there was a negative strong relationship, which is consistent with what was predicted, between what and what that failed to reach significance due to insufficient statistical power.

The ziggurat and water tasks are both thought to be hippocampally-dependent visio-spatial tasks (Faraji et al., 2007; Hodges, 1996; Morris, 1981), which require the association of an external cue with a given location in space (hidden platform in water task or goal ziggurat in the ziggurat task) relative to the rat's egocentric space (Hodges, 1996; Morris, 1981). As the rats that experienced SE had hippocampal damage,

impairments in both tasks were expected (Bortz, 2003; Detour et al., 2005; Hannesson & Corcoran, 2000; Hannesson et al., 2001; Hannesson et al., 2004; Helmstaedter, 2002; Letty et al., 1995), although, only an impairment in the ziggurat task was observed, with rats that experienced SE took longer to find the goal ziggurat when compared to the SA-treated controls (Table 8). No impairment was observed in the water task.

Table 8.

Summary of the main effects of group (p<.05) for the behavioural analyses for Experiment 1.

		Group Effect
Single Pellet Reaching Task	Total Reach	No
	Single Reach	No
Ladder Rung Walking Task	Number of Falls	No
	Number of Repositions	No
Object Discrimination Task	15 minutes Delay	No
	240 minutes Delay	No
Water Task	Latency	No
	Distance travelled	No
	Speed	No
Ziggurat Task	Latency	Yes
	Distance travelled	No
	Speed	No

This result is somewhat perplexing, until the nature of the two tasks is considered. The ziggurat task requires both vertical and horizontal navigation, whereas the water task requires only horizontal navigation (Faraji et al., 2007). Navigation in the ziggurat task is more complex and may require acquisition of multiple combinations of reference frames in the environment (O'Reilly & Rudy, 2001). As such, the ziggurat task may be more sensitive to smaller lesion sizes than the water task.

Previous studies suggest that kindling and the damage associated with KA-induced SE extends beyond the hippocampus to the perirhinal cortex (Bernasconi et al., 1999; Bernasconi et al., 2003; Kelly & McInetyre, 1996). Functionally, perirhinal cortex damage has been associated with impairments in object discrimination memory (Ennaceur & Aggleton, 1997). Unlike other studies (Hannesson et al., 2005; Lim et al., 2007), there was no discrimination memory impairment observed in the object discrimination task (Table 8). Several reasons may explain this finding. First, unlike kindling, in which a specific site, such as perirhinal cortex can be directly manipulated, KA-induced SE results in widespread variable damage. Thus, the same group of rats may show different amounts of damage to different sites in the brain. As extra-hippocampal damage was not measured in this study, it is unclear to what extent these areas were damaged and whether this damage was sufficient to lead to an impairment. Second, even though the objects that were used in this study were previously tested for discriminability, not even the SA-treated rats showed preference for the novel object. As previous studies either did not food deprive the rats (Hannesson et al., 2005; Lim et al., 2007) or only did so for a short period of time (Winters et al., 2004), the longer period of food-restriction in this thesis may have induced differences in behavior. That is, it is possible that the rats

were not directing their exploration towards the objects but rather they were searching their environment for food. Thus, given that my control rats did not exhibit the predicted behavior, it is difficult to interpret what the behaviour of the KA treated rats means.

Unlike other studies (Hernandez et al., 2002; Kubova et al., 2000), there were no motor impairments observed in either task used in my study (Table 8). However, it must be noted that Kubova et al. (2000) induced SE early in life and then motor skill later in adulthood. In contrast, in this study SE was induced in adulthood and motor skill was studied in adulthood. It has been suggested that SE has a different impact on the brain early in life and in adulthood (Lado et al., 2000; Sperber, 1991; Tremblay et al., 1984). Thus, even though Kobova et al. (2000) found an impairment in rotorod performance, no such impairment was detected in this study. Thus, the findings indicated that inducing SE and testing the rats in in adulthood does not lead to any motor impairments. Furthuremore, rotarod measures gross motor function while Experiment 1 was measuring fine motor function, which may explain the difference in the findings. As noted above, given the power issues, further study would be needed to confirm this fact.

All together, the findings of Experiment 1 indicated that SE is associated with a significant reduction in hippocampal volume that was positively correlated with the duration of SE. However, there was no significant correlation between the hippocampal volume loss and the number of spontaneous convulsions or the duration of spontaneous convulsions. Behaviourally, only the performance on the ziggurat task was significantly impaired.

EXPERIMENT 2. STRESS AND CONVULSIONS AND BEHAVIOUR

Introduction

Previous findings suggest that stress may cause hippocampal damage (McEwen & Magarinos, 1997; 2001; Watanabe et al., 1992). Stress-induced hippocampal damage has been associated with impairments in water task and radial-arm-maze (Aztiria, et al., 2007; Cilio et al., 2003; Detour et al., 2005; D'Hooge & De Deyn, 2001; Lai et al., 2006). Parallel to these findings, exposure to stress and stress hormones have been associated with worsening of seizure disorders (for example see Taher et al., 2005). To achieve a better understanding of the damage associated with KA-induced SE and the associated behavioural impairments, Experiment 2 was designed to look at the modulatory effects of stress and SE on hippocampal volume, frequency and duration of spontaneous convulsions, and a subset of behaviours chosen from Experiment 1.

Several lines of evidence suggest that stress may have a potential negative impact on seizure activity in humans, as many individuals with epilepsy show a positive association between the seizure frequency and stress (Schmid-Schonbein, 1998) and concentrations of stress hormones (Calabrese et al., 1993; Gallagher et al., 1984; Galimberti et al., 2005). Furthermore, the structural damage associated with stress, such as neuronal loss in hippocampus is very similar to those observed in SE (Bonilha et al., 2003; McEwen, 1999; Starkman et al., 1999). In addition, studies using non-human animals suggest that stress has an impact on susceptibility to seizures (Edwards et al., 1999; Karst et al., 1999; Salzberg, et al., 2007; Taher et al., 2005). All together, these findings suggest that exposure to stress negatively impacts a number of different facets of seizure disorders.

To replicate previous studies, as well as the results of Experiment 1, it was hypothesized that KA-treated rats that developed SE would have a reduced hippocampal volume when compared to SA-treated controls. Also, it was hypothesized that there would be a positive correlation between the duration of SE, number of convulsions and the amount of hippocampal volume loss. As there is a negative association between stress and hippocampal volume (Uno et al., 1989; Czeh et al., 2001), it was hypothesized that stressed SA-treated rats would show smaller hippocampal volume than non-stressed SA-treated rats.

As well, It was hypothesized that there would be an additive effect of SE and stress on hippocampal volume. Thus, it was hypothesized that rats that experienced SE and were exposed to stress exhibiting the lowest volumes of the hippocampus. However, rats that experienced SE and were not exposed to stress or stressed SA-treated rats would exhibit intermediate volumes. As was the case in Experiment 1, it was hypothesized that SA-treated controls would have the largest hippocampal volume.

With respect to spontaneous convulsions, it was predicted that stressed SA-treated rats would show a higher number of spontaneous convulsions than non-stressed SA-treated rats. Rats that experienced SE and were exposed to stress would exhibit the highest numbers of spontaneous convulsions. Intermediate levels were predicted for rats that experienced SE and were not exposed to stress and stressed SA-treated rats. As above, it was predicted that SA-treated controls would exhibit the lowest numbers of spontaneous convulsions.

With respect to cognitive behavior, the water task, the ziggurat task and object discrimination memory were examined. For the water task and the ziggurat task, it was predicted that stressed SA-treated rats would show longer latencies than non-stressed SA-treated rats. It was hypothesized that there would be an additive effect of SE and stress on and that the rats that experienced SE and stress and the rats that experienced SE and were not exposed to stress exhibiting the longest latencies. It was predicted that SA-treated controls would exhibit the shortest latency. For object discrimination memory, it was predicted that stressed SA-treated rats would show less of a preference towards the novel object than non-stressed SA-treated rats. As it was hypothesized that there would be an additive effect of SE and stress on preference towards the novel object, it was predicted that rats that experienced SE and were exposed to stress exhibiting the least preference towards the novel object. Similar to the other hypotheses, rats that experienced SE and were not exposed to stress and stressed SA-treated rats would exhibit intermediate preference towards the novel object. As always, the SA-treated controls would exhibit the greatest preference towards the novel object.

With respect to motor behaviours, there were some differences from Experiment 1, as the single pellet reaching task was omitted from Experiment 2. As single pellet reaching task requires extensive handling and training and food restriction during training and testing, both of which are associated with higher levels of stress and anxiety (Lapin, 1995). This would have introduced a confounding factor into a study that is examining the effects of stress. For the ladder rung walking task, it was predicted that stressed SA-treated rats would show higher number of falls and repositions than non-stressed SA-treated rats. As well, it was hypothesized that there would be an additive effect of SE and

stress on the number of falls and repositions, with rats that experienced SE and were exposed to stress exhibiting the highest numbers of falls and repositions. Intermediate levels were predicted for rats that experienced SE and were not exposed to stress and stressed SA-treated rats. As always, it was predicted that SA-treated controls would exhibit the lowest numbers of falls and repositions.

Methods

Subjects

Forty-one adult male Long–Evans hooded rats (Charles River) weighing 400–550 g at the beginning of the study were used as subjects. Rats were maintained in pairs in Plexiglas cages throughout the course of experiment. All experimental procedures were carried out during the light portion of the 12:12 h light/dark cycle. During testing periods, for the ziggurat task the rats were fed 15- 20 g of laboratory chow after daily testing sessions to maintain weights at 85–90% of free-feeding body weight. Water was available *ad libitum* throughout the experiment.

All rats were handled at least once every 48 hours throughout the experiment. All procedures were in accordance with the guidelines established by the Canadian Council of Animal Care as approved by the University of Lethbridge Animal Care Committee.

Rat Treatment

Each rat was assigned to one of three groups: Controls (1 ml/kg SA i.p., $n=13$); rats that received KA (10 mg/kg, i.p.) and had SE (KA+/+, $n=11$); and rats that received KA but did not have SE (KA+/-, $n=17$). Of the KA+/+ rats, 5 died due to excessive

convulsions. Of the KA+/- rats, all received a supplementary dose of KA (2 mg/kg, i.p.), although none developed SE and only exhibited immobilization and wet dog shakes (n=17).From the rats that survived (n=6) SE lasted for a few hours (M=10800 seconds; SD=3412.729).

Convulsion Monitoring

Convulsions were monitored by the experimenter and video recorded (Camera model: Canon Zr 500) as described in Experiment 1. Dependent measures were as described in Experiment 1.

Restraint Stressor

Within 48 hours after being injected with either KA or SA, the rats in the stress group (KA-/-=5; KA+/-=8; KA+/+=3) were placed in a transparent Plexiglas tube (6 cm inner diameter) of adjustable length (Figure 25). The length of the tube was adjusted to the rat's body size so that the base of the tail extended from the tube. Rats remained in the tube for 1 hour per day for 14 consecutive days. The time of day that the rat was placed in the tube varied randomly over the 14 days.
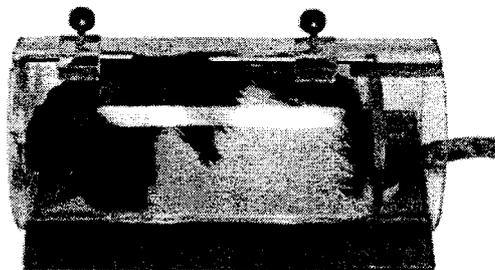
Figure 25. Photograph of a rat placed in a transparent Plexiglas tube, for restraint stress. Rats remained in the tube for 1 hour per day for 14 days.

Behavioural Assessment

Seventeen days after the rats had been injected with either KA or SA they were assessed on a battery of tests to detect changes in sensory and /or motor function. The order and sequence of testing was as described in Figure 26.

| Week I | Week II | | Week III | Week IV | | Week V | Week VI | Week VII | Week VIII |

Stress

KA Injection

No Stress

Stress

SA Injection

No Stress

Ladder Rung Walking Task

Object Discrimination Task Habituation

Object Discrimination Task Testing

Water Task Testing

Ziggurat Task Testing
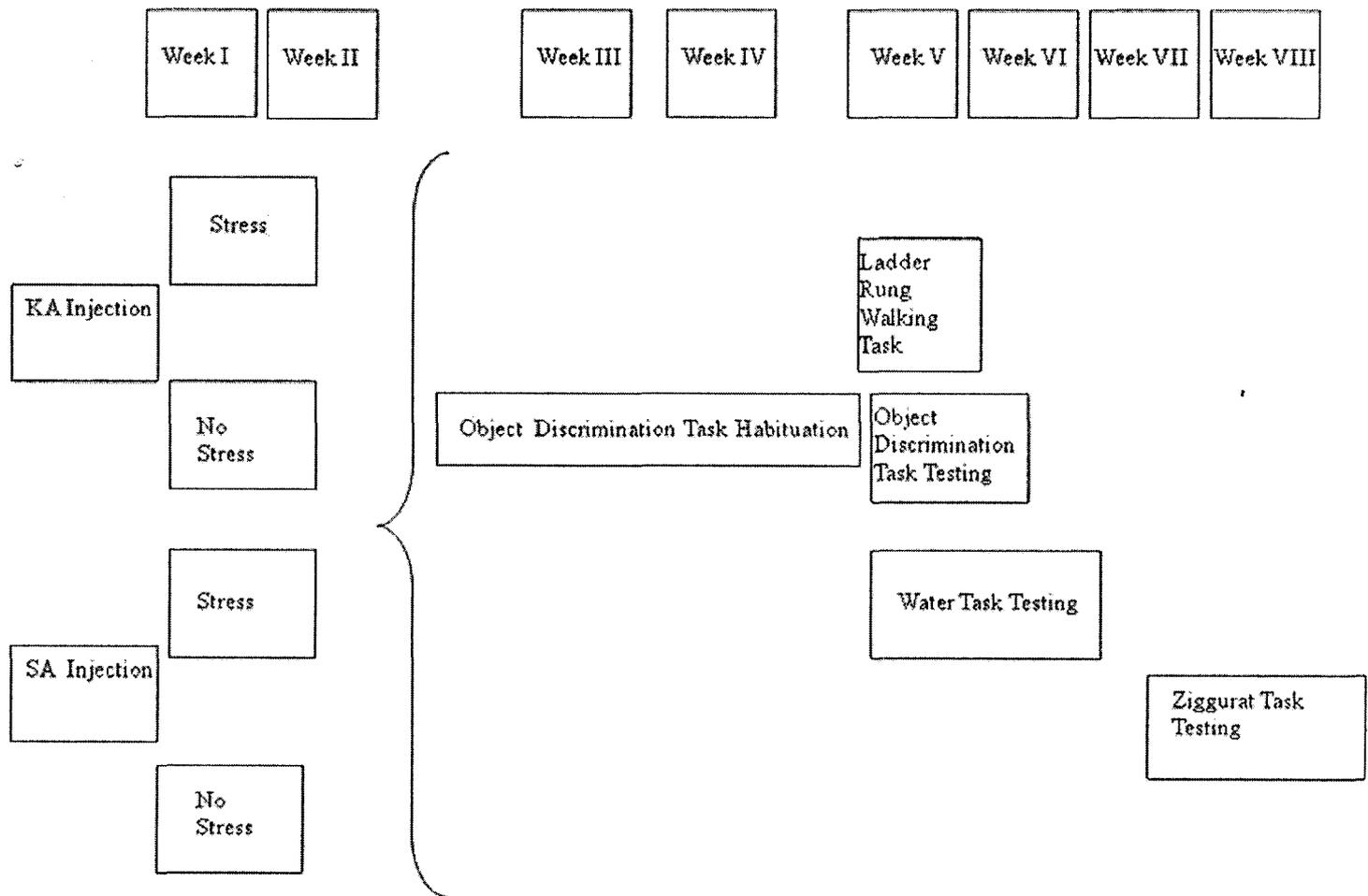
Figure 26. The time frame of the testing procedures in Experiment 2.

86

The ladder rung walking task and the object discrimination task were performed in the same manner as described in Experiment 1. The water task was performed identically, although the data were collected and analyzed using Ethovision 3 (Noldus). For these behavioural tests, the dependent measures were the same as in Experiment 1.

The ziggurat task was conducted largely the same as in Experiment 1. However, in Experiment 2, the learning and memory components of the test were doubled. That is, as in Experiment 1, rats were habituated for 4 days, but they were tested for 8 days; 4 days with a peripheral ziggurat location and 4 days with a central ziggurat location. Every other day, the goal ziggurat was located in a new position and rats had to find and learn the location of the goal ziggurat in the new place within 120 seconds (a reduction from 180 seconds in Experiment 1). Thus, as in Experiment 1, trials cycled between alternating "learning" days followed by a 'memory" days. The dependent measures were the same as in Experiment 1.

Euthanasia and Histology

Euthanasia and histology were performed as described in Experiment 1.

Results

Exclusions

Although 28 rats were injected with KA, only 11 developed SE. Of these 11 rats, 5 died. Of the rats that did not have convulsions, 5 of the rats (KA-/-, stress $n=2$; KA-/-, no stress $n=1$; KA+/-, stress $n=1$; KA+/-, stress $n=1$) had to be excluded from all analyses

due to problems during sectioning of the brain for the histological analysis. Thus, the

final assignment to group is shown in Table 9 with the total being $n=2$.

Table 9.

Final group assignment for the rats in Experiment 2.

	Stress	Number of rats
KA-/-	Yes	3
	No	3
KA+/-	Yes	7
	No	2
KA+/+	Yes	3
	No	3

Hippocampal Volume

Consistent with the Cavalieri method, hippocampal volume was calculated as

described in Experiment 1. The total number of sections were analyzed with ANOVA

with group (KA+/+, KA+/-, KA-/-) and stress (no stress, stress) as the between-subjects

measures.

For the number of sections, ANOVA failed to reveal a significant difference

between KA+/+ group ($M=10.666$; $SD=1.751$) and either the KA+/- group ($M=10.444$;

$SD=2.403$), or the KA-/- group ($M=10.666$; $SD=1.632$), $F(2,15)=0.052$, $p=.949$.

Similarly, ANOVA failed to reveal a significant difference between the stress group

($M=10.384$; $SD=2.063$) and no stress group ($M=10.875$; $SD=1.807$), $F(1,15)=0.207$,

$p=.656$; and there was no significant interaction between group and stress, $F(2,15)=0.985, p=.396$.

An ANOVA with group (KA+/+, KA+/-, KA-/-) and stress (no stress, stress) as the between subjects measures was performed on the hippocampal volume. The analysis failed to reveal a significant difference between hippocampal volume for the KA+/+ group ($M=101.520$ mm³; $SD=34.654$) and either the KA+/- group ($M=118.426$ mm³; $SD=34.902$), or the KA-/- group ($M=127.080$ mm³; $SD=24.421$), $F(2,15)=0.916, p=.421$. Similarly, ANOVA failed to reveal a significant difference between the stress ($M=116.215$ mm³; $SD=35.044$) and no stress rats ($M=115.830$ mm³; $SD=29.377$), $F(1,15)=0.002, p=.965$; and there was no significant interaction between group and stress, $F(2,15)=0.322, p=.730$.

To replicate the findings from experiment 1 the rats from no stress group were selected and independent sample t-test with group (KA+/+ and KA-/-) as an independent variable and hippocampal volume as a dependent variable was performed. The analysis failed to reveal a significant difference between the KA+/+ group ($M=92.800$; $SD=31.902$) and KA-/- ($M=109.260$; $SD=47.502$), $t(4)=1.622, p=.180$ (2 tailed).

As none of the rats from KA+/- group developed SE or spontaneous convulsions the rats belonging to two groups (KA+/- and KA-/-) were collapsed together, to measure hippocampal volume. Thus, with group (SE, no-SE) and stress (no stress, stress) as the between subjects measures were performed on the hippocampal volume. The analysis failed to reveal significant group differences between SE group ($M=101.520$ mm³; $SD=34.654$) and the no-SE group ($M=121.888$ mm³; $SD=30.468$), $F(1, 17)=1.935$,

$p=.182$. Also, the analysis failed to reveal a significant difference between the stress

. ($M=116.215$ mm³; $SD=35.044$) and no stress rats ($M=115.830$ mm³; $SD=29.377$),

$F(1,17)=0.033$, $p=.859$; and there was no significant interaction between SE and stress,

$F(1, 17)=0.822$, $p=.377$ (Figure 27).
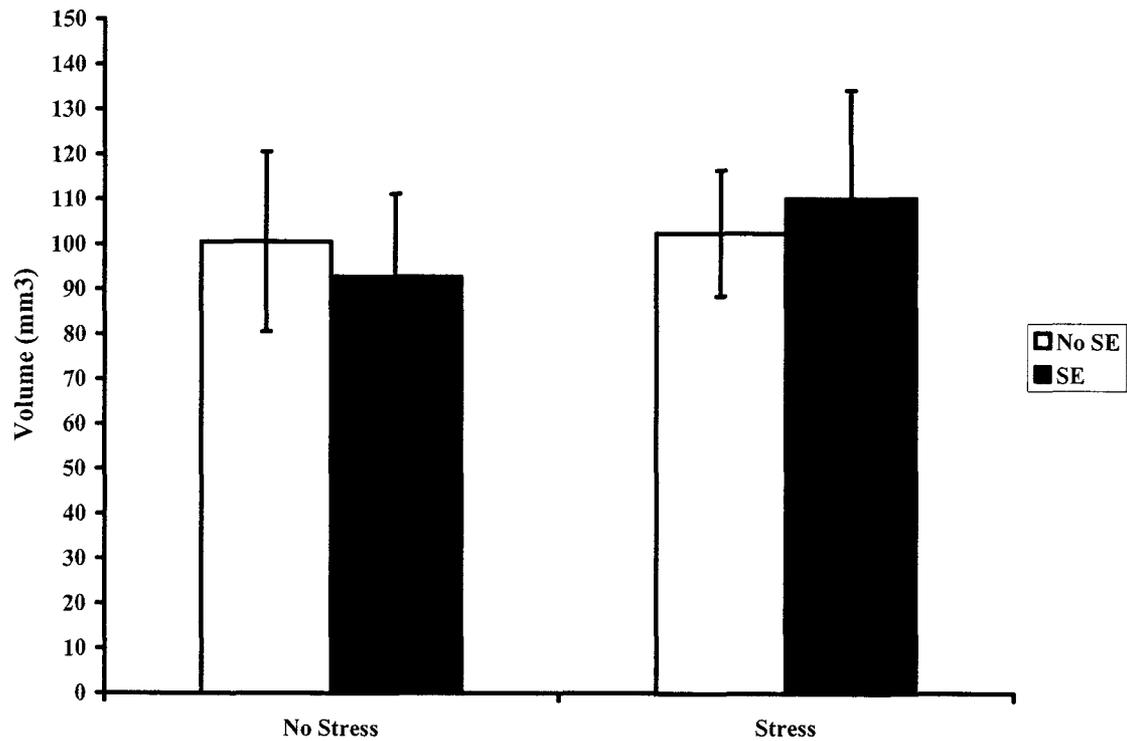
Figure 27. The non- significant interaction of SE and stress with hippocampal volume

(mm³). The bars represent the mean volume ± the SEM.

