STRUCTURAL ALTERATIONS IN THE HIPPOCAMPUS AND SPATIAL BEHAVIOR BY STRESS IN MALE AND FEMALE RATS: PROTECTION, AND RECOVERY IN WATER-BASED AND DRY-LAND TASKS

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To:

My father

Abstract

Stress-related cognitive changes are still a matter of debate. In some particular neuropathological conditions such as focal ischemia, cognitive functions have been shown to be significantly impaired. These conditions, however, may be improved by some factors such as steroid hormones. The purpose of the current thesis was to assess the structural and functional effects of corticosterone-related experiences on the hippocampus before and after endothelin-1 (ET-1)-induced stroke. We found corticosterone-related experiences enhance the hippocampal recovery, and improve its function in both wet and dry-land tasks after ET-1-induced focal stroke. Structural and functional effects of such experiences prior to the focal ischemia in the hippocampus, however, showed that stress, not corticosterone is a strong inhibitor for hippocampal recovery.

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Table of Contents

List of Tables and Figures

Page

1. Introduction

Stroke is the third leading cause of death in Western societies. Among survivors, stroke can cause an enormous disability. It is the disabilities that have the greatest impact on families, healthcare, and society. A stroke can have disastrous effects on motor functions and cognitive abilities. Motor and cognitive symptoms mostly depend upon the type of brain insult, the extent and location of area involved in the stroke, and the psychobiological background of patients. The residual deficits after stroke may impose significant limitations to the productivity and quality of life of patients.

Research on the neuropathology of stroke and post-stroke rehabilitation, either in humans or animals, is now aiming to understand neurobiological mechanisms underlying stroke-induced disabilities and to identify which specific therapeutic approaches are effective. Specifically, rodent stroke models provide the experimental backbone for determination of the mechanisms of cell death and neural repair *in vivo*, and for pre-clinical testing of neuroprotective compounds and therapies that enhance recovery. These studies in particular continue to shed light on the molecular, vascular, glial, neuronal, and behavioural events that are important to the functional recovery after a stroke. Thus, years of previous animal research have already resulted in a better understanding of what occurs in the brain following stroke and how the brain may reorganize in response to a given treatment.

Of the various animal stroke models that have been developed and characterized in the recent years, no one model alone may fully mimic human stroke because of the heterogeneity of the clinical situation. Currently, however, investigations are under way to develop strategies for modeling stroke in animals by more comprehensive measures of both motor and cognitive impairments and the subsequent recovery in response to treatments or improvement techniques. An increasing number of studies using animal models of stroke are focusing on identifying the fundamental neural substrates that support functional recovery after stroke. These approaches are slow to reach widespread acceptance, but successful interventions to produce functional recovery in animals reported in recent years offer hope for the future of human patients.

One of the most challenging functional manifestations after stroke is cognitive impairment. It is an extremely common consequence of stroke, so that the body of investigations in animal studies has expanded to studies that have implications for potential neuropharmacological interventions. These interventions target cognitive deficits or structural recovery of relevant structures of the brain. In this context, the hippocampus is important for at least two fundamental reasons: it is thought to play an important role in some cognitive processes, especially spatial and episodic memory, and it is highly plastic throughout life. Research on neuropharmacology of post-stroke recovery involving changes in hippocampus and in hippocampal-related cognitive processes may open new windows toward the effective treatment of stroke-induced pathological situations.

One of the broad goals of this thesis is to identify some of the stroke-related structural and functional deficits induced by endothelin-1 (ET-1), a potent vasoconstrictor that reduces local blood flow. Furthermore, this thesis will elucidate the processes of structural and functional changes after ET-1-induced hippocampal stroke in rats that have experienced stress or elevated stress hormones prior to or following the stroke.

In the following section, the current state of the literature in relation to hippocampal structure and function will be briefly reviewed. Then, the theories of hippocampal function, the hippocampus as a navigational system, and hippocampal neurogenesis will be specifically considered. In addition, the nature of stroke and stroke-related manifestations in human and animals will be discussed with respect to the main goal of this thesis. Finally, this thesis will outline general considerations for stress, psychobiology of stress and stress-related alterations in structure and function of the hippocampus for conducting research using rat models of stroke.

2. The Medial Temporal Lobe and the Hippocampus

2.1 The medial temporal lobe.

The medial temporal lobe (MTL) includes a system of anatomically related structures that are necessary for declarative memory (the aspect of human conscious memory that stores facts and events) (Squire et al., 2004). The MTL system consists of the hippocampal area (CA fields, dentate gyrus, and subicular complex) and the adjacent perirhinal, entorhinal, and parahippocampal cortices. One of the best links between the medial temporal lobe and memory was systematically documented in a patient, H.M. by Brenda Milner (1972). H.M. suffered from severe anterograde and retrograde amnesia following bilateral surgical removal of the medial temporal lobe. Extensive testing showed that H.M. was impaired on a broad range of memory functions, including recognition of previously presented words or figures, free recall of noun pairs, and memory for the position of objects. H.M. case and further works on the hippocampus suggested that this structure is responsible for many of the mnemonic operations of the medial temporal lobe (Squire et al., 2004; DeFelipe et al., 2007).

2.2 The hippocampus: Structure.

The term hippocampus (and sometimes the hippocampal formation) generally applies to the dentate gyrus (DG; fascia dentata), the Cornu Ammonis areas CA1-CA3 (and also CA4, which is frequently called the hilus and considered part of the DG), and the subiculum (Hendelman, 2006) (Figure 1).

The DG contains a layer of densely packed cells that is called the granule cell layer (GCL) and these cells serve as the primary excitatory neurons in the dentate gyrus (Jacobs et al., 2000). These cells have extensions into the CA3 region and due to their mossy appearance, are referred to as mossy fibers (MF; Isaacson 1982). Bordering the GCL is the subgranular zone (SGZ), which contain progenitor cells– it is these cells that are capable of producing the new neurons in the DG (Jacobs et al., 2000). The CA1, CA2, CA3 as well as CA4 fields make up the hippocampus proper. These areas of the hippocampus are primarily comprised of pyramidal cells. Information flow through the hippocampus proceeds from the DG to CA3 to CA1 to the subiculum. CA2 and CA4 in the hippocampus are anatomically very small areas and their functions are not fully investigated.

The perforant path is the major input to the hippocampus. The perforant path brings information primarily from entorhinal cortex (EC). The axons of the perforant path arise principally in layers II and III of the entorhinal cortex, with minor contributions from the deeper layers IV and V. Axons from layers II/IV project to the granule cells of the DG and pyramidal cells of the area CA3 (and to mossy cells, located in the hilus of the dentate gyrus), while those from layers III/V project to the pyramidal cells of the CA1 and the subiculum. Area CA3 combines this input from layers II/IV with signals from EC layer II and sends extensive connections within the area and also has extensive connections with area CA1 through a collection of fibers called the Schaffer collaterals. Area CA1, however, receives input from the CA3 subfield and EC layer III. CA1 projects to

the subiculum, EC as well as sending information along the aforementioned output paths of the hippocampus. The subiculum is the final stage in the pathway, combining information from the CA1 projection and EC layer III to also send information along the output pathways of the hippocampus. In the hippocampus, the main output pathways are the cingulum bundle and the fimbria/fornix, which arise from area CA1 and the subiculum. As noted previously, the DG granule cells give rise to the mossy fibers (MF).

The MF pathway extends from the DG to CA3 pyramidal cells, forming their major input. MF synapses on CA neurons are large aggregations of termini, with multiple transmitter release sites and post-synaptic densities. Multiple granule cells can synapse onto a single CA3 pyramidal cell. There are numerous subcortical inputs, mainly including the amygdala (specifically the anterior amygdaloid area, the basolateral nucleus, and the periamygdaloid cortex), the medial septum, and the thalamus (including the anterior nuclear complex, the laterodorsal nucleus, the paraventricular and parataenial nuclei, the nucleus reuniens, and the nucleus centralis medialis), the lateral preoptic and lateral hypothalamic areas, the ventral tegmental area, the raphe nuclei and the locus coeruleus. It is generally accepted that each of these areas has a distinctive functional role in information processing, but to date the specific contribution of each area in the hippocampal formation is poorly understood. (For an extensive review of the hippocampal anatomy we refer the reader to articles by Moser & Moser, 1998 and Ekstrom et al., 2003).

2.3 The hippocampus: Functions and theories.

The important question that “what does the hippocampus do?” has been asked many times in the past, most often in terms of how the hippocampus is involved in learning and memory. However, there is no consensus on a satisfactory answer yet and the nature of this involvement remains a matter of debate. The theoretical positions regarding the hippocampal involvement in memory differ principally in the nature of the information for which the hippocampus is essential. An important theory holds that the hippocampus is preferentially involved in the processing of and memory for one type of information, specifically, spatial information (O'Keefe & Nadel, 1978). Alternatively, it was suggested that the structure is more involved in particular learning and/or memory processes that are nonspecific for information type, for instance, working memory (Olton et al., 1979), configural learning (Sutherland and Rudy, 1989), relational memory (Cohen and Eichenbaum, 1993) and path-integration (McNaughton et al., 1996; Samsonovich & McNaughton, 1997; Whishaw et al., 1997). Several problems such as the lack of consensus on the exact area of the hippocampus, diversity of the surgical procedures, and behavioural methods have been considered to be the main sources of the existing inconsistency on the hippocampal involvement in the learning and/or memory functions (Jarrard, 1993, 1995).

The hippocampus and spatial memory. The discovery of hippocampal place cells, neurons that fire rapid bursts of action potentials when the rat enters a specific small region of an environment (O'Keefe and Dostrovsky, 1971),

prompted a theory that the hippocampus creates and stores cognitive maps and that its primary function is to support spatial memory (O'Keefe and Nadel, 1978). Cognitive map theory, basically, posits that the hippocampus constructs and stores allocentric (i.e. the representations of space based on topographical relations among places in environments) maps of the external world from the sensory inputs that it receives and the information from movements. O'Keefe and Nadel (1978) suggested that spatial behaviour is supported by two systems that are known as *taxon* and *locale* systems. While the taxon system is involved in associative or conditioning learning and performs its functions independently of the hippocampus, the locale system, or the province of the hippocampus produces a map-like representation of the external environment. The spatial map in the hippocampus may contain some information about the places, directions and the distances between them. Research over the past thirty-five years has confirmed that, at least, the rat hippocampus has an important role in spatial memory and navigation; however, characterizing the functions of the hippocampus for the cognitive map is a matter of controversy. Spatial memory and spatial-related tasks will be discussed in more detail later in Experiment 4.

The hippocampus and working memory. It has been suggested that the hippocampus involves in working memory that refers to the processes used for temporarily storing and manipulating information. In his original studies using the radial arm maze (RAM), Olton et al., (1977, 1979) showed that rats with hippocampal damage were not able to retain recently acquired information concerning arms already explored. Thus, working memory might be one aspect

of hippocampal function. This explains why animals with damage in the hippocampus could avoid going down arms that never contained food but still not remember which arms they had recently visited. Presumably, after training, the information about arms with-no-food was saved as long-term memory, but working memory was still required to avoid the arms where food had already been retrieved. The position on the hippocampus as a site for working memory later has been challenged by several investigations in which patients with hippocampal sclerosis did not show any problems with working memory (Cowey and Green, 1996).

The hippocampus and configural learning. Based on (but beyond) the elemental association learning principles, the configural association theory (Sutherland and Rudy, 1989; Rudy and Sutherland, 1995) proposes that the hippocampus plays a role in the long-term retention of associations that are based on the interrelations among cues (in the retention of the behavioural significance of combinations of stimuli but not of individual stimuli). According to this theory, relational or configural associations are required to solve learning problems where the reinforcement contingencies are essentially defined by some temporal, sequential, or spatial relationship between two or more stimuli. By contrast, simple associations alone are sufficient to solve problems where the reinforcement contingencies are defined by fixed, unambiguous relationships between individual stimulus elements and their reinforcement outcomes. Therefore, spatial or non-spatial problems in nature are typically solved by using a combination of two or more cues or features. Using this conjoining, the

hippocampus forms the configurational representation. There is substantial support for this theory (Davidson and Jarrard, 1992; Rickard et al., 2006); however, some failures have been reported to disrupt the performance of non-spatial configural tasks by hippocampal damage (Whishaw and Tomie, 1995).

The hippocampus and relational memory. The idea of relational memory (Cohen and Eichenbaum, 1993; Eichenbaum, 2004) is, basically, that highly processed sensory information comes into the hippocampus and nearby cortex, and memories are formed in a manner that links all the things happening at the same time. The relational theory also suggests that the hippocampus processes all manner of associations and sequences of events that comprise our daily lives, linking these into relational frameworks and making a declarative memory. Therefore, interconnectedness is a central feature of relational memory storage in order to make declarative memory that involves in conscious recollections of information about events. In general, while the cognitive map theory holds that the hippocampus preferentially processes the spatial relationships between locations in the environment, the relational theory posits that the hippocampus does not differentiate between spatial and non-spatial information but is instead specialized for relational processing.

The hippocampus and path integration. Specifically, the path integration theory (McNaughton et al., 1996; Whishaw et al., 1997) posits that the hippocampus is essential for path integration – the calculation of current location, past locations and future locations from one's own movements, and is not necessary for other forms of memory or spatial behaviour. The hippocampal

involvement in path integration is shown by an experiment in which rats are required to find food in a featureless environment. Once a rat has found the food, it takes it in a straight line back to its cage where it consumes it. The question of how the rat returns in a straight line to its home cage may characterize the role of the hippocampus in path integration. Although the rat may navigate by using the allocentric cues (e.g. pictures, doors, lights) in the room, it can also use egocentric cues (self-motion information), because even a blindfolded rat still runs straight to its home box with its newly found food morsel. Clearly, this blindfolded rat must have performed complex path integration as it moved about foraging, and from this it calculated the angle and length of the straight path back to its starting point. The key point in this context is that rats with hippocampal damage are incapable of returning by path integration, although they are able to make an accurate straight-line return journey by using allocentric cues (McDonald and White, 1995). This confirms that the hippocampus plays an important role in encoding relative spatial location, without reference to allocentric cues, by the integration of linear and angular self-motion. Importantly, these data do not rule out the possibility that it performs other functions.

Therefore, there is converging evidence for hippocampal participation in learning and/or memory. Furthermore, despite a general agreement on the hippocampal involvement in the formation of new memories about experienced events, different theories posit different functions for this structure. Meanwhile, the finding that the hippocampus has an essential role in spatial navigation is largely agreed upon.

2.4 The hippocampus: A navigational system.

Most animals must make intentional, planned movements to succeed in life. In nature, an animal such as the rat may have a safe place for pups and separate spots to search for food. In addition to information on a specific direction or pathway, all available sensory information and the distance to the target location determine the navigation strategy. In searching for food or running away from a predator, an internal or map-like representation of the surrounding environment formed from the previous experiences can be combined with available exteroceptive information to locate the way to the target or to a safe point. All sensory systems involved in a goal-directed movement give the brain information to find the best path from one spot to another. In particular, this information is the key source of spatial representations that allow the animal to engage in flexible navigation to a goal location from familiar or novel positions with equal capacity (Tolman, 1948). In this context and from an anatomical view, the hippocampus has been specifically reported to be specialized for all kinds of topographical representations or navigational computations, computations that allow the animals to solve difficult spatial problems in a given situation (O'keefe and Nadel, 1978; see also Muller et al., 1999 for review). Both the place cell discovery (O'Keefe and Dostrovsky, 1971; Muller and Stead, 1996) and functional measures in hippocampal lesion studies (Morris et al., 1982; Sutherland et al., 1982) demonstrate that this structure is intimately involved in spatial learning and/or memory.

In animal experiments at the functional level, the hippocampus-dependent spatial memory has been mostly measured by the Morris water task (MWT; Morris, 1984). The MWT represents a wet land that requires animals to locate a hidden platform employing the topographical relationships between visual, distal cues. For this aversively motivated task, a white or black pool is filled with water that is made opaque using dried milk or non-toxic paint. In order to measure spatial function in MWT, a platform, which is the goal, is positioned at a fixed location in the pool and rests just below the surface of the water so that it is not visible (hidden platform version). The animal is typically released from the side of the pool and searches for the hidden platform to allow it to escape from the cool water. With continued experience in the pool, the animal becomes very efficient at locating the hidden platform. Latency (time to find the hidden platform), path length, path speed, and probe performance are the most common measures of spatial performance reported in the MWT.

Additionally, it has been reported recently that rats with hippocampal damage show significant spatial deficits in the ziggurat task (ZT; Faraji et al., 2008), a dry-land task. The task consists of an open field containing a grid of 16 identical ziggurats (pyramid shaped towers) arranged at equal distances. One of the ziggurats is baited with a food reward. The task requires rats to navigate through the open field using a combination of distal and/or proximal cues in order to locate the food reward. The ability to acquire and recall the location of the goal (baited) ziggurat can be tested in consecutive training sessions. In the standard version of the ZT for measuring the spatial performance, the location of the goal

ziggurat must be changed every second day, requiring the rats to learn and/or remember the new locations.

Many kinds of hippocampal disruption in animal studies, including lesions (Sutherland et al., 1982; Gallagher and Holland 1992), aging (Deupree et al., 1991; Driscoll et al., 2005) and stroke (Driscoll et al., 2007; McDonald et al., 2008) have been reported to disrupt spatial performance.

2.5 The hippocampus: Neurogenesis.

The nature of hippocampal neurogenesis, its neuro-hormonal correlates and possible functional significance have lead us to look at neurogenesis here in a closer view. The adult brain neurogenesis story is not an entirely new one, but rather the re-opening of an old chapter, finding new meaning and new potential for a phenomenon that was largely ignored for many years. In most areas of the mammalian brain, the generation of neurons has classically been thought of mainly limited to the prenatal or neonatal periods. However, studies have shown that in the DG of several species, from birds to humans (Kaplan and Hinds, 1977; Barnea and Nottebohm, 1994; Kuhn et al., 1996; Kornack and Rakic, 1999; Eriksson et al., 1998) granule cells are produced postnatally. In fact, Altman and Das (1965) were the first to report neuron production in the DG of adult brains. Over a period of weeks these new cells are incorporated into the granule cell layer and had the morphology of mature granule cells. Since this early report, many investigations have provided further support that these new cells become neurons. Specifically, the newborn granule cells are capable of extending axonal

projections along the mossy fiber tract to their natural target area, the hippocampal CA3 region, and show all the ultrastructural and neurochemical features of neurons (Kuhn et al., 1996; van Praag et al., 2002). Despite strong evidence supporting the hippocamapal neurogenesis idea, the biological significance of adult neurogenesis still remains uncertain.

Overall, as a plastic structure in the medial temporal lobe, the hippocampus is intimately involved in several types of, mostly, spatial function. The newly generated neurons in adulthood may have a function in both cognition and brain repair. For example, new neurons are generated in the hippocampus after stroke and seizures, and in the cortex after a selective damage, suggesting that they may be involved in structural and functional recovery from ischemic injury. In the next section, we will briefly review the nature of stroke and the possible role of neurogenesis in the post-ischemic recovery in the brain.

3. Stroke

3.1 Stroke: An overview.

Stroke, is defined as loss or change in function resulting from an insufficient supply of blood to part of the brain or the rupture of blood vessels in the brain (Dirnagl et al., 1999). Stroke is the most common cause of adult disability in Canada and the United States (Health Canada, 2000; American Heart Association, 2003), and tragically there are few effective treatments that improve outcome. It has been estimated that stroke is the third leading cause of death in the Western world, after heart disease and cancer (Feigin, 2005; Yi et al., 2007)

and causes 10% of deaths worldwide (World Health Organization, 2004). The effects of a stroke mostly depend on where the brain was injured, as well as how much damage occurred.

In humans, stroke is a diverse neuropathological condition in terms of causes, manifestations, and anatomic sites of ischemia. Three types of stroke are generally reported in clinical patients: ischemic, hemorrhagic stroke or intracerebral hemorrhage, and subarachnoid hemorrhage (Graham et al., 2004). All these conditions exhibit different pathophysiologies and likey will require different therapeutic approaches. The most common type of stroke is ischemic stroke, accounting for 70-80% of cases (www.heartandstroke.ca. Accessed14/04/08). A clot or other blockage (fatty materials, calcium and scar tissue) in an artery leading to the brain generally causes ischemic stroke. Blood clots or vascular stenosis from plaque formation are common causes of arterial blockage. The arterial obstruction leads to local loss of oxygen and nutrients, resulting in dysfunction and necrosis of the brain tissue in that area. Hemorrhagic stroke, however, is the accumulation of blood anywhere within the skull vault. About 15-20% of strokes are hemorrhagic (http://www.nlm.nih.gov. Accessed14/04/08). Hemorrhage within or around the brain can also cause decreased blood flow and disruption of the blood-brain barrier that protects normal chemical balance in brain tissue, again leading to cell death. Hemorrhage can be secondary to trauma, congenital malformation, aneurysm, or disease. Subarachnoid hemorrhage also is a type of stroke caused by sudden rupture of an artery, but it differs from intracerebral hemorrhage in that location of

the rupture leads to blood filling the space surrounding the brain rather than inside of it. About 3% of clinical stroke cases fall into this category (Graham et al., 2004).

Because all these three types of stroke, particularly ischemic stroke, have a complex pathophysiology involving the interaction of many different cells and tissues such as neurons, glia, endothelium, and the immune system, these events are very difficult to mimic satisfactorily *in vitro*. Therefore, a large portion of stroke research is mostly conducted on animals such as rats.

3.2 Stroke: Animal models and inducing stroke in rats.

The specific goal in modeling human neurological conditions such as stroke in animals is the study of basic processes or potential therapeutic interventions in these diseases. Aside from that, animal models offer the opportunity to extend pathophysiological knowledge and to improve medical treatment of stroke in humans. This does not seem an easy task, because the same type of stroke may have different physiological manifestations across different species (Kleim et al., 2007). For this reason, a key to successfully modeling human neurological symptoms, such as those associated with stroke may be to first identify functional rather than physical similarities in neurological impairments. Furthermore, the complicated nature of the brain's function and its response to an injury are the major obstacles to evaluate cerebral ischemia and its physical consequences by merely *in vitro* systems. Preclinical and translational investigations into the causes, pathogenesis, and therapeutic intervention of stroke, therefore, employ

animal models in addition to other *in vitro* techniques and models (Graham et al., 2004).

Several animal models have been developed in rodents such as rats for the study of ischemic and hemorrhagic strokes. The advantages of using rats for stroke studies include the similarity of its intracranial circulation to that of man, the abundant neurochemical data derived from rat brain, and the relatively low animal cost which is important for large scale studies for statistical analysis (Chen et al., 1985; Buchan et al., 1992). In addition, rat models enable investigators the use of more complex experimental designs to examine neurochemical events such as time course of recovery and dose-response relationships that are not possible in non-human primate experiments (Kleim et al., 2007).

A number of models in rats are currently known to produce cerebral ischemia (see Hossman, 1998 for review; Beech et al., 2001). In addition to global ischemia models, either complete (e.g. decapitation and aorta/vena cava occlusion) or incomplete (e.g. hemorrhage or hypotension and hypoxic ischemia), most investigations in modeling stroke have been performed by inducing focal ischemia. Although global ischemia models, both complete and incomplete, tend to be easier to perform, they are less relevant to human stroke than the focal stroke models, because global ischemia is not a common feature of human stroke. The major models for focal ischemia are divided into techniques including middle cerebral artery occlusion (MCAO), photothrombosis, devascularization, and the injection of endothelin-1 (ET-1). Given the high

amount of the middle cerebral artery (MCA) in rats (Kleim et al., 2004), occlusion of the MCA is widely employed for inducing transient or permanent stroke in rats (Koizumi et al., 1986; Corbett et al., 2000; Colbourne et al., 2000; Wang et al., 2007). The procedure to induce MCAO involves restricting, temporarily or permanently, MCA blood flow to the brain sites such as cortex and striatum. Several factors including the rat strain and age, and the method, duration and location of MCAO may affect the subsequent lesion size, location and the level of impairment (Duverger and MacKenzie, 1988). Photothrombosis, however, involves the exposure of a vessel to appropriately filtered light in the presence of a potent photosensitizing dye (e.g. Rose-Bengal or photofrin) and inducing photo-oxidation by irradiating specific areas of tissue. The process of photochemically induced platelet aggregation was defined as photothrombosis (Watson et al., 1985). Because this model may induce very focal ischemic infractions with minimal surgical invasion, some studies recently showed that photothrombosis may provide a means for researchers to safely, easily and noninvasively induce reproducible ischemic lesions in specified regions of the neonatal cortex (Maxwell and Dyck, 2005). Devascularization, as an alternative animal model for focal ischemia, exhibits a progression of events consistent with the development of a delayed ischemia-like lesion. After cortical devascularization, either via electrocoagulation of surface vessels (Kleim et al., 2003) or by pial stripping (Whishaw 2000; Kirkland et al., 2008) blood flow is permanently interrupted resulting in areas of permanent focal ischemia whose volume is dependent upon the size of the vessel damaged (Herrera and Cuello,

1992; Bartnik et al., 2001). Specifically, pial devascularization results in the formation of focal damage consistent with that seen as a result of surface contusions or lacerations due to contact head injuries (Bartnik et al., 2005). However, producing mechanical damage in the underlying tissue and hemorrhagia have been suggested to be the main limitations of cortical devascularization (Kleim et al., 2007).

Focal ischemia can be also produced by ET-1 injected directly into brain tissue. ET-1 is a potent and long-acting vasoconstricting peptide (Yangisawa et al., 1988) that may play a role in the pathophysiology of a number of diseases. In modeling animal stroke, the injection of ET-1 into the target area reduces local blood flow to produce ischemic damage (Karhunen et al., 2006; Fuex et al., 1997). In addition to ischemic consequences, focal injection of ET-1 has been reported to induce ischemia-induced seizures in immature rats (Tsenov et al., 2007). There are a number of studies that have used the ET-1 model for focal ischemic damage in the forelimb motor region of the cortex (Windle et al., 2006), the hippocampus (Mateffyova et al., 2006; Driscoll et al., 2007; Tsenov et al., 2007; McDonald et al., 2008; Figure 2), sensorimotor cortex (SMC; Adkins-Muir and Jones 2003; Allred and Jones 2004), striatum (Ottani et al., 2003; Peeling et al., 2006), and white and grey matter (Hughes et al., 2003). Stereotaxic injection of ET-1 is a less invasive procedure compared to methods that expose an artery or introduce a suture into the lumen, yet it produces a pattern of ischemic damage similar to the more traditional stroke models (Sharkey et al., 1993).

The ET-1 model has several advantages. For instance, it has been suggested that stereotaxic injection of ET-1 can be delivered to conscious animals (Moyanova et al., 2003; Sharkey et al., 1993) and yields similar results to other focal ischemia models when investigating neuroprotective drugs (Sharkey et al., 1994). Furthermore, in the ET-1 stroke model, blood flow reduction is rapid, but not immediate (Macrae et al., 1993) and reperfusion occurs over several hours (Biernaskie et al., 2001; Macrae et al., 1993). This profile may be more representative of human stroke than the immediate reduction and reperfusion seen with the other animal models of ischemic stroke. Finally, intracerebral injection of ET-1 produces a localized and dose-dependent ischemic lesion with minimal edema (Windle et al., 2006).

3.3 Stroke: Post-ischemic recovery.

Many studies now confirm that new neurons are continuously formed in adult brains in many species including primates and humans (Barnea and Nottebohm, 1994; Kuhn et al., 1996; see also Wiltrout et al., 2007 for review). Moreover, studies have shown that traumatic and ischemic damage to the adult brain stimulate neurogenesis (Parent et al., 1997; Gu et al., 2000; Dempsey et al., 2003; Ernst and Christie, 2006). Accordingly, much recent attention has focused on the connection between neurogensis and post-ischemic structural recovery, increasing hope for functional improvement after a CNS insult.

Studies on neurogenesis following ischemia have shown that acute brain insults such as oxidative damage, seizures, traumatic injury, global and focal

ischemia significantly increase the progenitor cell proliferation in the adult brain specifically in DG (Dempsey et al., 2003; Yan et al., 2006; Rola et al., 2006). Even damaging the hippocampus indirectly by removing its afferent input from the enthorhinal cortex stimulates neurogenesis (Gama Sosa et al., 2004). This could also occur outside of the hippocampus. For instance, occlusion of the middle cerebral artery in rats not only enhances neurogenesis in the hippocampus, but also induces neural progenitors in the subventricular zone to migrate to the cerebral cortex and striatum (Jin et al., 2003). In addition, another interesting phenomenon in the context of post-ischemic recovery refers to the self-protective and remodeling dynamics in the brain by which the newly proliferated cells immigrate to the damaged regions of the brain, particularly following cerebral ischemia (Magavi et al., 2000; Arvidsson et al., 2002; Hicks et al., 2007). Whether such structural recovery or reorganizational dynamics result in actual functional improvement is still unclear, but the connection between neurogenesis and post-ischemic recovery as well as the potential neurobiological mechanisms are now a matter of intense study.

Major regenerative processes following global and focal ischemia in the CNS have been reported in the hippocampus. Generally, global ischemia causes an almost complete neuronal loss in the CA1 by 3-7 days after the insult (Imai et al., 2007). This can also occur after focal ischemia (Wang et al., 2004). However, following either global or focal ischemic insults, progenitor proliferation in the subgranular zone (SGZ), subventricular zone (SVZ), as well as DG was shown to be enhanced in rats (Zhang et al., 2001), mouse (Tureyen et al., 2004) and

monkeys (Koketsu et al., 2006). After global ischemia most studies showed an increased progenitor proliferation starting at 3–4 days, peaking at 7–10 days and returning to control levels by 3–5 weeks after global ischemia (Liu et al., 1998; Yagita et al., 2001; Iwai et al., 2002; Tonchev et al., 2003). In contrast, the profile of enhanced proliferation of progenitor cells after focal ischemia shows that it starts bilaterally in both SVZ and DG as early as 2 days, peaks at 1–2 weeks and returns to the base level by 3–4 weeks of reperfusion (Wiltrout et al., 2007). Post-ischemic neurogenesis may be even observed in the cerebral cortex following focal ischemia (Gu et al., 2000; Jiang et al., 2001).

3.4 Stroke: Neural bases of recovery.

It has been previously shown that CNS insults such as focal ischemia significantly enhance the progenitor cell proliferation in the adult brain (Gould et al., 1997). The newly proliferated progenitor cells, for instance, in the hippocampal sub-areas including DG will survive for at least 2-3 weeks after focal ischemia, but disappear (either die or migrate) within a week in the SVZ (Dempsey et al., 2003). In the first week after ischemia, these cells express doublecortin (DCX), a microtubule-associated phosphoprotein that has been utilized as a marker of newly born neurons and migrating neuronal phenotypes in the DG (Dempsey et al., 2003). In the DG, they either perish or migrate into the GCL. Most of the surviving newly formed DG cells differentiate into neuronal nuclei (NeuN)-positive [a mature neuronal marker] or calbindin-positive [a dentate granule cell marker] mature neurons by 3–4 weeks after ischemia

(Komitova et al., 2002; Jin et al., 2003; see also Wiltrout et al., 2007). Furthermore, in a well-designed study, Tanaka et al., (2004) showed that almost all enhanced green fluorescent protein-positive dividing cells after transient global ischemia in gerbils as marker for neurogenesis were found in the SGL. These cells proliferated and migrated to the GCL, expressing the developing neuronal markers polysialic acid and DCX, and differentiated to NeuN–positive or calbindin-positive mature granule cells at 30 days after transient global ischemia or sham-operation. The number of green fluorescent protein-positive cells in the GCL was significantly higher in the ischemic animals at 30 days than in sham-operated group (Tanaka et al., 2004).

These studies, however, do not draw a clear profile of full function of neurogenesis, and therefore further analysis is required to determine the mechanisms and functional roles of neurogenesis in the brain repair after stroke. The existence of enhanced neurogenesis in the adult hippocampus (e.g. dentate gyrus) after ischemic insults, either global or focal, may provide clues on the function of neurogenesis in the adult brain, and also offer hope that following brain injuries more plasticity exists in adult brain than might have been previously imagined. This capacity may reestablish functional connections between brain areas, structural reorganizations, increase functional capacities after stroke event.