Convulsions

All of the KA+/+ rats exhibited spontaneous convulsions ($M=8.17$ convulsions;

$SD=5.231$) during subsequent observation periods. Neither the KA+/- rats nor the KA-/-

rats exhibited spontaneous convulsions during the observation periods; as such, only the

KA+/+ rats convulsive behaviour was analyzed for this variable. Separate ANOVAs with

group (KA+/+, KA+/-, KA-/-) and stress (no stress, stress) as the between-subjects measures was performed on: duration of the initial episode of SE, total number of spontaneous convulsions, and the duration of spontaneous convulsions observed during the experiment.

For duration of the initial episode of SE, ANOVA revealed a significant difference between the KA+/+ group (M=10680.000 seconds; SD=3412.729) and either the KA+/- group (M=0.000 seconds; SD=0.0000), or the KA-/- group (M=0.000 seconds; SD=0.0000), $F(2,15)$=63.754, p<.001. Posthoc (Bonferroni) indicated that KA-/- group had significantly less (no convulsions) than KA+/+ group, p<.001. Also, KA+/- group had significantly less (no convulsions) than KA+/+ group, p<.001. However, the analysis failed to reveal difference between KA-/- group (no convulsions) than KA+/- group, p=1.000. Also, ANOVA failed to reveal a significant difference between the stress group (M=9840.000 seconds; SD=4501.199) and no stress group (M=11520.000 seconds; SD=2595.996), $F(1,15)$=0.397, p=.538; and there was no significant interaction between group and stress, $F(2,15)$=0.394, p=.681.

For total number of spontaneous convulsions, ANOVA revealed a significant difference between the KA+/+ group (M=8.166 convulsions; SD=5.231) and either the KA+/- group (M=0.000 convulsions; SD=0.0000), or the KA-/- group (M=0.000 convulsions; SD=0.0000), $F(2,15)$=15.645 , p<.001. Posthoc (Bonferroni) indicated that KA-/- group had significantly less (no convulsions) than KA+/+ group, p<.001. Also, KA+/- group had significantly less (no convulsions) than KA+/+ group, p<.001. However, the analysis failed to reveal significant difference between KA-/- group (no

91

convulsions) than KA+/- group, p=1.000. Also, ANOVA failed to reveal a significant difference between the stress group (M=9.333 convulsions; SD=6.028; maximum=15; minimum=3) and no stress group (M=7.000 convulsions; SD=5.292; maximum=13; minimum=3), $F(1,15)$=0.321, p=579; and there was no significant interaction between group and stress, $F(2,15)$=0.319, p=.731.

For the duration of spontaneous convulsions, ANOVA revealed a significant difference between the KA+/+ group (M=326.666 seconds; SD=214.514) and either the KA+/- group (M=0.000 seconds; SD=0.0000), or the KA-/- group (M=0.000 seconds; SD=0.0000), $F(2,15)$=14.015, p<.001. Posthoc (Bonferroni) indicated that KA-/- group had significantly less (no convulsions) than KA+/+ group, p<.001. Also, KA+/- group had significantly less (no convulsions) than KA+/+, p<.001. However, the analysis failed to reveal difference between KA-/- group (no convulsions) than KA+/- group, p=1.000. Also, ANOVA failed to reveal a significant difference between the stress group (M=333.330 seconds; SD=246.640; maximum=500; minimum=50) and no stress group (M=320.000 seconds; SD=232.542; maximum=550; minimum=85), $F(1,15)$=0.006, p=.940; and there was no significant interaction between group and stress, $F(2,15)$=0.006, p=.994.

Pearson product-moment correlations were performed among the hippocampal volume and: duration of the initial episode of SE; the number of spontaneous convulsions; and the total duration of spontaneous convulsions observed during the experiment. For the entire group of rats, there was a significant correlation between hippocampal volume and the duration of the initial episode of SE, $r(19)$=-.443, p=.022.

For the entire group of rats, there was significant correlation between hippocampal volume and the number of spontaneous convulsions, $r(19)=-.457$, $p=.037$. Also, for the entire group of rats, there was a significant correlation between hippocampal volume and the duration of spontaneous convulsions, $r(19)=-.507$, $p=.010$. However, as in Experiment 1, none of the KA+/- rats and the KA-/- rats had convulsions; thus, this correlation may be spurious.

As such, separate correlations were performed for the KA+/+ group alone. For the KA+/+ group, there was a correlation between hippocampal volume and the duration of the initial episode of SE, $r(4)=-.947$, $p=.002$. Also, for the KA+/+ group, the analysis revealed significant correlation between hippocampal volume and the number of spontaneous convulsions, $r(4)=-.706$, $p=.058$. Moreover, for the KA+/+ group, there was a significant correlation between hippocampal volume and the duration of spontaneous convulsions, $r(4)=-.862$, $p=.014$.

Behavioural Assessment

Ladder rung walking task. The number of repositions and falls were summed across the three trials and were analyzed with separate ANOVAs using group (KA+/+, KA+/-, KA-/-) and the stress (stress, no stress) as the between-subjects variables.

For total number of falls, ANOVA revealed a significant main effect of group, $F(2,15)=5.267$, $p=.019$ (Figure 28). Posthoc analysis (Bonferroni) indicated that group KA-/- group fell significantly more than KA+/- group, $p=.013$. However, the analysis failed to reveal a significant difference between KA+/+ group and KA+/- group, $p=.649$ or between KA-/- group and KA+/+ group, $p=.222$. Also, ANOVA failed to reveal a

93

significant difference between the stress group (M=12.153; SD=5.505) and no stress

group (M=10.750; SD=4.891), $F(1,15)$=2.673, p=.123; and there was no significant

interaction between group and stress, $F(2,15)$=1.891, p=.185.

Figure 28. The significant main effect of group for total number of falls in the ladder

rung walking task. The bars represent mean total number of falls ± the SEM.

For the number of hindlimb falls, ANOVA revealed a significant main effect of

group, $F(2,15)$=4.816, p=.024 (Figure 29). Posthoc analysis (Bonferroni) indicated that

KA-/- group fell significantly more than KA+/- group, p=.017. However, the analysis

failed to reveal a significant difference between KA+/+ group and KA+/- group, p=1.000

or between KA+/+ group and KA-/- group, p=.154. Also, ANOVA failed to reveal a

significant difference between the stress group (M=6.692; SD=3.326) and no stress group

(M=5.875; SD=2.948), $F(1,15)$=2.163, p=.162 and there was no significant interaction

between and stress, $F(2,15)$=0.853, p=.446.
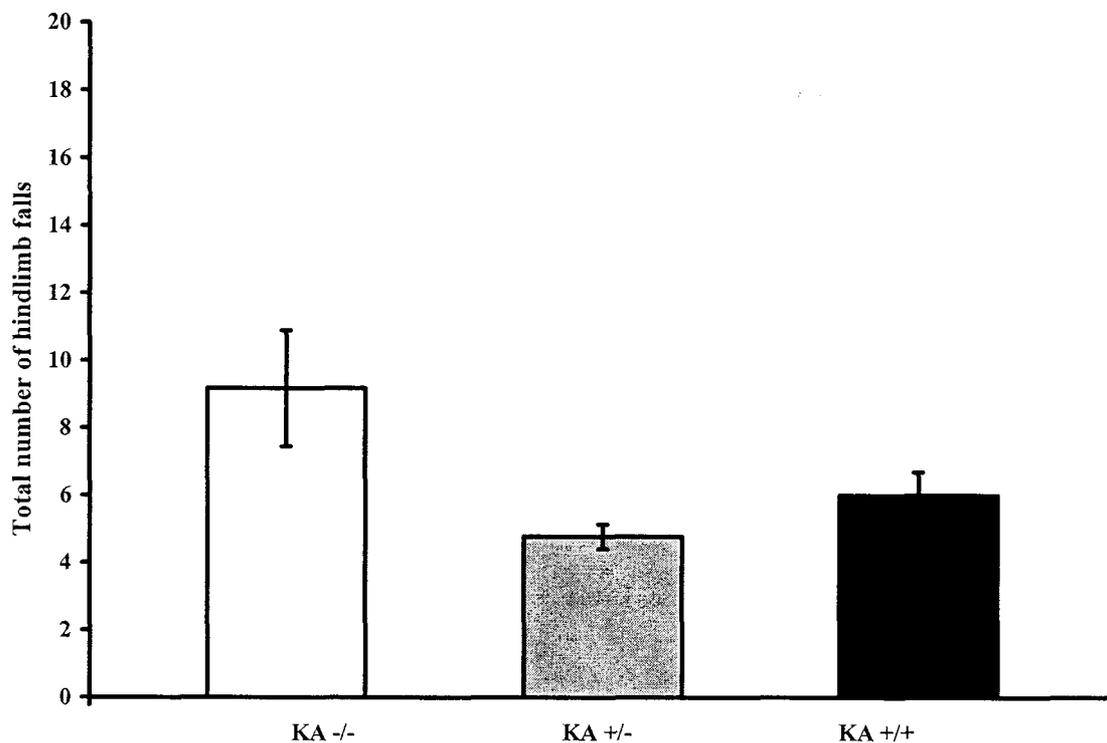
Figure 29. The significant main effect of group for total number of hindlimb falls in the

ladder rung walking task. The bars represent mean total number of falls ± the SEM.

For the total number forelimb falls, ANOVA failed to reveal a significant

difference between the KA+/+ group (M=5.500; SD=3.507), and either the KA+/- group

(M=4.000; SD=1.414) or the KA-/- group (M=6.833; SD=2.136), $F(2,15)$=2.985,

p=.081. Similarly, ANOVA failed to reveal a significant difference between the stress

group (M=5.461; SD=2.787) and no stress group (M=4.875; SD=2.232), $F(1,15)$=1.714,

p=.210; and there was no significant interaction between group and stress,

$F(2,15)$=2.400, p=.125.

For the total number of repositions, ANOVA failed to reveal a significant difference between the KA-/- group (M=4.666; SD=5.750), and either the KA+/- group (M=2.666; SD=2.397) or the KA+/+ (M=8.500; SD=5.167), $F(2,15)$=2.475, p=.118. Similarly, ANOVA failed to reveal a significant difference between the stress group (M=4.384, SD=4.682) and no stress group (M=5.750, SD=5.284), $F(1,15)$=0.014, p=.907; and there was no significant interaction between group and stress, $F(2,15)$=0.332, p=.723.

For the number of hindlimb repositions, ANOVA failed to reveal a significant difference between the group KA-/-(M=0.833; SD=2.041), and either the KA+/- group (M=0.777; SD=1.394) or the KA+/+ group (M=1.500; SD=2.345), $F(2,15)$=0.234 , p=.795. Similarly, ANOVA failed to reveal a significant difference between the stress group (M=0.923; SD=1.891) and no stress group (M=1.125; SD=1.807), $F(1,15)$=0.011, p=.917; and there was no significant interaction between group and stress, $F(2,15)$=1.139, p=.346.

For the number of forelimb repositions, ANOVA revealed a significant main effect of group, $F(2,15)$=4.153 , p=.037 (Figure 30). Posthoc analysis (Bonferroni) indicated that KA-/- group had significantly more repositions than KA+/- group, p=.035. However, the analysis failed to reveal a significant difference between KA+/+ group and KA+/- group, p=.820 or between KA+/+ group and KA-/- group, p=.322. On the other hand, ANOVA failed to reveal a significant difference between the stress group (M=3.461; SD=3.230) and no stress group (M=4.625; SD=4.103), $F(1,15)$=0.012,

p=.913; and there was no significant interaction between group and stress, $F(2,15)$=0.087, p=.918.
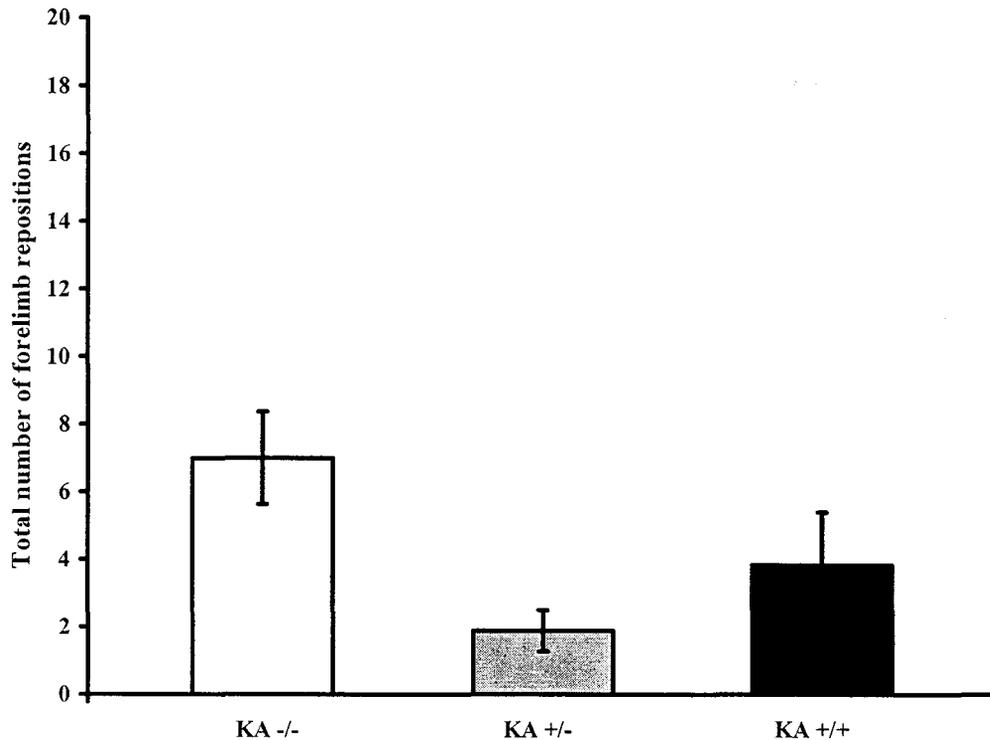
Figure 30. The significant main effect of group for total number of forelimb repositions in the ladder rung walking task. The bars represent mean of total number of reposition ± the SEM.

Pearson product-moment correlations were performed among the hippocampal volume and: the total number of repositions; and the total number of falls. For the entire group of rats, the analysis failed to reveal a significant correlation between the hippocampal volume and the total number of falls, $r(19)$=.254, p=.266. For the entire group of rats, the analysis failed to reveal a significant correlation between the hippocampal volume and the total number of repositions, $r(19)$=.309, p=.173.

As before, separate correlations were performed for the KA+/+ group alone. The analysis failed to reveal a significant correlation between the hippocampal volume and the total number of falls, $r(4)=.015$, $p=.978$ nor between the hippocampal volume and the total number of repositions, $r(4)=.405$, $p=.425$.

Object discrimination task. Using the discrimination index as a dependent variable, one sample t-test was performed comparing with the baseline zero. For 15-minute delay, the analysis revealed a significant effect $t(19)=3.163$, $p=.005$. Similarly, for 240-minutes, the analysis revealed a significant effect $t(19)=3.534$, $p=.002$.

Using the discrimination index as a dependent variable, separate ANOVAs with group (KA+/+, KA+/-, KA-/-) and stress (stress, no stress) as the between-subjects variables were performed for the trials that had a 15-minute delay and those which had a 240-minute delay.

For the 15-minute delay, ANOVA failed to reveal a significant difference between the KA+ /+ group ($M=-0.069$, $SD=0.427$), the KA+/- group ($M=0.399$, $SD=0.222$) and the KA -/- group ($M=0.361$, $SD=0.418$), $F(2,14)=2.565$, $p=.112$. Similarly, ANOVA failed to reveal a significant difference between the stress group ($M=0.0.284$, $SD=0.411$) and the no stress group ($M=0.244$, $SD=0.352$), $F(2,14)=0.006$, $p=.939$; and there was no significant interaction between group and stress, $F(2,14)=1.097$, $p=.361$.
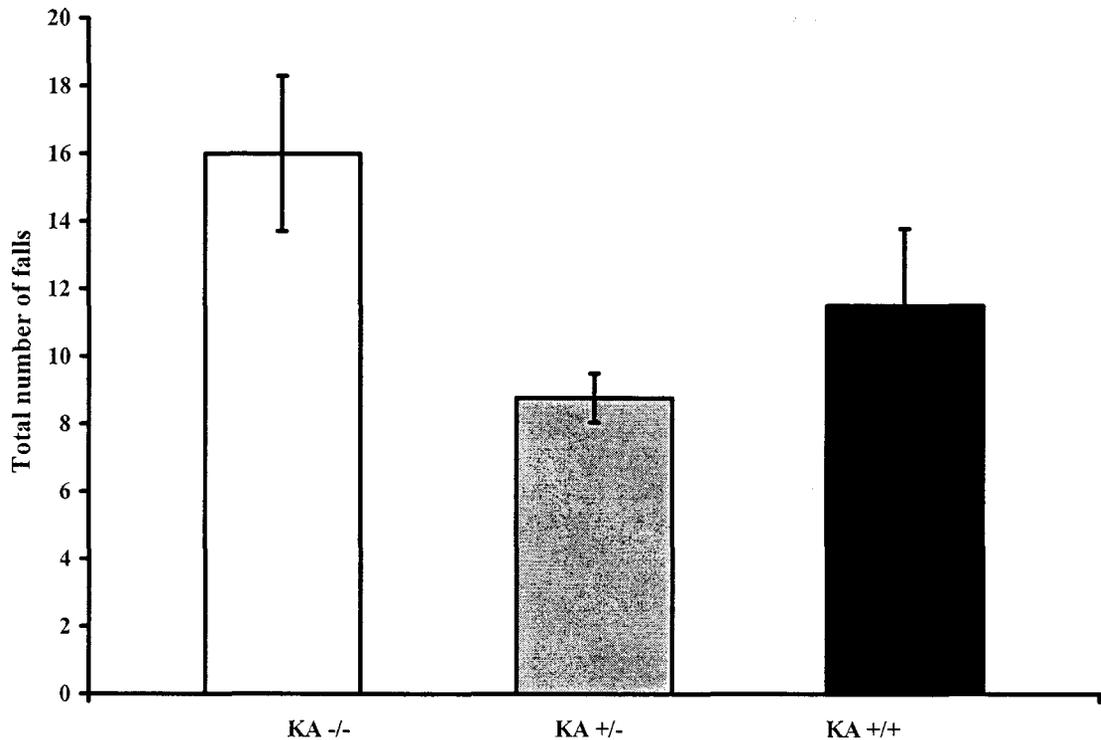
For the 240-minute delay, ANOVA revealed a significant main effect of group, $F(2,14)=17.443$, $p<.001$ (Figure 31). Posthoc analysis (Bonferroni) indicated that KA-/-

group discriminated significantly more than KA+/+ group, $p<.001$. Also, KA+/- group

discriminated significantly more than KA+/+ group, $p<.001$. However, the analysis failed

to reveal a significant difference between KA-/- group and KA+/- group, $p=1.000$. Also,

ANOVA failed to reveal a significant difference between the stress group ($M=0.142$;

$SD=0.392$) and no stress group ($M=0.333$; $SD=0.298$), $F(1,14)=2.590$, $p=.130$; and there

was no significant interaction between group and stress, $F(2,14)=1.459$, $p=.266$.
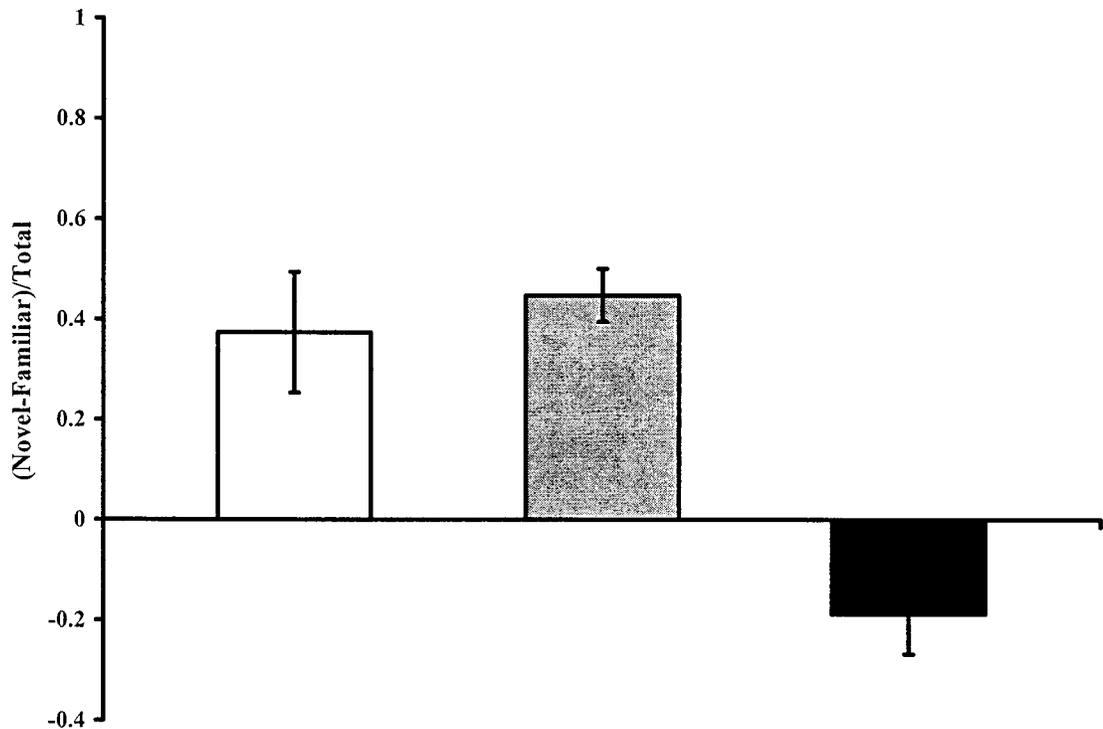
Figure 31. The significant main effect of group in the object discrimination task, for the

240-minute delay. The bars represent mean value for the discrimination index ± the SEM.

Pearson product-moment correlations were performed among the hippocampal

volume and the discrimination index for object discrimination task for: the 15-minute

delay; and the 240-minute delay. For the entire group of rats, the analysis failed to reveal

and stress, $F(6,45)=0.828$, $p=.554$; day, trial, and group, $F(78,585)=0.766$, $p=.929$; day, trial, and stress, $F(39,585)=0.978$, $p=.510$; day, trial, group, and stress, $F(78,585)=0.882$, $p=.753$; all failed to reach significance.
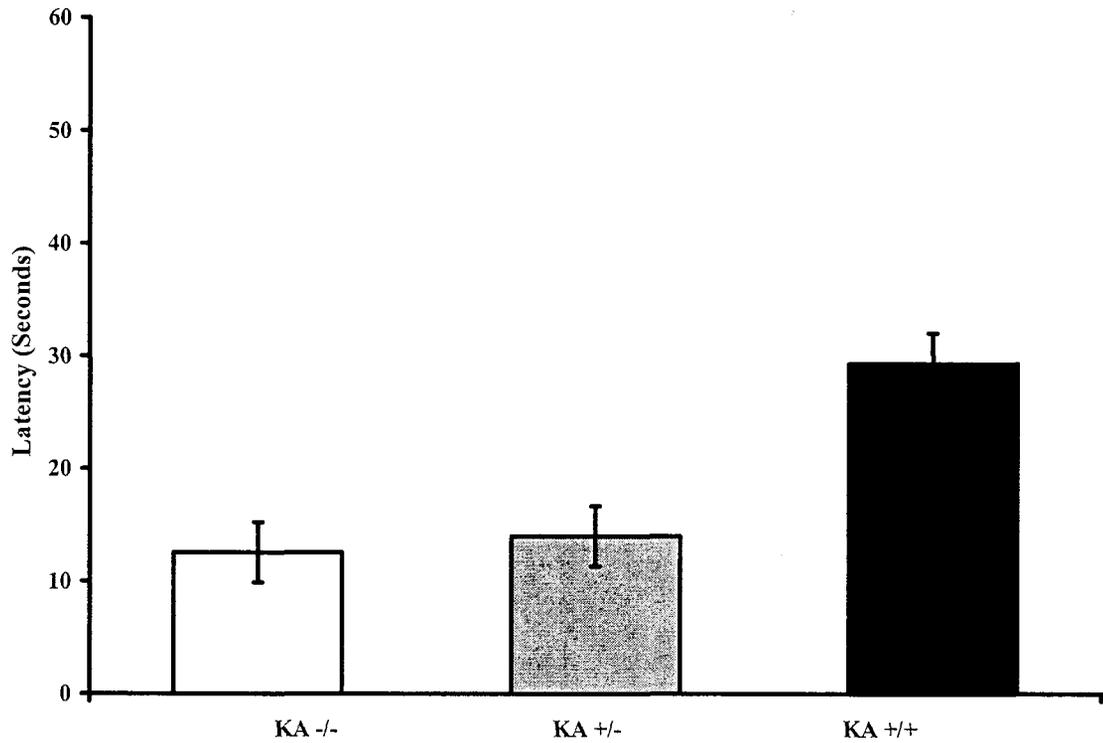
Figure 32. The significant main effect of group for the latency with which the rats found the platform in the water task. The bars represent mean latency (seconds) ± the SEM.

ANOVA revealed a significant main effect of day, $F(13,195)=9.483$, $p<.001$, a main effect of trial, $F(3,45)=19.678$, $p<.001$. However, these main effects were mediated by a significant interaction between day and trial, $F(39,585)=2.684$, $p<.001$ (Figure 33).

Figure 33. The significant interaction between day and trial for the latency with which

the rats found the platform in the water task. The symbols represent mean latency

(seconds) ± the SEM.

The latency was averaged across the four trials and planned comparisons

(Bonferroni) were performed on the learning days and the memory days. For the learning

days, planned comparisons were conducted between the two consecutive days that had

the same platform location. The analysis revealed a significant difference between:

between day 1 and day 2, $p=.049$; and between day 11 and day 12, $p<.001$. However, the

analysis failed to reveal a significant difference: between day 3 and day 4, $p=1.000$;

between day 5 and day 6, $p=1.000$; between day 7 and day 8, $p=.691$; between day 9 and

day 10, $p=.101$; and between day 13 and day 14, $p=1.000$.

For the memory days, planned comparisons (Bonferroni) were conducted on two consecutive days with the new platform location. For example, comparisons were made between: day 1 and day 3; day 3 and day 5; and so on. The analysis failed to reveal a significant difference between: day 1 and day 3, $p=.176$; day 3 and day 5, $p=1.000$; day 5 and day 7, $p=1.000$; day 7 and day 9, $p=1.000$; day 9 and day 11, $p=.672$; and day 11 and day 13, $p=1.000$.

To examine the simple main effect of trial, the latency was averaged across the fourteen days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed trial 1 was significantly longer than trial 2, $p=.007$; and trial 1 was significantly longer than trial 3, $p=.005$; and trial 1 was significantly longer than trial 4, $p<.001$. Also, trial 2 was significantly longer than trial 4, $p=.002$. However, the analysis failed to reveal a significant difference between trial 3 and trial 2, $p=.457$; and between trial 3 and trial 4, $p=.989$.

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the latency with which the rats found the platform for each day (averaged across trials). There was a significant correlation between the hippocampal volume and the latency with which the rats found the platform on day 4, however, the analysis failed to reveal any other significant correlation, for the entire group of rats. As before, separate correlations were performed for the KA+/+ rats alone among the hippocampal volume and the latency in which the rats found the platform, on a given day (averaged across trials) (Table 10). The analysis failed to reveal any significant

103

correlation between the hippocampal volume and the latency with which the rats found the platform, for the KA+/+ group.

Table 10.

Pearson product-moment correlations, for latency in which the rats found the platform in the water task.

	Entire group of rats		KA+/+ Group	
	Pearson Correlation	Sig. (2-tailed)	Pearson Correlation	Sig. (2-tailed)
Day1	-0.068	0.769	-0.082	0.878
Day2	-0.276	0.226	0.100	0.850
Day3	-0.205	0.373	0.293	0.573
Day4	**-0.451**	**0.040**	-0.605	0.203
Day5	-0.237	0.300	0.098	0.854
Day6	-0.163	0.479	-0.077	0.885
Day7	-0.340	0.132	-0.593	0.215
Day8	-0.372	0.097	-0.418	0.410
Day9	-0.164	0.477	-0.058	0.913
Day10	-0.149	0.519	-0.007	0.990
Day11	0.070	0.762	0.235	0.654
Day12	-0.033	0.888	0.043	0.935
Day13	-0.303	0.182	-0.720	0.107
Day14	-0.313	0.168	-0.499	0.313

Note. The correlation values for the entire group of rats (column 2) and the KA+/+ group alone (column 4). The significant values are identified in bold.

For the distance traveled, a repeated measures ANOVA was performed with day (days 1-14) and trial (trials 1-4) as within-subject measures and group (KA+/+, KA+/-, KA-/-) and stress (stress, no stress) as a between-subject measure. ANOVA failed to reveal a significant difference between the KA+/+ group (M=1.157 meters; SD=0.692) and the KA+/- group (M=0.756 meters; SD=0.199) and the KA -/- group (M=0.663 meters; SD=0.124), $F(2,15)$=2.309, p=.134. Similarly, ANOVA failed to reveal a

significant difference between the stress group (M=0.839 meters; SD=0.502) and the no

stress group (M=0.852 meters; SD=0.296), $F(1,15)$=0.036, p=.852. There was no

significant interaction between group and stress, $F(2,15)$=0.374, p=.694. Also, ANOVA

revealed a significant interaction between day and group, $F(26,195)$=1.573, p=.045

(Figure 34).
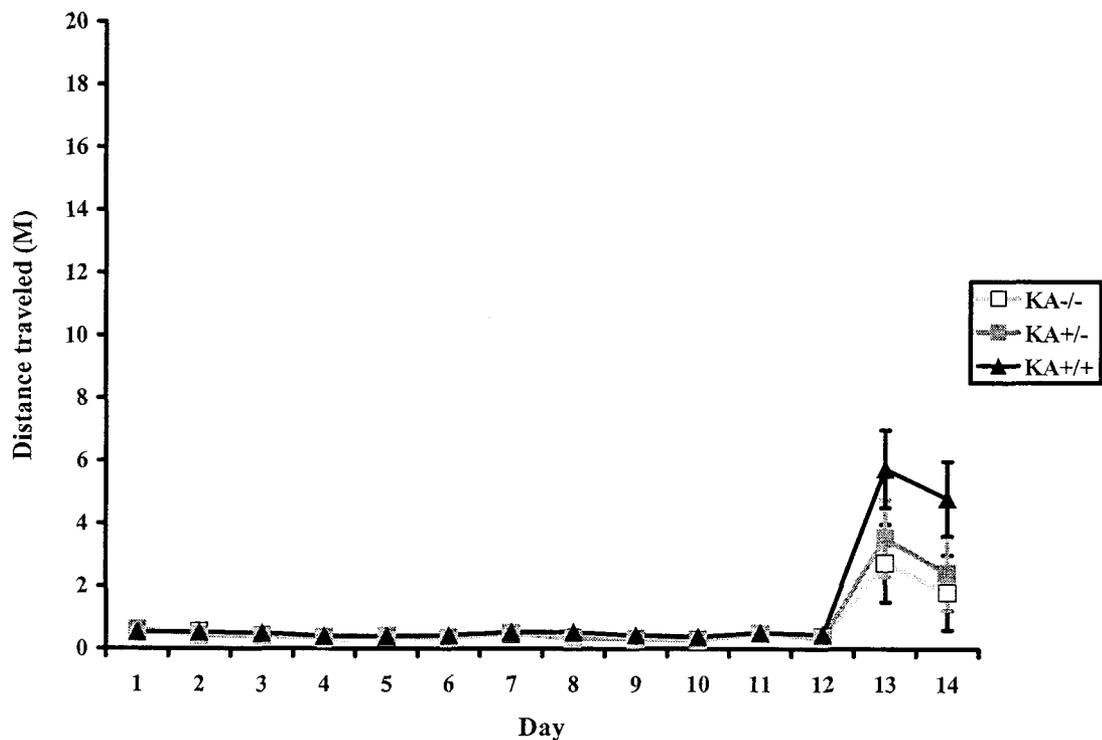
Figure 34. The significant interaction between day and group for distance which the rats

traveled to find the platform in the water task. The symbols represent mean distance

traveled (meters) ± the SEM.

Notably, the interactions between: trial and group, $F(6,45)$=0.774, p=.595; trial

and stress, $F(3,45)$=0.768, p=.518; day, trial, and group, $F(39,585)$=0.951, p=.599; day,

trial, and stress, $F(39,585)=1.118$, $p=.290$; trial, group, and stress, $F(6,45)=0.821$, $p=.560$; day, trial, group, and stress, $F(78,585)=0.710$, $p=.970$; all failed to reach significance.

ANOVA revealed a main effect of day $F(13,195)=19.908$, $p<.001$ and a main effect of trial $F(3,45)=9.045$, $p<.001$. However, this was mediated by a significant interaction between day and trial, $F(39,585)=3.087$, $p<.001$ (Figure 35).
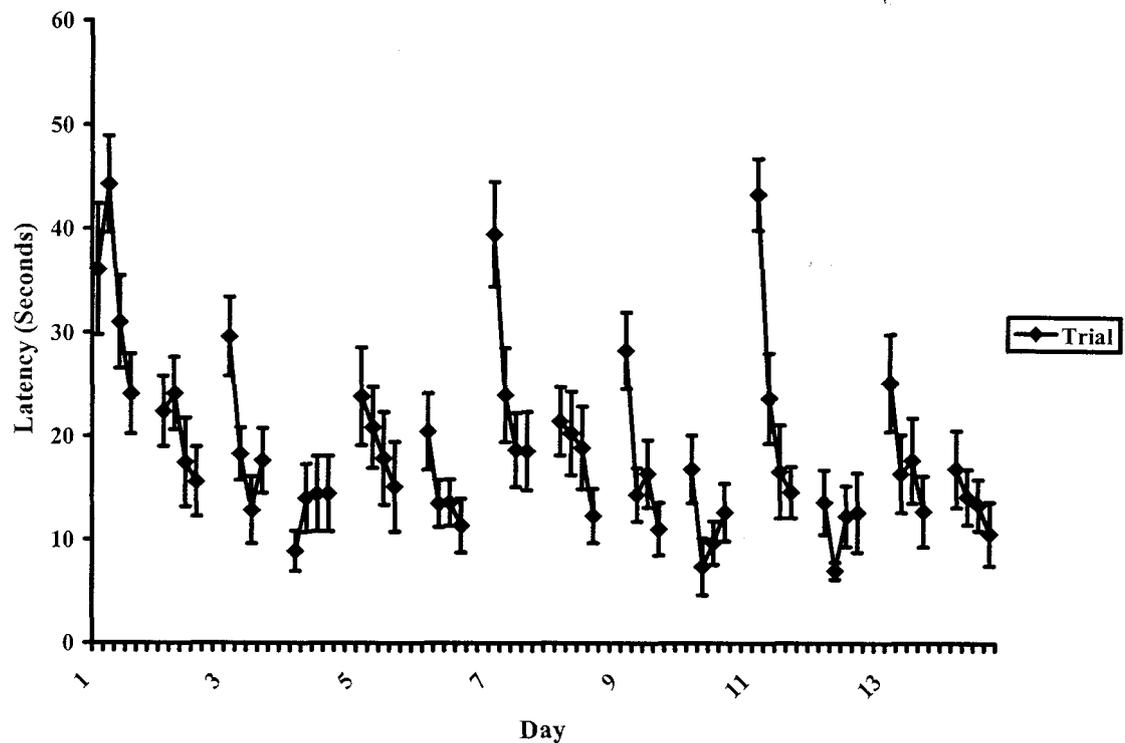

Figure 35. The significant interaction between day and trial for distance which the rats traveled to find the platform in the water task. The symbols represent mean distance traveled (meters) ± the SEM.

The distance traveled was averaged across the four trials and planned comparisons (Bonferroni) were performed on the learning days and the memory days.

106

For the learning days, planned comparisons were conducted between the two consecutive days that had the same platform location. The analysis revealed significant difference: between day 11 and day 12, $p=.008$. However, the analysis failed to reveal a significant difference between day 1 and day 2, $p=.964$; between day 3 and day 4, $p=.880$; between day 5 and day 6, $p=1.000$; between day 7 and day 8, $p=.113$; between day 9 and day 10, $p=1.000$; and between day 13 and day 14, $p=1.000$.

For the memory days, planned comparisons (Bonferroni) were conducted on two consecutive days with the new platform location. For example, comparisons were made between: day 1 and day 3; day 3 and day 5; and so on. The analysis revealed significant difference: between day 7 and day 9; between day 9 and day 11, $p=.020$; and between day 11 and day 13, $p=.014$. However, there was no significant difference between day 1 and day 3, $p=.089$; day 3 and day 5, $p=.960$; and day 5 and day 7, $p=.311$.

To examine the simple main effect of trial, the distance traveled was averaged across the fourteen days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed trial 1 was significantly longer than trial 3, $p=.024$; and trial 1 was significantly longer than trial 4, $p=.001$. However, the analysis failed to reveal a significant difference: between trial 1 and trial 2, $p=.226$; between trial 2 and trial 3, $p=1.000$; between trial 2 and trial 4, $p=.401$; and between trial 3 and trial 4, $p=.212$

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the distance which the rats traveled to find the platform, on a given day. As indicated in Table 11 the analysis failed to reveal any significant correlation between the hippocampal volume and the distance which the rats

107

traveled to find the platform, for the entire group of rats. As before, separate correlations were performed for the KA+/+ rats alone among the hippocampal volume and the distance which the rats traveled to find the platform, on a given day. As indicated in Table 11 the analysis failed to reveal any significant correlation between the hippocampal volume and the distance which the rats traveled to find the platform, for the KA+/+ group .

Table 11.

Pearson product-moment correlations, for distance traveled in which the rats found the platform.

	Entire group of rats		KA+/+ Group	
	Pearson Correlation	Sig. (2-tailed)	Pearson Correlation	Sig. (2-tailed)
Day1	0.339	0.133	-0.095	0.858
Day2	0.151	0.515	0.670	0.145
Day3	-0.062	0.791	0.502	0.310
Day4	-0.219	0.340	-0.556	0.252
Day5	-0.123	0.597	0.050	0.925
Day6	-0.146	0.527	0.042	0.938
Day7	-0.367	0.101	-0.628	0.182
Day8	-0.204	0.374	-0.497	0.315
Day9	-0.112	0.628	-0.080	0.881
Day10	-0.206	0.371	-0.002	0.997
Day11	-0.029	0.901	-0.200	0.704
Day12	-0.060	0.796	-0.276	0.597
Day13	-0.261	0.253	-0.698	0.123
Day14	-0.278	0.222	-0.498	0.315

Note. The correlation values for the entire group of rats (column 2) and the KA+/+ group alone (column 4). There are no significant values identified.

For the speed, a repeated measures ANOVA was performed with day (days 1-14) and trial (trials 1-4) as within-subject measures and group (KA+/+, KA+/-, KA-/-) and

stress group (stress, no stress) as a between-subject measure. ANOVA revealed a main

effect of group, $F(2,15) = 3.851$, $p < .001$ (Figure 36). Posthoc analysis (Bonferroni)

indicated that KA+/- group were significantly faster than KA+/+ group, $p = .036$.

However, analysis failed to reveal a significant difference between KA+/+ group and

KA-/- group, $p = .1000$ and between KA+/- group and KA-/- group, $p = 1.000$.
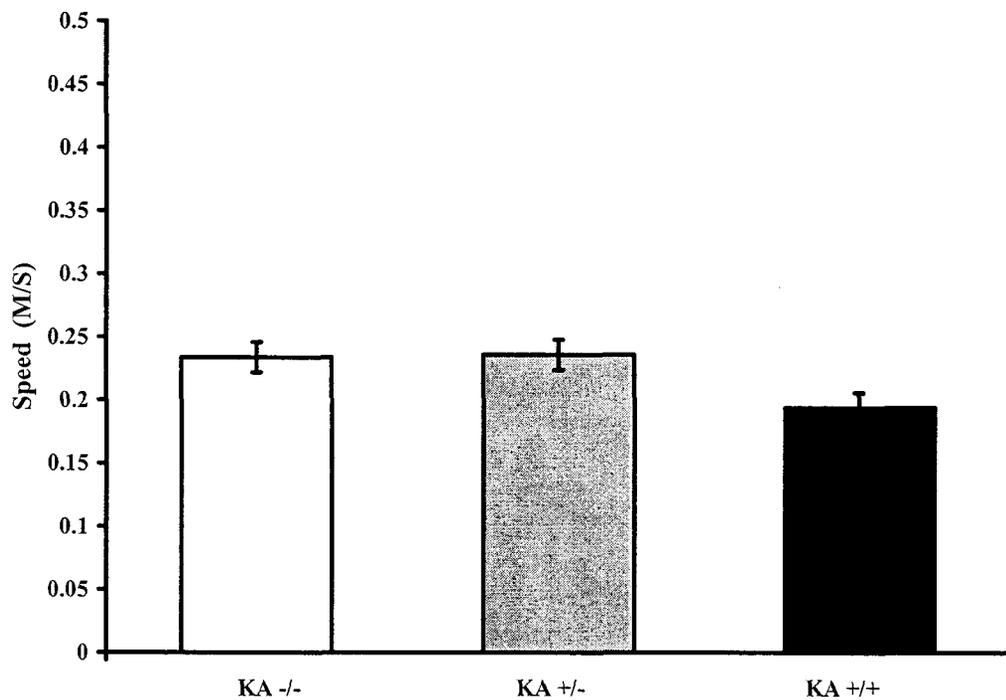
Figure 36. The significant main effect of group for the speed of which the rats found the

platform in the water task. The bars represent mean speed (meters/seconds) ± the SEM.

ANOVA failed to reveal a significant difference between the stress group

($M = 0.228$, $SD = 0.027$) and the no stress group ($M = 0.218$, $SD = 0.045$), $F(1,15) = 0.041$,

$p = .843$; and there was no significant interaction between group and stress,

$F(2,15) = 1.930$, $p = .180$.