3.5 Stroke: Factors that affect brain repair after ischemic insults.

In the process of post-ischemic recovery, neurogenesis can be basically

controlled at several major steps—precursor proliferation, migration, differentiation into neural phenotypes, integration and survival (Iwai et al., 2002; Parent, 2003). Many studies, however, focused on the first event, the neural progenitor proliferation after ischemia (Wiltrout et al., 2007). Normally, neurogenesis in the brain (e.g. in dentate granular layer) appears to be affected, at least in part, by factors such as aging, environmental interventions, exercise, genetic background, and stress (see Gage et al., 1998, for review). As mentioned above, accumulating evidence suggests that ischemic insults in the brain potently induce neurogenesis in proliferative areas of the adult brain. Although ischemia-induced neurogenesis may be regulated differently than neurogenesis in the non-ischemic brain, a rapidly increasing number of experiments are addressing this question that what factors affect neurogensis observed in post-ischemic situations in the brain.

Briefly, neurotrophic factors are the first candidates for mediating ischemia-induced neurogenesis (Parent, 2003). Of the various growth factors, epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF-2) are reported to play a key role in neurogenesis *in vitro* (Endoh et al., 1994; Kuhn et al., 1997; Yoshimura et al., 2001). Furthermore, it has been recently shown that adenoviral vector construct that produced constitutively expressed FGF-2 over longer time periods enhances neurogenesis at 3 months after ischemia in rats. This indicates that FGF-2 could play a significant role in long-term neural repair (Leker et al., 2007). Interestingly, increased progenitor proliferation in rats infused with FGF-2 and EGF is associated with enhanced post-ischemic memory formation and

retention (Nakatomi et al., 2002). Brain-derived neurotrophic factor (BDNF) is the second candidate for modulating ischemia-related neurogenesis as generate new neurons in the SVZ-olfactory bulb pathway (Kuhn et al., 1997; Benraiss et al., 2001), in the hippocampus (Takami et al., 1992), and in DG cells and CA1 and CA3 pyramidal neurons (Kokaia et al., 1995). Insulin-like growth factor (IGF-1) has been reported to have a significant role in neurogenesis after focal ischemia (Gluckman et al., 1992). But the most important issue about IGF-1-induced neurogenesis after focal ischemia is that IGF-1 promotes the survival of the newly formed cells so that other growth factors could induce the proliferation (Wiltrout et al., 2007). Other factors that have been shown to influence post-ischemic structural repair include nitric oxide (NO; Zhu et al., 2003; Sehara et al., 2006), dopamine (Borta and Hoglinger, 2007), hormones like estradiol (Suzuki et al., 2007), and experience of an enriched environment (Komitova et al., 2005).

Finally, it has been hypothesized that adrenal steroids (e.g. corticosterone) might result in neuroprotective consequences in some brain structures such as the hippocampus due to their anti-inflammatory and immunosuppressive effects (Quirarte et al., 1997; Grundy et al., 2000; Abraham et al., 2000; Melcangi et al., 2000; see also Molteni et al., 2001, for review). Three lines of evidence support the hypothesis of corticosterone-induced structural protection or repair in the brain following ischemic insults. First, although the increased expression of neurotrophins in the brain, particularly in the hippocampus, is an adaptive reaction for structural reconstruction following acute brain insults, their restorative functions are strongly regulated by glucocorticoids (Sun et al., 1993; Hansson et

al., 2000; Grundy et al., 2000; Grundy et al., 2001). For instance, Hansson et al., (2001) showed that bFGF gene expression in the hippocampus is positively modulated by GR actions on neurotrophic factor signaling. This clearly provides evidence for the active role of glucocorticoids in the restorative functions of neurotrophins particularly in the hippocampus after stroke. The second line in this context, however, is based on the findings that physiological concentration of glucocorticoids is crucial for neuronal survival as adrenalectomy (ADX) causes degeneration of hippocampal cells in SGC layers in the DG (Sloviter et al., 1989). Furthermore, because focal ischemia is known to induce chronic inflammation in adult rodent brain which inhibits neurogenesis (Ekdahl et al., 2003; Monje et al., 2003) and since corticosteroids are steroids endowed with powerful anti-edema and anti-inflammatory properties (Harbuz, 2002; Liu et al., 2007), the third hypothesis, therefore, has focused on the anti-inflammatory effect of corticosterone. Inflammation, basically, is the complex biochemical response of vascular tissues to harmful stimuli, such as pathogens and damaged cells. Because stroke causes necrosis, a type of unnatural death of cells, inflammation begines with cell swelling and disruption of the plasma membranes and organelle membranes. The release of intracellular content after plasma membrane rupture is the cause of inflammation in stroke-induced necrosis (Filbin, 2006).

In a well-documented mechanism, and influenced by the immune system specifically by cytokines after inflammation, the limbic-hypothalamo-pituitary-adrenal (LHPA) axis releases corticosterone. The negative feedback control of glucocorticoids is required for both CRH and neurotensin (NT) production in the

paraventricular nucleus (PVN) of the hypothalamus during inflammation (Loum-Ribot et al., 2006). The involvement of glutamate excitotoxicity in the neurodegeneration-enhancing actions of NT was recently analyzed by Antonelli et al., (2007). On the other hand, the complement system (a biochemical cascade in immune system which helps to clear pathogens from an organism) plays a significant role in post-ischemic inflammatory reactions. This system was recently shown to be a promoter of neurogenesis (Wiltrout et al., 2007) influenced by glucocorticoids (Nakano et al., 1986; Francis et al., 2003).

Taken together, little is known about the mechanisms of the contribution of neurotrophic factors and anti-inflammatory systems in the neuroprotective effects by corticosterone. However, there are some reasons to believe that glucocorticoids, in an intense and complicated biological dialogue with immune and other neuronal agents, may have an influential effect on post-ischemic recovery in the adult brain. For example, NO that increases infarct size and decreases neurogenesis after focal cerebral ischemia depending upon which isoform of NO synthase is the source of NO (Sun et al., 2005), is inhibited by glucocorticoids (Zhu et al., 2007). While there are some appropriate functional reasons for the contribution of glucocorticoids in post-ischemic recovery, the significance of the hypothesis of corticosterone-induced brain repair or protection after focal ischemia still needs to be more investigated. Corticosterone-related and stressful experiences are the target plans for future investigations on this challenging hypothesis.

4. Stress and Stress Hormones

4.1 Stress: Background and theoretical viewpoints.

The concept of stress has diverse applications. It is frequently associated with concepts of a psychological nature such as suffering, tension, distress, emotionality, fearfulness, and anxiety. These terms are mostly applied interdependently and sometimes as referring to the same phenomena, but their interpretation is not widely standardized. Generally, investigations on stress are challenged by the lack of direct measures. Because most of the stress-related concepts involve a subjective component, studies are obliged to infer such psychological states from their objective and measurable manifestations (physiology, endocrinology, behaviour, etc). Consequently, different research groups develop their studies on the basis of a number of assumptions that are often divergent and sometimes arbitrary. For instance, a given behavioural measure (e.g. exploration in novel environments) can lead to different interpretations regarding its psychological significance, depending on who uses it and in what context it is being used (Ramos and Mormede, 1997).

The term “stress“ has been traditionally defined as the nonspecific (or common) result of any demand upon the body (Selye, 1982). At the time that Selye formulated his theory (Selye, 1936), stress was thought to have an exclusively endocrine face. Influenced by his theoretical approach, noxious stimuli of physical or chemical nature were the primary stressors. Additionally, Selye was the first person who recognized the dual nature of stress. In the short

term, a stress state produces adaptive changes that help the animal respond to the stressor (e.g. mobilization of energy resources, inhibition of inflammation, and resistance to infection); in the long term, however, it produces changes that are maladaptive (e.g. enlarged adrenal glands due to overstimulation from the hypothalamus). He also found that animals and people, in reacting to stressors (*demands* in his definition), go through three stages, known as “General Adaptation Syndrome” or GAS (Selye, 1956). Briefly, during the first stage, the *alarm reaction*, the body prepares to cope with the stressor by increasing activity in the sympathetic nervous system and adrenal glands. Selye defined the alarm reaction as " the sum of all non-specific phenomena elicited by sudden exposure to stimuli to which the organism is quantitatively or qualitatively not adapted" (1946, p. 119). The nature of these stimuli or stressors is unimportant. He assumed an equivalent response to physical and psychological stimuli. The alarm stage is followed by the second stage. This is called *resistance* that involves coping, and attempts to reverse the effects of the alarm stage. The third stage is called *exhaustion*, which will be reached when the individual had been repeatedly exposed to the stressful situation and was incapable of showing further resistance. Although the GAS theoretical model was one of the first attempts to describe the reactions of organisms to the internal and external world (Bennett, 2000), Selye clearly regarded the individual as automatically responding to an external stressor by his model, and included only a minor role for psychological factors.

Selye’s theory of nonspecificity of stressors was later questioned by some

investigations that showed the biological consequences of stress are mainly dependent upon the cognitive/emotional involvement of the organism; stressors do not necessarily activate the neuroanatomical systems of stress if the animal does not perceive the situation as stressful (Mason, 1968, 1971). In this view, therefore, there is no unique physiological state that is stress specific. In other words, psychological processes are strong determinants of activation of the endocrine and sympathetic nervous systems. In human studies, an interesting question is why people may experience the same number and magnitude of events and yet have very different physiological and health outcomes. In partial answer this question, Lazarus and others (Lazarus & Folkman, 1984; Lazarus, 1993) suggested that different stressors can produce different responses in the same person because the person *interprets* the two stressors differently, a process called *cognitive appraisal*. Briefly, according to Lazarus, the cognitive appraisal involves two stages. The first stage is *primary appraisal* in which the individual initially appraises the event itself. Here, there are three possible ways that the event can be appraised: (*a*) irrelevant, (*b*) benign and positive, and (*c*) harmful and negative. The second stage is known as *secondary appraisal*. During this stage, the individual evaluates his or her personal and social resources that are available to manage a stressful circumstance and considers the action options that are needed. Generally, primary appraisal involves an appraisal of the outside world, and secondary appraisal involves an appraisal of the individuals themselves. Therefore, the cognitive appraisal theory predicts that the stress response per se is not harmful or pathological in itself but only when

an organism perceives a stressor as a harmful event and beyond his or her current management capabilities, the negative outcome(s) of stress will occur.

4.2 Stress: A working definition.

The most important issue resulting from the Selye's neuroendocrine perspective and Lazarus's cognitive approach is that they show how difficult it is to provide a universal definition of stress with operationally defined terms that can be applied equally to rodents and people. Clearly, the human stress response is influenced by a multitude of personality characteristics and life experiences that cannot be duplicated in animal investigations (Anisman and Merali, 1999). The Selye and Lazarus points of view, therefore, show that a definition must address: (1) that stress is not determined by the physical parameters of an environment, but by how an organism perceives and reacts to the stimulus, and (2) there is no distinctive physiological reaction that is stress specific. Furthermore, in recent years, there is a consistent finding across species showing that stressor controllability (Kim and Diamond, 2002) and the context of stressful experiences (de Kloet et al., 1999) have a profound influence on the impact of possible aversive outcomes of stress on physiology and behaviour.

Therefore, in a multidimensional view, as some authors (Anisman and Merali 1999; Kim and Diamond 2002) proposed, stress is a state or condition in which an organism is aroused by an aversive situation or stimulus (stressor). The magnitude of stress and its physiological outcomes are greatly influenced by the

organism's perception of its ability to control the presence or intensity of the stimulation. In other words, such stimuli, which are evaluated by a cognitive/emotional system may induce a variety of biological and behavioural changes in an attempt to maximize the probability of success over a demand (Ramos and Mormede, 1998).

4.3 Stress: Biopsychology.

The most extensively studied physiological stress systems have been: (1) the limbic-hypothalamo-pituitary-adrenal (LHPA) axis which stimulates the adrenal cortex to release glucocorticoids such as cortisol (in humans) or corticosterone (in rodents) into the bloodstream (Mason 1968a), and (2) the sympathetic-adrenomedullary (SAM) axis that influences the stress reaction by two different pathways working in parallel. One pathway is activated by nerve endings that trigger the release of epinephrine (EPI) from the chromaffin cells in the adrenal medulla into the bloodstream. The other pathway comprises the sympathetic nerve endings that provide essentially every organ in the body with norepinephrine (NE; Mason, 1968b).

Generally, the integrity of the LHPA axis is a crucial factor for the negative feedback on stress hormones. According to the glucocorticoid cascade hypothesis (Sapolsky 1989), which is based on animal studies, cortisol (corticosterone in rat) overexposure damages sites of negative feedback, particularly in the hippocampus. Because the hippocampus is a key mediator of the negative feedback onto the LHPA axis, it has been suggested that damage to

the hippocampus impairs the inhibition of the LHPA axis, leading to progressively higher cortisol levels and further hippocampal destruction (Aldwin et al., 2007). It should be noted that the glucocorticoid cascade model of stress and hippocampal damage has been challenged by some stereological studies that failed to detect significant structural/neuronal damage following prolonged hypercortisolism resulting from either aging, stress or corticosteroid administration (Rapp and Gallagher, 1996; Vollmann-Honsdorf et al., 1997; Leverenz et al., 1999).

The effects of glucocorticoids within the hippocampal formation are mediated in part by two classes of corticosteroid receptors: type Ⅰ, mineralocorticoid receptors (MRs) and type Ⅱ, glucocorticoid receptors (GRs). These receptors functionally complement each other to maintain homeostasis (Fuchs and Flugge, 2003). MRs bind cortisol or cortiosterone with higher affinity than the GR (Reul et al., 1987), resulting in a widespread occupation of this receptor under basal corticosteroid levels. In contrast, the GRs are extensively occupied only during times of high concentrations of circulating glucocorticoids (e.g. under stress). For this reason, GRs have been thought to be the primary mediator of feedback inhibitory control by the hippocampus.

Stress states can have biological consequences via pathways other than the LHPA axis and/or MRs- and GRs-involved neuronal alterations. The SAM axis is part of the sympathetic nervous system and is responsible for initiating the fight-or-flight response. Whereas the LHPA axis is primarily an endocrine system (i.e. hormones travel through the blood-system), the SAM axis has a

neuroendocrinological face, consisting of both neural and endocrine tissues (Harley, 2007). In the SAM axis, as mentioned above, stress stimulates nerves that directly innervate the adrenal medulla, which in turn release NE and EPI into the bloodstream. Stress may also activate NE release as a neurotransmitter from the locus coeruleus (LC) in the brain (Ebner and Singewald, 2007). This has widespread effects, including direct interactions with CRH in the central nucleus of the amygdala. Like the LHPA axis, the SAM axis has immunomodulatory effects. Whereas the LHPA axis inhibits the inflammatory response, the SAM axis activates it (Madden and Livnat, 1991). As nervous impulses directly stimulate the adrenal medulla, the SAM axis has much faster and immediate effects than the slower-acting LHPA axis. Basic effects of SAM hormones (NE and EPI) include increased heart rate, blood pressure, metabolic rate, and alertness (Aldwin et al., 2007).

It has been suggested that different neuronal circuits are activated depending upon the type of stressor (Lopez et al., 1999; Fuchs and Flügge, 2003). Limbic circuits connections (e.g. the hippocampus, amygdala, and prefrontal cortex) are sensitive to stressors such as restraint, fear, or exposure to a novel environment. These stressors commonly stimulate—before the initiation/inhibition of the stress response—an intralimbic processing of multisensory information, and this processing is strongly dependent upon previous experience. In contrast, physiological threats such as exposure to ether result in an activation of efferent visceral pathways that are directly relayed to the PVN of the hypothalamus. In this case, the rapid activation of brainstem and

hypothalamus circumvents the cognitive processing in limbic and cortical areas. Herman and Cullinan (1997) postulated two generalized stress pathways, the *systemic* and the *processive* pathway in order to differentiate between limbic-sensitive and limbic-insensitive stressors in mind. According to their hypothesis, integration of the hypothalamo–pituitary–adrenal stress response occurs by way of interactions between stress-sensitive brain circuitry and neuroendocrine neurons of the PVN in the hypothalamus. Stressors involving an immediate physiologic threat (or *systemic stressors*) are relayed directly to the PVN, probably via brainstem catecholaminergic projections. In this view, respiratory, cardiovascular, or immune stimuli represent systemic stressors that require immediate reactions for survival but no further interpretation from higher-order brain structures. On the other hand, stressors requiring interpretation by higher brain structures (or *processive stressors*) appear to be channeled through limbic forebrain circuits. Therefore, multimodal stimuli such as those resulting from psychological challenges are regarded as processive stressors. They need cortical processing and, depending upon previous experience or ongoing activation, the information is assembled within limbic structures to induce appropriate neuroendocrine and behavioural responses.

4.4 Stress: Neuronal and behavioural consequences.

Structural and functional consequences of stressful experiences are represented in two important research domains of animal studies. On the one hand, investigations have shown that stress may decrease neuronal plasticity

(Watanabe et al., 1992; Magariños and McEwen, 1995; Kim et al., 2007), neurogenesis, particularly in the hippocampus (Cameron et al., 1995; Galea et al., 1996; Guzman-Marin et al., 2007), and the number of neurons (Sapolsky et al., 1985; Hosseini-Sharifabad and Hadinedoushan, 2007). It has also been shown that stress and stress hormones can induce impairment in certain hippocampus-dependent forms of learning and/or memory (Luine et al., 1993; Diamond et al., 1999; Park et al., 2008).

4.5 Stress and Cognition.

Findings from both animal and human studies have shown that stress and stress hormones have major effects on cognition (Roozendaal, 1999). While sustained stressors rather have suppressive effects on cognition (McEwen and Sapolsky, 1995) mainly attributed to stress hormones (i.e. glucocorticoids), brief periods of stress usually enhance the formation of new memory. In addition, some findings indicate that stress and stress hormones are involved in regulating memory consolidation processes (de Kloet et al., 1998). For example, studies have shown that depending upon its severity and context, stress and steroid hormones can enhance hippocampal neural plasticity (Mocchetti et al., 1996) and improve its function (Roozendaal and McGaugh, 1996, Gina et al., 1997; Bowman et al., 2001; Buchanan and Lovallo, 2001; Beylin et al., 2003; Akirav et al., 2004). For example, Yarom et al., (2008) recently showed that exposure to acute forced swim stress increases neuronal plasticity in the DG in rats. Furthermore, Bartolomucci et al., (2002) indicated that specific memory

processes may not only remain intact, but indeed may be facilitated by chronic stress.

These findings suggest that although stress is a potent modulator of learning and/or memory processes, stress-related effects (including impairing and facilitating), either structurally or functionally are not the absolute consequences of stressful experiences. Instead, several factors such as source of stress, stressor duration, stressor intensity, stressor timing with regard to memory phase, and learning type (Sandi and Pinelo-Nava, 2007) determine when and how stress switches its effects on cognitive performance from impairment to enhancement.

Diverse stressors activate a wide spectrum of interacting neuronal and hormonal systems that guide behavioural and physiological responses. The brain's ability to perform functionally relevant adaptations following stressful or neuropathological challenges is due to its plasticity, which includes changes in size of brain nuclei, in morphology of neurons, neurotransmitter systems (Zills, 1992) and self-protective mechanisms for structural repair. It has been demonstrated that limbic brain structures, particularly the hippocampal formation, are strongly influenced by stress-induced activation of systems. This is the case for stroke events in the different regions of limbic system particularly the hippocampus. Therefore, in the context of neuropathological conditions, the correlations between stroke, protective effects of steroid hormones and structural reorganization in the brain may have strong potential to be translated into their pharmacological implications for efficient therapeutic interventions. Despite the

fact that investigations indicate that the structural and functional repair after focal ischemia are influenced by several factors (e.g. growth or neurotrophic factors, neurotransmitters, hormones), and some mechanisms underlying these events have been described, several questions still remain a matter of debate. Specifically, one question that is mostly linked to brain repair after focal ischemia is related to whether these alterations may be addressed in other types of strokes in animal studies. What is the extent and rate of the structural changes after a given ischemia? What region of the brain is more susceptible to and/or involved in these changes? Or do these changes simply occur in some specific regions of the brain? What factors may facilitate the recovery processes in the brain? In addition, the linkage between the structural recovery and functional improvements is questioned. In order to answer some of the questions in this context and to expand our current knowledge about focal ischemia induced by ET-1, and to better describe the changes occur after ET-1-induced hippocampal stroke and stress-related alterations in an animal model of stroke, we propose a series of studies in rats.

Objectives and hypotheses

The main objective of the present thesis is to evaluate the effects of stress and CORT on outcomes after focal hippocampal stroke. Based on prior work on focal ischemia (Hossmann, 1998; Sugo et al., 2002; Parent, 2003) and facilitative effects of glucocorticoids on brain function (Roozendaal, 2000; Beylin and Shors, 2003; Dunko et al., 2007), we hypothesized that stress or CORT may facilitate

the structural recovery and spatial performance after ET-1-induced focal stroke in the hippocampus. We set out to test several hypothses with eight complementary experiments. The logic and sequence of the experiments were as follows:

Study One (Experiments 1-3): Three experiments in this study were conducted to assess the effects of chronic stress on spatial performance in MWT before and after ET-1-induced hippocampal stroke. *Experiment 1:* The first experiment explored the behavioural consequences of chronic stress in spatial function within MWT. Chronic restraint stress was expected to have a suppressive effect on spatial performance. *Experiment 2:* In the second experiment, rats that received focal ischemic stroke by injection of ET-1 into the hippocampus were tested in the MWT. It was hypothesized that ET-1 would impair the rats function in the MWT. *Experiment 3:* Rats' spatial performance in MWT was investigated after stroke and stress in the third experiment. In the present experiment, it was expected that stress might facilitate spatial function due to the beneficial effects of glucocorticoids on the hippocampus after stroke.

We show that spatial performance in MWT is not affected by chronic stress. We also found that focal stoke in the hippocampus reduced the rats' spatial abilities and the suppressive functional effect of ET-1 reduced after chronic stress.

Study Two (Experiments 4-6): Study two aimed to develop a non-stressful, appetitively motivated task, the Ziggurat Task (ZT) for measuring spatial performance compared to the MWT. In addition to validate the ZT for spatial function in male and female rats, the present study investigated functional

alterations after hippocampal focal stroke and stress in both wet (MWT) and dry (ZT) lands. *Experiment 4:* The validity of the ZT for spatial performance was examined. It was hypothesized that the goal-directed behaviour in the ZT environment depends upon the integrity of the hippocampus. *Experiment 5:* In the fifth experiment, we aimed to investigate if focal stroke in the hippocampus induced by ET-1 can cause deficits in spatial performance within both MWT and ZT. In addition, alterations in plasma CORT as a validated indicator of the stress response were measured before, during and after MWT and ZT testing. We hypothesized that rats with hippocampal stroke will show spatial deficits in both tasks and the MWT testing would be significantly associated with elevated CORT compared to the ZT training. *Experiment 6:* Spatial performance of male and female rats in the ZT was investigated in experiment six. As a further step to validate the ZT in the present experiment, it was expected that males and females would show different profile of spatial navigation in MWT and ZT.

We found that the ZT is sensitive to neuronal loss in the hippocampus. In addition, rats with hippocampal focal stroke showed spatial impairment in both MWT and ZT. We have also reconfirmed that the MWT is a stressful task disclosed by significantly elevated plasma CORT when compared to a dry land such as the ZT. The sixth experiment that examined sex differences in both tasks, indicated that males performed significantly better than females in all indices of spatial navigation in the ZT. No significant sex difference was found in spatial navigation in MWT.

Study Three (Experiments 7-8): Study three investigated post- and pre-stroke effects of CORT-related experiences. *Experiment 7:* Experiment 7 explored the effects of stress and corticosterone on the severity of memory (measured by MWT and ZT) and anatomical pathology (measured by the Cavalieri method for volume estimation) produced by focal hippocampal strokes. We hypothesized that post-stroke CORT-related experiences enhance the structural and functional recovery in the hippocampus. *Experiment 8:* The focus of experiment eight was pre-stroke CORT-related experiences. Stress and CORT treatment were used prior to hippocampal focal stroke, and the structural and functional integrity of the hippocampus were assessed following CORT-related experiences and stroke. Enhanced structural damage and spatial deficits were expected to be reflected in the ZT due to the pre-stroke suppressive effects of glucocorticoids.

We found that post-stroke stress and CORT treatment significantly decreased the volume of hippocampal damage, and increasing CORT levels alleviates the hippocampal stroke-induced memory deficits. We have also show that both CORT and stress treatment prior to HPC ischemia caused visuo-spatial impairment. Chronic stress prior to hippocampal focal ischemia, however, was associated with more structural damages compared to the CORT+hippocampal stroke.

5. Study 1:

Hippocampal Function, Stress and Stroke

Stress-related cognitive changes are still a matter of debate. Although stress effects are often considered as deleterious to cognitive function, there are many instances in which neural structure and cognition are either facilitated by stress or not affected at all. Moreover, in some particular neuropathological conditions such as focal ischemia, cognitive functions have been shown to be significantly impaired. These conditions may be improved by some factors such as steroid hormones. Three experiments in this study were conducted to assess the effects of chronic stress on spatial performance in Morris water task (MWT) before and after stroke induced by the injection of endothelin-1 (ET-1) into the hippocampus, an animal model for hippocampal focal ischemia.

Experiment 1: *Chronic Restraint Stress and Spatial Performance in Morris Water Task (MWT)*

Background

Studies in animals and humans have reported divergent findings. Stress can produce enhancement, impairment, or no effect on spatial function (de Kloet et al., 1999; Sapolsky, 2000; Garcia et al., 2001; Payne et al., 2002; Yang et al., 2003). Much of the evidence in rodents shows that spatial performance is exquisitely sensitive to psychological stress (McEwen and Sapolsky, 1995). In particular, restraint stress has been shown to be associated with impairment in visuo-spatial learning in Morris water task (MWT), a widely used task to assess hippocampus-dependent learning and memory in rodents (Kitraki et al., 2004).

It is believed that because the hippocampus is enriched with two classes of corticosteroid receptors (mineralocorticoid receptors [MRs] and glucocorticoid receptors [GRs]), the adverse effects of stress on the hippocampus seem to be mediated largely by the lower-affinity GRs, which become heavily occupied with corticosteroids in response to stress (Kim and Diamond, 2002).

In addition to the adverse consequences of stress on spatial behaviour, there are many instances in which spatial cognition is not affected by stress (Warren et al., 1991; Beylin and Shors, 1998). Our motivation for assessing the relation between stress and spatial performance in the present experiment revolve around the fact that although there is some consensus in the literature that stress is a potent modulator of cognitive function in general, and its effects are

commonly regarded as deleterious to cognitive function, the stress-related spatial outcomes is still a point for further investigations because there are many evidences in which spatial performance is not influenced by different stress paradigms. Therefore, the current experiment investigates whether a given stress (chronic restraint stress paradigm) can impair spatial function in the MWT.

Material and Methods

Subjects

Fifteen adult male Long-Evans rats (Control, n=7; Stress, n=8) weighing 300-350 g, raised at the Canadian Centre for Behavioural Neuroscience Vivarium at the University of Lethbridge, were used. The animals were housed in pairs under a 12:12 h light/dark cycle with light starting at 07:30 h and temperature set at 22°C. All testing and training was performed during the light phase of the cycle at the same time of day. The animals received water *ad libitum*. All procedures were approved by the University of Lethbridge Animal Care Committee in compliance with the guidelines of the Canadian Council on Animal Care.

Blood samples

Blood samples were taken at baseline, the day prior to restraint stress. Blood sample were also taken 15 – 20 min after stress on the 21st day of treatment. Rats were transported individually to the surgical suite and anesthetized with 4% isoflurane. During the 3-4 min of anesthesia, 0.75 ml of blood was collected from a tail vein. Blood was sampled using a heparinized

butterfly catheter. Blood samples were then transferred to centrifuge tubes and plasma was obtained by centrifugation at 5000 rpm for 5 min. The plasma samples were stored at –20°C until analyzed for corticosterone (CORT) concentration using commercial radioimmunoassay kits (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, USA). All procedures for blood sampling were the same as that previously reported by Metz et al., (2005).

Restraint stress

For restraint stress (Figure 3), animals in the stress group were maintained in a transparent Plexiglas tube (6 cm inner diameter) of adjustable length, from 11:00 am to 12:00 am for 21 consecutive days. The tubes allowed the complete restraint of the animals while at the same time allowing them to breathe through perforated ends of the tube. The tubes maintained the animals in a standing position without compression of the body (Metz et al., 2005).

Apparatus: Morris water task (MWT)

In order to assess spatial performance of the animals, all rats in the experiments 1-3 were tested in the moving hidden platform version of the Morris water task (MWT, Morris, 1984). The MWT (Figure 4) consisted of a pool (1.5 m diameter) filled to within 20 cm of the top with water (21 ± 1 °C) that was rendered opaque by skim milk powder. The pool was located in a room rich with distal cues, which remained unobstructed throughout the duration of the experiment. During all hidden platform trials, the platform was submerged 1.5 cm

below the water surface. Each trial began with the rat being placed in the pool at one of the four cardinal compass positions around the perimeter of the pool according to a pseudo-random sequence. The maximum duration of each swim trial was 60 s. If a rat found the platform within this 60 s period, it was allowed to remain on the platform for 5 s. If the rat did not find the platform during the selected time, then it was placed onto the platform for 10 s by the experimenter. Following each swim trial, the rats were placed in a holding cage and allowed to rest for at 5 min before the start of the next swim trial. Animals in this experiment were tested in 8 trials per day for 10 consecutive days of training before and after stress. In this version of the task, the platform is moved to a new location every second day. In other words, the platform remains in the same position for two consecutive days. Because the location of the hidden platform was different every two days, all odd days were called different-platform days, and even days were called same-platform days.

The movements of the animals 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 (Travel Mate 225X).

Statistical analysis

The statistical analysis for all experiments was performed using SPSS 11.5.0 for windows (Standard Version, 1982-2002; SPSS Inc., USA). The results were subject to analysis of variance (ANOVA) for repeated measurements across testing sessions. Comparison means between groups were performed using

independent samples *t*-tests, and dependent samples *t*-tests for within-subject comparison. In all statistical analyses, a p-value of less than 0.05 was chosen as the significance level. All data are presented as mean +/- standard error.

Results

CORT levels

Figure 5 illustrates circulating levels of CORT as assessed from blood samples. Blood samples were assayed for levels of circulating CORT at baseline (a day prior to stress) and at post-treatment point (15-20 minutes after stress on day 21). As shown in Figure 5, rats that received restraint stress, showed an elevated levels of CORT. A dependent samples *t*-test conducted for baseline and chronic points showed a significant effect of stress on circulating CORT ($t = 2.12$, $p < 0.05$). This suggests that the restraint stress caused an increase in circulating CORT.

Behavioural results

Two behavioural indices, the latency and path speed in the MWT were analyzed for each odd (learning) and even (memory) day. Repeated measures ANOVA were conducted with day as a within-subjects variable, and treatment group (control, stress) as a between-subjects variable for each of the dependent measures. Latency and speed of spatial navigation in MWT served as the dependent variables.