However, ANOVA revealed a significant interaction between day and stress, $F(13,195)=1.752, p=.053$ (Figure 37) and a significant interaction between day, trial, and group , $F(78,585)=1.353, p=.030$ (Figure 38).
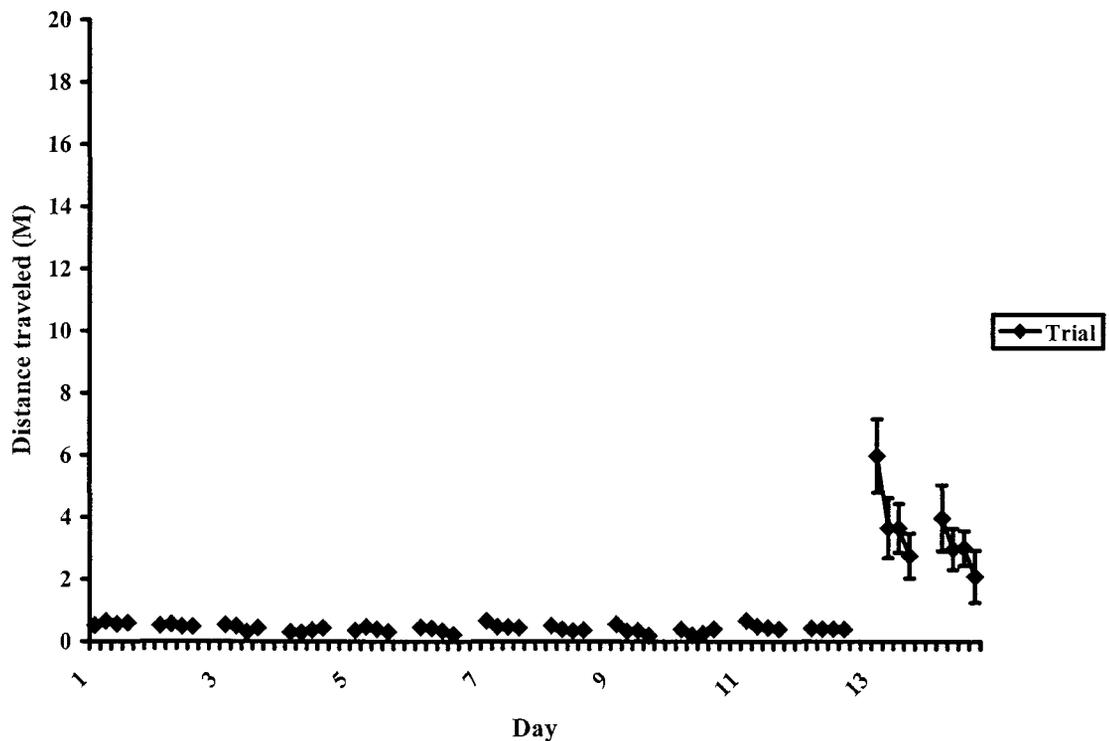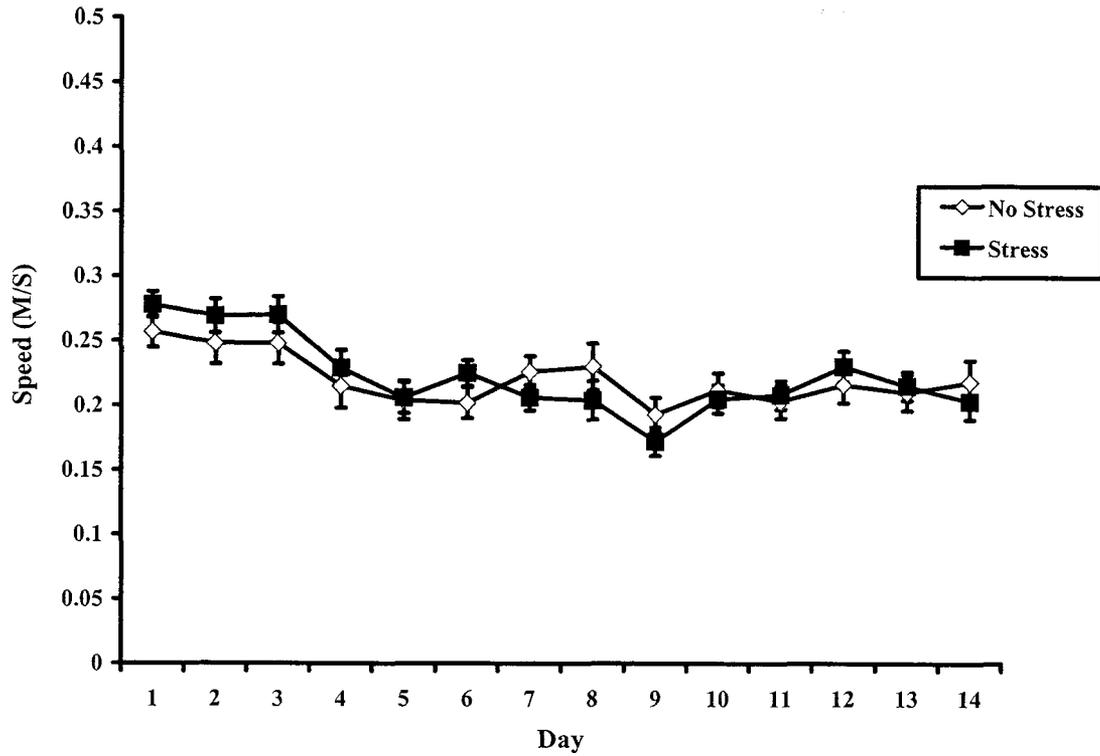

Figure 37. The significant interaction between day and stress for the speed of which the rats found the platform in the water task. The symbols represent mean speed (meters/second) ± the SEM.
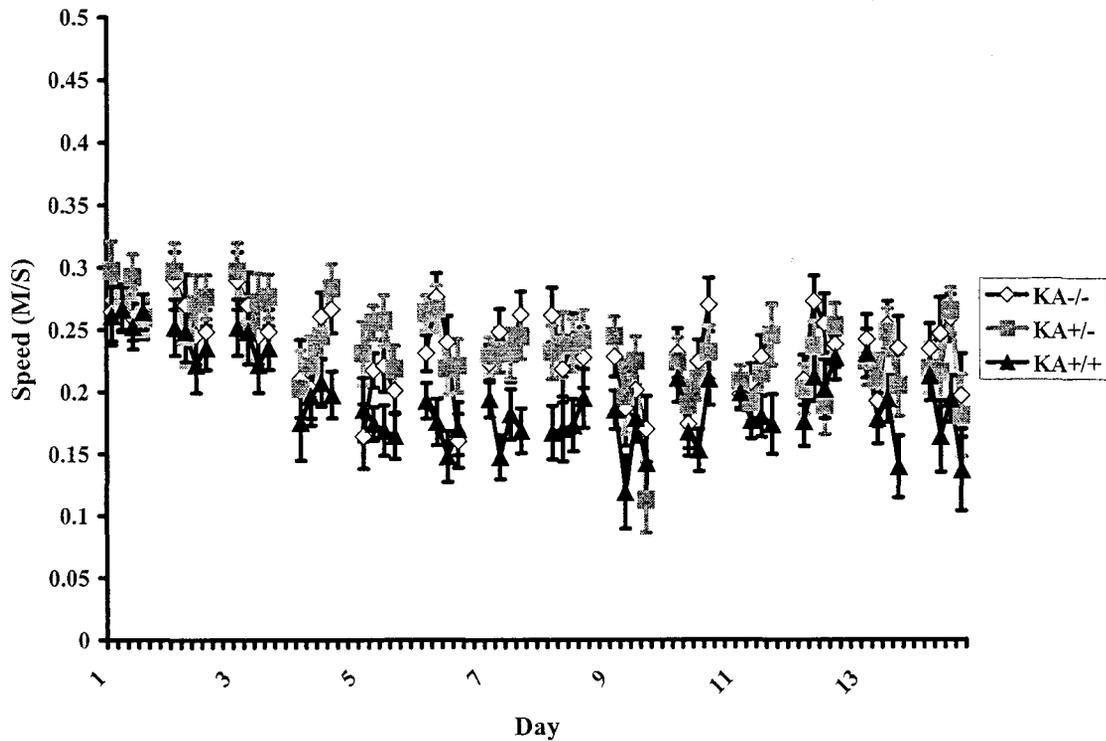
Figure 38. The significant interaction between day and trial and group for the speed of

which the rats found the platform in the water task. The symbols represent mean speed

(meters/second) ± the SEM.

Notably, the interactions between: day and group, $F(26,195)=1.085$, $p=.362$; day,

group, and stress, $F(26,195)=0.855$, $p=.671$; trial and group, $F(6,45)=1.430$, $p=.224$; trial

and stress, $F(3,45)=1.008$, $p=.398$, trial, group, and stress, $F(6,45)=1.685$, $p=.147$; day,

trial, and stress, $F(39,585)=1.002$, $p=.469$; and day, trial, group, and stress,

$F(78,585)=0.821$, $p=.860$; all failed to reach significance.

ANOVA revealed a significant main effect of day, $F(13,195)=12.923$, $p<.001$, a

main effect of trial, $F(3,45)=4.666$, $p=.006$. However, this was mediated by a significant

interaction between day and trial, $F(39,585)=4.911$, $p<.001$ (Figure 39).

111
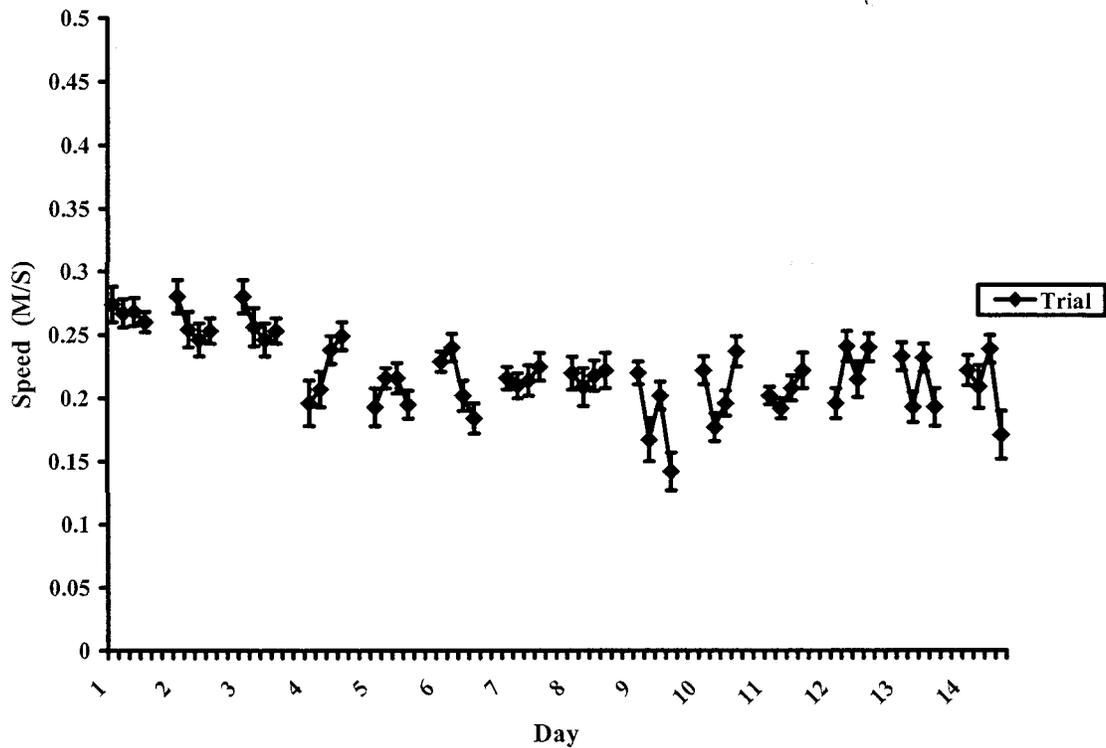
Figure 39. The significant interaction between day and trial for the speed of which the

rats found the platform in the water task. The symbols represent mean speed

(meters/second) ± SEM.

The speed was averaged across the four trials and planned comparisons

(Bonferroni) were performed on the learning days and the memory days. For the learning

days, planned comparisons were conducted between the two consecutive days that had

the same platform location. The analysis failed to reveal a significant difference: between

day 1 and day 2, $p=1.000$; between day 3 and day 4, $p=.109$; between day 5 and day 6,

$p=1.000$; between day 7 and day 8, $p=1.000$; between day 9 and day 10, $p=1.000$;

between day 11 and day 12, $p=1.000$; and between day 13 and day 14, $p=1.000$.

For the memory days, planned comparisons (Bonferroni) were conducted on two consecutive days with the new platform location. For example, comparisons were made between: day 1 and day 3; day 3 and day 5; and so on. The analysis revealed a significant difference between day 3 and day 5, $p<.001$. However, the analysis failed to reveal a significant difference: between day 1 and day 3, $p=1.000$; between day 5 and day 7, $p=1.000$; between day 7 and day 9, $p=.087$; between day 9 and day 11, $p=.228$; and between day 11 and day 13, $p=1.000$.

To examine the simple main effect of trial, the speed was averaged across the fourteen days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed a significant difference between trial 1 and trial 2, $p=.057$. However, the analysis failed to reveal a significant difference: between trial 1 and trial 3, $p=1.000$; between trial 1 and trial 4, $p=.099$; between trial 2 and trial 3, $p=.142$; between trial 2 and trial 4, $p=1.000$; and between trial 3 and trial 4, $p=.090$.

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the speed with which the rats traveled to find the platform, on a given day. As indicated in Table 12 the analysis failed to reveal any significant correlation between the hippocampal volume and the speed with which the rats traveled to find the platform, for the entire group of rats. As before, separate correlations were performed for the KA+/+ rats alone among the hippocampal volume and the distance which the rats traveled to find the platform, on a given day. As indicated in Table 12 the analysis failed to reveal any significant correlation between the

113

hippocampal volume and the speed with which the rats traveled to find the platform, for the KA+/+ group.

Table 12.

Pearson product-moment correlations, for the speed with which the rats found the platform.

	Entire group of rats		KA+/+ Group	
	Pearson Correlation	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Day1	0.049	0.833	-0.523	0.287
Day2	0.100	0.668	-0.015	0.977
Day3	0.161	0.486	0.103	0.846
Day4	0.429	0.052	-0.044	0.935
Day5	0.237	0.302	-0.068	0.899
Day6	0.154	0.506	-0.167	0.752
Day7	0.014	0.952	-0.757	0.082
Day8	0.129	0.577	-0.249	0.634
Day9	0.211	0.358	-0.119	0.823
Day10	-0.055	0.814	-0.318	0.539
Day11	-0.008	0.971	-0.269	0.606
Day12	0.006	0.979	-0.419	0.409
Day13	0.123	0.594	-0.262	0.616
Day14	0.123	0.595	-0.461	0.357

Note. The correlation values for the entire group of rats (column 2) and the KA+/+ group alone (column 4). There are no significant values identified.

Ziggurat task. For latency to find the goal ziggurat, a repeated measures ANOVA was performed with day (days 1-8) and trial (trials 1-8) as within-subject measures and group (KA+/+, KA+/-, KA-/-) and stress (stress, no stress) as a between-subject measure. ANOVA failed to reveal a significant difference for the latency to find the goal ziggurat KA+ /+ group (*M*=87.972 seconds; *SD*=34.923) and the KA+/- group (*M*=58.013 seconds; *SD*=16.851) and the KA -/- group (*M*=74.621 seconds; *SD*=25.843),

114

$F(2,15)=1.204, p=.327$. Similarly, ANOVA failed to reveal a significant difference between the stress group ($M=66.485$ seconds; $SD=28.140$) and the no stress group ($M=79.170$ seconds; $SD=26.002$), $F(1,15)=0.291, p=.598$; and there was no significant interaction between group and stress, $F(2,15)=2.078, p=.160$.

Notably, the interaction between: day and group, $F(14,105)=1.412, p=.160$; between day and stress, $F(7,105)=1.255, p=.280$; day, group, and stress, $F(14,105)=0.914, p=.547$; trial and group $F(14,105)=0.860, p=.604$; trial and stress, $F(7,105)=0.147, p=.994$; trial, group, and stress, $F(14,105)=0.930, p=.529$; day, trial, and group, $F(98,735)=0.937, p=.651$; day, trial, and stress, $F(49,735)=1.039, p=.403$; day, trial, group, and stress, $F(98,735)=0.984, p=.528$; all failed to reach significance.

ANOVA revealed a main effect of day, $F(7,105)=16.034, p<.001$ (Figure 40). The latency was averaged across the eight trials and planned comparisons (Bonferroni) were performed on the learning days and the memory days. For the learning days, planned comparisons were conducted between the two consecutive days that had the same goal ziggurat location. The analysis revealed day 1 was significantly longer than day 2, $p=.003$. However the analysis failed to reveal a significant difference: between day 3 and day 4, $p=1.000$; between day 5 and day 6, $p=.497$; and between day 7 and 8, $p=1.000$.
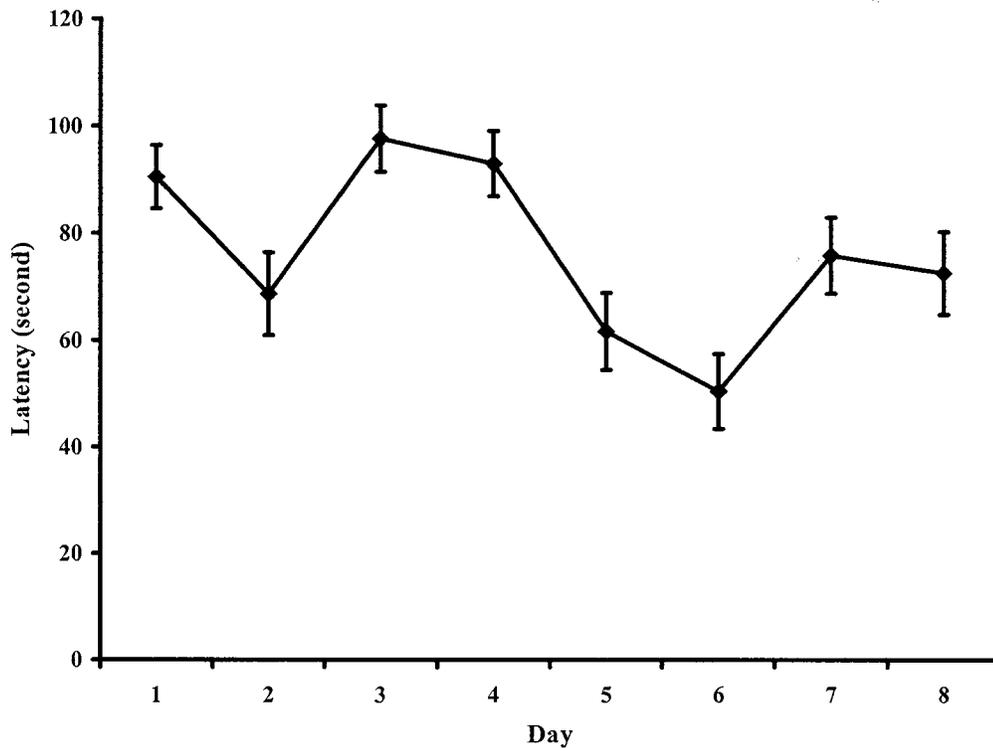
Figure 40. The significant main effect of day for latency with which the rats found the

goal in the ziggurat task. The symbols represent mean distance traveled (seconds) ± the

SEM.

For the memory days, planned comparisons (Bonferroni) were conducted on two

consecutive days with the new goal ziggurat location. For example, comparisons were

made between: day 1 and day 3; day 3 and day 5; and so on. The analysis revealed day 3

was significantly longer than day 5, $p=.004$. However, the analysis failed to reveal a

significant difference: between day 1 and day 3, $p=1.000$; and between day 5 and day 7,

$p=.485$.

Also, ANOVA revealed a main effect of trial, $F(7,105)=16.738, p<.001$ (Figure

41). To examine the simple main effect of trial, the latency was averaged across the eight

116

days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed trial1 was significantly longer than trial 4, $p=.002$; trial 1 was significantly longer than trial 5, $p=.017$; trial 1 was significantly longer than trial 6, $p=.020$; and trial 1 was significantly longer than trial 7 and trial 8, $p=.001$. Also, trial 2 was significantly longer than trial 4, $p<.001$; trial 2 was significantly longer than trial 5, $p=.012$; trial 2 was significantly longer than trial 6, $p=.011$; trial2 was significantly longer than trial 7 and trial 8, $p<.001$. As well, trial 3 was significantly longer than trial 8, $p=.025$. However, the analysis failed to reveal any other significant differences between the trials. Unlike the other measures, the interaction between day and trial failed to reach significance, $F(49,735)=1.107$, $p=.289$.
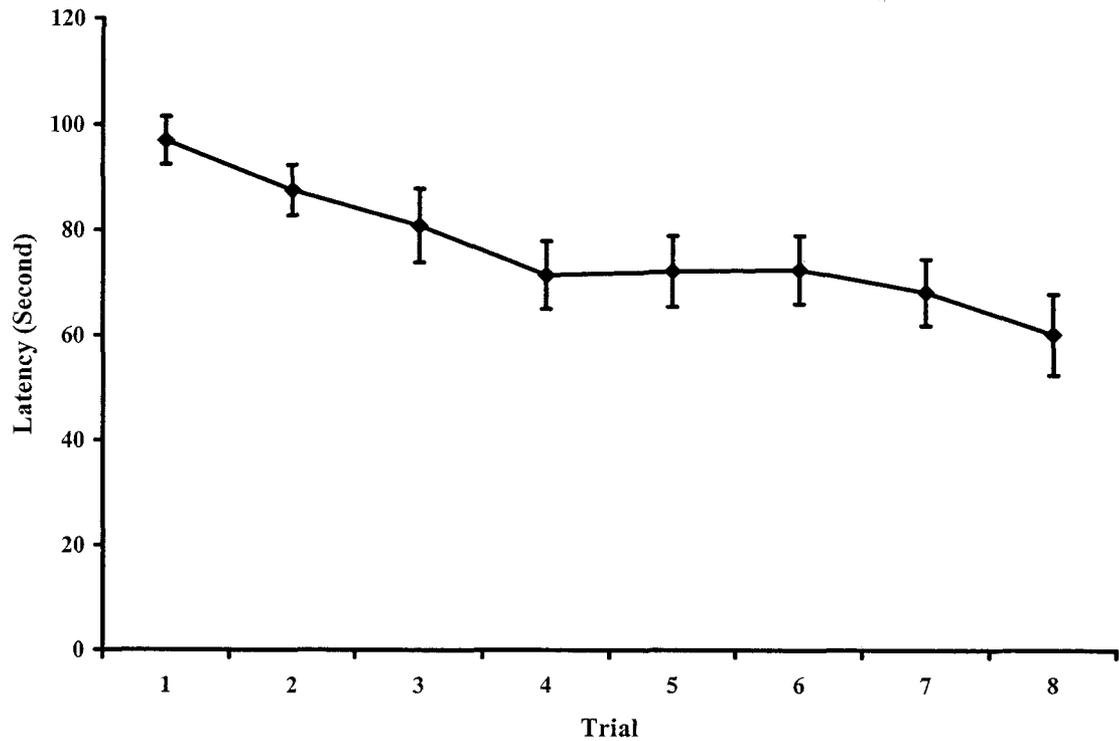
Figure 41. The significant main effect of trial for latency with which the rats found the

goal in the ziggurat task. The symbols represent mean latency (seconds) ± the SEM.

Pearson product-moment correlations were performed for the entire group of rats

among the hippocampal volume and the latency in which the rats found the goal ziggurat,

on a given day (Table 13). As indicated in Table 13 the analysis failed to reveal any

significant correlation between the hippocampal volume and the latency in which the rats

found the goal ziggurat, for the entire group. As before, separate correlations were

performed for the KA+/+ rats alone among the hippocampal volume and the latency in

which the rats found the goal ziggurat, on a given day (Table 13). There was a significant

correlation between the hippocampal volume and the latency in which the rats found the

goal ziggurat on day 3, however, the analysis failed to reveal any other significant

correlation between the hippocampal volume and the latency in which the rats found the goal ziggurat, for the KA+/+ group.