Latency: Figure 6 shows the average time spent to find the hidden platform in the MWT for both groups over 10 days of acquisition. A repeated measure ANOVA conducted for MWT testing before (pre-test) and after (post-test) stress revealed no significant effect of Group (pre-test, $p > 0.81$; post-test, $p > 0.63$) but significant effect of Day (pre-test: $F(1, 4) = 14.25$, $p < 0.05$; post-test: $F(1, 4) = 5.19$, $p < 0.05$). ANOVA also showed a significant effect of platform location for pre-test ($F(1, 1) = 9.28$; $p < 0.05$) and post-test ($F(1,1) = 6.49$; $p < 0.05$) trials and a significant effect of trial (pre-test: $F(1,7) = 37.61$, $p < 0.05$; post-test: $F(1,7)= 9.19$, $p < 0.05$). No effect of Group by Day interaction was found for pre-test ($p > 0.54$) and post-test ($p > 0.66$) trials. In addition, the interaction effect between Group and Trial for pre-test ($F(1, 70) = 2.25$; $p < 0.05$ and post-test ($F(1, 70) = 4.06$; $p < 0.05$) was significant. No significant interaction was found between Group and Platform Location (pre-test: $p > 0.57$; post-test: $p > 0.73$). Also, a comparison between last trial of different-platform days and first trial of same-platform days by dependent samples *t*-test indicated a significant difference in control group (pre-test: $t = 3.39$; post-test: $t = 1.47$; $p < 0.05$) and stress group (pre-test: $t = 1.19$; post-test: $t = 2.66$, $p < 0.05$). No significant difference was found between first and second trials on different-platform days in control and stress groups (all $p > 0.05$). Hence, the latency showed by the rats in the both groups before and after stress showed that spatial navigation measured by MWT was not affected by the restraint stress employed in this experiment. This clearly suggests that both control and stress rats were able to acquire and

retrieve the spatial information in a similar rate regardless of their experimental situation.

Path speed: Figure 7 reveals path speed in control and stress groups during 10 days acquisition. As it can be seen, the both groups showed relatively constant speeds across the 10 testing days in the task. An ANOVA conducted for post-stress speed in the MWT showed no significant main effect of group ($p > 0.60$).

Discussion

Despite the general consensus on the harmful effect of stress on spatial performance, we could not replicate such effect by the chronic restraint stress paradigm in male rats within the MWT. We found that the control and stress groups showed similar spatial performance indicated by the same rate of latency and speed in the MWT. Moreover, no habituation to the adverse effects of stress that is usually reported in the chronic paradigm of stress (Galea et al., 1997; Faraday, 2002) was found in the current experiment.

These results are consistent with previous studies showing that spatial performance is not affected by stress (Warren et al., 1991; Beylin and Shors 1998). Generally, several factors including the context in which corticosteroid-receptor activation takes place (de Kloet et al., 1999), stressor intensity, stress duration, source of stress, memory phase at which stress acts, stressor controllability and predictability, and gender may affect stress-related

consequences in spatial outcomes (Sandi and Pinelo-Nava 2007). However, in respect to our results a couple of possibilities should be specifically considered.

First, task demands may play a key role in the variation in MWT results. More specifically, even if restraint stress could affect the ability of the rats for spatial or mapping strategies (i.e. strategies to navigate to the platform using spatial information about the platform location and topographical relationship between distal cues), other strategies such as praxis strategy by which the rats can use a learned sequence of movement to locate the platform, and taxis strategy or the use of proximal cues in the task (D'Hooge and De Deyn 2001), may still enable the animals to show a productive spatial performance. Hence, the extent and nature of stress effects on performance in the MWT is strongly determined by task demands (e.g. indicators used to profile the spatial function) and the affected aspects of spatial navigation (e.g. utilized strategies to locate the platform).

The second possibility refers to the fact that corticotropin-releasing factor (CRF), in addition to its key role in stimulating pituitary ACTH secretion, may be the principal coordinating regulator of central stress responsiveness by influencing central serotonergic (Lowry et al., 2000) and catecholaminergic activities (Curtis et al., 1997). Furthermore, it has been reported that CRF have a direct effect on behavioural patterns such as locomotion (Lowry and Moore, 1991), neural survival after ischemia (Charron et al., 2008) and even learning (Wang et al., 2000). Therefore, simultaneous activation of CRF and ACTH systems likely cover the stress-induced behavioural alterations. This is based on

the fact that CRF in the brain may function as a fundamental behavioural activating system or may, at least sometimes, have a significant role in mediating behavioural responses to stress (Koob et al., 1993).

Third and most importantly, although many stressors can evoke dramatic neural and/or endocrine, and behavioural responses, the behavioural and endocrine stress responses can be dissociated (Koob et al., 1993), for the simple reason that multitude hormonal consequences of stress frequently affect multiple target tissues (Greenberg et a., 2002). In this perspective, many, if not all, of the hormones involved in stress responses possess, in addition to their direct (biological or behavioural) effects, collateral consequences that may or may not reinforce the direct or primary effect. It is likely that many of these other effects can provide the basis of mechanisms that might serve other, unrelated adaptive needs (Greenberg et al., 2002). Therefore, the concept of stress-sensitive behaviours and stress-related multiple biological reflections are the challenging issues that need further investigation particularly when they are interpreted only in the light of neuroendocrinological events.

Overall, it is well known that stress affects memory: facilitating, neutral as well as impairing influences have been described. These effects of stress on memory processes depend upon several factors that cause significant discrepancy between results among different investigations. Specifically, for our results in the present experiment and when memory has been shown to be unaffected by stress, task demands and selected criterion, collateral activity of some sub-systems in the neuroendocrinological axes, and the potentials for

dissociating behavioural and endocrine responses during stress should be regarded.

Experiment 2: *Hippocampal Focal Stroke and Spatial Performance in MWT*

Background

Amongst the animal models of focal ischemia used to investigate the physiopathology of stroke and effective therapies, one involves the delivery of endothelin-1 (ET-1) directly into the brain parenchyma by stereotaxic microinjection. ET-1 is an endogenous vasospasm-inducing peptide that has been identified as the most powerful known vasoconstrictor (Yanigasawa et al., 1988). The direct focal injection of ET-1 into selected brain regions in rats was recently suggested as an appropriate model of human focal stroke (Mateffyova et al., 2006). The injection directly into brain parenchyma produces less extensive ischemic damage and more limited neuronal loss than other non-selective procedures of rat models of stroke (Fuxe et al., 1997). Moreover, this procedure involves simpler surgical techniques and is not associated with postsurgical complications (Biernaskie et al., 2001). Therefore, as a suitable model for focal stroke, the intrahippocampal injection of ET-1 has been recently employed to investigate the functional and structural outcomes of ET-1-induced ischemic damage (Driscoll et al., 2007; McDonald et al., 2008). Because the hippocampus is a structure intimately involved in learning and memory function, and for the reason that strokes and other neuropathological conditions frequently cause

some learning and memory deficits, these studies focus on neurobehavioural outcomes of hippocampal focal ischemia (see Experiment 5 for detail).

Using ET-1 injection into the hippocampus the present experiment addresses the behavioural consequences of the hippocampal focal stroke. For this, we have tested the rats' spatial performance following ET-1-induced hippocampal stroke by employing the hidden platform version of MWT.

Material and Methods

Subjects

Thirteen adult male Long-Evans rats (Control, n=6; HPC stroke, n=7) weighing 350-420 g, raised at the Canadian Centre for Behavioural Neuroscience Vivarium at the University of Lethbridge, were used. The animals were housed in pairs under a 12:12 h light/dark cycle with light starting at 07:30 h and temperature set at 22°C. All testing and training was performed during the light phase of the cycle at the same time of day. The animals received water *ad libitum*. All procedures were approved by the University of Lethbridge Animal Care Committee in compliance with the guidelines of the Canadian Council on Animal Care.

Surgery

The procedure of ET-1 injection into the hippocampus was the same as that previously described by McDonald et al., (2008). The hippocampal formation was damaged by bilateral injections of a low concentration of ET-1 (7.5 pmol/0.5 μl;

0.1μl/min) dissolved in phosphate-buffered saline. Seven rats were anesthetized using 1.5% isoflurane inhalation. A midline incision was made in the scalp and periosteum. Rats received two injections of ET-1 in each hippocampus (Figure 8) through a 23-gauge cannulae attached to a Harvard infusion pump (model 22) and using the coordinates AP: - 4.1, - 5.3; ML: ± 3.0, 5.5; DV: - 3.7, - 6.3 in millimeters relative to the bregma-lambda distance. The cannulae were left in place for 5 min after each injection. The scalp was sutured after surgery and the animals were monitored until they became active before being returned to their home cages. Rats were allowed to recover for 4-5 days before the beginning of MWT testing.

Apparatus: Morris water task (MWT)

The MWT procedures used were identical to those described in Experiment 1 with the exception that the rats in the ET-1 group were given 4-5 days to recover before behavioural testing commenced. Moreover, both groups in this experiment were subjected to probe trial testing on the eleventh day of MWT, a transfer test that was performed to determine the extent to which the rats had learned about the location of the platform. For the purposes of the analysis on probe test day, the environment has been divided into four quadrants by the tracking system (HVS Image 2020) in which quadrants 1,2,3, and 4 were labeled for NE, SE (target quadrant in this experiment), SW, and NW respectively. The platform was removed from the pool and the rats were allowed to swim freely for one minute. The percentage of time that the animals spent in each quadrant of the task was

recorded.

Histology

All animals were sacrificed by an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 0.9% phosphate buffered saline followed by 4% paraformaldehyde. Each brain was removed from the skull and stored in 30% sucrose-formalin solution. The brains were then dissected out and 40_m coronal sections were cut on a cryostat microtome. Every forth section was mounted on glass slides and stained with cresyl violet. The stained sections were examined under a microscope (Zeiss, Germany) and images were captured using an AxioCam camera (Zeiss, Germany) to quantify the extent of the lesions. The amount of hippocampal lesion in each ischemic rat was estimated according to the Cavalieri method (Schmitz and Hof, 2005). Details for the assessment of lesion volume are presented in Experiments 4 and 5.

Results

Histological results

ET-1 produced tissue loss in the dorsal and ventral areas of the hippocampus in all rats of the ischemic group (Figure 9). The damage to the dorsal hippocampus was mostly limited to the CA1 and the DG. However, the extent of tissue loss in the ventral hippocampus was mainly restricted to the CA1 and CA2 fields. No damage was observed in the ventral DG. An independent samples *t*-test conducted on the percent tissue loss in the dorsal and ventral

hippocampus indicated significant difference between groups (Dorsal: t = 3.37, p < 0.05; Ventral: t = 6.09, p < 0.05).

Behavioural results

In addition to latency and path speed, probe trials were averaged and analyzed as indices for spatial navigation in the MWT.

Latency: Figure 10 (A) shows the average time spent to find the hidden platform (latency) for both control and hippocampal (HPC) groups over 10 days of acquisition. Although all rats showed a gradual decrease in the latency to locate the hidden platform, control rats located the platform more quickly than rats with hippocampal damage. An ANOVA conducted for the latency indicated a significant main effect of Group (F (1, 10) = 11.13, p < 0.05), Platform Location (F (1, 1) = 6.19, p < 0.05), Trial (F (1, 7) = 18.13, p < 0.05) and Day, (F (1, 4) = 8.89, p < 0.05). Tests of within-subjects effects showed no interaction between Group and Platform Location, and Group by Trial (p > 0.05). The interaction effect of Groups by Day was significant (F (1, 4) = 2.90, p < 0.05). Figure 10 (B) presents total mean latency for each group across the 10 days of testing in the task. Controls had shorter latencies to find the platform in the different- and same-platform days. An ANOVA showed a significant difference between different- and same-platform days for controls (F (1, 3) = 3.16, p < 0.05) but not for HPC group (p > 0.56). In addition, a comparison between last trial of different-platform and first trial of same-platform days by dependent samples t-test showed a significant difference in the control group (t = 1.49, p < 0.05) but not in the HPC group (p >

0.05). Dependent samples *t*-test also showed a significant difference between first and second trials on different-platform days in the control group ($t = 9.03$, $p < 0.05$) but not in the HPC group ($p > 0.05$). Therefore, although the general profile of spatial performance in the MWT discloses a gradual decreased latency for both groups, further analysis of different- and same-platform days shows that only the control group could acquire and retrieve the spatial location of the hidden platform.

Path speed: Figure 11A reveals path speed in control and HPC groups during 10 days acquisition. Although both groups showed relatively constant speeds across the 10 testing days in the task, no significant difference was found between groups ($p > 0.83$; ANOVA).

Probe trial: Figure 11B shows the percentage time spent in the testing and opposite quadrants of MWT during the probe trial. Spatial memory was indicated by significantly greater search time in training compared to the opposite quadrant of the task. Analysis of the 60 s of the probe performance revealed that rats in the control group spent a considerable proportion of their time (41.69%) searching in the target quadrant. The profile of time spending at the different quadrants and target quadrant (31.15%) for the HPC rats in the probe trial, however, was significantly different (F (1, 18) = 12.39, $p < 0.05$) suggesting that the HPC group did not acquire or retain a strong bias for the previous location of platform as compared to the control group. An independent samples *t*-test comparing the two groups in the target quadrant showed a significant difference ($t = 8.19$; $p < 0.01$). In summary, most control rats preferentially swam in the

quadrant 2 in which the platform had been presented during the previous training days.

Discussion

The present study utilized injections of ET-1 into the hippocampus as an analogue of ischemic stroke. We found that the hippocampal focal stroke induced by the injection of ET-1 produces spatial deficit within the MWT. Specifically, the spatial deficit in the task after the hippocampal stroke is the cognitive deficit resulting from the hippocampal stroke and may not be resulted by the locomotor consequence of the specific procedure of ET-1 injection.

Hippocampal cells are highly sensitive to stroke events (Sachdev et al., 2007) and any vascular insult could affect this structure leading to spatial cognition impairment. Because the tissue loss in the hippocampus following ET-1 injection in this experiment is mostly expected to occur in the dentate gyrus (DG), the observed behavioural decline supports the view that the DG plays a central role in learning and memory by processing and representing spatial information (Gallagher and Holland, 1992). An additional key issue about the DG's structure is that this structure is a plastic structure in which new neurons are born throughout the life of mammals (Derrick, 2007), although the functional impact of neurogenesis in the DG on physiology and behaviour is unclear. That the DG is structurally involved in neurogenesis, may open a window into brain recovery after some neuropathological conditions. Because studies have shown that the DG's neurogenesis is dependent, in some parts upon the presence of

glucocorticoids (Mocchetti et al., 1996; Grundy et al., 2000; Melcongi et al., 2000), our results in this experiment, therefore, provide one important hypothesis for future investigations: stressful situations or corticosterone treatment may enhance spatial performance particularly when the hippocampus is partially damaged by some neuropathological situations such as focal ischemia.

Consistent with the interpretation of the present results, the ET-1 ischemic damage in the hippocampus can mimic those deficits in spatial cognition that are caused by clinical strokes and are especially involved in the sub-regions of the hippocampus such as the DG. Furthermore, the ischemic outputs, structural or functional, in the hippocampus may be influenced by the neuroprotective consequences of glucocorticoid-related experiences, a hypothesis that is investigated in the next experiment.

Experiment 3: *Hippocampal Focal Stroke, Stress, and Spatial Performance in the MWT*

Background

It is believed that the hippocampus is a plastic structure with high density of adrenal steroid receptors. In the adult hippocampal DG, new neurons are produced, a process that refers to hippocampal neurogenesis. A great deal of animal research documents a facilitating effect of stress and corticosteroids on memory performance (Micco and McEven, 1980; Borrell et al., 1984; Buchanan and Lovallo, 2001). Specifically, it has been suggested that adrenal hormones

are associated with enhanced hippocampal plasticity (Mocchetti et al., 1996) and spatial memory (Roozendaal and McGaugh, 1996; Akirav et al., 2004). The stress-dependent memory enhancement view emphasizes that the physiology of memory formation and consolidation may involve stress hormones as endogenous positive modulators. Although many of the primary studies hypothesized that these processes will occur through the modulation of the steroid hormones in “neurotrophic” activities (Chao et al., 1998), the fundamental question of when and how stress switches its effects on cognitive performance from impairment to enhancement is still unanswered.

In the previous experiment, we demonstrated that ET-1-induced focal stroke in the hippocampus is associated with impairment of the hippocampal-dependent memory performance. Based on the hypothesis of the stress-dependent memory enhancement, the present experiment, however, is designed to answer the following question: Does stress have a facilitative effect on the hippocampal function after focal stroke?

Material and Methods

Subjects

Thirteen adult male Long-Evans rats weighing 370-450 g, raised at the Canadian Centre for Behavioural Neuroscience Vivarium at the University of Lethbridge, were used. The animals were housed in pairs under a 12:12 h light/dark cycle with light starting at 07:30 h and temperature set at 22°C. All testing and training was performed during the light phase of the cycle at the

same time of day. The animals received water *ad libitum*. All procedures were approved by the University of Lethbridge Animal Care Committee in compliance with the guidelines of the Canadian Council on Animal Care.

Surgery

All animals were subjected to the hippocampal injection of endothelin-1 (ET-1), and the surgical procedures used were identical to those described in Experiment 2. Animals were then randomly divided to two groups, HPC stroke (n=6) and HPC stroke + stress (n=7). Rats in HPC stroke + stress group were allowed to recover for 2-3 days before the beginning of blood sampling and restraint stress.

Blood samples

Blood samples procedures used were identical to those described in Experiment 1.

Restraint stress

The stress procedure used was identical to those described in Experiment 1 with the exception that the rats were manually vibrated for 10-15 seconds in every 15 minutes of stress phase in order to prevent the habituation effect of the given stress. Following the 21-days (1h/day) restraint stress, and in order to assess spatial performance of the animals, the both groups were tested in the moving hidden platform version of the Morris water task (MWT).

Apparatus: Morris water task (MWT)

The MWT procedures used were identical to those described in Experiment 1.

Results

CORT levels

Figure 12 illustrates circulating levels of CORT as assessed from blood samples. Blood samples were assayed for levels of circulating CORT at baseline (a day prior to stress) and post-treatment (15-20 minutes after stress on day 21). As it can be seen in Figure 12, rats that received restraint stress after focal stroke in the hippocampus showed an elevated level of CORT when compared with the HPC stroke-only group. Independent samples *t*-test comparing both groups for CORT in chronic point showed a significant difference between groups ($t = 6.29$, $p < 0.05$). Dependent samples *t*-test conducted for the HPC stroke + stress group also revealed a significant difference between baseline and chronic points in this group ($t = 9.86$, $p < 0.01$). Therefore, the restraint stress caused more changes in circulating CORT in the HPC stroke + stress group than HPC stroke-only group.

Behavioural results

Latency: Figure 13A shows the average time to find the hidden platform (latency) for both HPC stroke and HPC stroke + stress groups over 10 days of acquisition in the MWT. Similar to the previous experiments, all rats showed a gradual decrease in latency to locate the hidden platform regardless of their experimental situation. However, rats in HPC stroke + stress group located the

platform more quickly than rats with only HPC stroke. A repeated measure ANOVA indicated a significant main effect of Group (F (1, 11) = 58.55, $p < 0.05$), Platform Location (F (1, 1) = 3.20, $p < 0.05$), trial (F (1, 7) = 1.77, $p < 0.05$) and Day (F (1, 4) = 13.32, $p < 0.05$). No significant effects of Group by Platform Location, Group by Trial and Group by Day were observed (*all* $p > 0.05$). In addition, a comparison between last trial of different-platform and first trial of same-platform days by dependent samples t-test showed a significant difference in the HPC + stress group (t = 6.07, $p < 0.05$) but not in HPC-only group ($p > 0.05$). Dependent samples t-test also indicated a significant difference between first and second trials on different-platform days in the HPC + stress group (t = 2.13, $p < 0.05$) but not in the HPC-only group ($p > 0.05$). Moreover, an ANOVA showed a significant difference between different- and same-platform days for HPC stroke-only (F (1, 28) = 6.76, $p < 0.05$) and HPC stroke + stress groups (F (1, 36) = 2.17, $p < 0.05$; Figure 13B) suggesting that both groups could retrieve the spatial location of hidden platform. Therefore, our results show that the restraint stress after HPC stroke induced by ET-1 may decrease the time to find the hidden platform within MWT.

Path speed: The facilitated spatial performance in the MWT after stress might be due to the effect of stress on the rats' locomotion, thus we measured the rats' path speed in the MWT. Figure 14A reveals path speed in HPC stroke and HPC stroke + stress groups during 10 days acquisition in the MWT. Both groups showed gradually increased speeds across the 10 testing days in the task. No significant difference was found between groups ($p > 0.72$; ANOVA).

Hence, the observed facilitative effect of stress after HPC stroke on latency in the MWT likely do not result from an effect of restraint stress on locomotion in HPC stroke + stress group.

Probe trial: Results showing the percentage of time spent in each quadrant during the probe trial (60-s duration) are depicted in Figure 14B. Analysis of the probe performance revealed that rats in the HPC stroke + stress group spent a considerable proportion of their time (42.18%) searching in the target quadrant (quadrant 4). The profile of time spending at the different quadrants and target quadrant for the HPC stroke-only rats in the probe trial (33.79%), however, was significantly different (F (1, 33) = 2.89, $p < 0.05$) suggesting that the HPC stroke-only group did not acquire or retain as a strong bias for the previous location of platform as the rats in HPC stroke + stress group. An independent samples t-test comparing the two groups in the target quadrant showed a significant difference (t = 1.66; $p < 0.05$).

Discussion

The present experiment evaluated the effect of chronic daily stress on the magnitude of the spatial cognition deficit caused by ET-1-induced hippocampal ischemic stroke. The results indicate that rats with HPC stroke + stress take significantly less time to find the hidden platform in the MWT when compared to HPC stroke-only. Furthermore, the rats' spatial performance in the probe trial revealed that HPC stroke + stress rats spent significantly more time searching for the platform in the target quadrant relative to HPC stroke-only rats.

With regard to the nature of the hippocampal stroke and the improvement in spatial behaviour after stress, it can be drawn a line between the ontogeny of the ET-1-induced hippocampal ischemia and the mechanisms of the facilitative effects of stress on the hippocampal function. These facilitated functional changes may be attributed to the close biological dialogue between nervous and endocrine systems (Derrick, 2007). Moreover, our results in the present experiment may be interpreted in the light of the reports by which the enhanced memory after stress may be related to the regulatory effects of glucocorticoids on the expression of neurotrophins in the hippocampus (Grundy et al., 2001). A full description of the possible mechanisms involved in post-stroke enhancement of spatial function by steroid hormones or stress can be addressed in Experiment 7.

Concluding remarks

Taken together, we have found that spatial performance in the MWT is not subject to change after chronic restraint stress. This likely refers to procedural demands and possible collateral involvement of the CRF system in stress response. Using the ET-1 procedure to induce hippocampal focal ischemia, however, we observed that ET-1-related stroke in the hippocampus is associated with impaired spatial function in the MWT. Because the main target of this stroke has been the hippocampal DG, our findings confirm that this hippocampal sub-area is intimately involved in the spatial memory. In addition, because the DG is an extremely plastic area in the hippocampus, and this remarkable capacity for plasticity is attributed to the biological connections between the DG, steroid

hormones and neurotrophins, one can say that CORT or CORT-related experiences may enhance the structural recovery in the hippocampus and its function after stroke. Therefore, we have tested the hypothesis that stress may have a facilitative effect on hippocampal function after ischemic stroke in the MWT. We show that rats with HPC stroke who experience post-injury stress have better performance in the MWT. The improvement in spatial performance is apparent in shorter time to find the hidden platform and a greater proportion of searching time in the target quadrant in the no-platform probe trial. We conclude that corticosteroid-related experiences enhance the hippocampal function after HPC focal stroke.

The finding that stress enhances memory after stroke prompts the hypothesis that CORT may be responsible for the observed recovery in the hippocampal function. However, because the MWT procedure is an aversively motivated test that is stressful in itself and induces a strong stress response during testing (D'Hooge and De Deyn, 2001; Aguilar-Valles et al., 2005), a dry-land, less-stressful apparatus, the ziggurat task (ZT) has been designed and developed for assessing spatial performance (Faraji et al., 2008). The next study will focus on the validity of this new task and its application for the evaluation of spatial processes in male and female rats, stroke recovery.

6. Study 2:

The Validity of the Ziggurat Task (ZT) for Spatial Performance

The Morris water task (MWT) has been shown to be a stressful task for rats, and the ensuing endocrinological changes after being tested in the MWT may interfere with the acquisition processes. To reduce this kind of interference we designed the ziggurat task (ZT), a dry non-stressful task (compared to MWT) for measuring spatial performance. In addition to validating the ZT for spatial function in male and female rats, a second goal in this study was to address functional alterations after focal stroke and stress in both wet (MWT) and dry (ZT) lands.

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Experiment 4: *Widespread Hippocampal Damage and Spatial Performance in the Ziggurat Task (ZT)*

Background

Spatial behaviour refers to all behaviours with which animals guide all or parts of their bodies through space (Kolb and Whishaw, 2003). Spatial tasks, therefore, measure how organisms orient themselves in space. Although there has been an interesting history of progressive investigations into the spatial behaviours of animals, particularly rats, over the past three decades (see Redish, 2001 for review; Sutherland and Hamilton, 2004), many controversies remain as to which structures of the brain are involved and which instrument(s) are most appropriate for measuring various aspects of spatial behaviour.

Olton and others (Olton et al., 1976; Olton et al., 1977) first described the symmetric Radial Arm Maze (RAM) as an important tool for the study of spatial learning and memory. Since then we have been able to collect a significant amount of data in this area of study and are now better able to determine the processes in relevant neural systems that subserve performance in these tasks. As a dry-land task, the goal of the RAM is for food-restricted rats to learn the location of food at one or more arms. The RAM provides a constrained environment with a limited choice of routes for animals. Although this simple

route structure favours the emergence of certain search strategies, it has been suggested that the task is sensitive enough to measure both spatial reference and/or working memory (Olton and Pappas, 1979; Hodges, 1996).

Richard Morris (Morris, 1981; Morris, 1984) later opened a different window to the spatial performance proposing the use of an open field apparatus, a round water pool known as Morris Water Task (MWT). The animal has to discover and later swim to a small escape platform submerged underneath the surface of water. As an open field, the MWT allows the animals to move freely, enabling them to create a wider variety of search patterns and routes. Being geometric (Sutherland and Hamilton, 2004) and simple, its ability to differentiate between the spatial and non-spatial performances established the MWT as an important apparatus for the study of spatial learning and memory (acquisition and working memory). Nevertheless, the MWT is the target for some critiques for being an escape motivated task. For example, it is clearly stressful (D'Hooge and De Deyn, 2001; Block, 1999; Aguilar-Valles et al., 2005).

In both RAM and MWT, the animal can navigate to and remember locations using some combination of distal and/or proximal cues. While both tasks are designed to measure the animal's spatial memory and demonstrate a remarkable sensitivity to damage of many areas of the brain, especially the hippocampus, some investigators (Wenk, 1998; See also Whishaw and Kolb, 2005) believe that they reveal different profiles or features of spatial performance.

Since several cognitive components are engaged in spatial performance, different tasks reflect different functional competencies. Whishaw and Tomie

(1996), for instance, showed that in a dry-land maze there was no difference in the performance of rats and mice, whereas in a swimming-pool spatial task the performance of mice was inferior to that of rats. Similar findings were reported by Kimble and Whishaw (1993) revealing that the spatial performance of Brazilian gray, short-tailed opossums (Monodelphis domestica) was significantly different from that of the rats in both dry and wet tasks. Specifically, these contrasting findings may be attributable to the differences in the task difficulty (Dudchenko et al., 1997), training and testing procedures, the level of motivation, and the availability of spatial and associative cues between the two tasks (Hodges, 1996).

Kesner and others have proposed a dry-land task or cheeseboard in which animals investigate a flat dry land and learn the position of food reward placed on one of the several food wells using distal and/or proximal cues (Kesner et al., 1989; 1992). The cheeseboard task has several advantages over the MWT, such as the use of a positive reinforcement and lower effects on stress levels (Corwin et al., 1994).

The ZT incorporates the same advantages as the cheeseboard task, but also provides several more. First, the ZT is a greater analogue of the " natural " environment of animals regarding to the required types of searching behaviours compared to the other dry-land environments such as cheeseboard and RAM. The natural environment of animals is not merely even, and consequently the animal needs to utilize both vertical and horizontal navigation. In other words, the natural environment provides a combined even-uneven surface with many

different physical features or *reference frames* for rats. Thus, the animals are required to utilize more movement chains and additional sensory sources. Second, the investigation within an uneven, multi-featured environment, compared to an even, simple field requires more complicated cognitive strategies and representations, and arguably needs more active and deeper cognitive evaluation of the spatial relations between surrounding stimuli and a given destination or target (e.g. home or food). Thus, the ZT possibly presents an alternative picture from the animals' spatial performance. Last, a potential problem of flat and open fields such as cheeseboard for spatial testing is that they are likely more susceptible to lead the animal to show anxiety related behaviours. For instance, rats are less likely to investigate the centre of an open field, a phenomenon mediated, at least in part, by anxiety. In the ZT, large objects (i.e. ziggurats) may provide shelter or protection, reduce anxiety, and ultimately increase investigation and spatial navigation. Therefore, the ZT offers a different perspective on the assessment of spatial navigation and may lead to a more complete understanding of the neural basis of spatial memory.

Given these considerations, the principal goal of the present study is to introduce a new task that measures the spatial behaviour of rats within a dry, non-aversive environment. The task provides an open field for animals and will let them to freely navigate supported by a positive motivational condition. In order to validate the task we have compared groups of control and hippocampal damaged animals in ZT, and found the task is sensitive to neuronal loss in the

hippocampus. Thus, we showed that the goal-directed behaviour in the ZT environment depends on the integrity of the hippocampus.

Material and Methods

Subjects

Thirteen adult male Long Evans rats, weighing 350-420 g at the beginning of the experiment, raised at the University of Lethbridge, were used. Six rats received hippocampal damage and 7 rats served as unoperated controls. They were housed in pairs under a 12:12 h light/dark cycle with light starting at 07:30 h and temperature set at 22°C. All testing and training was performed during the light phase of the cycle. Rats were food-restricted prior to behavioural training and testing, and maintained at about 85% of their initial body weight throughout the experiment. Water was provided *ad libitum*. All procedures were performed in accordance with the guidelines of the Canadian Council for Animal Care.

Surgery

Rats were anesthetized with isoflurane (Janssen, Toronto, Ontario) in 0.8 L/min oxygen at 14.7 PSIA at 21°C (Benson Medical Industries, Markham, Ontario) and given buprenorphine (.07cc; .3mg/ml, s.c.; Schering-Plough, Hertfordshire,UK) as an analgesic. They were then placed in a stereotaxic frame (Kopf Instrument, Tujunga, CA) and a midline scalp incision was made to expose the top of the skull. The lesions were made by intrahippocampal infusions of N-methyl-D-aspartic acid (NMDA; 7.5 μg/μl: Sigma Chemical co., St. Louis, MO) at

10 sites bilaterally (see Table 1 for coordinates). The infusions were done sequentially through a 30-gauge injection needle attached to a 10 μl Hamilton syringe via polyethylene tubing (PE-50). At each site, a total volume of .4 μl was infused at a flow rate of .15 μl per minute. The injection needle was left in place for an additional 2.5-min following the injection to facilitate diffusion. As a prophylaxis against seizures, the rats were given a dose of diazepam (.1 cc; 10 mg/ml, i.p., Hoffman-La Roche, Mississauga, Ontario) immediately prior to the start of the NMDA infusions. Following the lesions, the scalp incision was closed using wound clips and rats were given a second dose of diazepam (.2 cc; 10 mg/ml, i.p.) upon awakening. All hippocampal damaged rats were allowed to recover for two weeks before testing began.

Apparatus: The Ziggurat Task (ZT)

All behavioural testing was carried out in a white rectangular room (3 × 5 m) with different distal cues (pictures and signs) on each wall. For data acquisition, a ceiling-mounted camera recorded the movements of the rats. The apparatus consisted of an open-field box (179 × 179 cm by 25 cm in height) constructed from white laminate, and resting on a 50 cm high table and measuring 179 cm square (Figure15A-D). The apparatus was located approximately 70 cm from the East and West wall of the room and 100 and 250 cm from the North and South wall respectively. The open field contained sixteen pyramidal ziggurats, arranged in a four by four matrix (Figure 16A). The ziggurats were identical and made of white styrofoam covered by clear duct tape. Each ziggurat (Figure 16B) was

designated by a number and had six levels with a total height of 21 cm. 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 reduced in size by 2 cm. The highest level, measured 11 cm square and a circular hole measuring 1.5 cm in diameter and 1 cm in depth in the center. The hole was of sufficient size to contain three to five 1 cm-long spaghetti (Tradizione). The distance between ziggurats was 11 cm. In order to minimize olfactory cues, both the box and ziggurats were cleaned with 5% alcohol after testing each group.

Behavioural procedures

Phase1 (training and habituation): Animals were weighed daily. They were food-restricted one week prior to habituation sessions and behavioural testing, and maintained at about 85% of their initial body weight throughout the experiment. The initial sessions involved training rats to search the ziggurats for food. On the first and second day, the rats were habituated by placing them individually into the apparatus for a 10 min session. The rats could freely explore the entire apparatus including all of the 16 ziggurats. Every ziggurat contained 3-5 small pieces of spaghetti. On day three, the rats were again placed in the apparatus for a 7 min session, but only half the ziggurats were baited. The same procedure was followed on day four with the exceptions that the animals were placed on a corner ziggurat, allowed to move within the task for 5 min, and only a quarter of the ziggurats were baited.

Phase 2 (Testing): The testing sessions were conducted over 10 days and began the day immediately following the last session of Phase 1. The cycle consisted of alternating “learning” days (odd days 1, 3, 5, 7, 9) and ‘memory” days (even days 2, 4, 6, 8, 10). On the odd days, the goal ziggurat was located in a new location, and rats had to find and learn the location of the goal ziggurat in the new place. The goal ziggurats remained in the same place on the even days. Two sets of ziggurats were defined in the environment. First, “start” ziggurats or ziggurats numbered 1, 4, 7, and 10 in each corner, and second, the rest of ziggurats or “goal” ziggurats. On the testing days, the rats, released from each starting point, could explore the environment, however only one goal ziggurat (peripheral or central) had 3-5 small pieces of spaghetti for each trial. During each testing day, testing took place in 8 trials with each of the 4 start locations sampled twice according to a pseudorandom order. A trial lasted for 120 s or until they found the goal ziggurat. To start, rats were placed facing the wall on the top of the starting ziggurat. On a typical trial the rat had to climb down the ziggurat, investigate the environment until it found the goal ziggurat whereupon it would eat the food (Figure 17).

The movements of the animals 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 (Travel Mate 225X; Figure 18).

It was expected that they would learn the location of the goal ziggurat on the first day and remember the location of the goal ziggurat on the second day. The location of goal ziggurat was not changed between days 1 and 2, but between

days 2 and 3 it was relocated. This procedure was replicated for days 5 and 6, 7 and 8, and days 9 and 10. Two procedures can be used to define the location of goal ziggurats: (1) *Peripheral-ziggurats procedure*. Using this procedure, animals have to locate one of the peripheral ziggurats (numbered 2, 3, 5, 6, 8, 9, 11, and 12) from each starting point on every two days. (2) *Peripheral-central ziggurats* or *mixed procedure*. Based on this procedure, animals locate a peripheral goal ziggurat on the first two days and a central goal ziggurat (numbered13, 14, 15, and16) on the next two days at a random position. Thus, according to procedure 2, rats needed to change the peripheral strategies to central strategies every two days. It should be pointed out that the data presented in this paper were collected by using the peripheral-central ziggurats procedure. Because there were no local cues that mark the location of the goal ziggurat, the animals' ability to find it depends on the animal's use of a configuration of external cues surrounding the ziggurat environment.

Errors: Normally, the animals are able to find the goal ziggurat using the distal and/or proximal cues following investigation of some non-goal ziggurats in the first trials. Each investigation of non-goal ziggurats (i.e., non-baited ziggurats) was considered an error. In other words, behaviours such as climbing onto incorrect ziggurats and touching the circular holes with the nose have been defined as "errors". In addition, rats in ZT can make two kinds of errors: (1) Errors type 1 in which rats investigate non-goal ziggurat once, and (2) Errors type 2 in which rats re-investigate non-baited ziggurats. This categorization of errors can

be useful for distinguishing processes related to working and reference memories.

Probe test: Probe trial-dependent behaviours were measured on the eleventh day as an additional measure for spatial memory performance. For the purposes of the analysis, the environment has been divided into four quadrants by the tracking system (HVS Image 2020) in which quadrants 1,2,3, and 4 were labeled for NE, SE, SW, and NW respectively. Each rat was given three consecutive 70-s probe trials, released from different starting points to reach the goal ziggurat. On the first trial, the goal ziggurat, located in the former location (quadrant 4 in NW) had 3-5 pieces of spaghetti. On the second and third trials, however, there was no food on it. Rats were allowed to navigate freely in the environment during the specified time. The percentage of time rats spent in trial two in each quadrant of the ziggurat task was recorded.

Cued-goal environment for non-spatial performance: Performance in the ZT may be affected by deficits in visual acuity, motor function, or some other non-cognitive factors, rather than a spatial learning and memory impairment *per se*. To assess this possibility, rats were tested in a cued navigation task that did not require learning and remembering a location in the ZT. Specifically, on day 12, an 11-trial block was completed by all animals in a cued goal configuration of the task. For this, a goal ziggurat covered with black tape was placed at a position not used during the former sessions.

Histology

After completion of behavioural testing, all animals were given an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 0.9% saline followed by 4% paraformaldehyde. The brains were dissected out and 40 μm coronal sections were cut on a cryostat microtome. Every fourth slice was mounted on glass slides and stained with cresyl violet. The stained sections were examined under a microscope to quantify damage.

Statistical analysis

Statistical analysis was performed using SPSS for Windows 11.5.0 (Standard Version, 1982-2002; SPSS Inc., USA) with repeated-measures analysis of variance (ANOVA). Differences in between-group comparisons were assessed with independent sample *t*-tests, with $p<0.05$ set as the significance level. All data are presented as mean +/- standard error of the mean.

Results

Histological results

The extent and nature of damage to the hippocampal system were measured using cresyl violet to stain cell bodies. Figures 19 and 20 illustrate the extent of the HPC lesions. The NMDA injections produced extensive tissue loss in all principle subfields of the HPC, dentate gyrus, for each lesion rat. Amongst all lesion rats, the damage to the dorsal HPC was almost complete. However, in 1 rat there was some minor unilateral sparing of dentate granule cells and CA

field pyramidal neurons in the most lateral portion of the HPC. The amount of damage to the ventral region of the HPC was also pronounced in each lesion rat, though there was some minor sparing of the CA fields in the most ventral region in most rats. There was minor damage to the anterior part of the subiculum, but the posterior region was spared in each rat. All lesion rats sustained damage to the posterior parietal cortex where the injection cannulae were inserted. Some rats also showed evidence of damage to the fimbria/fornix and 1 rat showed unilateral damage to the rhinal cortex.

Behavioural results

Latency: Figure 21 (A) shows the average time spent to find the goal ziggurat (latency) for both control and hippocampal groups on different- and same-platform days. Although all rats showed a gradual decrease in the latency to locate the goal ziggurat, control rats located the goal ziggurat more quickly than rats with hippocampal damage. Latencies to locate the goal ziggurat dropped from day 1 (60.97 ± 14.01 for controls and 79.27 ± 13.91 for HPC) to 10 (15.72 ± 6.07 for controls and 38.70 ± 5.12 for HPC) in both groups. Our results suggest that although both control and HPC rats can learn and remember during the testing days to locate the goal ziggurat, the acquisition is less efficient in the HPC rats. An ANOVA conducted for the latencies over the 80 trials of ziggurat-task testing revealed a significant main effect of group, (F (1, 11) = 6.13, p < 0.05) and day, (F (1, 8) = 1.44, p < 0.05), but no significant effect of group by day was observed (F (12, 19) = 0.16, p > 0.79). Panel B in figure 21 presents total

mean latency for each group across the 10 days of testing in the task. Controls had shorter latencies to find the goal ziggurat on different- and same-platform days. Comparison of the different- and same-platform days by paired *t*-test revealed a significant difference in controls (t = 3.13, $p < 0.05$), but not in damaged group. The two groups differed in latency on learning days and on memory days (Learning: unpaired *t*-test, t = 6.44, $p < 0.05$; Memory: unpaired *t*-test, t = 4.96, $p < 0.05$). Figure 21 (C) shows that control animals spent progressively less time to locate the goal ziggurat in both different- and same-platform days over the 8 trials. The HPC group, however, was substantially impaired on the same trials (Learning: F (1, 11) = 2.61, $p < 0.05$, ANOVA; Memory: F (1, 11) = 3.76, $p < 0.05$, ANOVA).

Path length: Examination of acquisition in terms of path length (distance traveled) to locate the goal ziggurat also revealed a significantly difference between groups. Both groups took progressively shorter move paths to find the goal ziggurat as training proceeded (Figure 22). However, rats in the HPC damaged group traveled greater distances to locate the goal ziggurat than did rats in the control group. An ANOVA conducted for the path length over 10 testing days showed a significant main effect of group (F (1, 11) = 9.00, $p < 0.05$) and a significant main effect of day (F (79, 1038) = 22.18, $p < 0.05$), but no significant interactive effect of group by day (F (12,19) = 0.31 $p > 0.62$).

Path speed: Examination of path speed during acquisition presented a further consequence of widespread damage of hippocampus in the ziggurat task. Hippocampal rats moved consistently faster than rats in control groups except on

days 6 and 10 (Figure 23). An ANOVA showed a significant main effect of group, (F (1, 11) = 2.19, $p < 0.05$) and a significant main effect of trial, (F (79, 1038) = 31.68, $p < 0.05$). Although one could say that an increased path speed may provide a major contribution to a reduced latency, it cannot be precluded the possibility that rats in faster group (here damaged rats) will necessarily perform more accurately.

Errors: Examination of acquisition in terms of the number of errors showed a gradual decrease in the numbers of errors of both groups during spatial navigation in ziggurat task (Figure 24). On the first day, the control group produced more errors than did lesion group. Conversely, the hippocampal group showed more errors on the rest of testing days relative to controls. The ANOVA showed a significant difference between groups in terms of the number of errors (F (1, 11) = 8.14, $p < 0.05$).

Probe trial: Figure 25 shows the percentage time spent in the testing and opposite quadrants of the ziggurat environment during the second probe trial. Spatial memory was indicated by significantly greater search time in training compared to the opposite quadrant of the task. Analysis of the 70 s of the probe trial revealed that rats in the control group spent a considerable proportion of their time (41.58%) searching in the quadrant of the ziggurat task in which the goal ziggurat had previously been baited. The profile of time spending at the different quadrants and target quadrant (11.65%) for the HPC rats in the probe trial, however, was significantly different (F (1, 11) = 4.18, $p < 0.05$) suggesting that they exhibited a more diffuse pattern of searching, with much less spatial

bias toward the former training quadrant. Independent *t*-test comparing the two groups in the target quadrant showed a significant difference ($t = 3.68$; $p < 0.01$). In summary, most control rats preferentially traveled in the quadrant 4 in which the target had been baited during the first trial and the testing days.

ELS Scale: This scale presents different possible profiles of spatial performances in ZT with a combination of errors (E), latency (L) and speed (S). (Figure 26). According to the ELS, animals show different spatial profiles in regard with the number of errors and the time they spent to find the goal ziggurat as well as their path speed during the navigation in the environment.

While control rats reveal a profile with low error, low latency, relatively high speed (Figure 26 A), rats with widespread hippocampal damage show at least three types of cognitive deficits (Figure 26, B-D). Panel B in figure 26 represents a profile of cognitive deficit in which HPC rats are characterized by averaged low errors, high latency and high speed. Rats with this profile usually show specific paths localized in peripheral and/or central pathways with a remarkable amount of returns in the specific ways (Figure 26, B1 and B2). A prominent picture of rats with this profile is a compulsive thigmotactic behaviour (spending times moving in the peripheral ways) prior to navigating in the alternative ways, and a stereotypic pattern searching in some specific central ways. In few cases, rats with this profile also show just thigmotactic behaviour without traveling in the central ways. Three rats with HPC damage were often showing this profile in the present study. Rats with cognitive deficits showed in panel C of figure 26, however, reveal a different cognitive performance. Averaged high error, latency and speed in this

profile are associated with a distributed, confused pattern of path (Figure 26, C1 and C2). Moving fast in the environment without spatial attention also causes a significantly long path length in the rats as observed in rats with the second profile too. The main picture of rats' navigation with this profile in the ziggurat task is a non-organized path with no focus on a specific central or peripheral way. Two HPC damaged rats showed this profile of cognitive impairment. Finally, panel D in figure 26 shows a different profile of cognitive deficit associated with low error, high latency and low speed (Figure 26, D1 and D2). This profile presents a type of cognitive disturbance covered by a motivational decline in which rats are not motivated enough to navigate in the environment although they are on the same food-restriction program. Rats with this profile usually show a short path length compared to the rats with the second and third profiles. Only one HPC animal in the present study showed this profile of spatial navigation in ZT.

Returns and path perseveration: Typically navigation within the ZT is a series of excursions (focused journeys taken for curiosity) and/or straight path tracks. Depending upon their location in the task and during the goal-directed movement, rats sometimes will show a relatively predictable profile of returns and path perseveration (Figure 27) by which they will select a different pathway in order to accomplish the task. *Returns* refer to the act of going or coming back mostly during the goal-directed navigation until the animal found the goal ziggurat. *Path perseveration*, on the other hand, means an uncontrollable repetition of the particular returns in given direction(s). For a normal rat in the ZT,

this profile comprises a small number of the returns in the first trials and days, an increased amount of returns in the middle trials and days, and a decreased number of returns in the last trials and days. These returns were characterized by several stops and by going back to a previous location along the same path (in-line return) or going back to a new path (out-line return). Examination of the number of the returns for both groups presented further information about spatial navigation in the ZT. An ANOVA conducted for the number of returns over 10 testing days showed a significant main effect of group (F (1, 11) = 3.29, $p < 0.01$) and a significant main effect of day (F (18, 311) = 9.92, $p < 0.01$), but no significant interactive effect of group by day (F (1,311) = 0.48, $p > 0.71$). As it can be seen in Figure 28, rats with widespread damage in the hippocampus had more returns than controls. Only on the first testing day did the number of returns for both groups overlap.

Cued-goal environment: A summary of latency (time spent to locate the cued goal), path length, path speed and number of errors over 11 cued-goal trials is illustrated in Figure 29. There was no significant difference between control and hippocampal damaged animals to find the cued goal for the entire block with respect to the average latency (25.18 vs. 28.45), path length (2.94 vs. 3.28), path speed (13.23 vs. 13.15) and the number of errors (5.72 vs. 8).

Discussion

The spatial performance in the ZT has been measured based on several behaviours in rats. Measurements included time spent to find the goal ziggurat,

path length, path speed, errors as well as behaviours in the probe trial and in a cued-goal environment. HPC rats showed significant deficits in all measures of spatial learning and memory in the ZT (non-cued goal environment) compared to normal rats. No significant difference was found between groups in the cued-goal environment. Additionally, the ELS scale revealed different profiles of spatial navigation in two groups with respect to error, latency and speed in the ziggurat task. Our analyses demonstrated that the task is able to differentiate between normal and impaired spatial navigation induced by widespread damage in the hippocampus. Furthermore, because the HPC rats showed significant spatial deficits in the ZT, the hippocampus is a key structure in spatial learning and memory.

It has been suggested that the hippocampus is a critical structure for spatial behaviour in either humans or animals (Sutherland et al., 1982; Morris et al., 1982; Angeli et al., 1993; Barnea and Nottebohm, 1994; Bohbot et al., 1998; Murray et al., 1998; Whishaw and Gorny, 1999; Astur et al., 2002; Wallace and Whishaw, 2003; Clark et al., 2005). For example, on a cellular level Shi and others (2006) recently showed that spatial learning and memory deficits after whole-brain irradiation are specifically associated with changes in NMDA receptor subunits in the hippocampus. Additionally, de Hoz and others (2005) found that spatial performance was proportional to the volume of hippocampus spared and independent of whether this was unilaterally or bilaterally located.

Despite many papers establishing the dependence of spatial behaviours on the hippocampus using different tasks and different versions of a specific task for

spatial performance, researchers have become increasingly aware that some of the tasks required the hippocampus, some of which did not (see Redish, 2001 for review). Our observations showed that the rats' goal-directed behaviours in the ZT are remarkably depended on the hippocampus. Several reasons support this conclusion.

The HPC rats took significantly more time to locate the goal ziggurat than control rats and their trial-by-trial searching behaviour over 10 days also showed that the HPC group had a significant impairment in finding the goal ziggurat compared to the control group. Regardless of the nature of cognitive map (for review see Eichenbaum et al., 1994 and Redish, 1999) in the ZT, latency or time spent to find a spatial goal in a specific laboratory environment is fundamentally based on animal's ability to make a set of spatial relations between the distal (allocentric) cues and a specific goal on the cognitive map. These established relations are not subjected to alter even if the location of the goal changed (Morris, 1984). In addition, for some open-field spatial tasks (e.g. MWT) it has previously been accepted that latency might be a valid measure of the hippocampal function (Morris, 1981; Sutherland et al., 1983; Brandeis et al., 1989; Benhamou et al., 1996; Bures et al., 1997; Good and Honey, 1997; Richmond et al., 1999; Bannerman et al., 1999; Clark et al., 2005) although several authors have recommended the use of path length (Lindner, 1997), cumulative distance to platform (Dalm, et al., 2000) and even, path directionality (Stewart and Morris, 1993) as the best indices of cognitive performance. Based on our observations, however, a combination of latency and other indices (i.e.

path speed and errors) are more informative about the rats' spatial behaviour in the ZT. Three specific issues should be considered for the latency in ZT. First, ZT is a dry-land task with a significant number of similar *reference frames* (e.g. ziggurats). Although reference frames (i.e. points, features, axes) allow an animal to efficiently organize trajectories in an environment (see Sutherland and Hamilton, 2004), their similarity may often confuse the animal to locate a specific spatial goal. Thus, being dry and having similar reference frames, established ZT more attractive but more difficult for animals to navigate than the other dry and wet-land tasks. These features may increase latency in ZT, even in normal rats. Second, like other dry-land tasks, ZT increases the likelihood of odor trail interference, which may enable animals to use a non-spatial navigation or odor-based strategy to locate the goal ziggurat, despite the absence of proximal visual cues. Third, our observations show that few rats rely on response chains and general algorithms (by choosing the same route) when they spatially navigate in the task and are particularly tested with peripheral goal procedure. Using some odorless foods such as spaghetti or raisin instead of banana flavored or sugar pellets to reinforce rats' behaviours and employing the peripheral-central goal or mixed procedure may often reduce the odor-based performance and prevent the effect of learning of response chains on the spatial performance in ZT.

Furthermore, path length and path speed, complementary measures of goal-directed performance in ZT showed that the HPC damaged rats not only traveled greater distances to find the goal ziggurat, but also they moved faster than rats in the control group. The greater traveled distance in the HPC rats, as it can be

observed in Figure 18, was associated with lack of a dynamic search strategy by which they are potentially able to reduce the latency in different ways. This behavioural confusion in the ZT can be probably related to their inability to switch from the spatial configuration of former goal towards a new goal prior to finding the goal ziggurat in the novel location. Both path length and speed may reflect the same cognitive deficit of navigation in ZT. Path speed in dry-land tasks, however, is probably an equivocal index of the navigation. On the one hand, it reveals cognitive element of spatial behaviour and on the other hand, it may represent the motivational component of spatial behaviour. In general, when a rat has learned the location of the goal in a spatial framework relative to different distal and/or proximal cues, he or she will typically move faster to find it in the subsequent trials than when the goal is not figured in a spatial relation. Additionally, when a rat moves very slowly in such a task and produces a long latency it probably suffers from a motivational decline rather than a spatial problem, particularly if the failure is associated with a decreased number of errors. Obviously, decreasing numbers of errors associated with a low path speed indicate that the organism is not prepared motivationally to navigate in the environment and to show its cognitive potentials. Rats with cognitive problems specifically in the ZT usually show a combination of relatively high errors and high speed, or low errors and high speed as we found in the present study. The finding that rats with widespread damage in the hippocampus move faster and demonstrate an increased tendency to explore the environment is well documented (Whishaw and Jarrard, 1995; Good and Honey, 1997; Richmond et

al., 1999; Bannerman et al., 1999). Hyperactivity after hippocampal damage is often reported for rats in activity chambers, elevated T-maze and water task. The observation that rats with widespread hippocampal damage show the same phenomenon in such open-dry spatial task is new.

Regarding to the animals' path speed in the cued environment on day twelve, the HPC rats were not significantly different from control group in the cued goal environment. Importantly, they did show hyperactivity compared to the controls in the non-cued goal environment over ten days of spatial testing. Thus, it would not be unreasonable to assume that a serious disorganization in the spatial relationship between different cues and the representation of a goal on the cognitive map may induce fast, non-focused searching behaviour in ZT. A remarkable impairment in visuomotor representation of the spatial goal may even lead the animals to show a profile of hyperactivity during spatial navigation within an open-field task such as ZT. Moving fast or hyperactivity in ZT, therefore, is probably related to the rats' cognitive disturbances, and the spatial nature of the task, and particularly to their spatial goal-directed behaviours.

Errors, however, present a distinct picture of spatial performance in the ZT. Our analysis showed that rats with HPC damage generally make more errors in the ZT relative to control rats. The observation that HPC damaged rats show significantly more errors in the ZT is on the line of those previous investigations that have shown HPC damaged rats present different types of errors in RAM and the similar dry tasks (Lopes Da Silva et al., 1986; Schacter et al., 1989; Leung et al., 1990; Ward et al., 1999; Pouzet et al., 1999). Ramos and Vaquero (2000), for

instance, showed that rats with lesions in hippocampus made significantly more errors than the control subjects in a four-arm plus-shaped maze. Theoretically, errors in the ZT are those undirected, disorganized searching behaviours, which are similar to the expected potential learned behaviour in a given trial, but they usually cause a long latency and path length. Errors provide the best index of the animals' spatial confusion. In other words, errors probably reflect the rats' failure to employ the *information* collected from self-movement, low-level sensory features, object identification and time to efficiently locate the goal ziggurat, because this information or the identity of cues usually controls the goal-directed movement or establishing the reference frames (see Sutherland and Hamilton, 2004). Errors in the ZT are defined by the behaviours such as climbing onto incorrect or non-baited ziggurats and touching the circular holes with nose. Thus, the decreasing profile of errors in HPC rats not only reflects the rats' spatial disorganization, but it may present a particular motivational status in which HPC rats lost their tendency to investigate the different ziggurats and find reinforcement after several failures. This aspect of error in hippocampal damaged rats within the ZT needs further investigation. Some one could say that errors might be a reflection of hyperactivity, but because there was no significant difference between control and HPC rats' errors in the cued environment on day twelve, it might be suggested that both increased errors and hyperactivity represent the rats' spatial confusion.

The ELS scale in the ZT may also provide an opportunity to look at the motivational processes and their role in the spatial navigation. Generally, the

principal function of the ELS scale is illustrating individual differences between hippocampal-damaged and control rats with respect to spatial and goal-directed behaviours in the ziggurat task. Furthermore, since most of brain damages may induce some motivational problems and sensorimotor deficits in one hand, and almost all dry-land tasks for spatial performance are not able quietly to separate the cognitive and motivational components of spatial navigation on the other hand, the ELS scale may be used to distinguish between the cognitive and motivational elements of spatial performance. Additionally, because different profiles of cognitive deficits in the ziggurat task may be correlated with a specific type and the extent of damage in the brain, the scale might be a valid measure for the correlational assessments of the brain impairments and their behavioural consequences.

Returns and path perseveration, on the other hand, present the dynamics of spatial performance in the ZT. At each return, rats tend to choose a new pathway in order to correct current direction toward the goal. Because each " return point " might be a " correction point ", the tendency to return or to repeat a specific path in a particular direction of the ZT may arise from the pursuit of a shift to a more promising route to the goal. Hence, the "number of returns " (and even probably the retraced segments) may reflect the "number of corrections" by which an animal employs (and revises) its pervious information regarding to the reference frames and movement control (see Sutherland and Hamilton, 2004 for review). In other words, this cognitive manipulation on the spatial information reflected by returns will drive the animal to choose a new pathway toward the spatial goal in

the context of a new spatial relation. It should be noted that the new spatial relation created or discovered by the animal is usually subjected to change (that is exhibited by the number and the type of returns) until the animal found the most effective relation. An effective spatial relation behaviourally results in low error, short latency, high speed and relatively straight trajectory toward the spatial goal in the ZT. Thus, a return to an earlier location provides the rat with an opportunity to shift its search to a new, more promising route.

The most challenging feature of the returns in the ZT, however, is that they are sometimes associated with path perseveration. Three rats in the HPC group presented path perseveration while neither of the rats in control group showed this specific profile of navigation in the ZT. Rats with this profile usually show some stereotypic paths localized in peripheral and/or central pathways with a remarkable amount of in-line returns (see Figure 27). In path perseveration, animals evidently show their failure to choose various pathways and to produce a distributed trajectory. In other words, during path perseveration they will inflexibly experience many returns in the same few pathways without shifting to more promising novel routes.

Path perseveration in the ZT, therefore, demonstrates at least three spatial failures: (1) the animal is unable to remember locations within the environment, (2) the animal has lost the use of cues that normally permit recognition of immediately sampled routes, and (3) the rat may have difficulty in actually switching or shifting to novel routes. In the present study, HPC rats seemed to be able to remember spatial locations, because they finally acquired and retrieved

the goal location in the ZT although they were significantly impaired on all functional measures.

In addition to the number of returns and path perseveration, the *type* of the returns can also be considered a distinctive index for the animal's spatial abilities. Returns in the ZT might happen in the pervious pathway (in-line return) or in a new pathway (out-line return). Although in-line returns in the ZT result shorter latency compared to out-line returns, control and HPC rats, both show the two types of the returns suggesting that the in-line and out-line returns are essential parts of the spatial navigation or goal-directed behaviour.

Noteworthy, we found of a considerable gradient in acquisition and retention in both controls and HPC rats across testing days, (Figure 21A), suggesting that rats with hippocampal damage still learned in the ZT. This latter finding is consistent with the view that extrahippocampal structures are also involved in spatial performance (Teixeira, et al., 2006; Moffat, et al., 2007). Indeed, multiple memory systems are believed to contribute to spatial memory (White and McDonald, 2002) and in some circumstances spatial learning and memory seems normal following extensive hippocampal damage (Lehmann, Clark, and Whishaw, 2007). Nevertheless, the hippocampus remains critical for optimal performance in the ZT because even on the last day of testing (day 10) the HPC rats were still impaired on all behavioural measures.

In summary, The performance by rats in the ziggurat task (ZT), a new task for spatial learning and memory, has been evaluated in the present experiment, comparing behaviour of normal rats and rats with using widespread hippocampal

damage. Rats with HPC damage revealed a significant deficit in the several measures of spatial performance compared to control group within the ziggurat task. As a dry land, the task provides an open-field environment for the rats during the spatial navigation. We also showed that the task could clearly differentiate between non-spatial (cued-goal) and spatial (non-cued goal) conditions. One of the advantages of the task is that it might be used in those experiments, which are focused on addressing the different spatial strategies of rats in a non-aversive, dry land. Several versions of the ZT (cued-goal, non-cued goal, single foil, and multi-foil environments) might be employed in this case. In other studies, the ZT has been used with some or all ziggurats in the environment. Additionally, if wet-land-task training in general increases the level of circulating corticosterone and then reduces neurogenesis in the hippocampus (Aztiria et al., 2007) even after four days of training (Mohapel et al., 2006), a dry-land task such as ZT might be a preferred choice for such investigations that are focused on spatial task training and HPC neurogenesis.

One of the main disadvantages of the device is its demands and duration so that the task requires longer time to train and test the subjects compared to the other open-field tasks such as MWT. Similar to the other dry-land tasks for spatial behaviours, its relative inability to have a complete control over the effect of odor trail interference or odor-based navigations, is another disadvantage of the ZT although under certain situations rats have been shown to use the odor-based strategies, even in wet-land tasks (D'Hooge and De Deyn, 2001).

Experiment 5: *Hippocampal Focal Stroke and Spatial Performance in Morris Water Task (MWT) and the Ziggurat Task (ZT)*

Background

The Morris water task (MWT) assesses spatial learning and memory in rodents using several measures across repeated trials, and spatial memory is assessed by preference for swimming in the area of the pool where the platform is hidden (Morris, 1984). Rats with hippocampal damage are impaired in hidden- but not in visible-platform MWT learning and memory. Interestingly, the impairment of spatial performance in hippocampal rats is linked to the volume of hippocampal damage particularly in dorsal regions. These lesions have also been shown to have more profound functional effects than ventral hippocampal lesions (Moser et al., 1993; 1995). However, testing in MWT as a gold standard for spatial performance is associated with increased activity in the LHPA axis (Engelmann et al., 2006) and consequently, elevated plasma corticosterone (Block, 1999; Aguilar-Valles et al., 2005; Mohapel et al., 2006; Aztiria et al., 2007) in rodents.

Our previous study demonstrated that the ziggurat task (ZT) is a sensitive and efficient dry task for measuring hippocampus-dependent spatial performance (Faraji et al., 2008). Several parameters, including latency to find the target, distance traveled, the number of visits to non-baited ziggurats (errors), and the number of returns were used as indices of learning and memory in rats with widespread hippocampal damage induced by N-methyl-D-aspartic acid (NMDA). Our results indicated that the NMDA injections, which produced extensive tissue

loss in all principal subfields of the HPC, caused significant impairment in spatial processes measured by the standard version of the ZT. Because no information exists about the effects of partial HPC damage on spatial performance in the ZT, the aim of this study was to determine if focal stroke in the hippocampus induced by ET-1 can cause deficits in spatial performance within both MWT and ZT. In addition, plasma CORT was measured before, during and after MWT and ZT testing as an indicator of stress level.

Material and Methods

Subjects

Twelve adult male Long-Evans rats (Control, n=6; HPC Stroke, n=6) weighing 300-370 g, raised at the Canadian Centre for Behavioural Neuroscience Vivarium at the University of Lethbridge, were used. The animals were housed in pairs under a 12:12 h light/dark cycle with light starting at 07:30 h and temperature set at 22°C. All testing and training was performed during the light phase of the cycle at the same time of day. Rats were food-restricted prior to behavioural training and testing in the ZT, and maintained at about 85% of their initial body weight throughout the experiment. The animals received water *ad libitum*. All procedures were approved by the University of Lethbridge Animal Care Committee in compliance with the guidelines of the Canadian Council on Animal Care.

Surgery

Animals in the HPC focal stroke group received hippocampal injections of ET-1, and the surgical procedures used were identical to those described in Experiment 2.

Blood samples

Blood sample procedures used were identical to those described in Experiment 1 with the exception that blood samples were taken at baseline, and on days 6 and 10 of MWT and the ZT testing.

Apparatus: Morris water task (MWT) and ziggurat task (ZT)

Morris water task (MWT): The MWT procedures used were identical to those described in Experiment 1.

Ziggurat task (ZT): The ZT procedures used were identical to those described in Experiment 4 with the exception that only the standard version of the ZT has been employed for assessing spatial behaviour in the present experiment.

Histology

All animals were sacrificed by an overdose of sodium pentobarbital (100 mg/kg i.p.) and perfused transcardially with 0.9% phosphate buffered saline followed by 4% paraformaldehyde. Each brain was removed from the skull and stored in 30% sucrose-formalin solution. The brains were then dissected out and

40_m coronal sections were cut on a cryostat microtome. Every forth section was mounted on glass slides and stained with cresyl violet. The stained sections were examined under a microscope (Zeiss, Germany) and images were captured using an AxioCam camera (Zeiss, Germany) to quantify the extent of the lesions. The amount of hippocampal lesion in each ischemic rat was estimated according to the Cavalieri method (Schmitz and Hof, 2005). This method allows for volume estimation based on the integration of the areas of a defined reference space. In practice, the Cavalieri method requires an initial random cut through the reference space of interest, with subsequent cuts at consistent intervals, i.e., systematic-uniform-random sampling. In this Experiment, five images were captured, corresponding approximately to –2.3, –3.3, –4.3, –5.3 and –6.3 mm relative to bregma. After capturing an image of each section under 1× and 10× magnification, a systematic sampling grid with an area per point of 20,000 pixels was randomly thrown over each image and the number of points hitting intact hippocampal tissue were counted. Grids were generated using imageJ software (http://rsb.info.nih.gov/ij/). The total number of hits in each rat was then divided by the average number of hits obtained by three control rats. The complement proportion was used as the percentage hippocampal lesion estimate (Lehmann et al., 2007).