Table 13.

Pearson product-moment correlations for latency with which the rats found the goal ziggurat.

	Entire group of rats		KA+/+ Group	
	Pearson Correlation	Sig. (2-tailed)	Pearson Correlation	Sig. (2-tailed)
Day 1	-0.049	0.833	-0.459	0.360
Day 2	-0.176	0.447	-0.551	0.257
Day 3	-0.293	0.197	**-0.890**	**0.018**
Day 4	-0.041	0.860	-0.318	0.538
Day 5	-0.144	0.533	-0.388	0.447
Day 6	-0.301	0.185	-0.529	0.281
Day 7	-0.099	0.669	-0.521	0.289
Day 8	-0.170	0.460	-0.631	0.179

Note. The correlation values for the entire group of rats (column 2) and the KA+/+ group alone (column 4). The significant values are identified in bold.

For the distance traveled, a repeated measures ANOVA was performed with day (days 1-8) and trial (trials 1-8) as within-subject measures and group (KA+/+, KA+/-, KA-/-) and stress (stress, no stress) as a between-subject measures. ANOVA failed to reveal a significant difference between KA+/+ group (M=6.807 meters; SD=3.446) and the KA+/- group (M=6.557 meters; SD=1.676) and the KA -/- group (M=6.283 meters; SD=0.934), $F(2,15)$=0.140, p=.871. Similarly, ANOVA failed to reveal a significant difference between the stress group (M=6.545 meters; SD=1.648) and the no stress group (M=6.559 meters; SD=2.788), p=.998; and there was no significant interaction between group and stress, $F(2,15)$=0.629, p=.546. ANOVA revealed a main effect of day

$F(7,105)=4.393$, $p<.001$ and a main effect of trial, $F(7,105)=19.662$, $p<.001$. However, this was mediated by a significant interaction between day and trial, $F(49,735)=1.366$, $p=.052$ (Figure 42).
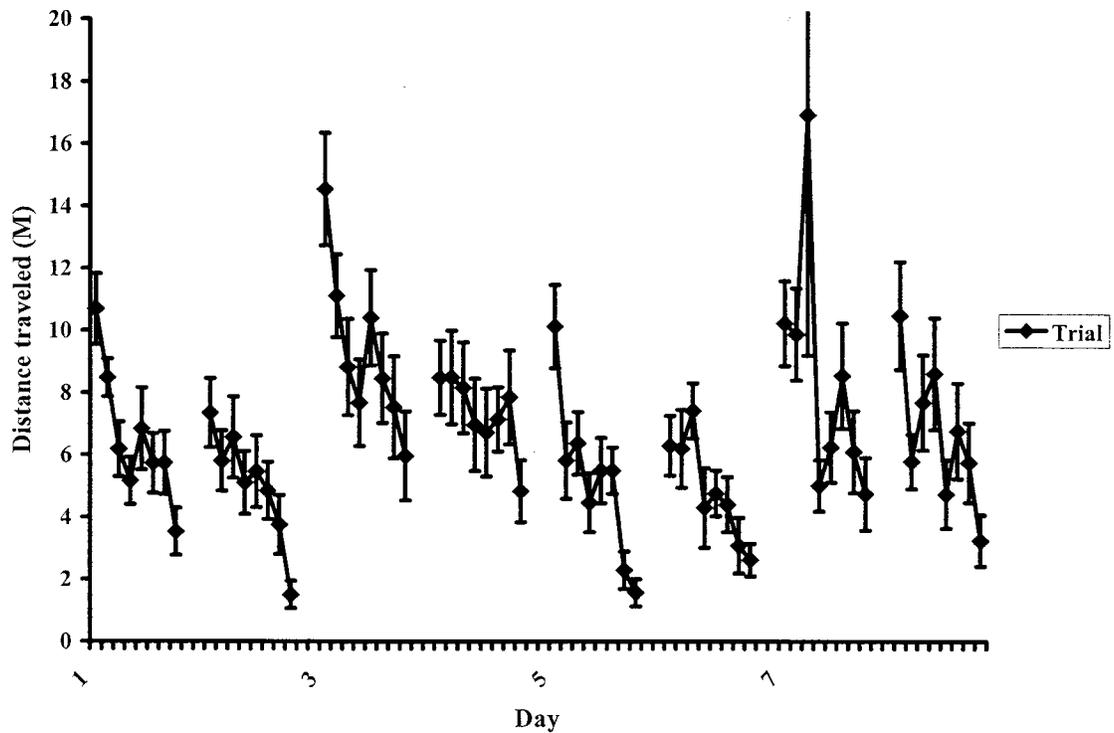
Figure 42. The significant interaction between day and trial for distance in which the rats traveled to find the goal in the ziggurat task. The symbols represent mean distance traveled (meters) ± the SEM.

Also, ANOVA revealed a significant interaction between day, trial, and group, $F(98,735)=1.420$, $p=.007$ (Figure 43) and a significant interaction between trial, group, and stress, $F(14,105)=1.946$, $p=.029$ (Figure 44). Notably, of interaction between: day and group, $F(14,105)=0.358$, $p=.983$; day and stress, $F(7,105)=0.634$, $p=.726$; day, group, and stress, $F(14,105)=0.851$, $p=.613$; trial and group, $F(14,105)=0.763$, $p=.706$;

trial and stress, $F(7,105)=0.704$, $p=.669$; day, trial, and stress, $F(49,735)=1.159$, $p=.217$;

day, trial, group , and stress, $F(98,735)=1.147$, $p=.169$; all failed to reach significance.
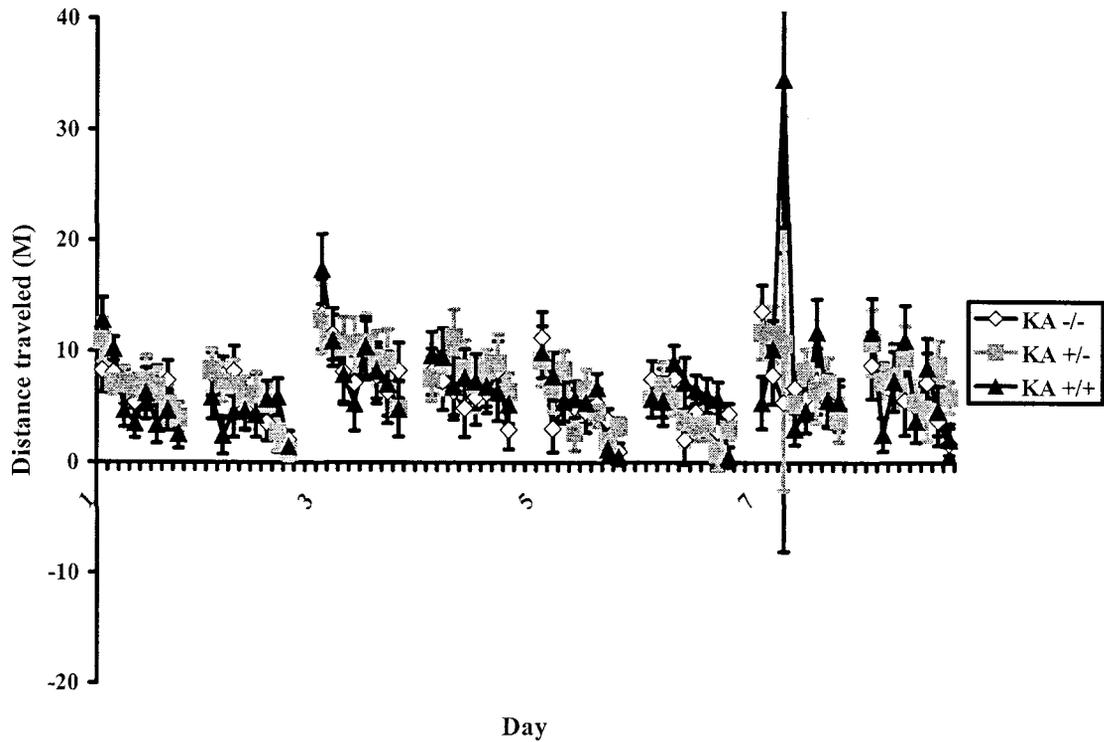
Figure 43. The significant interaction between group, day, and trial for distance in which

the rats traveled to find the goal in the ziggurat task. The symbols represent mean

distance traveled (meters) ± the SEM.
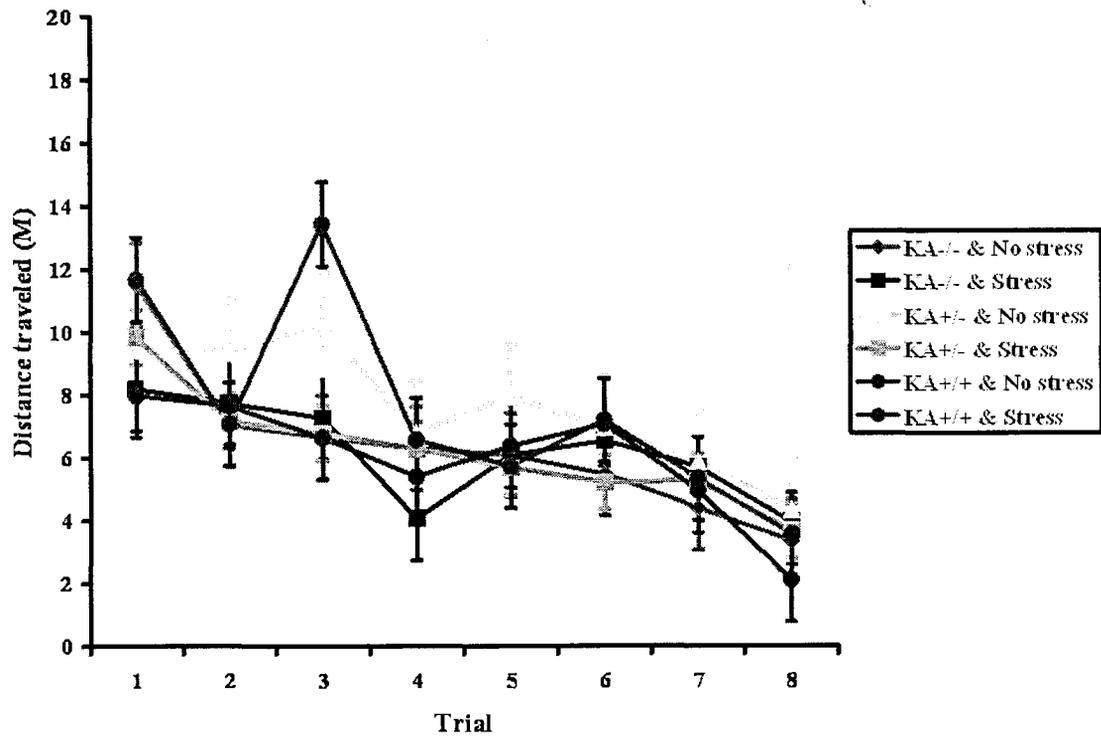
Figure 44. The significant interaction between, stress, group and trial for distance in which the rats traveled to find the goal in the ziggurat task. The symbols represent mean distance traveled (meters) ± the SEM.

The distance traveled was averaged across the eight trials and planned comparisons (Bonferroni) were performed on the learning days and the memory days. For the learning days, planned comparisons were conducted between the two consecutive days that had the same goal ziggurat location. The analysis failed to reveal a significant difference: between day 1 and day 2, $p=.204$; between day 3 and day 4, $p=.641$; between day 5 and day 6, $p=1.000$; and between day 7 and day 8, $p=1.000$.

For the memory days, planned comparisons (Bonferroni) were conducted on two consecutive days with the new goal ziggurat location. For example, comparisons were

made between: day 1 and day 3; day 3 and day 5; and so on. The analysis failed to reveal a significant difference: between day 1 and day 3, $p=.289$; between day 3 and day 5, $p=.066$; and between day 5 and day 7, $p=1.000$.

To examine the simple main effect of trial, the distance traveled was averaged across the eight days and planned comparisons (Bonferroni) were performed among all trials. The distance traveled in trial 1 was significantly longer than trial 2, $p=.004$; trial 1 was significantly longer than trial 4, trial 5, trial 7, and trial 8, $p<.001$; and trial 1 was significantly longer than trial 6, $p=.003$. Also, trial 2 was significantly longer than trial 4, trial 7, and trial 8, $p<.001$; trial 2 was significantly longer than trial 5, $p=.007$. As well, trial 3 was significantly longer than trial 7, $p=.043$; and trial 3 was significantly longer than trial 8, $p=.006$. Moreover, trial 4 and trial 6 were significantly longer than trial 8, $p=.001$; trial 7 was significantly longer than trial 8, $p=.015$; and trial 5 was significantly longer than trial 8, $p<.001$. However, the analysis failed to reveal any other significant differences between the trials.

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the distance which the rats travel to find the goal ziggurat, on a given day (Table 14). The analysis failed to reveal any significant correlation between the hippocampal volume and the distance which the rats travel to find the goal ziggurat, for the entire group of rats. As before, separate correlations were performed for the KA+/+ rats alone among the hippocampal volume and the distance which the rats travel to find the goal ziggurat, on a given day (Table 14). The analysis

failed to reveal any significant correlation between the hippocampal volume and the distance which the rats travel to find the goal ziggurat, for the KA+/+ group.

Table 14.

Pearson product-moment correlations for distance traveled with which the rats found the goal ziggurat.

	Entire group of rats		KA+/+ Group	
	Pearson Correlation	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Day 1	-0.109	0.639	0.300	0.564
Day 2	-0.137	0.553	0.157	0.767
Day 3	-0.263	0.250	-0.241	0.646
Day 4	-0.055	0.812	-0.065	0.903
Day 5	0.187	0.417	0.407	0.423
Day 6	-0.417	0.060	-0.646	0.166
Day 7	-0.229	0.317	-0.325	0.530
Day 8	-0.070	0.764	-0.161	0.760

Note. The correlation values for the entire group of rats (column 2) and the KA+/+ group alone (column 4). There are no significant values identified.

For the speed, a repeated measures ANOVA was performed with day (days 1-8) and trial (trials 1-8) as within-subject measures and group (KA+/+, KA+/-, KA-/-) and stress (stress, no stress) as a between-subject measures.

ANOVA failed to reveal a significant difference between KA+ /+ group (M=0.094 meters/second; SD= 0.058) and the KA+/- group (M=0.124 meters/second; SD=0.022) and the KA -/- group (M=0.096 meters/second; SD=0.031), $F(2,15)$=0.585, p=.569. Similarly, ANOVA failed to reveal a significant difference between the stress group (M=0.113 meters/second; SD=0.031) and the no stress group (M=0.098

meters/second; $SD=0.050$), $F(1,15)=0.116$, $p=.738$; and there was no significant interaction between group and stress, $F(2,15)=0.809$, $p=.464$.

Notably, the interaction between: day and group, $F(14,105)=.646$, $p=.820$; trial, group, and stress, $F(14,105)=1.704$, $p=.066$; day and stress, $F(7,105)=.481$, $p=.847$; day, group, and stress, $F(14,105)=.485$, $p=.937$; trial and group, $F(14,105)=.769$, $p=.671$; trial and stress, $F(7,105)=.984$, $p=.447$; day, trial, and stress, $F(49,735)=1.086$, $p=.323$; all failed to reach significance.

ANOVA revealed a main effect of day $F(7,105)=8.669$, $p<.001$ and a main effect of trial, $F(7,105)=6.452$, $p<.001$. However, this was mediated by a significant interaction between day and trial, $F(49,735)=2.013$, $p<.001$ (Figure 45).
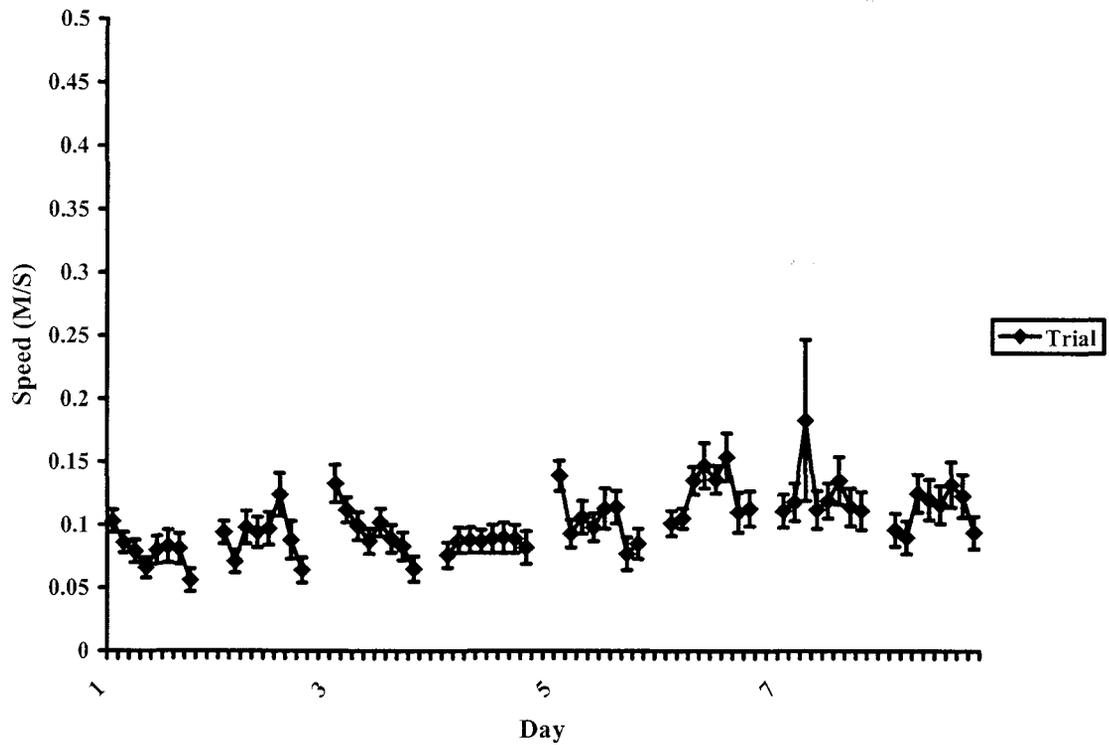
Figure 45. The significant interaction between day and trial for speed in which the rats found the goal in the ziggurat task. The symbols represent mean speed (meters/second) ± the SEM.

Also, ANOVA revealed a significant interaction among day, trial, and group, $F(98,735)=1.432$, $p=.006$ (Figure 46), and a significant interaction among day, trial, group, and stress, $F(98,735)=1.271$, $p=.048$ (Figure 47).
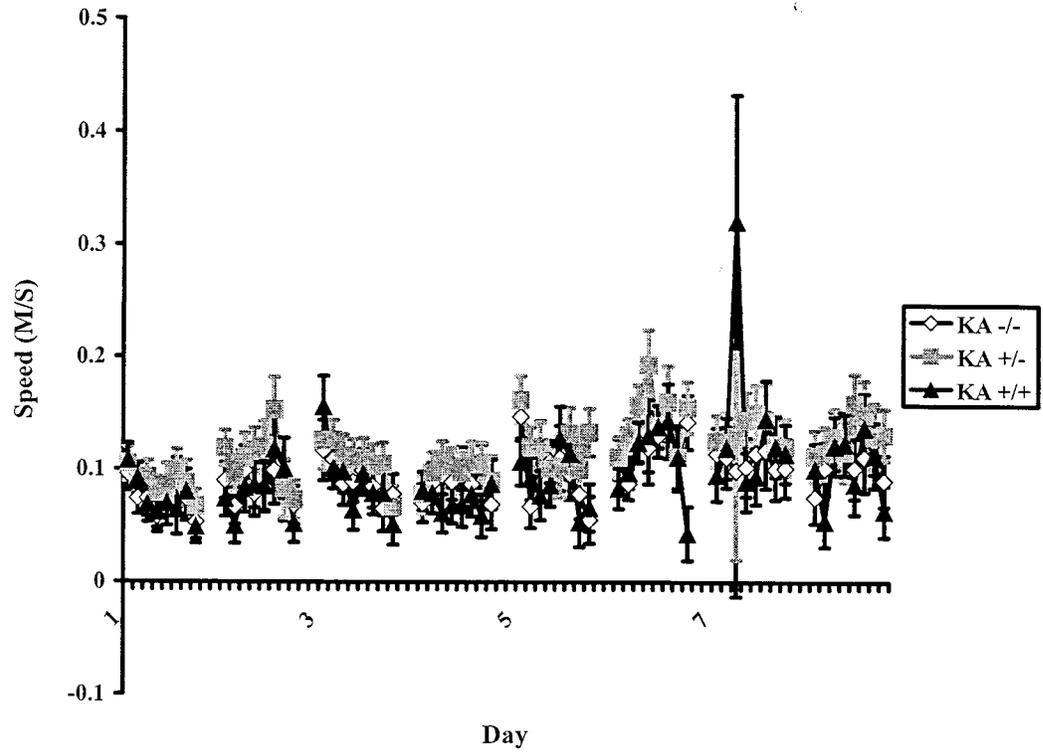
Figure 46. The significant interaction between group, day, and trial for speed in which

the rats found the goal in the ziggurat task. The symbols represent mean speed

(meters/second) ± the SEM.
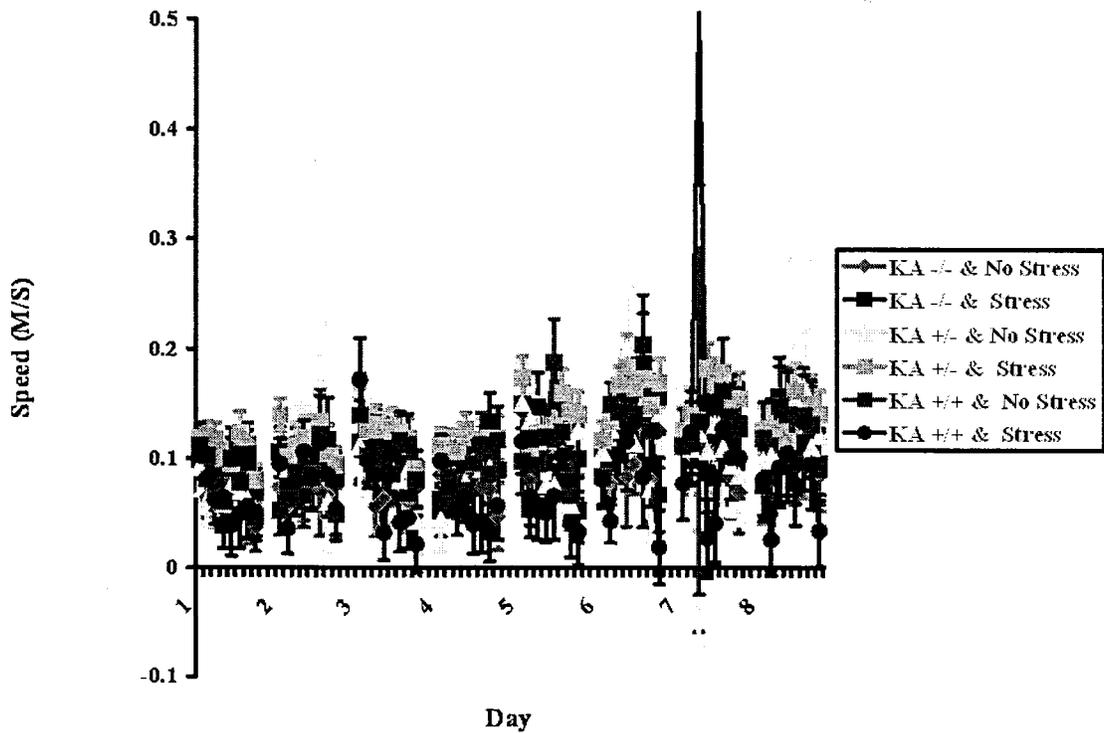
Figure 47. The significant interaction between day, trial, group, and stress for speed in which the rats found the goal in the ziggurat task. The symbols represent mean speed (meters/second) ± the SEM.