Results

Histological results

Figures 30 and 31 illustrate the amount of ET-1 damage to the hippocampus in the HPC stroke group. ET-1 produced tissue loss in the dorsal and ventral areas of the hippocampus in all rats of the ischemic group. While damage to the dorsal hippocampus was mostly limited to the CA1, the DG, the hilus and the proximal part of CA3, the extent of tissue loss in the ventral hippocampus was mainly restricted to the CA1 and CA2 fields. No damage was observed in the ventral DG. An independent samples *t*-test conducted on the percent tissue loss in the dorsal and ventral hippocampus indicated significant difference between groups (Dorsal: $t = 1.82$, $p < 0.05$; Ventral: $t = 5.19$, $p < 0.05$).

CORT levels

Figure 32 illustrates circulating levels of CORT as assessed from blood samples. Blood samples were assayed for levels of circulating CORT at baseline (a day prior to the MWT and ZT testing), days 6th and 10th of the testing. As shown in Figure 32 (A), both groups that received MWT testing showed elevated levels of CORT on day 6. ANOVA showed no significant main effect of group ($p > 0.53$) and day ($p > 0.61$) and group by day interaction ($p > 0.92$). Dependent samples *t*-test conducted for baseline and day 6 in each group showed a significant difference between baseline and day 6th in both groups (control, $t= 8.33$, $p < 0.05$; HPC stroke, $t = 3.28$, $p < 0.05$) indicating that the MWT was clearly stressful at the acute time point of testing. Both groups dropped back to almost the baseline concentration of CORT on day 10th. Panel B in Figure 32,

however, shows a different profile of CORT response to the ZT testing. Both control and HPC groups equally showed a decreased CORT concentration on days 6th and 10th. No significant effect of group ($p > 0.59$; ANOVA), day ($p > 0.53$, ANOVA) and group by day ($p > 0.96$, ANOVA) was found in the CORT response to the ZT testing. Figure 32 (C) compares the CORT concentrations in the MWT and ZT. Both groups responded to the MWT by a significantly elevated level of CORT at both early and later times relative to the ZT (day 6^{th}: $t = 11.83$, $p < 0.05$; day 10^{th}: $t = 9.06$, $p < 0.05$; independent samples *t*-test). Taken together, the results show that MWT is a stressful task when compared to the ZT, and that the stressful effect of MWT is reflected more at the early time point (day 6^{th}) than the later time point (day 10^{th}) of MWT testing.

Behavioural results

Latency: Figure 33 shows the average time to find the hidden platform in the MWT (A) and the goal ziggurat in the ZT (B) for both groups over the acquisition (learning) and retrieving (memory) days. A repeated measure ANOVA conducted for the latencies over 80 trials of MWT and the ZT testing revealed a significant main effect of group ($F = 6.19$, $p < 0.05$, MWT; $F = 13.66$, $p < 0.05$, ZT), platform location ($F = 11.87$, $p < 0.05$, MWT), trial ($F = 3.51$, $p < 0.05$, MWT) and day ($F = 3.51$, $p < 0.05$, MWT; $F = 7.07$, $p < 0.05$, ZT). The interaction effects of group by platform location ($F = 11.62$, $p < 0.05$), group by trial ($F = 12.49$, $p < 0.05$) and group by day ($F = 12.89$, $p < 0.05$) in MWT were significant. In addition, a comparison between last trial of different-platform and first trial of same-platform

days by dependent samples *t*-test for MWT testing showed no significant difference in both groups ($p > 0.05$). However, a significant difference between first and second trials on different-platform days was found in control ($t = 1.66$, $p < 0.05$) but not in HPC group ($p > 0.05$).

Path speed: Path speed during acquisition in the MWT and the ZT is presented in Figure 34 (A and B). Both groups showed relatively constant speeds across the 10 testing days in MWT and the ZT. No significant main effect of group was found in the MWT ($p > 0.66$) and the ZT ($p > 0.78$). Therefore, the observed effects on the latency within both tasks likely are related to the cognitive consequences of the ET-1-induced ischemia in the hippocampus.

Probe trial: The percentage time spent in the testing and opposite quadrants of MWT and the ZT during the probe trial is depicted in Figure 35 (A and B). Analysis of the 60 s of the probe performance in both tasks revealed that rats in the control group spent a considerable proportion of their time searching in the target quadrant, relative to the HPC group that did not acquire or retain a strong bias for the previous location of the hidden platform or goal ziggurat. An independent samples *t*-test comparing the two groups in the target quadrant showed a significant difference (MWT, $t = 8.19$; $p < 0.05$; ZT, $t = 4.77$; $p < 0.05$).

Discussion

The results of the present experiment support our prediction that rats with partial damage in the hippocampus show significant impairment in both wet and dry lands (MWT & ZT) for spatial performance. More specifically, in Experiment 4

we previously found that the ZT is not only sensitive to widespread damage in the hippocampus, this experiment shows that spatial deficits in rats with hippocampal partial damage may be also shown in the ZT. We additionally, reconfirm that the MWT is a stressful task disclosed by significantly elevated plasma CORT when compared to a dry land such as the ZT.

Spatial performance in the MWT has been shown to be impaired after hippocampal lesions in many studies (Morris et al., 1982; Sutherland et al., 1982; Barnea & Nottebohm, 1994; Bohbot et al., 1998; Murray et al., 1998; Whishaw and Gorny, 1999; Astur et al., 2002; Wallace and Whishaw, 2003). Spatial function also was impaired in the ZT after complete hippocampal damage (Faraji et al., 2008).

Here we show that the ZT task is also sensitive to ET-1-induced partial damage in the hippocampus; a procedure that induces hippocampal focal ischemia. Because the observed damage mostly occurred in the DG in the dorsal hippocampus, our behavioural results in the ZT are in the line with the finding that DG, particularly in the dorsal hippocampus is involved in spatial function (Gothard et al., 2001; Cimadevilla et al., 2005; Potvin et al., 2007).

We have confirmed that the MWT is a stressful task as indicated by plasma concentrations of CORT. This is a particularly crucial issue when researchers plan to evaluate the involvement of the LHPA system in stress-related alterations of spatial learning and/or memory processes. Our results showing the increased CORT in the MWT are consistent with previous findings (Engelmann et al., 2006). Because neurendocrinological changes during the MWT testing may

intervene in spatial processes, a non-stressful task for spatial performance helps to prevent, or at least reduce this kind of interference. Our results in this experiment indicate that the ZT testing can provide a non-stressful opportunity (compared to MWT) for animal in which the organization of the task and its procedural demands are not associated with increased activity of the LHPA axis and increased concentration of CORT.

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Experiment 6: *A Characterization of Spatial Performance by Male and Female Long-Evans Rats in MWT and the ZT*

Background

Spatial performance is generally based on the ability to encode, store and retrieve mainly visual information regarding route navigation and object locations (Postma et al., 2004). Sex is a major variable that appears to be a source of individual differences in spatial function. Studies investigating sex differences in spatial function have provided some of the most reliable findings in psychological research. Substantial evidence in human studies suggests that men outperform women in spatial navigation tasks (Sandstrom et al., 1998; Saucier et al., 2002; Driscoll et al., 2005). Furthermore, Astur et al. (1998) showed that male and female humans display robust and reliable differences in spatial performance within a virtual Morris water task (vMWT).

In animal research, however, sex differences in spatial tasks are not consistently found across studies. Generally, spatial performance in rodents such as rats is assessed in MWT (Morris, 1981) and radial arm maze (RAM; Olton and Samuelson, 1976). The MWT, as originally described, consists of a circular swimming pool filled with opaque water in which a small platform is hidden. The rats will use the topographical relationship between distal cues and pool wall in

order to locate the hidden platform and escape from water. Although some studies employing MWT show superior performance by male rats compared to females (Perrot-Sinal et al., 1996; Roof and Stein, 1999; Saucier et al., 2007), no sex differences were found in others (Bucci et al., 1995; Voikar et al., 2001). Similarly, in the RAM, in which food-restricted rats learn the location of food in baited arms, inconsistent findings concerning sex differences have been reported (Einon, 1980; Juraska et al., 1984; van Haaren et al., 1987; see also Astur et al., 2004; Bimonte et al., 2000). Generally, sex differences in spatial performance stems, at least in part, from organizational effects of sex hormones (Burkitt et al., 2007), age (Bucci et al., 1995), diet type (Endo et al., 1994), rearing conditions (Juraska et al., 1984), and task-dependent procedures and parameters (Roof and Stein, 1999; Rahman et al., 2005).

The present study employed the MWT and the ziggurat task (ZT; Faraji et al., 2008) in order to compare sex differences in spatial function in rats assessed in a simple open field with minimal within-maze cues and a task with abundant within-maze cues. Structural differences (e.g. physical features, and task demands and procedures) between water-based and dry land tasks (Hodges, 1996) can be potential reasons for sex differences in spatial behaviour in the MWT and ZT. For instance, although search-to-platform area determines the degree of reliance on spatial versus non-spatial strategies in both arenas, MWT requires rats to swim toward a submerged escape platform in an open field with minimum landmarks. In the ZT, however, rats are required to navigate from start locations of a dry open arena to locate a baited ziggurat. Compared to MWT, the

ZT is an environment with more landmarks and complex route system. In humans, it has been previously shown that male superiority in spatial function (for example, on a map) may be attributable to sexually dimorphic route description (MacFadden et al., 2003). For this, we predicted that males outperform females on the ZT due to sex differences in route learning and the complex route system of the ZT.

These features (e.g. abundant within-maze cues and complex route system) of the ZT may provide a complex environment for spatial navigation in which goal-directed behaviour is dependent upon how and what *strategies* are utilized by rats to accomplish a given trial in the task. The task-dependent nature of spatial behaviour in the present study refers to the effect of the organizational differences between MWT and ZT on spatial function of male and female rats.

Therefore, two major reasons have been specifically considered to select the ZT in the present study. First, the task is a new dry-land task designed to evaluate spatial behaviour in rats, and is known to be sensitive to damage to the hippocampus, a sexually dimorphic structure in rats related to cognition. Second that the ZT is an open and dry-land task with distinctive structural characteristics, and likely with different cognitive demands than the MWT. The results in the present study indicate that male and female rats use different strategies to perform in these two tasks, and that males outperform females in ZT testing. Males' superiority in spatial behaviour in the ZT, not MWT reflects the effect of task-dependent demands and procedures on spatial performance.

Material and Methods

Subjects

Six adult male and six adult female Long-Evans rats (5 months of age), weighing 320-400 g, raised at the Canadian Centre for Behavioural Neuroscience Vivarium at the University of Lethbridge, were used. The animals were housed in pairs under a 12:12 h light/dark cycle with light starting at 07:30 h and temperature set at 22°C. All testing and training was performed during the light phase of the cycle at the same time of day. The animals received water *ad libitum*. Animals were food-restricted prior to baseline training and testing in the ZT, and maintained at about 85% of their free-feeding body weight. To maintain body weight, rats were given an additional amount of food in their home cage at least 3 h after completion of the behavioural training and testing. All procedures were approved by the University of Lethbridge Animal Care Committee.

Apparatus

Morris water task (MWT): The MWT procedures used were identical to those described in Experiment 1 with the exception that animals were tested in 8 trials per day for 8 consecutive days of training.

Ziggurat task (ZT): The ZT procedures used were identical to those described in Experiment 2 with the exception that the animals navigated in the environment for 60 sec or until they found the goal ziggurat, and that the testing sessions were conducted over 14 days (9 days for spatial and 5 days for non-spatial performance). The movements of the animals in the both environments

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 (Travel Mate 225X; Figure 36).

In addition, because performance in the ZT may be affected by deficits in visual acuity, motor function, or some other non-cognitive factors, rather than a spatial learning and/or memory impairment *per se*, rats were tested in a cued version of the ZT that did not require learning and remembering a location. Specifically, on day 10, an 8-trial block was completed by all animals in a cued goal configuration of the task. For this, a goal ziggurat covered with black tape was placed at a position not used during the former sessions. In the cued version of ZT, rats learn that only black ziggurat has food. However, in single foil testing or *matching to sample version* of ZT that had two black ziggurats, rats were required to learn and remember only one black ziggurat was true goal, and thus had food. The second black ziggurat was not baited. Rats were tested in the environment for two days; one for learning and the other for memory (Days 11 and 12). Finally, an additional two-days testing of the *multi-foil version* of ZT was conducted on days 13 and 14. In the multi-foil variation of the ZT, only half the ziggurats were black and the animals had to learn and remember that only one black or white ziggurat had food.

Results

Six behavioural indices, the latency, path length, path speed, percentage of time spent in target quadrant on the probe trial day, errors and returns have been averaged and analyzed for each odd (learning) and even (memory) day. Repeated measures analysis of variance (ANOVA) was conducted with day as a within-subjects variable, and group (males and females) as a between-subjects variable for each of the dependent measures. Latency, path length, path speed, percent time spent in the target quadrant, errors and returns served as the dependent variables.

Behavioural results

Morris Water Task

Results showing all behavioural measures including latency to reach the hidden platform, path (swim) length, path speed and probe trial performance for both male and female rats in the MWT over the different- and same-platform days are depicted in Figure 37, Panels A-D.

Latency: Inspection of Panel A reveals that latencies to locate the hidden platform in MWT dropped from day 1 to 8. The latency by the rats in both groups decreased over 8 days of testing in the MWT suggesting that all rats, regardless of their gender were able to acquire and retrieve the spatial information in a similar rate. Repeated measure ANOVA showed a significant effect of Day (F (1, 3) = 2.09, $p < 0.05$), Platform Location (F (1, 1) = 7.18, $p < 0.05$) and Trial (F (1, 7) = 12.68, $p < 0.05$), but no effect of Group ($p > 0.05$). Tests of within-subjects

effects indicated a significant interaction between Groups and Platform Location (p = 0.66), Groups and Day (p = 0.57), and Groups and Trial (p = 0.86). In addition, a comparison between last trial of different-platform and first trial of same-platform days by dependent samples t-test showed no significant difference in males and females ($p > 0.05$). Moreover, no significant difference between first and second trials on different-platform days was found in both sexes ($p > 0.05$).

Path length: Examination of acquisition in terms of path length (distance traveled) to locate the hidden platform also revealed that males and females took progressively shorter move paths to find the platform as training proceeded (Figure 37 B). Mean path lengths (in meter) on the first and last day of testing were as follows: Males, 9.26 ± 1.02 and Females, 10.33 ± 0.93 (first day) and males, 1.63 ± 0.24 and females, 1.74 ± 0.22 (last day). No significant difference was found in the path length by groups (F (1, 36) = 1.19, $p > 0.05$).

Path speed: Examination of path speed during acquisition, however, showed that females had a constantly faster navigation in the environment than males as testing proceeded (Figure 37 C). Additionally, except on days 1 and 2, both groups showed a relatively flat speed line across 8 testing days in the MWT. ANOVA showed no significant difference between male and female rats in path speed within the MWT (F (1, 36) = 2.18, $p > 0.05$).

Probe trial: Results showing the percentage of time spent in each quadrant during the first half the probe trial (30-s duration) are depicted in Figure 37 D. Rats in the both groups showed similar preference to spend time in the target

quadrant (quadrant two; SE) within the MWT. No significant difference was found in the percentage time spent in the target quadrant between groups (F (1,11) = 8.56, $p > 0.05$).

Ziggurat Task

Spatial performance

The behavioural measures in the ZT, however, revealed a different profile of spatial performance for male and female rats. Figure 38 shows the mean latencies during testing (A), path length (B), path speed (C) and the percentage of time male and female rats spent in each quadrant (D) of the ZT for spatial function.

Latency: Inspection of Panel A reveals that male rats located the goal ziggurat more quickly than female rats. Latencies to locate the goal ziggurat dropped from day 1 to 8 in both groups. An ANOVA conducted for the latencies over the 64 trials of ziggurat-task testing revealed a significant main effect of group, (F (1,11) = 11.42, $p < 0.05$) and day, (F (1,16) = 2.83, $p < 0.05$), but no significant effect of group by day was observed (F (2,32) = 9.17, $p > 0.61$).

Path length: Panel B shows that females traveled more than males to investigate the goal ziggurat in the ZT. Mean path lengths (in meter) on the first and last day of ZT testing were as follows: Males, 3.92 ± 0.48 and Females, 6.3 ± 0.44 (first day) and males, 2.45 ± 0.44 and females, 5.65 ± 0.46 (last day). An ANOVA conducted for the path length over 8 testing days showed a significant main effect of group (F (1,11) = 6.91, $p < 0.05$) and a significant main effect of

day (F (1,16) = 3.33, p < 0.05), but no significant interactive effect of group by day (F (2,32) = 10.19, p > 0.87).

Path speed: Examination of path speed during acquisition presented a further consequence of sex difference in the ZT. Although both groups were moved slower in the ZT than MWT, as it was almost the case for the animals' speed in the MWT female rats moved consistently faster than rats in male group within the ZT (Panel C). An ANOVA showed a significant main effect of group, (F (1,11) = 2.12, p < 0.05) and a significant main effect of day (F (1,16) = 7.44, p < 0.05). No interaction was found between these factors (F (2,32) = 2.83, p > 0.58). Although an increased path speed may provide a major contribution to a reduced latency, our results show that it cannot be precluded the possibility that rats in faster group (here females) will necessarily perform more accurately because their latency to find the goal ziggurat reflected a poor spatial function compared to males in the ZT.

Probe trial: Panel D shows the percentage time spent in the testing and opposite quadrants of the ziggurat environment during the second probe trial. Like MWT, spatial memory in the ZT was indicated by significantly greater search time in training compared to the opposite quadrant of the task. Analysis of the first half of the probe trial (30-s duration) revealed that rats in the male group spent a considerable proportion of their time (46.4% ± 3.16) searching in the quadrant of the ZT in which the goal ziggurat had previously been baited. The profile of time spending at the different quadrants and target quadrant for female rats (28.19% ± 3.49) in the probe trial, however, was significantly different (F

(1,11) = 5.06, $p < 0.05$) suggesting that they exhibited a more diffuse pattern of searching, with much less spatial bias toward the former training quadrant. Independent sample *t*-test comparing the two groups only in the target quadrant showed a significant difference ($t = 2.39$; $p < 0.01$).

Errors: Examination of acquisition in terms of the number of errors showed a gradual decrease in the numbers of errors of males, but not of females during spatial navigation in the task (Figure 39). Although the both groups presented an almost similar profile of errors on the first second days of spatial investigation, males produced less errors (17 errors on the first day vs. 4 errors on the last day) than did females as testing proceeded. Conversely, the female group showed more errors (13 errors on the first days vs. 37 errors on the last day) relative to males on the rest of testing days. The ANOVA showed a significant difference between groups in terms of the number of errors (F (1,102)= 8.19, p <0.05).

Returns: Results showing the number of returns are depicted in Figure 40. Although the profile of returns in both groups shows that they were involved in some return-based investigation even on the last days of testing in the same pattern, the number of returns in female group was significantly more than male group suggesting that the spatial investigation or goal-directed navigation in the ZT for females was much more difficult than males. An ANOVA conducted for the number of returns over 8 testing days showed a significant main effect of group (F (1,11) = 3.29, $p < 0.01$) and a significant main effect of day (F (1, 16) = 4.43, $p < 0.01$), but no significant interactive effect of group by day (F (2,32) = 2.83, $p > 0.05$).

Non-spatial performance

Cued-goal environment: Figure 41 shows a summary of latency in the cued version (Panel A) and a single foil or matching to sample version of ZT (Panel B). Latency in the multi-foil version of ZT in which animals must learn and remember that only one black or white ziggurat has food is also depicted in Panel C. It should be noted that the multi-foil field may measure some spatial aspects of the investigation in the ZT, but it has been employed as a non-spatial field in the current study. Inspection of the latency for the both groups revealed no systematic differences between male and female rats (p = 0.77 for cued goal; p = 0.86 for single foil; p = 0.71 for multi-foil field). Hence, our results show that sex differences between male and female rats in the spatial performance can be addressed only in the spatial variation of the ZT, and the observed differences between groups in the ZT may reveal some distinctive features of spatial function in the animals.

Discussion

The present study found that adult male and female rats show no significant difference in performance in the MWT. Specifically, male and females in this study rapidly learned the location of the hidden platform during place learning, and there was no sex difference in the latency, path length, path speed and probe trial performance. More comparative investigation with the same animals using the ZT, however, revealed that males performed significantly better than females in all indices of spatial navigation in the ZT. Males' latency,

path length and path speed in the spatial (non-cued) version of the ZT showed that they found the goal ziggurat faster than did females. In the probe trial, male rats spent significantly more time in the target quadrant of the environment. Interestingly, the same profile of sex difference in the ZT was found in the number of errors and returns. However, in navigation to the visible goals in the different cued versions of the task, there were no significant differences between males and females. This indicates that the differences observed between the two groups in locating the spatial goal ziggurat are not likely to be induced by motivational, sensory, or locomotor differences between the male and female rats. Hence, our results show that sex differences between male and female rats in the spatial performance can be addressed only in the spatial variation of the ZT, and the observed differences between groups in the ZT may reveal some distinctive features of spatial function in the animals.

In most research paradigms that are employed to investigate spatial behaviours, an animal is required to accomplish a specific task by reaching a food reward or escaping from a potentially harmful environmental stimulus. MWT and RAM are commonly used to examine the spatial function in rats. Depending upon their experimental demands and structural features, the tasks may provide some distinct results about sex differences in spatial processing. In the current study, however, we employed the MWT and the ZT to evaluate difference between male and female rats in spatial learning. The tasks do not assess spatial learning in the same manner; the MWT can reflect the rats' spatial performance

in an open, wet land, and the ZT may present a profile of their spatial processing in an environment with more landmarks and complex route system.

Extensive evidence in human and animal studies suggests that males outperform females in spatial navigation tasks. For instance, it has been recently reported that males had significantly shorter search latency than females in a virtual 8-arm RAM (Rahman and Koerting, 2008). This sexual dimorphism in spatial function has been also shown in male and female rats in MWT (Blokland et al., 2006). However, findings demonstrating male superiority in spatial behaviour in MWT (Roof, 1993; Kolb and Cioe, 1996; Kavaliers et al., 1998; Sherren et al., 1999; Doucette et al., 2007; Saucier et al., 2008) mostly relied upon latency or time measures to locate the hidden platform. This measure, however, is relatively poor indicator of learning accuracy and may be simply reflect differences in swimming speed. For this, it has been suggested that the male advantage in the spatial learning may have resulted from some procedural mistakes (Bucci et al., 1995). The performance of males and females within MWT in this study, however, is very similar to that obtained by previous studies (Gallagher et al., 1993; Bucci et al., 1995; Berger-Sweeney et al., 1995; Healy et al., 1999) showing no spatial superiority in male rats. Locomotor activity has been suggested as the main source for the observed sex difference in spatial function within the MWT in some previous animal studies, but our data in this study show that the profile of spatial memory in MWT provided by male and female rats may not be caused by different levels of locomotor activity *per se*. Moreover, the probe trial performance that is a sensitive measure for accuracy of

spatial search in the tasks such as MWT, indicated that males are not more proficient than females in place learning in the water task.

In the dry land task, however, our data for spatial behaviour revealed a large sex difference between male and female rats. A similar male superiority in spatial performance in the dry tasks has been reported in rodent species (see Williams and Meck, 1991 for review). For instance, male CD-1 mouse performed significantly better than females in the dry-land version of RAM (LaBuda et al., 2002) but not in it's wet-land version (Markowski et al., 2001). The discrepancy between the results in the ZT and MWT may be due to the distinctive task procedures; they do not assess spatial processing in the same manner. Their procedural demands reinforce different strategy selection during spatial navigation. Specifically, the ZT differs from MWT in two important ways. First, the ZT employs appetitive motivation while the MWT relies on aversive motivation. This probably provides a specific motivational condition to the animals when they navigate in the tasks. Second, it seems that wet-land tasks such as the MWT impose a stressful situation on the animals (Block, 1999; Aguilar-Valles et al., 2005) that induce a particular sustained emotional condition compared to the dry-land tasks like the ZT. These procedural issues should be specifically considered when one investigates sex difference in spatial navigation within wet and dry fields, because sex-related difference in the tasks may be reflected either by the effect of reward strategies on rats' spatial navigation or the rats' emotional conditions in the environment. Hence, the often-cited male advantage in spatial

processing appears strongly task-dependent and specific to the psychological and cognitive processes involved in different tasks.

On the other hand, despite the task-dependent nature of sexual dimorphism in spatial cognition in this study, one can speculate that the difference observed in the profile of spatial function in the tasks may be related to neuroanatomic differences that might particularly support spatial navigation in male subjects. For example, sex-specific patterns of spatial function and hippocampal size are positively correlated (Jacobs et al., 1990). Furthermore, it has been previously noted that the sex differences are correlated with a difference in the dentate gyrus of the hippocampus, and better spatial performance is strongly correlated with an increased size of the dentate gyrus cell layers (Roof and Havens, 1992). Because the integrity of the dentate gyrus represents a crucial factor for normal spatial processing in rats (Gallagher and Holland, 1992; Gothard et al., 2001), it would not be unreasonable to assume that sex differences, at least in some tasks, may be attributable to differences in the organization of the hippocampus, a structure intimately involved in spatial function. It has been suggested that evolution gives an adaptive value to these differences in behaviour and their neural substrates (Jacobs et al., 1990).

The distinctive structure of the ZT may also disclose another feature of the correlation between the neuronal organization and different spatial capabilities in male and female rats. As noted previously (Faraji et al., 2008), the ZT represents an open field arena with several ziggurats and routes, and the animal is required to (1) navigate within the environment, (2) direct his/her search toward the goal

ziggurat, and (3) form visuospatial-memory traces of the field and its objects based on the room–centered and body-centered representations. It has been suggested that the room-centered representations are integrated mainly in the hippocampus and the body-centered representations are encoded in the posterior parietal cortex (Gron et al., 2000). On the other hand, unlike MWT, animals' navigation within the ZT is likely to involve more complex processing of routes and landmarks. Moreover, for a successful navigation in the ZT, compared to MWT, rats should be more able to synthesize and organize separate elements perceived in the environment into multiple routes. These task-specific demands require an accurate judgment of direction, orientation and distance. Therefore, the sex difference in performance in ZT, probably reflects a substantial difference in (1) the route-learning strategies or the use of egocentric information to control the movement toward the goal in the environment and allocentric strategies to construct a memory of spatial location, and (2) the ability for visuospatial scanning and tracking in a complex navigation.

In conclusion, the results of the current study demonstrated the better performance in male compared to female rats in spatial memory within the ZT. No significant difference has been found between the same animals in MWT. This variation appears to be more dependent on the task-related procedures and reconfirm the hypothesis of task-dependent sexual dimorphism in spatial cognitive processing.

Concluding remarks

Morris water task by itself represents a stressor. This is mostly related to the physical demands of the task (i.e. the immersion of the animals into the water). If the hyperactivity of the limbic-hypothalamo-pituitary-adrenal (LHPA) axis results in releasing CORT in rats, and if the MWT testing is associated with significant activation of the LHPA system, a dry environment may reduce the task-specific neuroendocrinological changes. The ziggurat task (ZT) is a sensitive task for measuring spatial performance in rats with either complete or partial HPC damage. In addition to its potentials to present a clear profile of spatial learning and/or memory, the ZT provides an additional measure of the rats' motivational condition during the spatial navigation. Male and female rats have been shown to be involved in different visuo-spatial strategies and consequently show significant difference in spatial performance within the ZT, while in the MWT they do not. This task-specific picture of spatial function is likely due to the physical organization of the ZT.

Now that we have established that both complete and partial damages in the hippocampus may be measured by the ZT as a non-stressful task (compared to MWT) for spatial performance, we can proceed with assessment of hippocampal alterations after ET-1-induced focal ischemia and stress or CORT treatment.

7. Study 3:
Hippocampal Focal Stroke, Stress, Corticosterone and Spatial Performance

The data in Studies 1 and 2 indicate that rats with hippocampal focal stroke induced by ET-1 show significant functional deficits in both the MWT and ZT. Spatial performance after hippocampal stroke was significantly improved by chronic restraint stress paradigm. In the present study, however, we evaluated the effects of stress and exogenous corticosterone treatment administered after ET-1-induced focal stroke in the hippocampus. Structural and functional effects of corticosterone and stress treatment prior to the focal ischemia have also been investigated.

Modified from a paper submitted to *Brain Research*, by Jamshid Faraji, Hugo Lehmann, Gerlinde Metz and Robert J. Sutherland in June 25th, 2008. Ms. No.: BRES-D-08-01104.

Experiment 7: *Hippocampal Post-stroke Recovery Induced by Stress and Corticosterone*

Background

As a suitable model for focal stroke in the hippocampus, intrahippocampal injection of ET-1 has been recently employed to investigate the functional and structural outcomes of ischemic damages. Mateffyova et al. (2006), for instance, showed that injection of ET-1 into the hippocampus of immature rats results in neuronal death, epileptic seizures, and cognitive impairments later in life. Additionally, aged rats are more vulnerable to cell death and learning deficits, compared to the young rats, following the hippocampal mini-stroke induced by ET-1 (Driscoll et al., 2007). The rationale for these studies to investigate the neurobehavioural outcomes of hippocampal focal ischemia are clear: (1) strokes and the other neuropathological conditions frequently cause some learning and memory deficits, and (2) the hippocampus is a structure intimately involved in the processing, learning and storage of new information. Therefore, these recent findings may shed some light on the nature of the neurological disorders such as cerebral ischemia, stroke and possibly temporal lobe epilepsy that affect primarily the hippocampus and hippocampal-dependent cognitive functions.

The hippocampus is selected for the present stroke research for two additional reasons: the structural plasticity and the high density of adrenal steroid receptors in the hippocampus. Studies have shown that in the adult hippocampal dentate gyrus (DG) new neurons are produced. Both the number of proliferating cells and the number of cells produced in the DG are affected by a variety of stimuli including exercise, drugs, aging, hormones, stress (Hayes et al., 2002), and even stroke (Liu et al., 1998) and seizures (Parent et al., 1997). Although the biological significance of cell growth and plasticity in the hippocampus is still unknown, it has been proposed that they are recruited for purposes of hippocampal memory function (Zhang et al., 2008; Trejo et al., 2008) and brain repair (see Kuhn et al., 2001 and Kempermann, 2002, for review).

The hippocampus also represents a key structure in the stress response. There are two types of corticosteroid receptors, mineralocorticoid receptors (MR or type Ⅰ) and glucocorticoid receptors (GR or type Ⅱ) in the brain, and both receptor types are found in the hippocampus (de Kloet et al., 1999). In rats GRs, with low affinity for corticosterone, are rich in the hippocampus (McEwen and Sapolsky, 1995) and fully activated when corticosteroid levels rise significantly, for instance, after stressful experiences (Lupien et al., 2005). Evidence suggests that hippocampal function is extremely sensitive to stress and its hormonal consequences. Despite the disruptive effects of corticosteroid hormones (e.g. corticosterone) on the hippocampus and its function following stress (Sapolsky et al., 1986; Roozendaal, 2002), it has also been suggested that adrenal hormones are associated with enhanced hippocampal plasticity (Mocchetti et al., 1996) and

memory formation (Roozendaal and McGaugh, 1996, Gina et al., 1997; Bowman et al., 2001; Buchanan and Lovallo, 2001; Beylin et al., 2003; Akirav et al., 2004). The stress-dependent enhancement view emphasizes that the physiology of memory formation and consolidation may involve stress hormones as endogenous positive modulators. Many of the primary studies hypothesized that these processes will occur through the modulation of the steroid hormones in "neurotrophic" activities (Mocchetti et al., 1996; Chao et al., 1998). However, the fundamental question of when and how stress switches its effects on cognitive performance from impairment to enhancement is still unanswered.

The hippocampus provides a useful model for studying the neurobehavioural changes after focal ischemic damages and an opportunity to investigate the hypothesis that stress and/or stress hormones may result in structural and functional protective effects following some neuropothological situations.

The present study was designed to answer the following questions: (1) Is ET-1-induced focal stroke in the hippocampus associated with the impairment of the hippocampal-dependent memory performance? (2) What is the extent and pattern of damage caused by ET-1 in the dorsal and ventral hippocampus? (3) Do stress or corticosterone affect cognitive or structure outcomes after focal hippocampal stroke? To answer these questions, the effects of focal hippocampal stroke were characterized using two spatial tasks, the Morris Water Task (Morris, 1981) and the Ziggurat Task (Faraji et al., 2008). The former task measures the rats' spatial function in a wet-land using aversive motivation,

whereas the latter task provides a dry-land environment using appetitive motivation to investigate the rats' spatial goal-directed behaviours. The extent of tissue loss after focal hippocampal stroke was measured in each rat using the Cavalieri method for volume estimation (Schmitz and Hof, 2005).

Material and Methods

Subjects

Twenty-seven adult male Long-Evans rats, weighing 350-420 g, raised at the Canadian Centre for Behavioural Neuroscience Vivarium at the University of Lethbridge, were used. The animals were housed in pairs under a 12:12 h light/dark cycle with light starting at 07:30 h and temperature set at 22°C. All testing and training was performed during the light phase of the cycle at the same time of day. The animals received water *ad libitum*. Animals were food-restricted prior to baseline training and testing in the Ziggurat Task, and maintained at about 85% of their initial body weight throughout the experiment. To maintain body weight, rats were given an additional amount of food in their home cage at least 3 h after completion of the behavioural training and testing. All procedures were approved by the University of Lethbridge Animal Care Committee in compliance with the guidelines of the Canadian Council on Animal Care.

Rats were divided into four groups: control (n= 7), ET-1 (n= 6), ET-1+ stress (n= 7), and ET-1+ corticosterone (n= 7). Rats in each ET-1 group received injections of ET-1 bilaterally into the hippocampus. In order to assess the

baseline levels of circulating CORT, all groups were subjected to blood sampling before the stress and CORT administration on day 7 following the ET-1 lesion. Rats in ET-1+ stress group were then placed in Plexiglas tubes in their home cage for 1 h/day for 16 consecutive days starting from day 8. Group ET-1+ corticosterone received CORT instead of stress. All four groups were again subjected to blood sampling on day 24 (the last day of stress or CORT treatments, respectively). Morris water task and the Ziggurat-task training and testing were performed from day 29 to day 56. Following all behavioural tests, the rats were perfused and the brains processed for histological analysis to determine lesion extent and location.

Surgery

All animals in ET-1 groups were subjected to the hippocampal injection of endothelin-1 (ET-1), and the surgical procedures used were identical to those described in Experiment 2. Rats were allowed to recover for 4-5 days before the beginning of blood sampling, restraint stress and CORT administration.

Blood samples

Blood samples were taken at baseline, the day prior to restraint stress or CORT administration. Blood sample were also taken 15 – 20 min after stress and one hour after CORT administration on the 16th day of treatment. Blood samples procedures used were identical to those described in Experiment 1.

Restraint stress

For restraint stress, the animals in the group ET-1+stress were maintained in a transparent Plexiglas tube (6 cm inner diameter) of adjustable length, from 10:30 am to 11:30 am for 16 consecutive days. The stress procedure used was identical to those described in Experiment 1.

CORT administration

Each animal in the group ET-1+corticosterone was orally administered 0.5mg/kg CORT (Sigma-USA) daily. The CORT was mixed with 0.35 mg crushed banana-flavored pellets and one drop peanut oil between 10:30 and 11:30 am for 16 consecutive days. All rats readily consumed the mixture.

Apparatus

Morris water task (MWT): The MWT procedures used were identical to those described in Experiment 1 with the exception that animals were tested in 8 trials per day for 11 consecutive days of training.

Ziggurat task (ZT): The ZT procedures used were identical to those described in Experiment 2 with the exception that only the standard version of the ZT has been employed for assessing spatial behaviour in the present experiment. The movements of the animals (Figure 42) including latency and path speed within MWT and ZT 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).

Histology

The histology procedures used were identical to those described in Experiment 5 with the exception that five images were captured, corresponding approximately to –1.88, –2.80, –3.80, – 4.80 and –5.80 mm relative to bregma.

Statistical analysis

The statistical analysis for all experiments was performed using SPSS 11.5.0 for windows (Standard Version, 1982-2002; SPSS Inc., USA). The results were subject to analysis of variance (ANOVA) for repeated measurements across testing sessions. Comparison means between groups were performed using independent samples *t*-tests, and dependent samples *t*-tests for within-subject comparison. In all statistical analyses, a p-value of less than 0.05 was chosen as the significance level. All data are presented as mean +/- standard error.

Results

Histological results

Figures 43 and 44 illustrate the amount of ET-1 damage to the hippocampus in the ET-1 group and ET-1+corticosterone/stress groups. In addition, Figure 45 describes the average amount of damage in each ischemic group. ET-1 produced tissue loss in the dorsal and ventral areas of the hippocampus in all rats of the ischemic groups. Damage to the dorsal hippocampus in the ET-1-only group was more extensive than in the other two

ischemic groups, including substantial tissue loss in the CA1 field and dentate gyrus (DG). Damage to the dorsal hippocampus in the ET-1+ corticosterone and ET-1+stress groups was very small and predominantly limited to the immediate location of the ET-1 injection. Hence, it seems that corticosterone treatment and stress caused less tissue damage after ET-1 administration in the dorsal hippocampus.

The extent of damage to the ventral hippocampus was more consistent across groups. All the ischemic groups had substantial tissue loss in the CA1 and CA3 fields. No damage was observed in DG. An ANOVA conducted on the percent tissue loss in the dorsal hippocampus indicated a significant effect of group ($F = 6.11$, $p < 0.05$). *Post hoc* comparisons showed that the ET-1-only group had significantly more hippocampal damage than both the ET-1+corticosterone+stress groups ($p < 0.05$). No significant differences were found between ischemic corticosterone and ischemic stressed groups for the dorsal hippocampal damage ($p > 0.05$). In the ventral hippocampus, the amount of damage did not significantly differ amongst ischemic groups ($p = 0.63$).

In sum, the severity of the ET-1-induced hippocampal damage in the ET-1-only group was higher than in ET-1+corticosterone/stress groups. Most tissue damage observed in ET-1 group occurred in the dorsal hippocampus CA3 and DG. The beneficial effect of stress and corticosterone administration on ET-1-induced damage was observed only in the dorsal hippocampus.

CORT levels

Figure 46 illustrates circulating levels of CORT as assessed from blood samples. Blood samples were assayed for levels of circulating CORT at baseline (a day prior to restraint stress or CORT administration) and at post-treatment point (15-20 minutes after stress and 1 hour following CORT treatment on day 16). As shown in Figure 46, rats that received daily oral CORT and restraint stress following HPC stroke, showed an elevated levels of CORT. Restraint stress caused more changes in circulating CORT than oral administration of CORT (t = 3.69, p < 0.05). There was a significant difference between the baseline and chronic points in the both groups (t = 2.18, p < 0.05 for ET-1+corticosterone; t = 1.39, p < 0.01 for ET-1+stress).

Behavioural results

Two behavioural indices, the latency and path speed in the MWT and the ZT were analyzed for each odd (learning) and even (memory) day. Repeated measures ANOVA were conducted with day as a within-subjects variable, and treatment group (control, ET-1, ET-1+corticosterone, and ET-1+stress) as a between-subjects variable for each of the dependent measures. Latency and speed of spatial navigation in both areas served as the dependent variables.

Latency: Figure 47 shows the average time spent to find the hidden platform in the MWT (A) and the goal ziggurat in the ZT (B) for all groups over the acquisition (learning) and retrieving (memory) days. A repeated measure ANOVA conducted for the latencies over 80 trials of MWT and the ZT testing

revealed a significant main effect of group (F = 3.28, p < 0.05, MWT; F = 9.06, p < 0.05, ZT) and day (F = 8.32, p < 0.05, MWT; F = 5.14, p < 0.05, ZT). Specifically, in MWT no significant effect of Trial (p > 0.05), and no interaction effect of Group by Day (p > 0.1) and Group by Platform Location (p > 0.61; MWT) was found. The interaction between Groups by Trial, however, was significant (F = 1.56, p < 0.05, MWT). In addition in both tasks the latencies on the odd days (when the platform or goal ziggurat moved to a new location) were higher than the even days (when the platform or goal ziggurat remained in the same location as the pervious day) for all groups. The ET-1-only group in the both tasks, however, had significantly higher latencies compared to the other ischemic groups and controls (F = 2.19, p < 0.05, MWT; F = 1.44, p < 0.05, ZT). No significant difference was found in the latency by the ET-1+corticosterone and ET-1+stress groups within MWT and the ZT (p = 0.83, p = 0.66 respectively). The latency by the rats in all groups decreased over 10 days of testing in both dry and wet task, suggesting that regardless of their experimental situation all rats were able to acquire and retrieve the spatial information in a similar rate. A comparison between last trial of different-platform and first trial of same-platform days by dependent samples t-test for MWT testing showed a significant difference only in the ET-1 + corticosterone group. Dependent samples t-test also showed no significant difference between first and second trials on different-platform days in MWT in any group (p > 0.05).

Path Speed: Path speed during acquisition presented different profiles in the MWT and the ZT (Figure 48). Although all groups showed relatively constant

speeds across the 10 testing days in MWT, the speeds progressively increased in the ZT. A post-hoc ANOVA for multiple comparisons showed that rats in the ET-1+corticosterone group moved significantly faster than the other groups, but only in the MWT ($p < 0.05$). No significant main effect of group was found in the ZT ($F = 0.36$ $p > 0.61$). An ANOVA also indicated a significant main effect of day ($F = 3.12$, $p < 0.05$) and group by day interaction ($F = 9.55$, $p < 0.05$). No other significant differences emerged.

Discussion

The current findings demonstrate that ET-1-induced stroke in the hippocampus impairs spatial learning and memory in two tasks. Chronic stress or CORT administration reduced the severity of these symptoms after stroke. Indeed, our results show that the partial ischemia induced in the hippocampus by ET-1 had significant structural effects. In the ET-1-only group, rats showed clear structural damage in the hippocampus that was accompanied by a significant decline of cognitive performance in the spatial tasks. The most dramatic structural consequences in the dorsal hippocampus occurred in the CA1 and DG areas. In the ventral hippocampus, however, mostly the area CA1 was affected by damage following the ET-1 injection. Particularly the ET-1-only group exhibited significant spatial memory deficits compared to the other groups in both wet and dry spatial tasks. Our results also revealed that the ET-1+corticosterone and ET-1+stress groups performed significantly better than the ET-1-only group in both the MWT and ZT. Hippocampal volumetric assessment showed that the

post-stroke stress and corticosteroid treatment significantly decreased the amount of damage in the dorsal CA1 and DG regions of the hippocampus. Taken together, these findings suggest that hippocampal partial stroke by ET-1 can induce structural and functional deficits and that increasing corticosteroid levels alleviates the hippocampal tissue loss and related memory deficits.

ET-1-induced hippocampal ischemia

The present study utilized injections of ET-1 into the hippocampus as an analogue of ischemic stroke. ET-1 is an endogenous vasospasm-inducing peptide that has been identified as a very potent vasoconstrictor (Yanigasawa et al., 1988). The focal injection of ET-1 into targeted brain regions in rats has been suggested to be an appropriate model of human focal stroke (Gilmoure et al., 2004; Mateffyova et al., 2006). ET-1 injection was suggested to induce selective and constrained ischemic neuronal loss when compared to other non-selective procedures of rat model of stroke (Fuxe et al., 1997). Moreover, this procedure involves simpler surgical techniques and is associated with very few postsurgical complications (Biernaskie et al., 2001).

Behavioural consequences of HPC ischemia induced by ET-1

Generally, neurons in the hippocampus are highly sensitive to stroke events (Sachdev et al., 2007). This suggests that any vascular insults could affect this area leading to learning and memory deficits (Driscoll, 2007; McDonald et al., 2008). The behavioural decline following the damage occurred in the dorsal CA1

and DG, in the current experiment, supports the view that these areas play an important role in learning and memory by processing and representing spatial information (Gallagher and Holland, 1992; Conrad and Roy, 1995; Tsien et al., 1996; see also Squire et al., 2004 for review). CA1-involved spatial representation has been demonstrated in several experiments (Ferbinteanu and Shapiro, 2003; Bower et al., 2005) and it has been shown that this area and the DG clearly integrate activity during the spatial functions (Gothard et al., 2001). Consistent with the interpretation of the present results, the ET-1 ischemic damage in the hippocampus can mimic those deficits in spatial cognition that are caused by clinical strokes and are especially involved in the sub-regions of the hippocampus such as CA1 and DG.

Moreover, it is important to note that we selected path speed, in addition to latency, as a further indicator for spatial performance in our behavioural reports for a simple reason that both stress- and ischemia-induced hyperactivity are previously reported (Strekalova et al., 2005; Plamondon et al., 2008). On the other hand, latency, a traditional measure for spatial function in some spatial tasks (e.g. the MWT) is relatively poor indicator of learning accuracy and can be simply affected by differences in swimming/path speed. Our results present a distinctive profile of speed in both wet and dry lands, and are in line with finding that neither stress, nor ischemic insult can increase path speed within spatial tasks (McDonald et al., 2008). That is, the latency to locate the platform in the MWT and the goal ziggurat in the ZT showed by different groups was not affected by rats' speed during the spatial navigation.

Dentate gyrus (DG) and post-ischemic alterations by steroid hormones

The most prominent anatomical feature of the DG in the hippocampus, in addition to its key role in the spatial performance, is that this area in the hippocampus is extremely plastic (see Lledo et al., 2006 and Taupin, 2006 for review). For example, it has been suggested that the DG has the capacity for the numerous types of plasticity that use diverse mechanisms and are thought essential for the storage of information in the hippocampus (Derrick, 2007). The possibility of the cell regeneration and structural plasticity in the hippocampus especially in the DG may provide evidences demonstrating an intense biological dialogue between the nervous and endocrine systems. Little is known about the mechanisms of this interaction. Nevertheless, a great deal of research has focused on the hypothesis that the adrenal steroids might be key modulators of the structural changes in the hippocampus.

In the context of the brain structural changes and adrenal steroids, a few papers have concluded that there is no effect of stressful experiences or stress hormones on stroke outcome (Storey, 1985; Macko et al., 1996). Others have provided support of a relationship between these experiences and detrimental outcome of stroke in both human (Fassbender et al., 1994) and mice (De Vries et al., 2001; Sugo et al., 2002). For instance, these studies demonstrated increased lesion volume in animals treated with chronic stress (Sugo et al., 2002). These changes are generally attributed to the destructive effects of glucocorticoids. However, it can be speculated that adrenal steroids might also result in neuroprotective consequences in some brain structures such as the

hippocampus due to their immunosuppressive effects (Quirarte et al., 1997; Grundy et al., 2000; Abraham et al., 2000; Melcangi et al., 2000; see also Molteni et al., 2001, for review). According to this view, the increased expression of neurotrophins in the hippocampus is an adaptive reaction for structural reconstruction following acute brain insults and their restorative functions are strongly regulated by glucocorticoids (Grundy et al., 2001). These adaptive reactions are normally reported in the hippocampus after traumatic brain injuries (TBIs), cerebral strokes, epilepsy and ischemic damages (Parent and Lowenstein, 1997; Parent et al., 1997; Liu et al., 1998; Taupin, 2006; See also Wiltrout et al., 2007 for review).

Possible mechanisms underlying glucocorticoid-induced recovery in the hippocampus after ischemia

Our results show that the structural reorganization and behavioural protective effect seen in the ET-1+corticosterone/stress groups may not be attributed to only those adaptive neural reconstructions because these changes (e.g. ischemia-evoked structural reorganization) were not found in the ET-l-only group in this study. Rather, the current findings suggest that the structural and consequently the functional recovery in the ET-1+corticosterone/stress groups might be due to the possible effects of the adrenal steroids on the structure, function, and survival of the hippocampal neurons. Because the level of glucocorticoid receptor (GR) expression in the hippocampus is highest in the CA1, lowest in the CA3, and intermediate in the DG (Chao et al., 1998), these

effects of glucocorticoids on the neuronal structure and function in the hippocampus may be mediated through their action on the receptors in these areas (Abraham et al., 2000).

It should be noted that glucocorticoids play this important regulatory role in a close cooperation with basolateral amygdala (BLA) and the hippocampus (Quirarte et al., 1997) through the neurohormonal interaction between the adrenal cortical system and neurotrophic factors such as BFGF and BDNF (Hansson et al., 2000; Grundy et al., 2000). The interactions of the basolateral amygdala (BLA) with the hippocampus in mediating glucocorticoid effects on memory (Roozendaal et al., 1999; Roozendaal, 2000; Roozendaal et al., 2001) may extend the view to the observed facilitated learning following stress and CORT treatment in this study. The hypothesis of BLA function in memory suggests that the amygdala, which has a moderate density of GRs, participates in the influence of glucocorticoids on memory consolidation. This anatomical feature enables BLA to integrate hormonal and neuromodulatory influences on memory consolidation. Because the amygdala is involved in autonomic and humoral stress responses, it is likely that these responses feed back to the hippocampus and enable glucocorticoids to enhance memory storage (Roozendaal, 2000). Even for the structural alterations, it has been suggested that the BLA activation is essential for inducing glucocorticoid-mediated plasticity in the hippocampus (Quirarte et al., 1997). In any case, it seems that glucocorticoids not only play a key role in enhancing memory, they are also essential mediators to link between the amygdala and hippocampal activities

during glucocorticoid-related experiences and recovery processes in the hippocampus.

Substantial evidence, in the recent years, has described the roles of neurotrophins such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and basic fibroblast growth factor (bFGF)) in neural development and pathology. Because the hippocampus is the site of synthesis of many of the gene family of neurotrophins (Grundy et al., 2001), evidence suggests that the neurotrophic receptors such as NGF are important targets for corticosterone-related effects (Lindholm et al., 1994) and the administration of glucocorticoids may increase NGF mRNA levels in the hippocampus (Scully and Otten, 1995). To date, relatively little is known about the contribution of neurotrophic factors in the neuroprotective effects of corticosterone. However, several studies have shown that restraint stress modulates BDNF mRNA in different areas of the brain including the hippocampus (Givalois et al., 2001; Rage et al., 2002; Li et al., 2007). BDNF plays an important role in regulation of cholesterol metabolism for synapse development (Suzuki et al., 2007) and structural rearrangements of axons and dendrites particularly in the DG (Binder, 2007). Additionally, corticosterone, although with a selective action on growth factor expression (Gubba et al., 2004), can regulate bFGF gene expression in the hippocampus through GRs activities on the neurotrophic factor signaling (Hansson et al., 2000; Hansson et al., 2001). BDNF and NGF mRNA expression in the hippocampus is also modulated by corticosterone (Grundy et al., 2000; Grundy et al., 2001). Hence, the contribution of corticosterone and, at least in part, some

neurotrophins such as BDNF and NGF may be the central process involved in the hippocampal structural and behavioural reorganization after stroke.

Furthermore, one of the other novel aspects of this experiment involves the "late" administration of corticosterone and stress having opposite effects on stroke outcomes achieved when these manipulations occur prior to stroke (Sugo et al., 2002; Mc Donald et al., 2008). The view that the adrenal steroids possess trophic activity following stroke is supported by two different research lines 1) physiological concentration of glucocorticoids is crucial for neuronal survival as adrenalectomy accelerates degeneration of hippocampal cells (Sloviter et al., 1989) and 2) adrenal steroid hormones play an important role in mediating induction of NGF and bFGF mRNA evoked by seizure episodes (Sun et al., 1993). Therefore, by providing direct evidence that corticosterone and stress regulate the structural and prevents functional impairments after the hippocampal stroke, our findings support the hypothesis that some of the facilitative effects observed in cognitive performance after stress and some neurorpathological conditions may be attributed to events linked to the contribution of corticosterone and neurotrophic factors in the brain's selected areas.

Summary

The present results show that rats with partial stroke in the hippocampus induced by ET-1 have a significant loss of cells in the hippocampus and a deficit in hippocampal-mediated spatial behaviours. These deficits can be alleviated by chronic stress or corticosterone administration. The post-stroke recovery

supports the view that corticosterone, a major glucocorticoid, may be a potent mediator leading the hippocampus to structural and functional recovery after some ischemic insults. Corticosterone may enhance neuronal plasticity and repair in the hippocampus via an increased efficacy of neurotrophic factors. Modulation of corticosterone action holds significant potential for efficient therapies for a variety of ischemic disorders in the brain.

Experiment 8: *Corticosterone and Stress Treatment Prior to the Hippocampal Stroke, and Spatial Performance in the ZT*

Background

Two different (permissive and suppressive) actions of glucocorticoid hormones secreted by adrenal cortex during stressful experiences have been considered. On the one hand, glucocorticoids protect the brain against adverse events, induce structural recovery and are essential for cognitive performance (de Kloet et al., 1999; Roozendaal, 2000). On the other hand, the central action of corticosteroids has mostly been portrayed as damaging and disruptive to learning and memory (Sapolsky et al., 2000). Generally, it is believed that corticosteroid effects on cognition and related brain regions can turn from protective (adaptive) into non-protective (maladaptive), when actions via the two corticosteroid-receptor types (MRs and GRs) are imbalanced for a prolonged period of time (de Kloet et al., 1999). In these conditions, chronic stress and glucocorticoids may reduce hippocampal dendritic complexity (Watanabe et al., 1992; Conrad et al., 1999; Kleen et al., 2006; McLaughlin et al., 2007) and can

even cause hippocampal cell death (Landfield et al., 1978; Uno et al., 1989; Sapolsky, 2005, McDonald et al., 2008).

Because, little is known about the contribution of corticosterone-dependent challenges before the ischemic insults in structural alterations, the main purpose of this experiment is to determine whether a history of chronic stress and glucocorticoid elevations modulate DG damage after ET-1-induced HPC focal stroke. A second purpose of this study is to investigate the effects of chronic glucocorticoid elevations before HPC focal stroke on spatial learning and memory. Studies investigating the effects of chronic glucocorticoid exposure on spatial performance, although not directly relevant to the context of stroke research, have reported mixed results, with some studies showing intact spatial memory under conditions that should produce hippocampal damage (Luine et al., 1993; Magariños et al., 1998; Coburn-Litvak et al., 2003, Conrad et al., 2007), whereas other investigations show spatial memory deficits (Dachir et al., 1993; McLay et al., 1998). Therefore, the following study included spatial memory assessment after chronic stress and glucocorticoid challenges, and focal stroke in the hippocampus.

Material and Methods

Subjects

Twenty-six adult male Long-Evans rats, weighing 330-360 g, raised at the Canadian Centre for Behavioural Neuroscience Vivarium at the University of Lethbridge, were used. The animals were housed in pairs under a 12:12 h

light/dark cycle with light starting at 07:30 h and temperature set at 22°C. All testing and training was performed during the light phase of the cycle at the same time of day. The animals received water *ad libitum*. Animals were food-restricted prior to baseline training and testing in the Ziggurat Task, and maintained at about 85% of their initial body weight throughout the experiment. To maintain body weight, rats were given an additional amount of food in their home cage at least 3 h after completion of the behavioural training and testing. All procedures were approved by the University of Lethbridge Animal Care Committee in compliance with the guidelines of the Canadian Council on Animal Care.

Rats were divided into four groups: control (*n*= 6), HPC stroke (n= 7), CORT + HPC stroke (n= 7) and Stress + HPC stroke (n=6). Rats in CORT and stress groups received daily CORT and restraint stress before the ET-1-induced stroke in the hippocampus. Bilateral injection of ET-1 into the hippocampus was used to induce HPC focal stroke. In order to assess baseline levels of circulating CORT, all groups were subjected to blood sampling one day before and on day 16th of the CORT and stress treatment. All four groups were subjected to the Ziggurat-task training for spatial performance. Following all behavioural tests, the rats were perfused and the brains processed for histological analysis to determine lesion extent and location.

Blood samples

Blood samples were taken at baseline, that is, the day prior to CORT and stress treatment. Blood samples were also taken one hour after CORT and stress on day 16 of treatment. Blood sample procedures used were identical to those described in Experiment 1.

CORT administration

Each animal in the CORT + HPC stroke group was orally administered 0.5 mg/kg CORT (Sigma-USA) daily (16 consecutive days) before the injection of ET-1 into the hippocampus. All CORT administrations procedures used were identical to those described in Experiment 8.

Restraint stress

The stress procedure used was identical to the one described in Experiment 1 with the exception that the rats were manually vibrated for 10-15 seconds every 15 minutes of that stress phase in order to prevent the habituation effect of the given stress. Following the 16-days (1h/day) of restraint stress, and in order to assess spatial performance of the animals, all groups were trained and tested in the standard or non-cued version of the ziggurat task (ZT) for spatial performance.

Surgery

All animals except controls were subjected to bilateral hippocampal injection of ET-1, and the surgical procedures used were identical to those described in Experiment 2. Rats were allowed to recover for 4-5 days before the beginning the ZT testing.

Apparatus

Ziggurat task (ZT): The ZT procedures used were identical to those described in Experiment 4 with the exception that the testing sessions were conducted over 9 days.

Histology

The histology procedures used were identical to those described in Experiment 2 with the exception that five images were captured, corresponding approximately to –2.12, –2.80, –3.60, – 4.30 and –5.20 mm relative to bregma.

Results

Histological results

Figures 49 and 50 illustrate the amount of ET-1 damage to the hippocampus in all HPC stroke groups. ET-1 produced tissue loss in the dorsal and ventral areas of the hippocampus in all rats of the ischemic groups. There was no sign of neural damage in the hippocampus of any the rats in the control group. In all ischemic groups, ET-1-induced damage to the dorsal hippocampus

was mostly limited to the CA1 and the DG, and the extent of tissue loss in the ventral hippocampus was usually restricted to the CA1 and CA2 fields. No detectable neural death was observed in the ventral DG. Rats in the stress + stroke group, however, showed extensive tissue loss in most regions of the hippocampus particularly CA1 and the DG when compared with the CORT+HPC stroke group. The damaging effects of CORT and stress were not found in the ventral hippocampus. An ANOVA conducted on the percent tissue loss in the dorsal hippocampus showed a significant effect of group ($F = 6.11$, $p < 0.05$) as the stress+stroke group showed more neuronal loss in the dorsal hippocampus than the CORT+Stroke, HPC stroke-only and control groups. Furthermore, ET-1-induced ischemia caused significant cell damage in the ventral hippocampus ($F = 3.47$, $p < 0.05$), no significant difference was found between ischemic groups in terms of the extent of the neural damage ($p > 0.93$). That is, only the dorsal hippocampus was affected by stress state prior to the stroke.

CORT levels

Figure 51 illustrates circulating levels of CORT as assessed from blood samples. Blood samples were assayed for levels of circulating CORT at baseline, day 16th of CORT and stress treatment. As it can be seen, rats in the CORT+HPC stroke and stress+HPC stroke groups, showed elevated levels of CORT on day 16. Dependent samples *t*-test conducted for baseline and day 16 in each group showed a significant difference between the CORT values of baseline and chronic time points in the CORT+HPC group ($t = 6.19$, $p < 0.05$)

and stress+HPC stroke (t = 2.78, p < 0.05). Both exogenous CORT administration and restraint stress produced significantly elevated levels of circulating CORT.

Behavioural results

Latency: Figure 52 (A) shows the average time spent to find the goal ziggurat in the ZT for all groups over the acquisition (learning) and retrieving (memory) days. A repeated measure ANOVA conducted for the latencies over 64 trials of the ZT testing revealed a significant main effect of Group (F = 2.99, p < 0.05), Day (F = 11.41, p < 0.05) and Group by Day interaction (F = 6.58, p < 0.05). *Post hoc* comparison (Tukey HSD) revealed significant differences between the stress + HPC stroke group when compared with the control group (p < 0.03), HPC stroke-only group (p < 0.04) and CORT+HPC stroke group (p < 0.04). That is, the latency to locate the goal ziggurat by the stress + stroke group was different (slower) from all of the other groups.

Path speed: Path speed during acquisition in the ZT is presented in Figure 52 (B). All three groups showed relatively constant speeds across the 8 testing days in the ZT. No significant main effect of group was found in the task (p > 0.73) suggesting that the observed behavioural deficits in the ZT following CORT and stress treatment and ET-1 injection may be attributed to the cognitive outcomes of the rats' exposure to CORT, stress and ET-1-induced ischemia in the hippocampus.

Probe trial: the percentage time spent in the testing and opposite quadrants of the ZT during the probe trial is depicted in Figure 53. Analysis of the 60 s of the probe performance in the ZT revealed that rats in the control group spent a considerable proportion of their time searching in the target quadrant. A repeated measure ANOVA conducted for the percentage of time spent in each quadrant within the ZT showed no significant main effect of group ($p > 0.70$), quadrant ($p > 0.05$), but a significant interaction between groups by quadrant ($F = 2.18$, $p < 0.05$). However, *post-hoc* comparisons showed that the HPC stroke groups receiving CORT or stress spent significantly less time in the target quadrant than both the controls ($p < 0.009$) and the HPC stroke-only group ($p < 0.03$). This finding is consistent with the idea that both groups did not acquire or retain a strong bias for the previous location of the goal ziggurat as compared to the control and HPC stroke–only groups. No significant difference was found between control and HPC groups ($p > 0.57$) and between CORT and stress+ HPC stroke groups ($p > 0.81$).

Taken together the behavioural results (latency, speed and probe performance) within the ZT in this experiment show that both CORT and stress+ HPC stroke rats had difficulty in acquiring and/or storing spatial information in the ZT regardless of whether it is associated with more significant structural damage in the hippocampus.

Discussion

The results in the present experiment indicate that focal stroke localized to the hippocampus using ET-1 had different effects, both structurally and functionally. Furthermore, chronic restraint stress prior to HPC focal stroke was associated with significantly more structural damage compared to the CORT+ HPC stroke. Both CORT and stress treatment prior to HPC stroke caused an enhanced spatial impairment in the ZT. These results show that a chronic history of stress that is associated with either chronically elevated circulating CORT or high emotionality and anxiety endangers or sensitizes hippocampal cells to the damaging consequences of focal ischemia. These harmful effects of stressful experiences on the hippocampus even impair the post-stroke hippocampal-dependent behaviours.

It is known from Experiment 7 that post-stroke exposure to stress and CORT may induce structural recovery in the hippocampus and improve spatial performance. In contrast, exposure to stress or CORT prior to stroke presents a different picture. In the literature, chronic exposure to gluococorticoids or stress has been shown to be associated with exacerbation of several neurological disorders (Zigmond and Stricker 1984; Sugo et al., 2002; Kirkland et al., 2008). For instance, McDonald et al. (2008) recently showed that rats with hippocampal focal stroke that had previously experienced stress showed enhanced hippocampal cell death and spatial deficits when compared to a non-stressed group with the same kind of hippocampal stroke. This finding is consistent with the present results at the structural level. Interestingly, our results for the

CORT+HPC stroke group shows that CORT can not induce a significant enhancement damage in the hippocampus while it is associated with spatial behavioural deficits. In the line of our results, there are some reports that concluded that there is no effect of CORT-related experiences on stroke outcome (Macko et al., 1996). Our results, however, showed that chronic stress prior to HPC stroke could enhance cell death. This clearly suggests that the alternative effects of stress on the brain may be occurred through a different endocrinological axis (e.g. non-adrenocorticotropin-mediated mechanism; De Souza and Van Loon 1982) other than what is usually reported. Stress-induced high emotionality, anxiety, frustration particularly after uncontrollable and unpredictable stress as well as fearful experiences during stress can simply make stressed animal more susceptible to devastating structural changes than when it experiences only elevated plasma CORT. A distinguishable profile of stress and CORT effects on the basolateral amygdala (BLA) has been previously shown (Kavushansky and Richter-Levin, 2006). Therefore, it seems a constricted approach to a stress state when stress and its consequences are defined based upon only LHPA activity or elevated plasma CORT.