The speed was averaged across the eight trials and planned comparisons (Bonferroni) were performed on the learning days and the memory days. For the learning days, planned comparisons were conducted between the two consecutive days that had the same goal ziggurat location. The analysis revealed rats found the goal significantly faster during day 5 than day 6, *p*=.008. However, the analysis failed to reveal significant difference: between day 1 and day 2, *p*=.229; between day 3 and day 4, *p*=.870; and between day 7 and day 8, *p*=1.000.

128

For the memory days, planned comparisons (Bonferroni) were conducted on two consecutive days with the new goal ziggurat location. For example, comparisons were made between: day 1 and day 3; day 3 and day 5; and so on. The analysis failed to reveal a significant difference between day 1 and day 3, $p=.087$; between day 3 and day 5, $p=1.000$; and between day 5 and day 7, $p=1.000$.

To examine the simple main effect of trial, the speed was averaged across the eight days and planned comparisons (Bonferroni) were performed among all trials. The analysis revealed trial 3, trial 4, trial 5 and trial 6 all were significantly faster than trial 8. Also, trial 6 was significantly faster than trial 7. However, the analysis failed to reveal any other significant differences between the trials.

Pearson product-moment correlations were performed for the entire group of rats among the hippocampal volume and the speed in which the rats found the goal ziggurat, on a given day (Table 15). The analysis failed to reveal any significant correlation between the hippocampal volume and the speed in which the rats found the goal ziggurat, for the entire group of rats. As before, separate correlations were performed for the KA+/+ rats alone among the hippocampal volume and the speed in which the rats found the goal ziggurat, on a given day (Table 15). The analysis failed to reveal any significant correlation between the hippocampal volume and the speed in which the rats found the goal ziggurat, for the KA+/+ group.

Table 15.

Pearson product-moment correlations for speed in which the rats found the goal

ziggurat.

	Entire group of rats		KA+/+ Group	
	Pearson Correlation	**Sig. (2-tailed)**	**Pearson Correlation**	**Sig. (2-tailed)**
Day 1	-0.078	0.736	0.328	0.525
Day 2	0.066	0.775	0.480	0.336
Day 3	-0.149	0.519	0.130	0.807
Day 4	-0.040	0.864	-0.045	0.932
Day 5	0.105	0.652	0.242	0.645
Day 6	0.016	0.944	0.119	0.823
Day 7	-0.199	0.387	-0.155	0.769
Day 8	0.015	0.948	0.099	0.852

Note. The correlation values for the entire group of rats (column 2) and the KA+/+ group

alone (column 4). There are no significant values identified.

Discussion

The goal of this experiment was to examine the potential role of stress on

convulsions and to determine if there was an additive effect of stress and SE on

hippocampal volume and behaviour. It was predicted that the effects observed in

Experiment 1 would be replicated; that is, relative to SA-treated controls, the rats that

received KA-induced SE would exhibit decreases in hippocampal volume, increased

numbers of spontaneous convulsions and would exhibit deficits in the ziggurat task.

Replication of Experiment 1

Although not significant, rats that had experienced SE without stress showed a

reduction in hippocampal volume when compared to SA-treated controls. This difference

might have reached significance if greater numbers of rats had experienced SE. In this

experiment 28 rats were injected with KA and only 11 developed SE. Of these 11 rats, 5 died. Even though KA solution in SA was made fresh prior to the injections, some of the KA that was used in this experiment was purchased at an earlier date, which may explain the reduction in drug efficacy in induction of SE (Sperk, 1985; 1994).

It should be noted that the KA+/- group was not an intended group; rather they resulted from a large number of rats failing to exhibit SE. Moreover, none of the rats in the KA +/- group exhibited spontaneous convulsions or a reduction in hippocampal volume. These findings suggest that the development of SE followed by a reduction of hippocampal volume is the main factor that later on leads to development of spontaneous convulsions.

There was no significant association between the number of spontaneous convulsions and the hippocampal volume loss. As mentioned in Experiment 1, the rats were only monitored for spontaneous convulsions for one hour per day, which may not have detected a potentially existing association. However, as mentioned in Experiment 1, this explanation is somewhat unlikely, as the monitoring was distributed throughout the day. Finally, just like Experiment 1, there were problems with statistical power due to small sample size.

Unfortunately, the rats that had experienced SE without stress did not exhibit statistically significant deficits in the ziggurat task, although, the mean latency to find the goal ziggurat was longer in KA+/+ group than the KA-/- group (Table 16). This could be as a result of two main reasons. First, there was a larger total number of rats that were to be tested in Experiment 2 ($n=26$) compared to Experiment 1 ($n=17$); thus, due to time

limitations for testing, there were changes to the protocol of the Experiment 2. These changes include a reduction in number of trials per day from 10 trials in Experiment 1 to 8 trials in Experiment 2. Also, to be more consistent with published research on the ziggurat task (Faraji et al., 2007) the number of testing days was doubled from 4 days to 8 days in the second experiment. These changes in the protocol may have led to differences in findings of Experiment 1 and 2. Second, a very small sample size in each group (especially in Experiment 2) may have resulted in insufficient statistical power to detect any significant effect.

Table 16.

Summary of the behavioural analysis for Experiment 2. Significant main effect of group, significant main effect of stress, significant interaction between group and stress (p<.05).

		Main Effect of Group	Main Effect of Stress	Interactions Between Group and Stress
Ladder Rung Walking Task	Number of Falls	Yes	No	No
	Number of Repositions	Yes	No	No
Object Discrimination Task	15 minutes Delay	No	No	No
	240 minutes Delay	Yes	No	No
Water Task	Latency	Yes	No	No
	Distance travelled	No	No	No
	Speed	Yes	No	No
Ziggurat Task	Latency	No	No	No
	Distance travelled	No	No	No
	Speed	No	No	No

Consistent with the hypothesis, but unlike Experiment 1, the rats that had experienced SE showed a deficit in the water task, taking longer to find the hidden

platform. However, the deficits in the water task were cofounded by the speed with which the rats swam to find the platform. Thus the deficit found in the water task in Experiment 2 may be a result of motor dysfunction as the KA+/+ were significantly slower than KA+/-.

Consistent with the hypothesis, but unlike Experiment 1, the rats that had experienced SE showed a deficit in object discrimination memory only at the long delay (240- minute). However, unlike the first experiment in which the results may have been biased due to chronic food restriction, the rats were only food restricted for the ziggurat task and not for the other tasks during Experiment 2. Thus, in Experiment 2 the rats directed their exploration towards objects rather than searching the environment for food (Table 16). Consistent with previous studies, these findings suggest that there may be extra hippocampal damage (i.e. perirhinal cortex) in rats that experienced SE, which leads to impaired object discrimination. However, histological confirmation is required to support this possibility.

The rats that had experienced SE exhibited motor deficits on the ladder rung walking compared to the SA-treated controls. Curiously, the SA-treated controls had a higher number of repositions than the KA+/- rats (Table 16). It is possible that the rats that had experienced SE were slower and thus more cautious while walking across the ladder.

Stress Effects

Unlike previous studies (Bremner et al., 1995; Schmid-Schonbein, 1998; Uno et al., 1986), there was no effect of stress on the number of spontaneous convulsions or

hippocampal volume. Moreover, rats that experienced stress did not show any impairment in any of the behavioural tasks.

Several reasons may explain these findings. First, unlike this study, in all of the reviewed literature, the rats were exposed to stress or were administered corticosterone just prior to seizure induction. Thus, differences in the timing of the exposure to the stressor may affect the frequency of convulsions. In addition, the type of stressor may have influenced the intensity of the stress response. In all of the reviewed literature, the rats were exposed to either corticosterone or maternal separation. However, in this study the rats were exposed to 14 days of restraint stress (1 hour/day/14 days) beginning 48 hours after the induction of SE. It is not clear, though, whether the stressor was stressful, as the rats initially were housed in cages that had plastic tubes in them as enrichment that were removed from the cages 2 weeks prior to KA injections. Although these tubes were much wider than the restraint stress tubes and were open on both sides this may have reduced the effectiveness of the restraint as a stressor.

Combined Effect of Status Epilepticus and Stress

Based on previous studies (Lai et al., 2006; Letty et al., 1995), it was expected that the rats that experienced SE and were exposed to stress be the most impaired in cognitive and motor tasks. However, there were no impairments found. This may be due to the fact that no structural changes (i.e. reduction in hippocampal volume) could be found and therefore, no functional changes could be observed. Thus, unlike the expectation, stress did not worsen the effects of SE on any observed behaviours. Further, this was compounded by a very small sample size and thus insufficient statistical power.

135

GENERAL DISCUSSION

The aim of the first study in this thesis was to characterize the behavioural impairments and hippocampal pathology associated with SE. The second experiment was conducted to examine the potential role that stress had on hippocampal volumes, the severity of convulsions, and associated behavioural change. It was found that SE reduced hippocampal volume. Results for spatial, motor and object discrimination tasks were more variable. Thus, some key effects were replicated and others were not (Table 17).

Table 17.

Comparison between behavioural findings for Experiment 1 and Experiment 2, significant main effect of group, p<.05.

	Behavioural Task	Main Effect of Group
Experiment 1	Ladder rung walking task	No
	Object discrimination task	No
	Water task	No
	Ziggurat task	Yes
Experiment 2	Ladder rung walking task	Yes
	Object discrimination task	Yes
	Water task	Yes
	Ziggurat task	No

Effects of Status Epilepticus

It has been demonstrated that rats that experience SE show a significant reduction in hippocampal volume. Analysis of the histological data suggests that there was a significant hippocampal volume loss as a result of SE in Experiment 1 and a reduction that did not reach significance in Experiment 2. These differences could be due to several reasons. First, the duration of SE was longer in Experiment 1 (M=18000 seconds; SD=3086.357) than Experiment 2 (M=10680.000 seconds; SD=3412.729). It must be noted that in Experiment 1, this reduction in hippocampal volume was positively associated with the duration of SE. Second, previous research suggested that there is a positive association between the number of spontaneous convulsions and reduction in hippocampal volume. The findings suggest that there was no association between the hippocampal volume loss and the number of spontaneous convulsions; in fact, the data suggest that hippocampal volume was only affected by the duration of SE. It must be noted that the rats were only monitored rats one hour per day and thus the number of spontaneous convulsions may have been underestimated. Furthermore, this result may reflect insufficient statistical power to observe the previously reported results.

The hippocampus is heavily involved in spatial memory (Eichenbaum, 1999; 2000), which was measured with the water task (Morris, 1984) and the ziggurat task (Faraji et al., 2007). In the first experiment, there was impairment in the latency with which the rats found the target ziggurat, but no impairments were detected in the water task. The opposite pattern of results was observed in Experiment 2. The inconsistencies could be explained perhaps by the nature of the ziggurat task being more sensitive to subtle hippocampal damage, an effect not observed in Experiment 2. However, the

observation of a deficit in the water task in Experiment 2 remains curious, especially as no significant decrease in hippocampal volume was observed. It must be noted that the effect in the water task was confounded with an increase in the speed with which the rats swam, which further complicates interpretation.

SE has been associated with extra-hippocampal damage (Sperk, 1994). For instance, damage to perirhinal cortex, a structure which is implicated in object discrimination memory, was observed in rats which had experienced SE, but only in Experiment 2 and only at the long delay (Jutila et al., 2001; Lim et al., 2007). Given that extra-hippocampal damage was not measured in either Experiment 1 or 2, it cannot be confirmed that Experiment 2 was associated with perirhinal damage, although the behaviour is suggestive.

SE has been associated with impairments in motor function (Kubova et al., 2000), although only Experiment 2 observed changes in motor function. However, the data suggest that SA-treated rats that fell more often than KA+/- rats in the ladder rung walking task. This could be due to the fact that the rats which has experienced SE walked slower on the ladder and thus fell less and made less repositions relative to the control.

Effects of Stress

Prolonged exposure to stress has been associated with reductions in hippocampal volume (Czeh et al., 2001) and cellular atrophy of the hippocampal CA1 region in rats (McEwen, 1999). However, unlike the expectation, no effect of stress on hippocampal volume was found. As explained earlier, this may have been due to use of inadequate stressor, small sample size and insufficient statistical power.

Stress is known to affect behaviour. The findings suggest that rats that were exposed to stress did not show impaired spatial or object discrimination memory. However, perhaps, absence of structural damage could explain the lack of impairments in spatial memory. It must also be considered that the stressor may have been inadequate, that I had small sample size and insufficient statistical power.

Combined Effects of Status Epilepticus and Stress

Unlike my prediction, there was no combined effect of SE and stress on hippocampal volume or on any of the behaviours that were examined. This was not unexpected as there was no effect of stress or SE by themselves on most measures. Two reasons may explain these findings. However, as mentioned previously, the absence of structural damage could explain the lack of impairments in spatial memory. It must also be considered that the stressor may have been inadequate; there was a small sample size and insufficient statistical power.

In conclusion, several points could be deduced from the findings. First, SE results in structural changes in hippocampus, which is evident in the volume loss. Second, the consequence of these structural changes is sufficiently large to be detected behaviourally in tests such as the ziggurat. Third, procedural changes during training and testing rats (such as food restriction) may alter the behavioural outcome in tasks such as object discrimination.

Improvements and Limitations

Several things could have improved this project. First, KA was injected i.p. Thus, any procedure that increased the absorption of the drug, such as food restriction, prior to

injection of KA may have increased the epilepstogenic effect of KA (Sperk, 1985; Sperk 1994). Moreover, the drug efficacy decreases with time (Sperk, 1985; Sperk 1994). Thus, purchase of a fresh drug prior to each experiment is essential.

Second, it would be preferable if the high mortality rate associated with administration of a single large dose KA could be reduced. Buckmaster & Dudek (1997) suggest that administering the drug gradually rather than one single large dose increases the chances of survival drastically.

Third, to study the compound effects of stress and SE on behavioural impairments, the rats were exposed to a stressor (i.e. restraint) every day. However, as noted it is unclear as to the extent to which restraint was stressful for my rats. Perhaps it would be more informative if corticosterone was administered. Administration of corticosterone may produce less variability in response to the stressor, and greater similarity in the physiological response.

Fourth, testing procedures, such as similar trial number, similar day number, similar duration of trial for the ziggurat task and water task, should have been kept as comparable as possible during the two experiments. This would have allowed for better comparisons between the two experiments. Furthermore, within the same group of rats, tasks that have similar requirements should be used. For example, food restriction that is required for the single pellet reaching task may have affected the results of the ziggurat and water tasks as well as the object discrimination task in the first experiment.

Finally, fifth, it must be noted that very small samples were used in both experiments. An increase in sample size, especially for the second experiment, would allow for better statistical power and perhaps more reliable group effects.

Implications for Seizure Disorders

The findings suggest that there is a reduction in hippocampal volume as a result of SE and that the extent of damage is positively correlated with the duration of SE. Furthermore, only the rats which experienced SE developed spontaneous convulsions after the latent phase. Thus, to minimize the amount of damage associated with SE, it should be treated as a medical emergency and controlled as quickly as possible with anticonvulsants (Gates et al., 1990).

Some degree of impairment in spatial ability, and some varying impairments in recognition memory at longer delays were observed. Also, some varying impairments in motor function were observed. However, it must be noted that none of these were associated with changes in hippocampal volume or convulsive severity. Thus this thesis is unable to speak to whether or not SE is associated with changes in cognitive behaviour.

There was no effect of stress on hippocampal volume or any of the examined behaviours. Perhaps, only severe types of stress, such as those seen in PTSD, lead to hippocampal volume change and thus behavioural impairments. However, unlike other studies in which the rats were exposed to stress prior to SE, the fact that the rats were exposed to stress after experiencing SE, may have influenced my results. In addition, it is not unexpected that there were no effect of the SE and stress combined as there were no structural changes of the stress exposure alone.

141

Future Directions

One of the temporal lobe structures that is affected by TLE is the amygdala (Bernasconi, et al., 2003). The amygdala is involved in fear and anxiety behaviours (Davis, 1992). Anxiety disorders are common in individuals with TLE (Torta & Keller, 1999), with a prevalence rate of about 10-25% (Jacoby et al., 1996; Torta & Keller, 1999). However, from my studies, it is unclear whether in fact higher levels of anxiety have led to these impairments or not. Using other animal models it has been shown that rats with TLE show higher level of anxiety (Adamec & Young, 2000; Kalynchuk et al., 1997; Kalynchuk et al., 1998 a; Kalynchuk et al., 1998 b; Kalynchuk et al., 1999; Kalynchuk, 2000; Kalynchuk et al., 2001; Kemppainen et al., 2006). It would be interesting to know if there are any associations between amygdala damage and SE-induced impairments. Thus, further investigations are required in order to identify the role of amygdala in SE-induced behavioural impairments.

Another structure that is being affected by SE is the perirhinal cortex. The impairments observed in object discrimination at the 240-minute delay were attributed to possible perirhinal cortex damage. However, since the perirhinal cortex damage was not measured, the extent of damage is unclear. Thus, further investigations are required in order to identify the role of the perirhinal cortex in SE-induced behavioural impairments.

The second part of this thesis involved investigating the role of stress on SE-induced damage and its associated behavioural impairments. Unlike previous findings, there were neither hippocampal damage nor impairments observed in any of the examined behaviours, although, it must be noted that the rats were exposed to stress after

SE rather than concurrently with SE. A plausible question is then does the timing of the stress affect the extent of damage and subsequent behavioural outcome? Another concern is that it is possible that the stressor was not intense enough. Thus, the next question to be asked is how the intensity of stress modulates convulsions and subsequently affects on behaviour.

CONCLUSION

TLE is often accompanied by reductions in hippocampal volume (Lee, 1995; Tasch et al., 1999; Van Paesschen et al., 1997), behavioural impairments such as disturbances in memory function (Bortz, 2003; Gilliam et al., 2003; Giovagnoli & Avanzini, 1999; Glogau et al., 2004; Griffith et al., 2006; Hermann et al., 1997) and mood disorders (Helmstaedter et al., 2004; Lambert & Robertson, 1999). However, the nature of these impairments in individuals with TLE is not very well understood. Using the KA model of SE, the potential hippocampal volume change and the associated behavioural changes were investigated. The findings suggest that there are variable impairments in spatial ability, object discrimination and motor coordination. Thus, some of the behavioural impairments that are observed in individuals with TLE may be a result of the neuropathology of convulsive disorders.

There are several lines of evidence suggesting that stress may play a significant role during the process of epileptogenesis (Herman & Cullinan, 1997; Herman et al., 2003; Krugers et al., 1995; Sheline et al., 1996). However, unlike the expectations, there was no effect of stress on hippocampal volume or on the examined behavioural tasks. Moreover, there was no compound effect of SE and stress on hippocampal volume or the examined behavioural tasks. This may be due to the type and intensity of the stressor that was used. It must be noted that the very small sample that was used resulted in insufficient statistical power to observe even large effects. Thus, further investigations are required to identify the factors influencing the effect of SE and stress on hippocampal volume and subsequent behavioural alterations.

144

REFERENCES

Abrahams, S., Pickering, A., Polkey, C., & Morris, R. (1997). Spatial memory deficits in patients with unilateral damage to right hippocampal formation. *Neuropsychologia, 35,* 11-24.

Adamec, R., & Young, B. (2000). Neuroplasticity in specific limbic system circuits may mediate specific kindling induced changes in animal affect–implications for understanding anxiety associated with epilepsy. *Neuroscience and Biobehavioral Reviews, 24,* 705–723.

Aikia, M., Kalviainen, R., & Riekkinen, P. (1995). Verbal learning and memory in newly diagnosed partial epilepsy. *Epilepsy Research, 22,* 157-164.

Alessio, A., Damasceno, B., Camargo, C., Kobayashi, E., Guerreiro, C., & Cendes, F. (2004). Differences in memory performance and other clinical characteristics in patients with mesial temporal lobe epilepsy with and without hippocampal atrophy. *Epilepsy and Behavior, 5,* 22–27.

Altshuler, L., Rausch, R., Delrahim, S., Kay, J., & Crandall, P. (1999). Temporal lobe epilepsy, temporal lobectomy, and major depression. *Journal of Neuropsychiatry Clinical Neuroscience, 11,* 436–443.

Aztiria, E., Capodieci, G., Arancio, L., & Leanza, G. (2007). Extensive training in a maze task reduces neurogenesis in the adult rat dentate gyrus probably as a result of stress. *Neuroscience Letters, 2*(416), 133–137.

Babb, T., Brown, W., Pretorius, J., Davenport, C., Lieb, J., & Crandell, P. (1984). Temporal lobe volumetric cell densities in temporal lobe epilepsy. *Epilepsia, 25*(6), 729-740.