More interestingly, our results provide a unique opportunity to compare pre- and post-stroke consequences of CORT-related experiences. Previously, in Experiments 3 and 8 we showed that post-stroke exposure to either CORT or stress might enhance the structural and behavioural recovery. However, in the present study we confirm that pre-stroke exposure to the CORT or more particularly stress is associated with more tissue loss in hippocampus. Although

post-stroke facilitative effects of CORT-related experiences were only shown in recent years (see Wiltrout et al., 2007 for review), there are some well-documented reports that stress and CORT may endanger nervous cells prior to the ischemic insult (Sugo et al., 2002; McDonald et al., 2008). These destructive effects are mostly attributed to compromising antioxidant enzyme defenses (McIntosh et al., 1998), or inhibitive effects of CORT on local cerebral glucose utilization and glucose transport in neurons and consequently ATP depletion in the neurons (Sugo et al., 2002). In addition, it has been shown that pre-ischemic exposure to stress may affect infarct size by suppressing endogenous expression of *bcl-2* (DeVries et al., 2001). The *bcl-2* protooncogene promotes cell survival and protects against apoptosis and cellular necrosis in numerous neurodegenerative disorders, including stroke.

Our results clearly emphasize the existence of both destructive and facilitative effects of CORT-related experiences on hippocampal structure and function following focal stroke. The discrepancy between different studies and conclusions is still a matter of discussion (de Kloet et al., 1999; Roozendaal, 2002). The inconsistency in conclusions among previous investigations may be due to factors such as small sample size in some of the studies, differences in mean age of subjects, and the methods associated with reporting and rating of stressful events (Sugo et al., 2002). Clearly, additional studies are needed to assess the impact of stressful or CORT-related experiences on HPC ischemia outcome.

Concluding remarks

ET-1-induced focal stroke in the hippocampus impairs spatial learning and memory in both wet and dry lands. Focal stroke was also found to be accompanied by tissue loss in the hippocampus. However, chronic stress or CORT treatment reduced the severity of these structural and functional consequences after stroke. CORT-induced recovery after HPC focal ischemia may occur because glucocorticoids have been shown to play a key role in regulation of growth or neurotrophic factors. Glucocorticoids may play an important regulatory role with a close cooperation of basolateral amygdala and the hippocampus through the neurohormonal interaction between the adrenal cortical system and neurotrophic factors as well as their anti-inflammatory effects. Conversely, pre-stroke exposure to stress enhances tissue loss in the hippocampus, possibly by increasing cell death, and impairs spatial performance. No deleterious effect of pre-ischemic exposure to CORT was found on the HPC tissue loss.

8. General Discussion

The previous chapters illustrated a profile of extraordinary diversity of glucocorticoid actions in the brain after focal ischemia and during CORT-related experiences. Collectively, three major results can be inferred from our experiments:

First, "*stroke, but not restraint stress (1 h/day; 21 days), may impair spatial function. Post-stroke stress, however, reduces the stroke-induced spatial deficits."* From a theoretical perspective, stressors are actual or perceived challenges to an organism's ability to meet its actual or perceived needs. On the one hand, these challenges are inevitable, or a necessary part of life, as Selye (1976) noted. On the other hand and from a clinical viewpoint, they sometimes have to be avoided due to their direct or indirect impact on the organism's well-being. Avoidance of or coping with these challenges is determined by several internal or external factors. These factors (e.g. the context, magnitude, duration, frequency, and the nature of stressor), in fact, change the expected absolute effects of stressful challenges to some unexpected relative consequences. For example, McLaughlin and others (2007) showed that only rats exposed to the 6 h/21 days restraint paradigm exhibited neuronal deficits in hippocampus.

Laboratory rats live in environments that are different from their natural habitat and demands, but the stressful challenges and coping strategies of these rats should also be interpreted in the above mentioned approach. Stressors may impact the animal current homeostasis in acute, sequential, episodic, chronically intermittent, sustained, or anticipated forms (Sapolsky et al., 2000). Several

strategies may be evoked by the physical arrangement of the environment, the animal's perception, physical capabilities and experience of their effectiveness in coping when it encounters a stressful challenge. For instance, the environmental input in general and the context of a learning task that activate certain neuronal pathways are likely to influence steroid-receptor-mediated changes in limbic activity (de Kloet et al., 1999). For example, exogenous corticosterone administered immediately after training facilitates spatial performance in the MWT (Sandi et al., 1997). A decrease in the water temperature that produces a CORT response also improves cognitive performance. The contextual or environmental input, therefore, sometimes determines the level of the involvement of LHPA-related outputs in, for example, memory function.

Previous findings like our results in Experiment 1 that show spatial function is not affected by stress (Warren et al., 1991; Beylin and Shors 1998) clearly are a challenge for the traditional concepts of stress-sensitive behaviours (Gesamte, 1975; Ohl and Fuchs, 1998; Greenberg, 2002). The effects of stress on behaviour, in their perspectives, are an absolute consequence of hormones acting directly on specific neural pathways mediating actions. Subsequently, what may be seen in the relation of stress and behaviour is a type of unconditioned behavioural sensitivity to stress. This perspective is clearly not consistent with our current knowledge. For example, it is now well-documented that the same hormone can have opposite effects and the same stressors can evoke different patterns of an endocrine response (Sapolsky et al., 2000; Greenberg et al., 2002). Furthermore, because the actions of stress hormones

on the target tissue may be constrained by the activity of other hormones or by environmental circumstances, stress responses typically appear to have built on different elements of the stress-related axes. Therefore, the absolute levels of neurotransmitters or hormones typically observed in stress investigations may not necessarily matter in the production of significant and adaptive outcomes. On a more general note, this explanation helps to understand and accept the entity of the great diversity of adaptive behavioural patterns in the light of the dynamics and flexibility of neuroendocrine stress output (see Sapolsky et al., 2000 for more details).

In any case, our results in Experiment 1, either related to the complicated and diverse actions of stress hormones or due to the effects of the given stressor on the different goal-directed strategies in the MWT, suggest that stress may have different behavioural reflections, at least in the context of the hippcampal function; this makes the concept of stress-sensitive behaviour a matter of further investigation and debates.

For alterations resulting from any structural damage (e.g. accidents, diseases, aging, etc.) to the brain, however, the story yields a simpler profile. For instance, in neuropathological conditions, stroke is itself a stressor, at least because the activation of LHPA axis is among the first measurable physiological responses to cerebral ischemia (Sugo et al., 2002). Although little is known about the contribution of LHPA hormonal outputs during ischemia, we simply assume that since the ischemic insults usually result in inflammation in the brain (Ekdahl et al., 2003; Monje et al., 2003), this association can be translated (e.g. through

the well-known anti-inflammatory effects of corticosteroids) into some adaptive results for the ischemic brain. Therefore, we predicted the impaired spatial performance induced by the HPC focal ischemia in the Experiment 2, has potentially a chance to be improved by a model of stress or low-dose CORT administration after a stroke. This experimental condition increases the basal concentration of CORT, although other neurohormonal changes (e.g. nerve growth factors) should not be ignored. In Experiment 3, we were able to demonstrate that chronic CORT-related experiences after a stroke can significantly enhance behavioural recovery.

It is difficult to estimate how much the observed results are hippocampus-specific or may occur in other systems. Swim stress has been shown to change sensory-motor performance of rats in different behavioural paradigms (Metz et al., 2001). In addition, Kirkland et al. (2008) recently demonstrated that both pre- and post-lesion chronic restraint stress has a strong inhibitory effect on behavioural recovery after focal stroke in the motor cortex (see also Metz, 2007 for review). This profile, however, seems different from the hippocampus, perhaps because of the striking capacity of hippocampus for structural flexibility.

The hippocampus has been recently selected for stroke investigations because: (1) the hippocampus is a structure intimately involved in a well characterized processes of learning and storage of new information, (2) strokes and other neuropathological conditions frequently cause some learning and memory deficits, (3) in the adult hippocampal DG new neurons are produced, and (4) the structure is a major target of stress hormones, having one of the

highest concentrations of receptors for corticosteroids in the mammalian brain. Based on our results in the first study, one can at least conclude that the hippocampus is susceptible to the structural and functional recovery after ischemia and CORT-related experiences. Meanwhile, it remains to be further investigated whether the post-ischemic enhancement of recovery by steroid hormones also applies to other brain structures especially those involved in neurogenesis, learning and memory, and stress response.

Second, " *MWT, but not the ZT, is stressful. In addition, the ZT is sensitive to complete or partial hippocampal damage".* After more than 25 years of use in behavioural neuroscience and the investigations of spatial performance with non-human animals, primarily rodents, the Morris water task (MWT) still remains a valuable standard tool for the analysis of spatial processes. In addition, a computer-generated environment, the virtual Morris water task (vMWT) has also been developed to study human spatial learning and memory (Astur et al., 1998; Hamilton and Sutherland, 1999).

A strength of the MWT, in addition to its relative simplicity, is that it can be utilized to study a variety of different navigational systems. However, as mentioned by several authors (Block, 1999; D'Hooge and de Deyn, 2001; Aguilar-Valles et al., 2005), one of the main disadvantages of the task is that it requires the animals to navigate in an aversive environment (i.e. water). This may cause considerable stress when an animal is locating the hidden platform in the water. Although a reasonable water temperature has been suggested that can minimize stress, Iivonen et al., (2003) concluded that even at 24º C, the

MWT testing renders animals hypothermic, which may confound the test results. Our results about LHPA axis hyperactivity in the MWT measured by plasma CORT confirmed the stressful feature of MWT testing. This effect has not been observed in ZT testing in which the CORT concentration tended to be reduced during the animals' navigation. The increased activity of LHPA axis and the ensuing endocrinological alterations induced by the common water task protocol may potentially interfere with the spatial processes.

More interesting, although the MWT induced a significant rise in CORT concentrations after 6 days of training, the CORT concentration significantly decreased on repeated exposures by day 10. This finding is consistent with studies on stress tolerance (Spencer and McEwen 1997) or habituation (Galea et al., 1997; Faraday, 2002) effects in chronic stress paradigms, suggests that the nature of LHPA involvement in the water task and the accompanying CORT response is different than its involvement in the restraint stress experience in which even after 21 days (see Experiment 2, for example) the CORT concentration was not reduced. Our results clearly indicate that the involvement of LHPA axis in the stress response and habituation effect on CORT concentration in a chronic stress paradigm depends upon the source of stress. Moreover, in a comparative viewpoint, MWT represents a source of stress, at least in short time, it may be perceived a controllable stress condition in which rats are able to escape from water if they learn the location of the platform, while restraint stress and its adverse consequences are not controllable. Therefore, the difference between the CORT profile on chronic time points in MWT and restraint

has likely a root in cognitive evaluation processes (controllability versus uncontrollability) in rats.

Our current findings add to the body literature that reports the ZT may be an effective alternative task when investigators are concerned about the activity of LHPA axis and its interference with spatial or other processes. We have previously shown that the ZT was a sensitive task for hippocampal damage (Faraji et al., 2008). One of the most important characteristics of this task for spatial performance is that it provides a multi-featured dry arena in which the rats' navigation does not depend on aversive motivation. Moreover, unlike some maze-shape tasks (e.g. the radial arm maze [RAM]) for spatial processes, rats' navigation within the ZT is measured by their free goal-directed behaviours in a non-constricted environment. Using the ZT, one can study a variety of learning and memory strategies (for example, strategies that are dependend upon within-maze cues and/or rout learning) that are difficult to be addressed in constricted environments such as RAM as well as in wet, open fields like MWT.

The ELS scale that presents different possible profiles of spatial performance in ZT with a combination of errors (E), latency (L) and speed (S) (see Experiment 4), may help to draw a clear profile of spatial processes that are mostly embedded with motor, motivational, and sensory components in the spatial tasks. Spatial ability, in these conditions, sometimes can be confounded by some irrelevant factors during navigation within the spatial tasks that are not theoretically significant for experimental goals but show themselves in the final behavioural outcomes. A good example of this situation is found in lesion studies

particularly using stroke, in which cognitive deficits typically reflect a complicated compound of spatial, motivational, motor and sensory impairments (Ryan et al., 2006). The ELS scale in the ZT enables researchers to change this potentially confounded profile to a relatively clear picture of spatial ability.

Our findings about the rats' speed during spatial navigation should be specifically considered. We selected path speed, in addition to latency, as a further indicator for spatial performance in all of our behavioural reports for the simple reason that both stress-induced hyperactivity (Strekalova et al., 2005) and ischemia-induced hyperactivity (Plamondon et al., 2008) have been previously reported. Our results present a different profile of speed in both wet and dry lands, and they are in line with the finding that neither stress nor ischemic insult can increase path speed within these tasks (McDonald et al., 2008). That is, the latency to locate the platform in the MWT and the goal ziggurat in the ZT by ischemic and stress groups was not affected by the rats' speed or hyperactivity during the spatial navigation.

Third, "*Post-ischemic exposure to stress and CORT treatment alleviates the HPC ischemic outcomes. Pre-ischemic exposure to stress, however, enhances tissue loss in the hippocampus and impairs hippocampus-dependent functions."* The opposing effects of CORT-related experiences in our experiments not only reflect to the diversity of glucocorticoid actions in the stress response (Sapolsky et al. 2000), but also provide some firm evidence for the dynamic interplay of neuroendocrine and behavioural mechanisms (Johnson et al., 1992). The most important aspect of our results is that they provide a provocative hypothesis in

which the facilitative effects of glucocorticoids after focal stroke and CORT-related experiences may occur through mechanisms that are potentially different than the neurobiological mediators underlying destructive consequences of stress prior to stroke.

More specifically, for post-ischemic facilitative effects of CORT-related experiences a couple of possibilities are apparent. First, there could be a close relation between glucocorticoid activity and the neurotrophic receptors such as NGF that are important targets for CORT-related effects (Lindholm et al., 1994). Furthermore, it has been reported that the administration of glucocorticoids may increase NGF mRNA levels in the hippocampus (Scully and Otten, 1995). This biological dialogue between glucocorticoids and NGFs has been also shown between stress hormones and BDNF mRNA in different areas of the brain including the hippocampus after restraint stress (Givalois et al., 2001; Rage et al., 2002; Li et al., 2007). Because neurotrophins (e.g. BDNF) play an important role in the regulation of cholesterol metabolism for synapse development (Suzuki et al., 2007) and structural rearrangements of axons and dendrites particularly in the DG (Binder, 2007), the glucocorticoid-neurotrophic axis is one of the most important pathways to play a key role in the neuro-protective consequences of steroid hormones after focal ischemia in the hippocampus. Therefore, the increased expression of neurotrophins in the brain particularly in the hippocampus is an adaptive reaction for structural reconstruction following brain insults and their restorative functions are strongly regulated by glucocorticoids (Sun et al., 1993; Hansson et al., 2000). Second, our results may be interpreted

in the light of the fact that corticosteroids are steroids endowed with powerful anti-edema and anti-inflammatory properties (Harbuz, 2002). Basically, focal ischemia is known to induce a long-lasting inflammation that inhibits neurogenesis in adult rodent brain (Ekdahl et al., 2003; Monje et al., 2003). The inflammation, however, is significantly relieved by glucocorticoids through the complement system (a biochemical cascade in immune system which helps to clear pathogens from an organism) that plays a role in post-ischemic inflammatory reactions and was recently shown to be a promoter of neurogenesis (Wiltrout et al., 2007). Hence, in addition to their active cooperation with nerve growth factors, glucocorticoids may play their restorative roles after stroke events through reducing the stroke-induced inflammation in the brain.

The harmful effects of pre-stroke stress and glucocorticoids, provide a contrasting profile of effects in hippocampal stroke. It is commonly believed that stress is a key factor in the etiology of stroke although there are some reports that have concluded that there is no effect of emotional factors on stroke incidence (Macko et al., 1997). Despite disagreement regarding the effects of prior exposure to stress on stroke incidence, several studies have provided evidence suggesting that high emotionality before a stroke is associated with elevated concentrations of glucocorticoid hormones and adversely affects stroke outcome (DeVries et al., 2001; Sugo et al., 2002; McDonald et al., 2008). Our findings in Experiment 8 are in line with this idea. We show that pre-ischemic chronic stress increases tissue loss after focal stroke in the hippocampus and can impair hippocampus-dependent behavioural functions. These detrimental

effects are attributed to several neurological events following exposure to stress. For example, it has been shown previously that glucocorticoids may have an inhibitory effect on cerebral glucose utilization (Kadekaro et al., 1988) and glucose transport in hippocampal neurons (Horner et al., 1990). Glucocorticoids and stress, also, can greatly exacerbate infarct size by suppressing post-ischemic *bcl-2* expression in the brain. The *bcl-2* promotes cell survival and protects against apoptosis and cellular necrosis in stroke (DeVries et al., 2001).

Our results, on the other hand, revealed a unique feature of stress effects on hippocampal structure and function when compared with the effect of CORT. Rats that had previously experienced stressful episodes showed enhanced tissue loss and functional deficit that did not happen in the rats with pre-stroke exogenous CORT. This observation suggests that only stressed rats are vulnerable to insults to the hippocampus. Therefore it may be important to distinguish between effects of stress versus CORT on stroke outcomes.

Concluding remarks

The results reviewed here emphasize: (1) the behavioural dynamics in dry- and wet-land tasks particularly after stressful episodes and hippocampal stroke, and (2) the importance of CORT-related experiences in stroke outcome. We have demonstrated that behavioural deficits caused by HPC focal ischemia can be regulated by stress hormones. This interesting picture of functional recovery might be also seen in rats that were exposed to oral CORT suggesting that both stress and CORT treatment alleviate the hippocampal recovery following focal

ischemia. Conversely, pre-stroke stressful history may be deleterious to stroke outcomes showing that the profile of structural and functional changes influenced by CORT-related experiences in the HPC focal ischemia is strongly dependent upon the “ time ” of the incidence of these experiences.

9. Future Directions

Despite the interesting outcome of the present experiments, there are many questions that remain unanswered. As we look closer at structural and functional alterations in the different experiments and the potential underlying mechanisms affected by glucocorticoid-related experiences, new questions emerge. Are pre- or post-stroke changes induced by CORT gender-specific? Is it possible to replicate the HPC post-ischemia recovery for other regions of the brain or is there any other potential target site in the brain for these alterations? Are these changes applicable to the motor system? In post-ischemic regulation of glucocorticoids, which one is more important: glucocorticoid-neurotrophic axis or glucocorticoid-complement system for anti-inflammatory responses? Is the observed recovery in ischemic hippocampus by steroid hormones a dose-dependent event? Is the recovery dependent upon the type of the induced stroke?

In a clinical viewpoint, many more issues are still a matter of discussion. For example, both post-stroke rehabilitation with exercise (Chouinard et al., 2006) and growth factor treatment especially for focal strokes (Ren and Finklestein, 2005) seem to be glucocorticoid-dependent events (Hansson et al., 2001; van Praag, 2008). From this perspective, our results suggest that steroid hormones are potentially strong mediators of post-stroke clinical interventions. However, these biological relations and their clinical applications need to be further investigated to develop more efficient therapeutic procedures.

Moreover, since stroke recovery is a process that occurs during days to weeks following stroke, a time window of any steroid-based intervention needs to be established to enhance the possibility of structural and functional recovery. This certainly depends upon more investigations on the potential efficacy of different doses, the time and methods of the delivery of CORT.

Finally, our results about post-ischemic interventions by steroid hormones showed some significant improvement in the hippocampus. It is not, however, clear if these changes are really “recovery-based“ or “compensation-based“ alterations. We mostly used the term “recovery“ or “tissue loss“ to describe such structural alterations in this thesis without any exact cellular observations. Hence, cellular analyses of recovery or compensation processes should be a focus of future studies. Such studies will help to develop better strategies for clinical applications and for reducing cognitive deficits in stroke patients.

Overall, although many other questions regarding the brain function under stress conditions, the nature and application of ET-1-induced focal ischemia in animal studies as well as pre-ischemic and post-ischemic alterations by CORT-related experiences still remain to be answered, our preliminary results on these crucial issues may open new windows into the better understanding of stroke and stroke-relevant conditions.

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11. Tables and Figures

Table 1. Injection coordinates relative to Bregma (in mm) for NMDA lesions of the hippocampus for male and female rats.

Antcroposlcrior (AP)		Mediolateral (ML)		Dorsoventral (DV)	
Male	Female	Male	Female	Male	Female
−3.1	−3.0	±1.0		**−3.6**	−3.6
−3.1	−3.0	±2.0		**−3.6**	−3.6
−4.1	−4.0	±2.0		**−4.0**	−4.0
−4.1	−4.0	±3.5		**−4.0**	−4.0
−5.0	−4.9	±3.0		**−4.1**	−4.1
−5.0	−4.9	±5.2		−7.3	−7.2
−5.0	−4.9	±5.2		−5.0	−5.0
−5.8	−5.7	±4.4		**−4.4**	−4.4
−5.8	−5.7	±5.1		−7.5	−7.3
−5.8	−5.7	±5.1		−6.2	−6.0

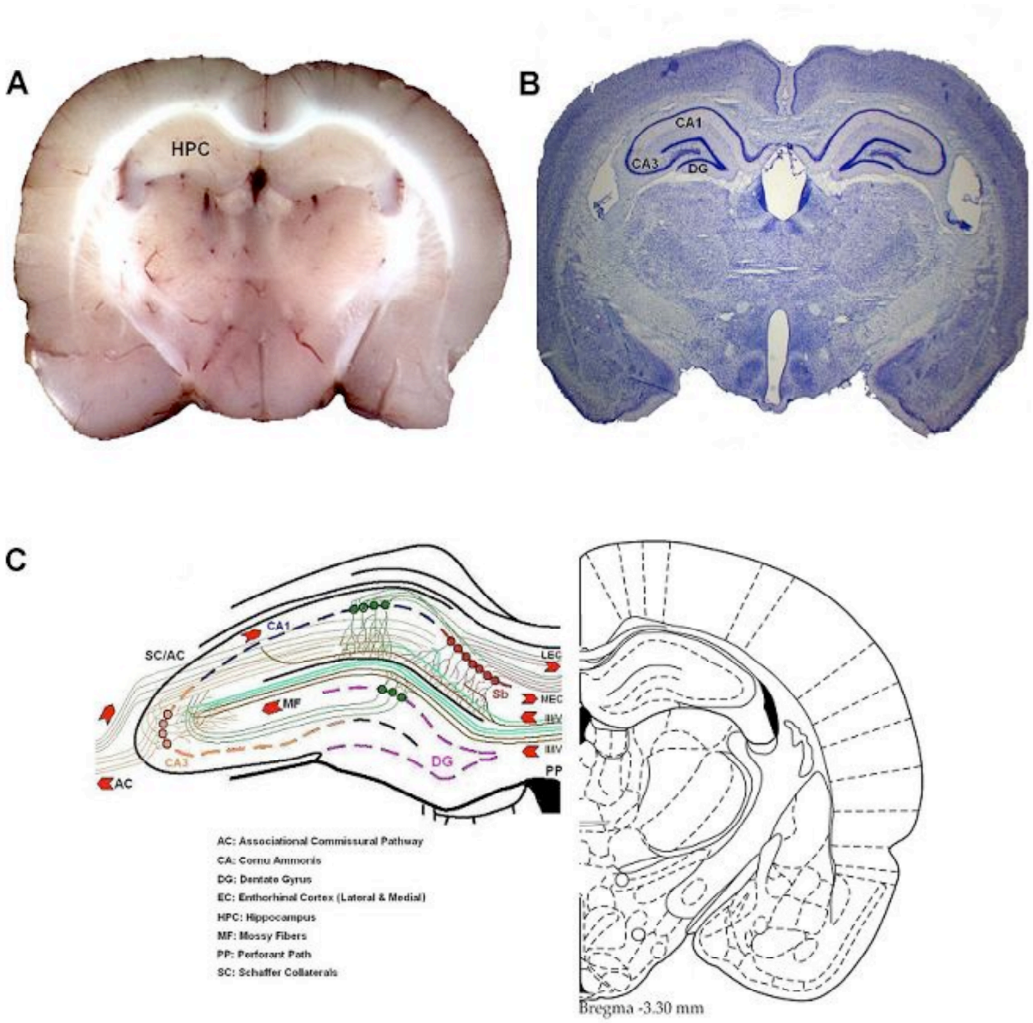


Figure 1. The hippocampus (HPC) and hippocampal formation. (A) representative coronal section of the hippocampus, with (B) Cresyl violet staining. (C) The hippocampus forms a network with inputs from the Lateral and Medial Entorhinal Cortex (LEC and MEC) that forms connections with the Dentate Gyrus (DG) and Cornu Ammonis (CA3) region via the Perforant Path (PP). PP is the major input to the hippocampus. CA3 region also receives input from the DG through the mossy fibers (MF). Atlas plates are from Paxinos and Watson (1997).

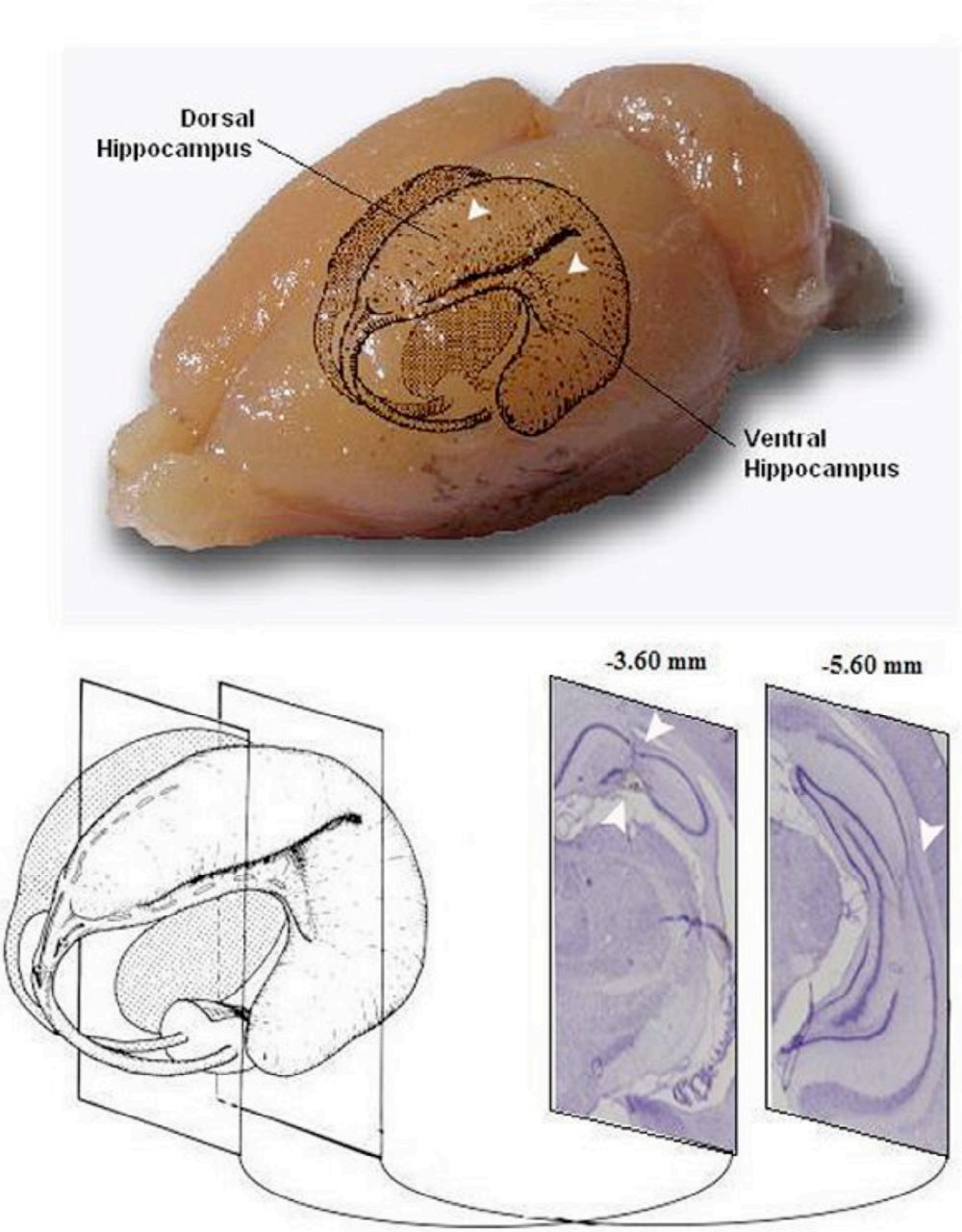


Figure 2. Schematic diagram of the hippocampus in rat brain (top). The white arrows show approximate points of ET-1 injections into the dorsal and ventral hippocampus, and subsequent cell loss induced by focal ischemia (down).

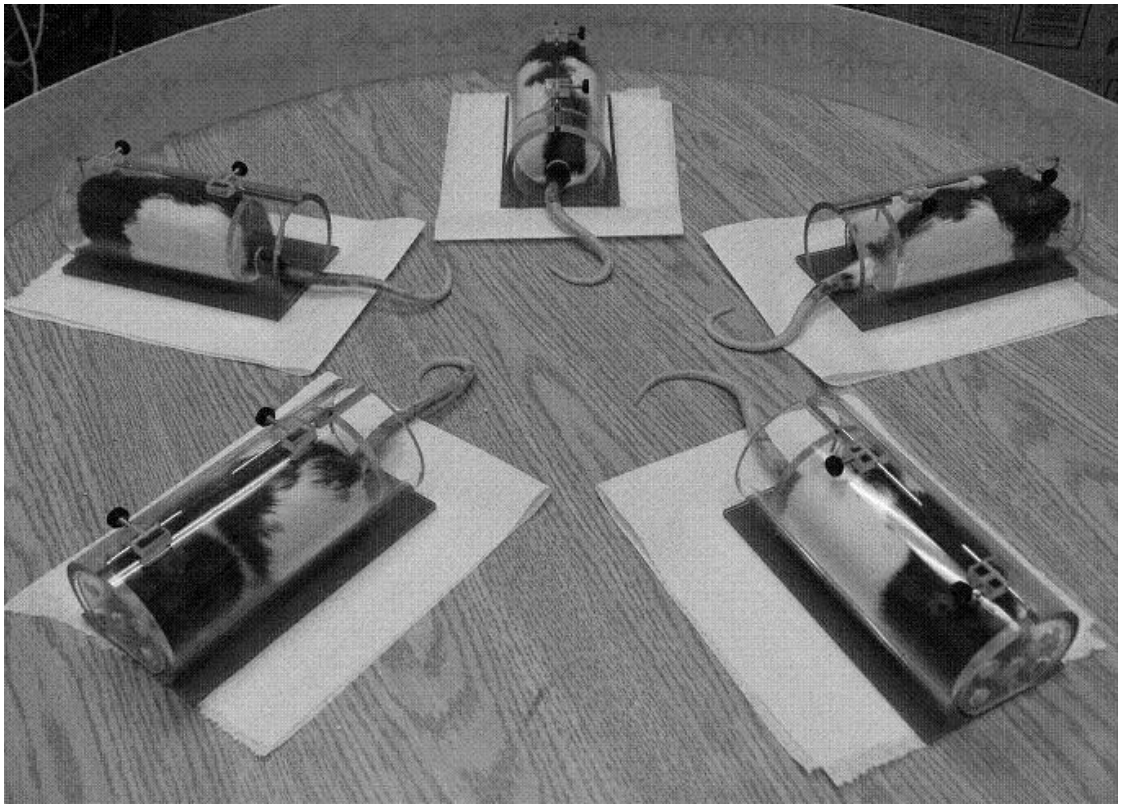


Figure 3. Restraint stress. Animals in stress groups were maintained 1h/day for 21 consecutive days in the transparent Plexiglas tubes of adjustable length.