Babb, T., Kupfer, W., Pretorius, J., Crandall, P., & Levesque, M. (1991). Synaptic reorganization by mossy fibers in human epileptic fascia dentata. *Neuroscience, 42,* 351–363.

Baram, T., & Hatalski, C. (1998). Neuropeptide-mediated excitability: a key triggering mechanism for seizure generation in the developing brain. *Trends in Neuroscience, 21,* 471–476.

Barry, J., Huynh, N., & Lembke, A. (2000). Depression in individuals with epilepsy. *Current Treatment Options in Neurology, 2,* 571–585.

Belanoff, J., Gross, K., Yager, A., & Schatzberg, A. (2001). Corticosteroids and cognition. *Journal of Psychiatry Research, 35*, 127–145.

Ben Ari, Y., & Lagowska, J. (1978). Epileptogenic action of intra-amygdaloid injection of kainic acid. *Comptes Rendus Hebdomadaires des Séances de l'Académie des Sciences, 287*, 813–816.

Ben-Ari, Y., Tremrlay, E., Ottersen, O. P., & Naquet, R. (1979). Evidence suggesting secondary epileptogenic lesions after kainic acid: pre-treatment with diazepam reduces distant but not local damage. *Brain Research, 165*, 362-365.

Ben-Ari, Y., Tremrlay, E., Riche, D., Ghilini, G., & Naquet, R. (1981). Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculline and pentetrazole: metabolic mapping using the deoxyglucose method with special reference to the pathology of epilepsy. *Neuroscience, 6*, 1361-1391.

Ben-Ari., Y. (1985). Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. *Neuroscience, 2*(14), 375-403.

Bernasconi, N., Bernasconi, A., Andermann, F., Dubeau, F., Feindel, W., & Reutens, D. (1999). Entorhinal cortex in temporal lobe epilepsy. A quantitative MRI study. *Neurology, 52*, 1870-1876.

Bernasconi, N., Bernasconi, A., Caramanos, Z., Andermann, F., Dubeau, F., & Arnold, D. (2000). Morphometric MRI analysis of the parahippocampal region in temporal lobe epilepsy. *Annals of the New York Academy of Sciences, 911*, 495–500.

Bernasconi, N., Bernasconi, A., Caramanos, Z., Antel, S., Andermann, F., & Arnold, D. (2003). Mesial temporal damage in temporal lobe epilepsy: a volumetric MRI study of the hippocampus, amygdala and parahippocampal region. *Brain, 126*, 462–469.

Bernasconi, N., Natsume, J., & Bernasconi, A. (2005). Progression in temporal lobe epilepsy: differential atrophy in mesial temporal structures. *Neurology, 65*, 223–228.

Blumer, D., Montouris, G., & Hermann, B. (1995). Psychiatric morbidity in seizure patients on a neurodiagnostic monitoring unit. *The Journal of Neuropsychiatry and Clinical Neuroscience, 7*, 445–456.

Bonilha, L., Kobayashi, E., Rorden, C., Cendes, F., & Li, L. (2003). Medial temporal lobe atrophy in patients with refractory temporal lobe epilepsy. *Journal of Neurology, Neurosurgery and Psychiatry, 74*, 1627–1630.

Bortz, J. (2003). Neuropsychiatric and memory issues in epilepsy. *Mayo Clinic Proceedings, 78*, 781-787.

Bremner, J., Randall, P., Scott, T., Bronen, R., & Seibyl, J. (1995). MRI based measurement of hippocampal volume in patients with combat related posttraumatic stress disorder. *American Journal of Psychiatry, 152*, 973-981.

Brown, M., & Aggleton, J. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nature Reviews Neuroscience, 2*, 51–61.

Buckmaster, P., & Dudek, F. (1997). Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *Journal of Comparative Neurology, 385*, 385–404.

Butterbaugh, G., Rose, M., Thomson, J., Roques, B., Costa, R., Brinkmeyer, M., Olejniczak, P., Fisch, B., & Carey, M. (2005). Mental health symptoms in partial epilepsy. *Archives of Clinical Neuropsychology, 20*, 647–654.

Calabrese, V., Gruemer, H., Tripathi, H., Dewey, W., Fortner, C., & de Lorenzo, R. (1993). Serum cortisol and cerebrospinal fluid beta-endorphins in status epilepticus. Their possible relation to prognosis. *Archives of Neurology, 50*(7), 689-693.

Cavalheiro, E., Riche, D., & Legallasalle, G. (1982). Long term effects of intrahippocampal kainic acid in rats: a method for inducing spontaneous recurrent seizures. *Electroencephalography and Clinical Neurophysiology, 53*, 581-582.

Cendes, F., Andermann, F., Gloor, P., Lopes-Cendes, I., Andermann, E., Melanson, D., Jones-Gotman, M., Robitaille, Y., Evans, A., & Peters, T. (1993). Atrophy of mesial structures in patients with temporal lobe epilepsy. cause or consequence of repeated seizures. *Annals of Neurology*, 34-79.

Cendes, F., Andermann, F., Carpenter, S., Zatorre, R., Cashman, N. (1995). Temporal lobe epilepsy caused by domoic acid intoxication: evidence for glutamate receptor-mediated excitotoxicity in humans, *Annals of Neurology, 37*, 123–126.

Cilio, M., Sogawa, Y., Cha, B., Liu, X., Huang, L., & Holmes, G. (2003). Long-term effects of status epilepticus in the immature brain are specific for age and model. *Epilepsia, 44*, 518–528.

Cockerell, O., Moriarty, J., Trimble, M., Sander, J., & Shorvon, S. (1996). Acute psychological disorders in patients with epilepsy: a nation-wide study. *Epilepsy Research, 25*, 119–131.

Corey, L., Pellock, J., & DeLorenzo, R. (2004). Status epilepticus in a population based Virginia twin sample. *Epilepsia, 2*(45), 159-165.

Coulter, D., McIntyre, D., & Löscher, W. (2002). Animal models of limbic epilepsies: what can they tell us? *Brain Pathology, 12*, 240–256.

Coyle J. T. (1983). Neurotoxic action of kainic acid. *Journal of Neurochemistry, 41*, 1-11.

Coyle, J. T., Bird, S. J., Evans, R. H., Gulley, R. L., Nicklas, W. J., & Olney, J. W. (1981). Excitatory amino-acid neurotoxins: selectivity, specificity and mechanisms of action. *Neuroscience Research Program Bulletin, 19*, 1-427.

Coyle, J. T., Molliver, M. R., & Kuhar, M. J. (1978). In situ injection of kainic acid: a new method for selectively lesioning neuronal cell bodies while sparing axons of passage. *The Journal of Comparative Neurology, 180*, 301-318.

Czeh, B., Michaelis, T., Watanabe, T., Frahm, J., de Biurrun, G., van Kampen, M., Bartolomucci, A., & Fuchs, E. (2001). Stress induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by prevented by anti-depressant treatment with tianeptine. *Proceedings of the National Academy of Sciences, 98*, 12796–12801.

Davis, M. (1992). The Role of the Amygdala in Fear and Anxiety. *Annual Review of Neuroscience, 15*, 353-375.

de Kloet, E. R., Oitzl, M. S., & Joels, M. (1999). Stress and cognition: Are corticosteroids good or bad guys? *Trends in Neuroscience, 22*, 422-426.

de Kloet, E. R., Vreugdenhil, E., Oitzl, M. S., & Joels, M. (1998). Brain corticosteroid receptor balance in health and disease. *Endocrine Reviews, 19*, 269-301.

de Lorenzo, R., Hauser, W., & Towne, A. (1996). A prospective population- based epidemiologic study of status epilepticus in Richmond, Virginia. *Neurology, 46*, 1029-1035.

de Lorenzo, R., Pellock, J., Towne, A., & Boggs, J. (1995). Epidemiology of status epilepticus. *Journal of Clinical Neurophysiology, 12*, 316-325.

de Quervain, D., Roozendaal, B., & McGaugh, J. (1998). Stress and glucocorticoids impair retrieval of long-term spatial memory. *Nature, 394*, 787–790.

de Quervain, D., Roozendaal, B., Nitsch, R., McGaugh, J., & Hock, C. (2000). Acute cortisone administration impairs retrieval of long-term declarative memory in humans. *Nature Neuroscience, 3*, 313-317.

de Rogalski Landrot, I., Minokoshi, M., Silveira, D., Cha, B., & Holmes, G. (2001). Recurrent neonatal seizures: relationship of pathology to the electroencephalogram and cognition. *Developmental Brain Research, 129*, 27–38.

de Giorgio, C., Tomiyasu, U., & Treiman, D. (1992). Hippocampal pyramidal cell loss in human status epelipeticus. *Epilepsia, 1*(33), 23-27.

Detour, J., Schroeder, H., Desor, D., & & Nehlig, A. (2005). A 5 month period of epilepsy impairs spatial memory, decreases anxiety, but spares object recognition in the lithium philocarpine model in adult rats. *Epilepsia, 4*(46), 499-508.

Devinsky, O., & Vasquez, B. (1993). Behavioral changes associated with epilepsy. *Neurologic Clinics, 11*, 127–49.

D'Hooge, R., & De Deyn, P. (2001). Applications of the Morris water maze in the study of learning and memory. *Brain Research Reviews, 36*, 60-90.

Dodrill, C. (1986). Correlates of generalized tonic–clonic seizures with intellectual, neuropsychological, emotional, and social functions in patients with epilepsy. *Epilepsia, 27*, 399–411.

Dodrill, C. (2002 a). Progressive cognitive decline in adolescents and adults with epilepsy. *Progress in Brain Research, 135*, 399–407.

Dodrill, C. (2002 b). Neuropsychological effects of seizures. *Epilepsy and Behavior, 5 (Suppl. 1)*, S 21–S 24.

Dodrill, C., & Batzel, L. (1986). Interictal behavioral features of patients with epilepsy. *Epilepsia, 27*(Suppl.2), S 64–S 76.

Du, F., Whetsell, W. J., Abou-Khalil, B., Blumenkopf, B., Lothman, E., & Schwarcz, R. (1993). Preferential neuronal loss in layer III of the entorhinal cortex in patients with temporal lobe epilepsy. *Epilepsy Research, 16*(3), 223-233.

Dupont, S., Van de Moortele, P., Samson, S., Hasboun, D., Poline, J., Adam, C., Lehéricy, S., Le Bihan, D., Samson, Y., & Baulac, M. (2000). Episodic memory in left temporal lobe epilepsy: a functional MRI study. *Brain, 123*, 1722–1732.

Edwards, H., Burnham, W., Mendonca, A., Bowlby, D., & MacLusky, N. (1999). Steroid hormones affect limbic afterdischarge thresholds and kindling rates in adult female rats. *Brain Research* (838), 136–150.

Edwards, H., Vimal, S., & Burnham, W. (2002). The effects of ACTH and adrenocorticosteroids on seizure susceptibility in 15-day-old male rats. *Experimental Neurology* (175), 182–190.

Eichenbaum, H. (2000). A cortical-hippocampal system for declarative memory. *Nature Reviews Neuroscience, 1*, 41–50.

Eichenbaum, H. (1999). The hippocampus and mechanisms of declarative memory. *Behavioural Brain Research, 103*, 123-133.

Engel, J. (2001). A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: report of the ILAE Task. *Epilepsia, 42*(6), 796-803.

Engel, J. (1996). Introduction to temporal lobe epilepsy. *Epilepsy Research, 26*, 141–150.

Engelberts, N., Klein, M., van der Ploeg, H., Heimans, J., Ader, H., & van Boxtel, M. (2002). Cognition and health-related quality of life in a well-defined subgroup of patients with partial epilepsy. *Journal of Neurology, 249*, 294-299.

Ennaceur, A., & Aggleton, J. (1997). The effects of neurotoxic lesions of the perirhinal cortex combined to fornix transection on object recognition memory in the rat. *Behavioural Brain Research, 88*, 181–193.

Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. I: Behavioural data. *Behavioural Brain Research, 31*, 47– 59.

Faraji, J., Lehmann, H., Metz, G., & Sutherland, R. (2007). Rats with hippocampal lesion show impaired learning and memory in the ziggurat task: A new task to evaluate spatial behavior. *Behavioural Brain Research, 189*, 17-31.

Fendler, K., Karmos, G., & Telegdy, G. (1961). The effect of hippocampal lesion on pituitary–adrenal function. *Acta physiologica Scandinavica, 20*, 293–297.

French, J., Williamson, P., Thadani, V., Darcey, T., Marson, R., Spencer, S., & Spencer, D. (1993). Characteristics of medial temporal lobe epilepsy: I. Results of history and physical examination. *Annals of Neurology, 34*, 774–780.

Galimberti, A., Flavia, M., Francesco, C., Carla, A., Luca, C., Massimo, C., Vittoriana, P., & Giovanni, M. (2005). Seizure frequency and cortisol and dehydroepiandrosterone

sulphate levels in women with epilepsy receiving antiepileptic drug treatment. *Epilepsia,* *4*(45), 517-523.

Gallagher, B., Murvin, A., Flanigin, H., King, D., & Luney, D. (1984). Pituitary and adrenal function in epileptic patients. *Epilepsia, 25*(6), 683-689.

Gates, J. R., & Cruz-Rodriguez, R. (1990). Mesial temporal sclerosis: Pathogenesis, Diagnosis, and Management. *Epilepsia, 31*(*Suppl 3*), S 55-S 66.

Gilliam, F., Hecimovic, H., & Sheline, Y. (2003). Psychiatric comorbidity, health, and function in epilepsy. *Epilepsy Research* (*Suppl 4*), *4,* S 26-S 30.

Giovagnoli, A., & Avanzini, G. (1999). Learning and memory impairment in patients with temporal lobe epilepsy: relation to the presence, type, and location of brain lesion. *Epilepsia, 40,* 904–911.

Giovagnoli, R., Erbetta, A., Villani, F., & Avanzini, G. (2005). Semantic memory in partial epilepsy: verbal and non-verbal deficits and neuroanatomical relationships. *Neuropsychologia, 43,* 1482–1492.

Glogau, S., Ellgring, H., Elger, C., & Helmstaedter, C. (2004). Face and facial expression memory in temporal lobe epilepsy patients: preliminary results. *Epilepsy and Behavior, 1*(5), 106-112.

Golden, G., Smith, G., Ferraro, T., Reves, P., Kulp, J., & Fariello, R. (1991). Strain differences in convulsive response to the excitotoxin kainic acid. *NeuroReport, 2,* 141-144.

Goddard, G., McIntyre, D., & Leech, C. (1969). A permanent change in brain function resulting from daily electrical stimulation. *Experimental Neurology , 25,* 295-330.

Good, M., & Honey, R. (1997). Dissociable effects of selective lesions to hippocampal subsystems on exploratory behavior, contextual learning, and spatial learning. *Behavioral Neuroscience, 111*(3), 487-493.

Griffith, H., Richardson, E., Pyzalski, R., Bell, B., Dow, C., Hermann, B., & Seidenberg, M. (2006). Memory for famous faces and the temporal lobe: Functional imaging findings in temporal lobe epilepsy. *Epilepsy and Behavior, 9,* 173–180.

Grippo, A., Pelosi, L., Metha, V., & Blumharrdt, L. (1996). Working memory in temporal lobe epilepsy: an event related potential study. *Electroencephalogrphy and Clinical Neurophysiology, 99,* 200–213.

Gröticke, I., Hoffmann, K., Löscher, W. (2008). Behavioral alterations in a mouse model of temporal lobe epilepsy induced by intra-hippocampal injection of kainite. *Experimental Neurology, 211,* 1-13.

Gurvits, T., Shenton, M., Hokama, H., Ohta, H., Lasko, N., Gilbertson, M., Orr, S., Kikinis, R., Jolesz, F., McCarley, R., & Pitman, R. (1996). Magnetic resonance imaging study of hippocampal volume in chronic, combat-related posttraumatic stress disorder. *Biological Psychiatry, 40,* 1091–1099.

Hannesson, D. K., Mohapel, P., & Corcoran, M. E. (2001). Kindling of the dorsal hippocampus selectively disrupts spatial learning/short-term memory. *Hippocampus, 11,* 275-286.

Hannesson, D., & Corcoran, M. (2000). The mnemonic effects of kindling. *Neuroscience and Biobehavioral Reviews, 24,* 725–751.

Hannesson, D., Howland, J., Pollock, M., Mohapel, P. W., & Corcoran, M. (2001). Dorsal hippocampal kindling produces a selective and enduring disruption of hippocampally mediated behavior. *Journal of Neuroscience, 21*(12), 4443-4450.

Hannesson, D., Howland, J., Pollock, M., Mohapel, P., Wallace, A., & Corcoran, M. (2005). Anterior perirhinal cortex kindling produces long-lasting effects on anxiety and object recognition memory. *European Journal of Neuroscience, 4*(21), 1081-1090.

Hannesson, D., Wallace, A., Pollock, M., Corley, S., Mohapel, P., & Corcoran, M. (2004). The relation between extent of dorsal hippocampal kindling and DMTP performance in the Morris water maze. *Epilepsy Research, 58,* 145-154.

Hauser, W., Annegers, J., & Kurland, L. (1993). Incidence of epilepsy and unprovoked seizures in Rochester, Minnesota: 1935-1984. *Epilepsia, 3*(34), 453-468.

Hauser, W., Annegers, J., & Kurland, L. (1991). Prevalence of epilepsy in Rochester, Minnesota: 1940-1980. *Epilepsia, 32,* 429-445.

Heiderstadt, K., McLaughlin, R., Wright, D., Walker, S., & Gomez- Sanchez, C. (2000). The effect of chronic food and water restriction open field behavior and serum corticosterone levels in rats. *Laboratory Animals, 34,* 20– 28.

Helmstaedter, C. (2002). Effects of chronic epilepsy on declarative memory systems. *Progress in Brain Research, 135,* 439-453.

Helmstaedter, C., & Kockelmann, E. (2006). Cognitive Outcomes in Patients with Chronic Temporal Lobe Epilepsy. *Epilepsia, 47(Suppl 2),* 96–98.

Helmstaedter, C., & Kurthen, M. (2001). Memory and epilepsy: characteristics, course, and influence of drugs and surgery. *Current Opinion in Neurology, 14*(2), 211-6.

Helmstaedter, C., Kurthen, M., Lux, S., Reuber, M., & Elger, C. (2003). Chronic epilepsy and cognition: a longitudinal study in temporal lobe epilepsy. *Annals of Neurology, 4*(54), 425-432.

Helmstaedter, C., Sonntag-Dillender, M., Hoppe, C., & Elger, C. (2004). Depressed mood and memory impairment in temporal lobe epilepsy as a function of focus lateralization and localization. *Epilepsy and Behavior, 5*(5), 696-701.

Herman, J. P., Figueiredo, H., Mueller, N. K., Ulrich-Lai, Y., Ostrander, M. M., Choi, D., & Cullinan, W. (2003). Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamopituitary- adrenocortical responsiveness. *Frontiers in Neuroendocrinology, 24*, 151-180.

Herman, J., & Cullinan, W. (1997). Neurocircuitry of stress: central control of the hypothalamic-pituitary-adrenocortical axis. *Trends in Neuroscience, 20*, 78–84.

Herman, J., Schafer, M., Young, E., Thompson, R., Douglass, J., Akil, H., & Watson S. (1989). Evidence for hippocampal regulation of neuroendocrine neurons of the hypothalamo– pituitary–adrenocortical axis. *Journal of Neuroscience, 9*, 3072–3082.

Hermann, B., Seidenberg, M., & Bell, B. (2000). Psychiatric comorbidity in chronic epilepsy: identification, consequences, and treatment of major depression. *Epilepsia, 41*(*Suppl 2*), S 31–S 41.

Hermann, B., Seidenberg, M., Schoenfeld, J., & Davies, K. (1997). Neuropsychological characteristics of the syndrome of mesial temporal lobe epilepsy. *Archives of Neurology, 54*, 369-376.

Hernandez, M., Sauerwein, H., Jambaqué, I., De Guise, E., Lussier, F., Lortie, A., Dulac, O., & Lassonde, M. (2002). Deficits in executive functions and motor coordination in children with frontal lobe epilepsy. *Neuropsychologia, 4*(40), 384-400.

Hodges H. (1996). Maze procedures: the radial-arm and water maze compared. *Cognitive Brain Research, 3*, 167-181.

Hort, J., Brozek, G., Komarek, V., Langmeier, M., & Mares, P. (2000). Inter-strain differences in cognitive functions in rats in relation to status epilepticus. *Behavioural Brain Research, 112*, 77–83.

Jacobson, L., & Sapolsky, R. (1991). The role of the hippocampus in feedback regulation of the hypothalamic pituitary adrenocortical axis. *Endocrine Reviews, 12*, 118-134.

153

Jacoby, A., Baker, G., Steen, L., Potts, P., & Chadwick, D. (1996). The clinical course of epilepsy and its psychological correlates: findings from a U.K. community study. *Epilepsia, 37*, 148–461.

Joels M. (2001). Corticosteroid actions in the hippocampus. *Journal of Neuroendocrinology, 13*, 657-669.

Jokeit, H., & Ebner, A. (1999). Long term effects of refractory temporal lobe epilepsy on cognitive abilities: a cross sectional study. *Journal of Neurology, Neurosurgery, and Psychiatry, 67*, 44-50.

Jutila, L., Ylinen, A., Partanen, K., Alafuzoff, I., Mervaala, E., Partanen, J., Vapalahti, M., Vainio, P., & Pitkänen, A. (2001). MR volumetry of the entorhinal, perirhinal, and temporopolar cortices in drug-refractory temporal lobe epilepsy. *AJNR. American Journal of Neuroradiology, 22*, 1490-1501.

Kalynchuk, L. (2000). Long-term amygdala kindling in rats as a model for the study of interictal emotionality in temporal lobe epilepsy. *Neuroscience and Biobehavioral Reviews, 24*, 691–704.

Kalynchuk, L., Davis, A., Gregus, A., Taggart, J., Chris Dodd, C., Wintink, A., & Marchant, E. (2001). Hippocampal involvement in the expression of kindling-induced fear in rats. *Neuroscience and Biobehavioral Reviews, 25*, 687–696.

Kalynchuk, L., Pearson, D., Pinel, J., & Meany, M. (1999). Effect of Amygdala Kindling on Emotional Behavior and Benzodiazepine Receptor Binding in Rats. *Annals of the New York Academy of Sciences, 877*, 737-741.

Kalynchuk, L., Pinel, J., & Treit, D. (1998 a). Long-term kindling and interictal emotionality in rats: effect of stimulation site. *Brain Research, 779*(1-2), 149-157.

Kalynchuk, L., Pinel, J., Treit, D., & Kippin, T. (1997). Changes in emotional behavior produced by long-term amygdala kindling in rats. *Biological Psychiatry, 41*(4), 438-451.

Kalynchuk, L., Pinel, J., Treitj, D., Barnes, S., McEachern, J., & Kippin, T. (1998 b). Persistence of the interictal emotionality produced by long-term amygdala kindling in rats. *Neuroscience, 85*(4), 1311–1319.

Karst, H., de Kloet, E., & Joels, M. (1999). Episodic corticosterone treatment accelerates kindling epileptogenesis and triggers longterm changes in hippocampal CA1 cells, in the fully kindled state. *European Journal of Neuroscience, 11*, 887–898.

Keenan, P., Jacobson, M., Soleymani, R., Mayes, M. S., & Yaldoo, D. (1996). The effect on memory of chronic prednisone treatment in patients with systemic disease. *Neurology, 47*, 1396–1403.

Kelly, M., & McIntyre, D. (1996). Perirhinal cortex involvement in limbic kindled seizures. *Epilepsy Research, 26*, 233–243.

Kemppainen, E., Nissinen, J., & Pitkänen, A. (2006). Fear Conditioning Is Impaired in Systemic Kainic Acid and Amygdala-stimulation Models of Epilepsy. *Epilepsia, 5*(47), 820–829.

Knigge, K. (1961). Adrenocortical response to stress in rats with lesions in hippocampus and amygdala. *Proceedings of the Society for Experimental Biology and Medicine, 108*, 18–21.

Knigge, K., & Hays, M. (1963). Evidence of inhibitive role of hippocampus in neural regulation of ACTH release. *Proceedings of the Society for Experimental Biology and Medicine, 114*, 67–69.

Kotloski, R., Lynch, M., Lauersdorf, S., & Sutula, T. (2002). Repeated brief seizures induce progressive hippocampal neuron loss and memory deficits. *Progress in Brain Research, 135*, 95–110.

Krugers, H., Knollema, S., Kemper, R., Ter Horst, G., & Korf, J. (1995). Down-regulation of the hypothalamo-pituitary-adrenal axis reduces brain damage and number of seizures following hypoxia/ischaemia in rats. *Brain Research, 690*, 41-47.

Kubova, H., Haugvicova, R., Suchomelova, L., & Mares, P. (2000). Does status epilepticus influence the motor development of immature rats? *Epilepsia, 41*(*Suppl 6*), S64-S69.

Lado, F., Sankar, R., Lowenstein, D., & Moshe, S. (2000). Age-dependent consequences of seizures: relationship to seizure frequency, brain damage, and circuitry reorganization. *Mental Retardation and Developmental Disabilities Research Reviews, 6*, 242–52.

Lah, S., Lee, T., Grayson, S., & Miller, L. (2006). Effects of temporal lobe epilepsy on retrograde memory. *Epilepsia, 3*(47), 615–625.

Lai, M., Holmes, G., Lee, K., Yang, S., Wang, C., Wu, C., Tiao, M., Hsieh, C., Lee, C., & Huang, L. (2006). Effect of neonatal isolation on outcome following neonatal seizures in rats–the role of corticosterone. *Epilepsy Research, 68*, 123–136.

Lai, M., Liou, C., Yang, S., Wang, C., Hung, P., Wu, C., Tung, Y., &.Huang, L. (2002). Recurrent bicuculline-induced seizures in rat pups cause long-term motor deficits and increase vulnerability to a subsequent insult. *Epilepsy and Behavior, 3*, 60-66.

Lambert, M., & Robertson, M. (1999). Depression in epilepsy: etiology, phenomenology, and treatment. *Epilepsia, 40(Suppl 10)*, S 21–S 47.

Lapin, I. P. (1995). Only controls; effects of handling, sham injection, and intraperitoneal injection of saline on behavior of mice in an elevated plus-maze. *Journal of Pharmacological and Toxicological Methods, 34*, 73-77.

Lee, N., Tien, R., Friedman, A., Felsberg, G., Crain, B., Hulettec, C., Osumi, A., Smith, J., Vanlandingham, K., & Radtke, R. (1995). Fast spin-echo, magnetic resonance imaging-measured hippocampal volume: correlation with neuronal density in anterior temporal lobectomy patients. *Epilepsia, 36*, 899-904.

Lee, P., Grimes, L., & Hong, J. (1989). Glucocorticoids potentiate kainic acid-induced seizures and wet dog shakes. *Brain Research, 480*, 322–325.

Lespinet, V., Bresson, C., N'Kaoua, B., Rougier, A., & Claverie, B. (2002). Effect of age of onset of temporal lobe epilepsy on the severity and the nature of preoperative memory deficits. *Neuropsychologia, 40*, 1591–600.

Letty, S., Lerner-Natoli, M., & Rondouin, G. (1995). Differential impairments of spatial memory and social behavior in two models of limbic epilepsy. *Epilepsia, 36*, 973–82.

Lim, C., Turner, L., & Heinrichs, S. (2007). Short-term social recognition memory deficit and atypical social and physiological stressor reactivity in seizure-susceptible El mice. *Seizure, 16*, 59-68.

Logroscino, G., Hesdorffer, D., Cascino, G., Hauser, W., Coeytaux, A., Gallopades, B., Morabia, A., & Jallon P. (2005). Mortality after a first episode of status epilepticus in the United States and Europe. *Epilepsia, 46(Suppl 11)*, 46–48.

Löscher, W. (2002). Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. *Epilepsy Research, 50*, 105–123.

Löscher, W. (1997). Animal models of intractable epilepsy. *Progress in Neurobiology, 53*, 239–258.

Löscher, W., & Schmidt, D. (1993). New drugs for the treatment of epilepsy. *Current Opinion in Investigational Drugs, 2*, 1067-1095.

Löscher, W., & Schmidt, D. (1994). Strategies in antiepileptic drug development: is rational drug design superior to random screening and structural variation? *Epilepsy Research, 17,* 95-134.

Lothman, E. W., & Bertram, E. H. (1993). Epileptogenic effects of status epilepticus. *Epilepsia, 34(Suppl 1),* S 59– S 70.

Lowenstein, D. H. (2005). Seizures and epilepsy. In Harrisson Principles of Internal Medcine (pp. 2357-2371). McGrow-Hill.

Luine, V., Spencer, R., & McEwen, B. (1993). Effects of chronic corticosterone ingestion on spatial memory performance and hippocampal serotonergic function. *Brain Research, 616,* 65–70.

Luine, V., Villegas, M., Martinez, C., & McEwen, B. (1994). Repeated stress causes reversible impairments of spatial memory performance. *Brain Research, 639,* 167–170.

Magarinos, A., & McEwen, B. (1995). Stress induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. *Neuroscience, 69,* 83-88.

Mathern, G., Cifuentes, F., Leite, J., Pretorius, J., & Babb, T. (1993). Hippocampal EEG excitability and chronic spontaneous seizures are associated with aberrant synaptic reorganization in the rat intrahippocampal kainate model. *Electroencephalography and Clinical Neurophysiology, 87,* 326–339.

McEwen, B. (1999). Stress and hippocampal plasticity. *Annual Reviews in Neuroscience , 22,* 105-122.

McEwen, B., & Magarinos, A. (2001). Stress and hippocampal plasticity: implications for the pathophysiology of affective disorders. *Human Psychopharmacology, 16,* S7–S19.

McEwen, B., & Magarinos, A. (1997). Stress effects on morphology and function of the hippocampus. *Annals of the New York Academy of Sciences, 821,* 271–284.

McIntyre, D., & Kelly, M. (2000). The parahippocampal cortices and kindling. *Annals of the New York Academy of Sciences, 911,* 343–354.

Metz, G., & Whishaw, I. (2002). Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. *Journal of Neuroscience Methods, 115*(2), 169-179.

Moore, P., & Baker, G. (2002). The neuropsychological and emotional consequences of living with intractable temporal lobe epilepsy: implications for clinical management. *Seizure, 11*, 224-230.

Morimoto, K., Fahnestock, M., & Racine, R. (2004). Kindling and status epilepticus models of epilepsy: rewiring the brain. *Progress in Neurobiology, 73*, 1–60.

Morris, R. (1984). Developments of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods, 1*(11), 47–60.

Morris, R. (1981). Spatial localization does not require the presence of local cues. *Learning and Motivation, 12*, 239–60.

Nadler, J., & Cuthbertson, G. (1980). Kainic acid neurotoxicity toward hippocampal formation: dependence on specific excitatory pathways. *Brain Research, 195*, 47-56.

Nadler, J., Evenson, D., & Cuthbertson, G. (1981). Comparative toxicity of kainic acid and other acidic amino acids toward rat hippocampal neurons. *Neuroscience, 6*, 2505-2517.

Nadler, J., Perry, B., Gentry, C., & Cotman, C. (1980). Degeneration of hippocampal CA3 pyramidal cells induced by intraventricular kainic acid. *The Journal of Comparative Neurology, 192*, 333-359.

Newcomer, J., Selke, G., Melson, A., Hershey, T., Craft, S., Richards, K., & Alderson, A. (1999). Decreased memory performance in healthy humans induced by stress-level cortisol treatment. *Archives of General Psychiatry, 56*, 527-533.

O'Brien, C., Bowden, S., Bardenhagen, F., & Cook, M. (2003). Neuropsychological correlates of hippocampal and rhinal cortex volumes in patients with mesial temporal sclerosis. *Hippocampus, 13*, 892–904.

O'Leary, D., Lovell, M., Sackellares, C., Berent, S., & Giordani, B. (1983). Effects of age of onset of partial and generalized seizures on neuropsychological performance in children. *The Journal of Nervous and Mental Disease, 171*, 624–629.

O'Leary, D., Seidenberg, M., Berent, S., & Boll, T. (1981). Effects of age of onset of tonic–clonic seizures on neuropsychological performance in children. *Epilepsia, 22*, 197–204.

O'Reilly, R., & Rudy, J. (2001). Conjunctive representations in learning and memory: principles of cortical and hippocampal function. *Psychological Reviews, 108*(2), 311-345.

O'rourke, D., Saykin, A., Gilhool, J., Harley, R., O'Connor, M., & Sperling, M. (1993). Unilateral hemispheric memory and hippocampal neuronal density in temporal lobe epilepsy. *Neuroscience, 32*, 574-580.

Oitzl, M., & de Kloet, E. (1992). Selective corticosteroid antagonists modulate specific aspects of spatial orientation learning. *Behavioral Neuroscience, 106*, 62–71.

Oitzl, M., Reichardt, H., Joels, M., & de Kloet, E. (2001). Point mutation in the mouse glucocorticoid receptor preventing DNA binding impairs spatial memory. *National Academy of Sciences, 98*, 12790-12795.

Olton, D., & Samuelson, R. (1976). Rememberance of places passed: Spatial memory in rats. *Journal of Experimental Psychology. Animal Behavior Processes, 2*, 97–116.

Onley, J., Fuller, T., & deGubareff, T. (1979). Acute dendrotoxic changes in the hippocampus of kainate treated rats. *Brain Research, 176*, 91-100.

Pedersen, B., & Dam, M. (1986). Memory disturbances in epileptic patients. *Acta Neurologica Scandinavica, 74(suppl 109)*, 11-14.

Piazzini, A., Turner, K., Chifari, R., Morabito, A., Canger, R., & Canevini, M. P. (2006). Attention and psychomotor speed decline in patients with temporal lobe epilepsy: A longitudinal study. *Epilepsy Research, 72(2-3)*, 89-96.

Pirttila, T., Pitkänen, A., Tuunanen, J., & Kauppinen, R. (2001). Ex vivo MR microimaging of neuronal damage after kainate-induced status epilepticus in rat: correlation with quantitative histology. *Magnetic Resonance in Medicine : Official Journal of the Society of Magnetic Resonance in Medicine, 46*, 946–954.

Pitkänen, A., & Sutula, T. (2002). Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. *Lancet Neurology, 1*, 173–181.

Pulsipher, D., Seidenberg, M., Jones, J., & Hermann, B. (2006). Quality of life and comorbid medical and psychiatric conditions in temporal lobe epilepsy. *Epilepsy and Behavior, 9*, 510–514.

Roberts, A., & Keith, L. (1994 a). Mineralocorticoid receptors mediate the enhancing effects of corticosterone on convulsion susceptibility in mice. *The Journal of Pharmacology and Experimental Therapeutics, 270*, 505–511.

Roberts, A., & Keith, L. (1994 b). Sensitivity of the circadian rhythm of kainic acid-induced convulsion susceptibility to manipulations of corticosterone levels and mineralocorticoid receptor binding. *Neuropharmacology, 33*, 1087–1093.

Roozendaal, B., Griffith, Q., Buranday, J., de Quervain, D., & McGaugh, J. (2003). The hippocampus mediates glucocorticoid-induced impairment of spatial memory retrieval: dependence upon the basolateral amygdala. *Proceedings of the National Academy of Sciences, 100*, 1328-1330.

Salmenperä, T., Kälviäinen, R., Partanen, K., & Pitkänen, A. (2000). Quantitative MRI volumetry of the entorhinal cortex in temporal lobe epilepsy. *Seizure, 9*, 208–215.

Salzberg, M., Kumar, G., Supit, L., Jones, N., Morris, M., Rees, S., & O'Brien, T. (2007). Early Postnatal stress confers enduring vulnerability to limbic epileptogenesis. *Epilepsia, 48*(11), 2079-2085.

Sapolsky, R., Krey, L., & McEwen, B. (1985). Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *Journal of Neuroscience, 5*(5), 1222–1227.

Sapolsky, R., Krey, L., & McEwen, B. (1984). Glucocorticoid-sensitive hippocampal neurons are involved in terminating the adrenocortical stress response. *Proceedings of the National Academy of Sciences of the United States of America, 81*, 6174–6177.

Sapolsky, R., Zola, M., Morgan, S., & Squire, L. (1991). Inhibition of glucocorticoid secretion by the hippocampal formation in the primate. *Journal of Neuroscience, 11*, 3695-3704.

Sapolsky, R., Romero, L., Munck, A. (2000). How do glucocorticoids influence the stress-response? Integrating permissive, suppressive, stimulatory, and preparative action. *Endocrine Reviews, 21*, 55-83.

Schmid-Schonbein, C. (1998). Improvement of seizure control by psychological methods in patients with intractable epilepsies. *Seizure, 7*, 261–270.

Schmitz, C., & Hof, P. (2005). Design-based stereology in neuroscience. *Neuroscience, 130*(4), 813-831.

Schwarcz, R., & Witter, M. (2002). Memory impairment in temporal lobe epilepsy: the role of entorhinal lesions. *Epilepsy Research, 50*, 161-77.

Shorvon, S. (1996). The epidemiology and treatment of chronic and refractory epilepsy. *Epilepsia*, S1- S3.

Silberman, E., Sussman, N., Skillings, G., & Callanan, M. (1994). Aura phenomena and psychopathology: A pilot investigation. *Epilepsia, 35*, 778–784.

Spencer, D., & Spencer, S. (1994). Hippocampal resections and the use of human tissue in defining temporal lobe epilepsy syndromes. *Hippocampus, 4*, 243–249.

Sperber, E., Haas, K., Stanton, P., & Moshe, S. (1991). Resistance of the immature hippocampus to seizure-induced synaptic reorganization. *Developmental Brain Research, 60*, 88–93.

Sperk, G., Lassmann, H., Baran, H., Seitelberger, F., & Hornykiewicz, O. (1985). Kainic acid-induced seizures: Dose relationship of behavioural, neurochemical and histopathological changes. *Brain Research, 338*, 289-295.

Sperk, G. (1994). Kainic acid seizures in the rat. *Progress in Neurobiology, 42*(1), 1-32.

Squire, L., & Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science, 253*, 1380–1386.

Starkman, M., Gebarski, S., Berent, S., & Schteingart, D. (1992). Hippocampal formation volume, memory dysfunction, and cortisol levels in patients with Cushing's syndrome. *Biological Psychiatry, 32*, 756-765.

Starkman, M., Giordani, B., Gebarski, S., Berent, S., Schork, M., & Schteingart, D. (1999). Decrease in cortisol reverses human hippocampal atrophy following treatment of Cushing's disease. *Biological Psychiatry, 46*(12), 1595-1602.

Sutula, T., Lauersdorf, S., Lynch, M., Jurgella, C., & Woodard, A. (1995). Deficits in radial arm maze performance in kindled rats: Evidence for long-lasting memory dysfunction induced by repeated brief seizures. *Journal of Neuroscience, 15*, 8295-8301.

Suzuki, W., Zola-Morgan, S., Squire, L., & Amaral, D. (1993). Lesions of the perirhinal and parahippocampal cortices in the monkey produce long-lasting memory impairment in the visual and tactual modalities. *Journal of Neuroscience, 13*, 2430–2451.

Tabachnick, B., & Fidell, L. (2001). *Using multivariate statistics* (4th ed.). Boston, MA: Allyn & Bacon (pp. 34-35).

Taher, T., Salzberg, M., Morris, M., Rees, S., & O'Brien, T. (2005). Chronic low-dose corticosterone supplementation enhances acquired epileptogenesis in the rat amygdala kindling model of TLE. *Neuropsychopharmacology, 30*, 1610–1616.

Talmi, M., Carlier, E., Bengelloun, W., & Soumireu-Mourat, B. (1995). Synergistic action of corticosterone on kainic acid-induced electrophysiological alterations in the hippocampus. *Brain Research, 704*, 97–102.

Tanaka, S., Kondo, S., Tanaka, T., & Yonemasu, Y. (1988). Long-term observation of rats after unilateral intra-amygdaloid injection of kainic acid. *Brain Research, 463*, 163-167.

Tanaka, T., Tanaka, S., Fujita, T., Takano, K., Fukuda, H., Sako, K., & Yonemasu, Y. (1992). Experimental complex partial seizures induced by a microinjection of kainic acid into limbic structures. *Progress in Neurobiology, 38*, 317-334.

Tasch, E., Cendes, F., Li, L.M., Dubeau, F., Andermann, F., & Arnold, D. (1999). Neuroimaging evidence of progressive neuronal loss and dysfunction in temporal lobe epilepsy. *Annals of Neurology, 45*, 568–576.

Teitelbaum, J., Zatorre, R., Carpernter, S., Gendron, D., Evans, A., Gjedde, A., & Cashman, N. (1990). Neurologic sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. *The New England Journal of Medicine, 322,* 1781–1787.

Torta, R., & Keller, R. (1999). Behavioral, psychotic, and anxiety disorders in epilepsy: etiology, clinical features, and therapeutic implications. *Epilepsia, 40(Suppl 10)*, S2–S20.

Tremblay, E., Nitecka, L., Berger, M., & Ben Ari, Y. (1984). Maturation of kainic acid seizure-brain damage syndrome in the rat. I. Clinical, electrographic and metabolic observations. *Neuroscience, 13*, 1051– 1072.

Tuunanen, J., Lukasiuk, K., Halonen, T., & Pitkänen, A. (1999). Status epilepticus-induced neuronal damage in the rat amygdaloid complex: distribution, time-course and mechanisms. *Neuroscience, 94*(2), 473-495.

Uno, H., Tarara, R., Ross, T., Else, J., Suleman, M., & Sapolsky, R. (1989). Hippocampal damage associated with prolonged and fatal stress in primates. *Journal of Neuroscience, 9*, 1709-1711.

Van Paesschen, W., Revesz, T., Duncan, J., King, M., & Connelly, A. (1997). Quantitative neuropathology and quantitative magnetic resonance imaging of the hippocampus in temporal lobe epilepsy. *Annals of Neurology, 42*(5), 756–766.

Vannest, J., Szaflarski, J., Privitera, M., Schefft, B., & Holland, S. (2008). Medial temporal fMRI activation reflects memory lateralization and memory performance in patients with epilepsy. *Epilepsy and Behavior, 12*(3), 410-418.

Victoroff, J. (1994). DSM-III-R psychiatric diagnoses in candidates for epilepsy surgery: lifetime prevalence. *Neuropsychiatry, Neuropsychology and Behavioural Neurology, 7*, 87–97.

Waber, D., Carpentieri, S., Klar, N., Silverman, L., Schwenn, M., Hurwitz, C., Mullenix, P., Tarbell, N., & Sallan, S. (2000). Cognitive sequelae in children treated for acute lymphoblastic leukemia with dexamethasone or prednisone. *Journal of Pediatric Hematology/Oncology, 22,* 193-6.

Watanabe, Y., Gould, E., & McEwen, B. (1992). Stress induces atrophy of apical dendrites of hippocampus CA3 pyramidal neurons. *Brain Research, 588,* 341-344.

Weiss, G., Castillo, N., & Fernandez, M. (1993b). Amygdala kindling rate is altered in rats with a deficit in the responsiveness of the hypothalamo-pituitary-adrenal axis. *Neuroscience Letters, 157,* 91–94.

Weiss, G., Lucero, K., Fernandez, M., Karnaze, D., & Castillo, N. (1993a). The effect of adrenalectomy on the circadian variation in the rate of kindled seizure development. *Brain Research, 612,* 354–356.

Whishaw, I., & Jarrard, L. (1995). Similarities vs. differences in place learning and circadian activity in rats after fimbria-fornix section or ibotenate removal of hippocampal cells. *Hippocampus, 5*(6), 595-604.

Wieser, H. (2004). ILAE Commission report. Mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia, 45,* 695–714.

Winters, B.D., Forwood, S.E., Cowell, R.A., Saksida, L.M., Bussey, T.J. (2004). Double dissociation between the effects of peri-postrhinal cortex and hippocampal lesions on tests of object recognition and spatial memory: heterogeneity of function within the temporal lobe. *Journal of Neuroscience, 24,* 5901–5908.

Woolley, C., Gould, E., & McEwen, B. (1990). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Research, 531,* 225–231.

Zentner, J., Wolf, H., Helmstaedter, C., Grunwald, T., Aliashkevich, A., Wiestler, O., Elger, C., & Schramm, J. (1999). Clinical relevance of amygdala sclerosis in temporal lobe epilepsy. *Journal of Neurosurgery, 1*(91), 59-67.