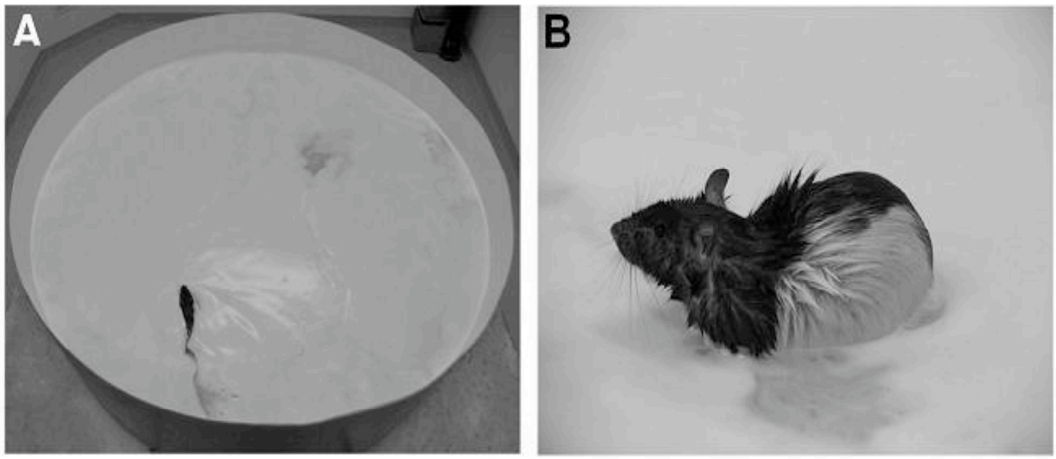


Figure 4. Morris Water Task (MWT; A & B), a wet-land task involving a circular pool with white interior. The task requires rats to escape from the water by locating a hidden platform.

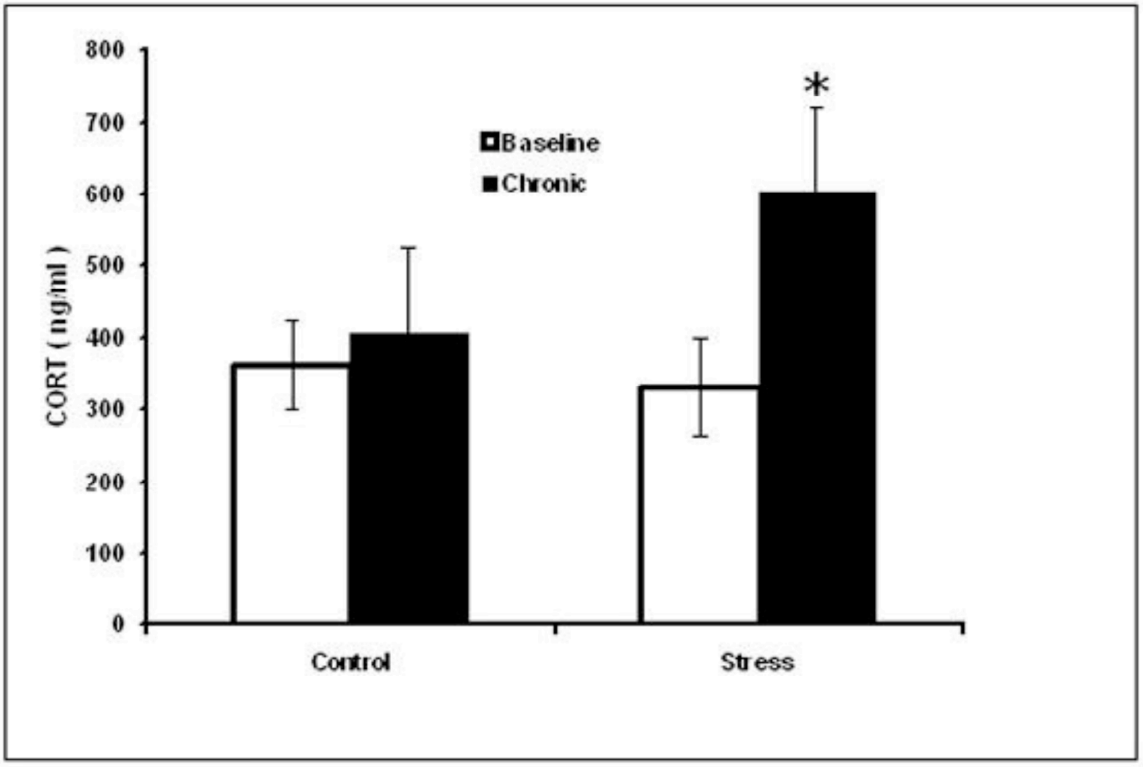


Figure 5. Plasma CORT concentration prior to (baseline) and at chronic levels (day 21 of daily treatment) of daily restraint stress. Circulating CORT levels were significantly higher after stress in the stress group. * $p < 0.05$; dependent samples *t*-test for within-subject comparison. Error bars show ± SEM.

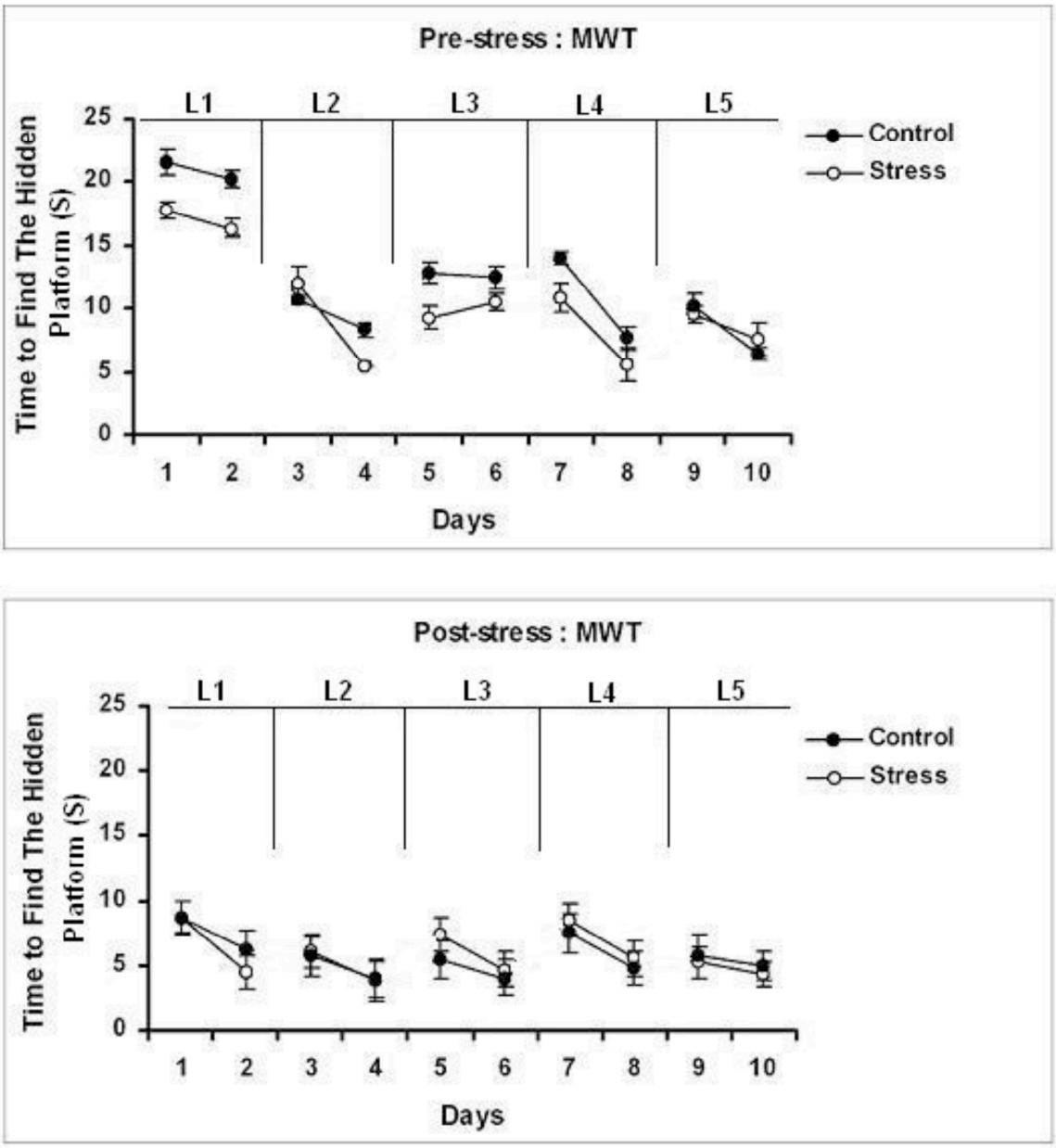


Figure 6. Average latency to find the hidden platform on different- and same-platform days in pre-test and post-test (before and after stress) within MWT. No significant effect of stress was found in pre- and post-stress latency. Error bars denote average ± SEM for each group. L: Location of platform.

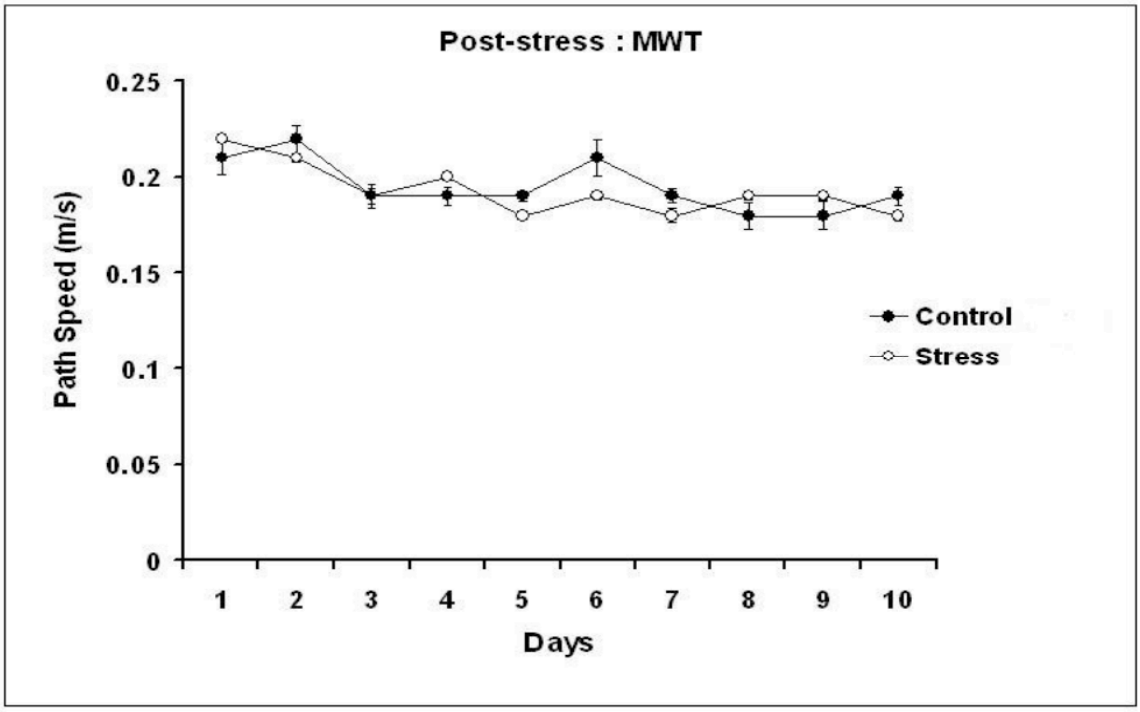


Figure 7. Path speed averaged across 10 days of post-stress testing in MWT. Both groups showed relatively constant speeds across the 10 testing days in the task. Error bars show ± SEM.

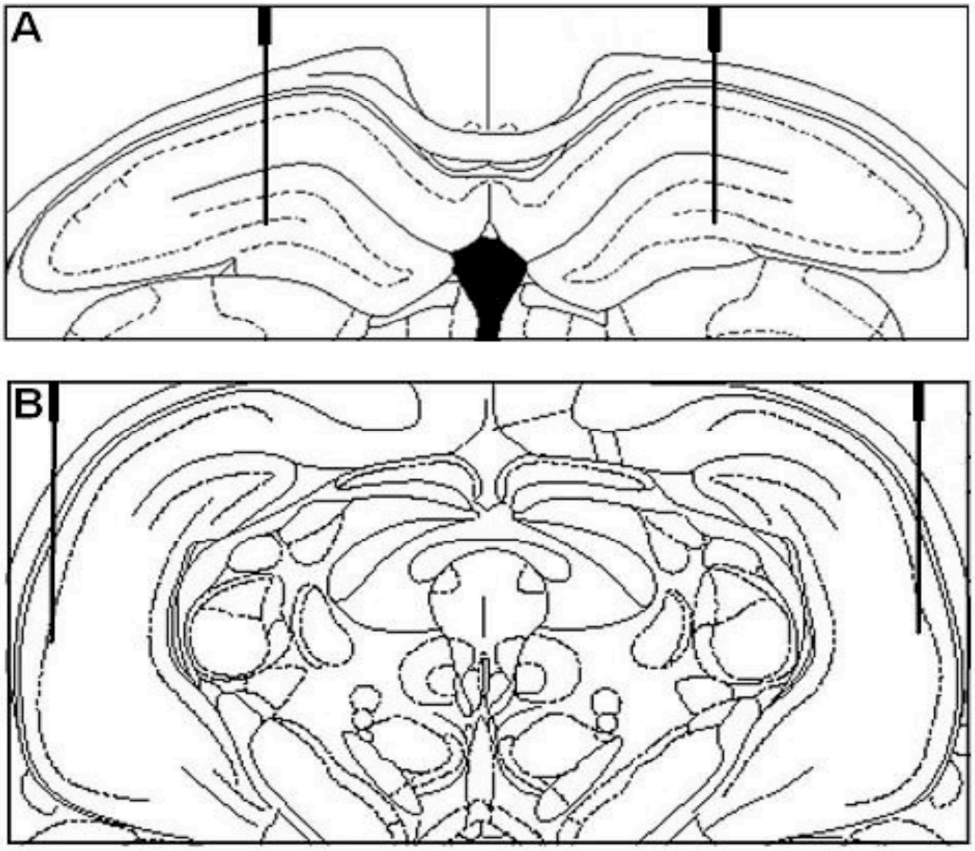


Figure 8. Illustration of the injection site for endothelin-1 (ET-1). Rats received two injections in each hippocampus (A – dorsal region &B – ventral region). Atlas plates are from Paxinos and Watson (1997) approximately to –3.60 mm (A) and –5.60 mm (B) relative to bregma.

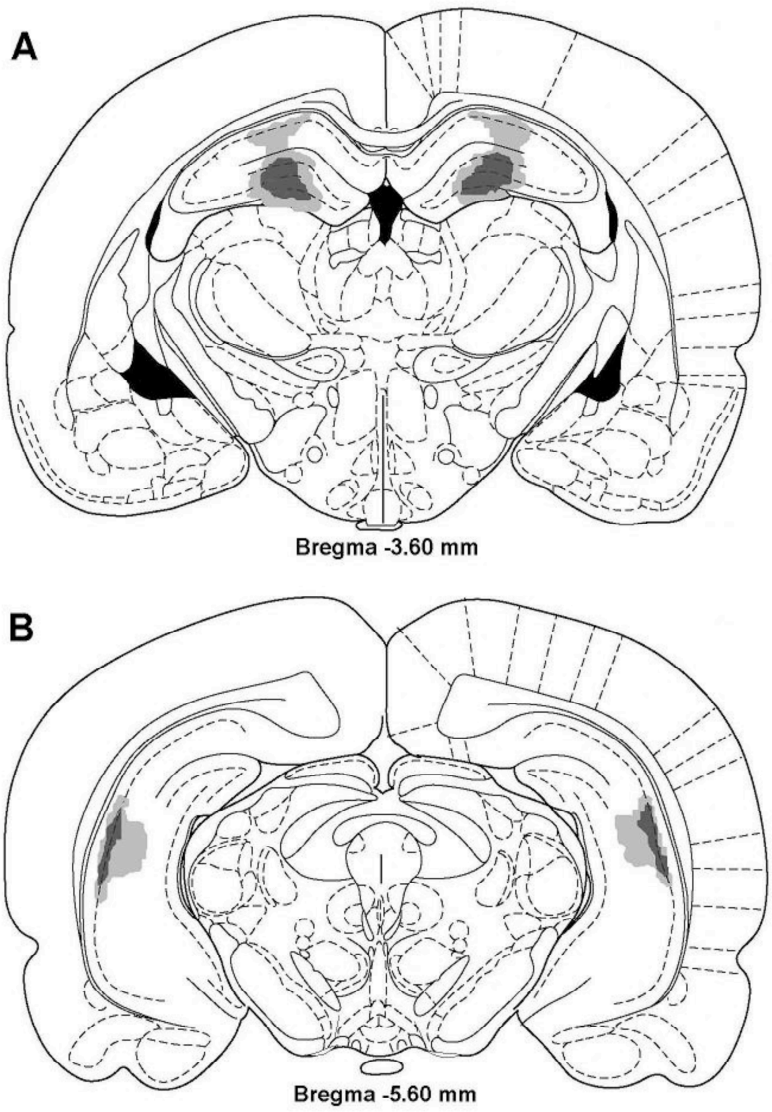


Figure 9. Schematic of lesions of (A) the dorsal and (B) ventral hippocampus induced by ET-1. Dark and light gray shading indicate the maximal and minimal neuronal loss common to all subjects after ET-1 injection. Atlas plates are from Paxinos and Watson (1997) approximately to –3.60 mm and –5.60 mm relative to bregma.

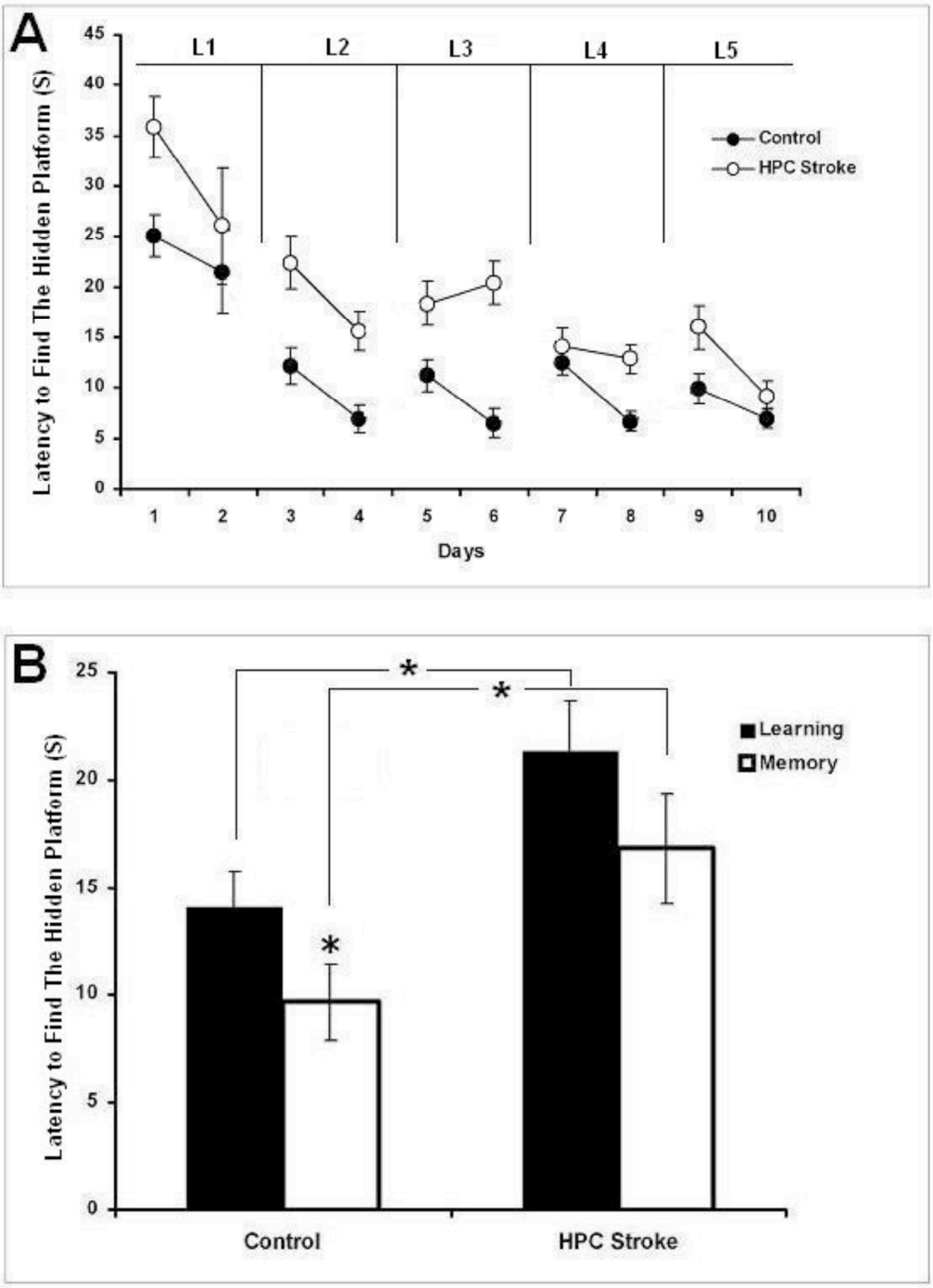


Figure 10. Testing in MWT. (A) Latency to find the hidden platform during 10 days of testing. (B) Average latency for both control and HPC (ET-1) groups on the different- and same-platform days. Error bars denote average ± SEM for each group. Asterisks indicate significance: * $p < 0.05$; ANOVA. L: Location of platform.

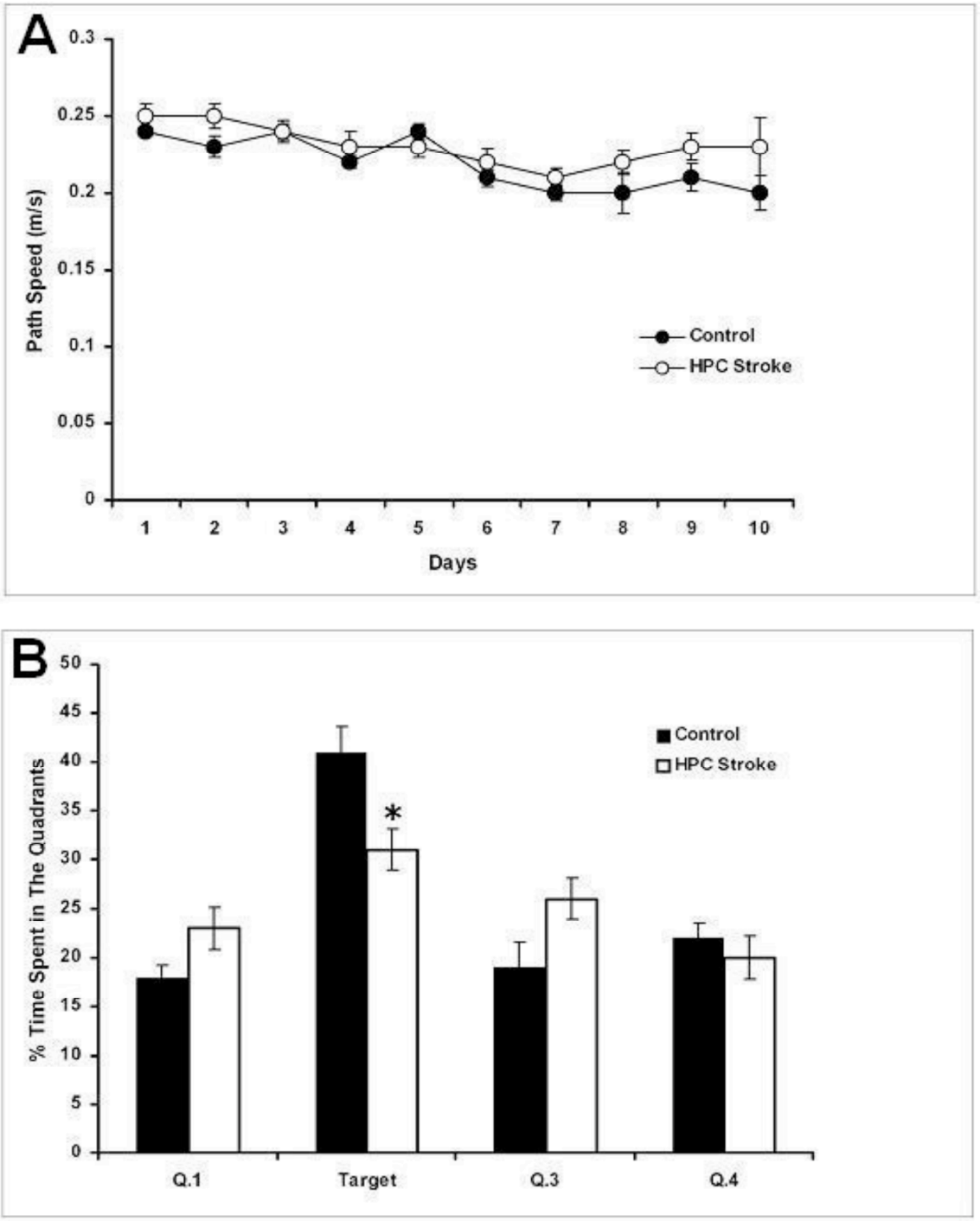


Figure 11. (A) Mean path speed averaged across 10 days of testing in MWT. No significant difference was found between groups in path speed during spatial navigation. (B) The mean percentage of time spent in the four quadrants of MWT during the 60 s of the probe trial conducted on day 11. Controls spent significantly more time searching for the platform in the target quadrant relative to the HPC group. * $p < 0.05$; Independent samples *t*-test. Error bars show ± SEM.

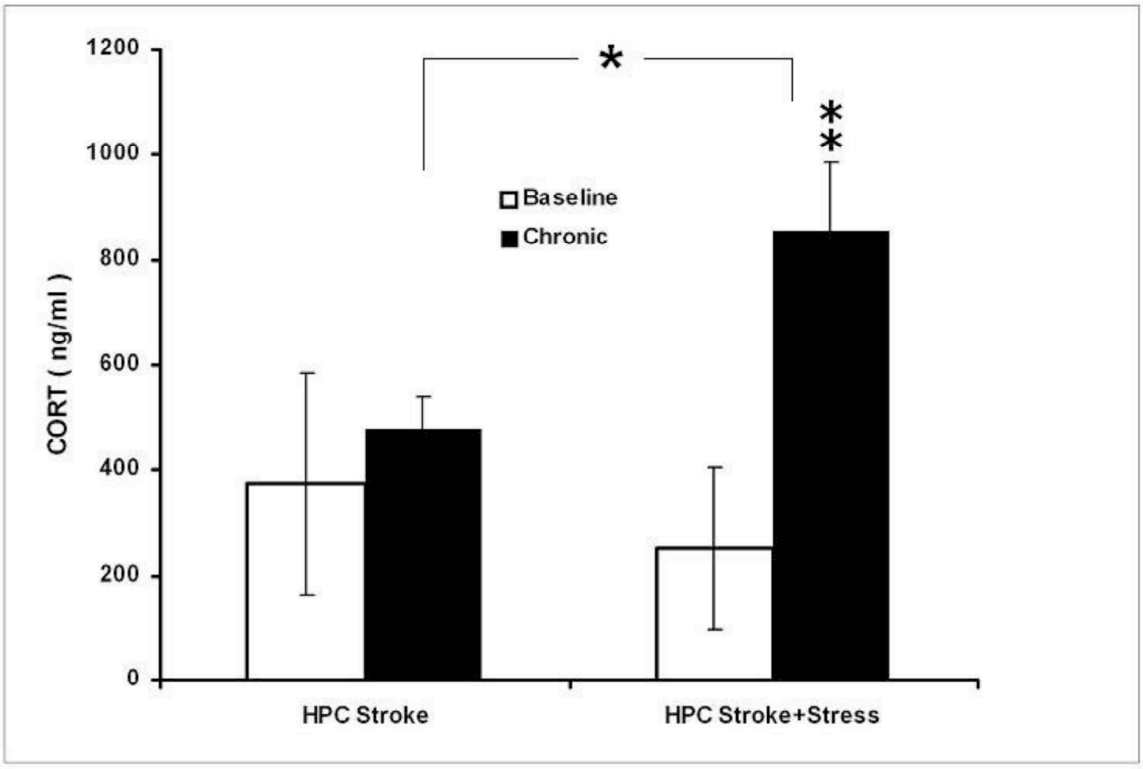


Figure 12. Plasma CORT concentration prior to (baseline) and at chronic levels (day 21of daily treatment) of daily restraint stress. Circulating CORT levels were significantly higher after stress in the HPC stroke + stress group. * $p < 0.05$ and ** $p < 0.01$ independent and dependent samples *t*-test. Error bars show ± SEM.

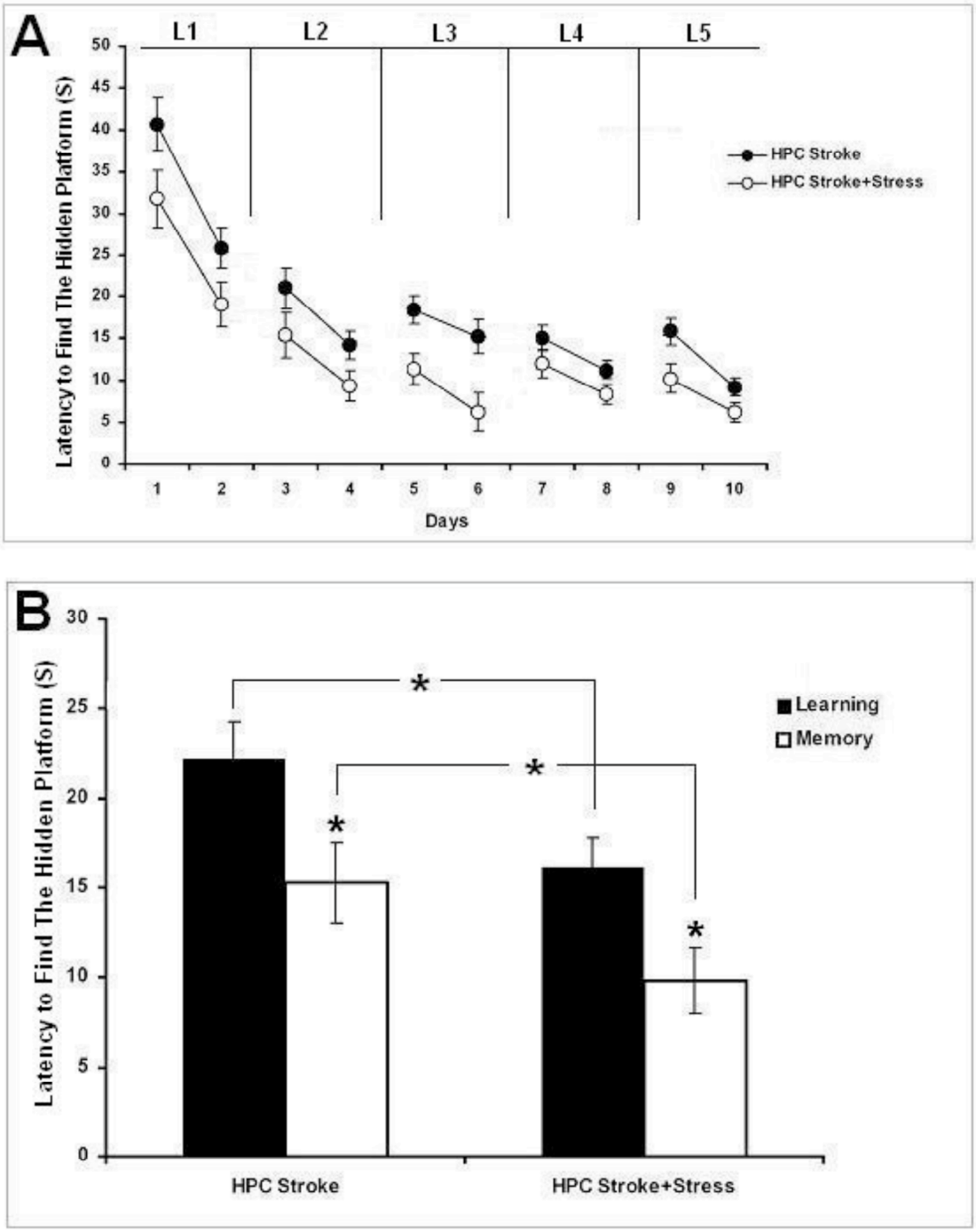
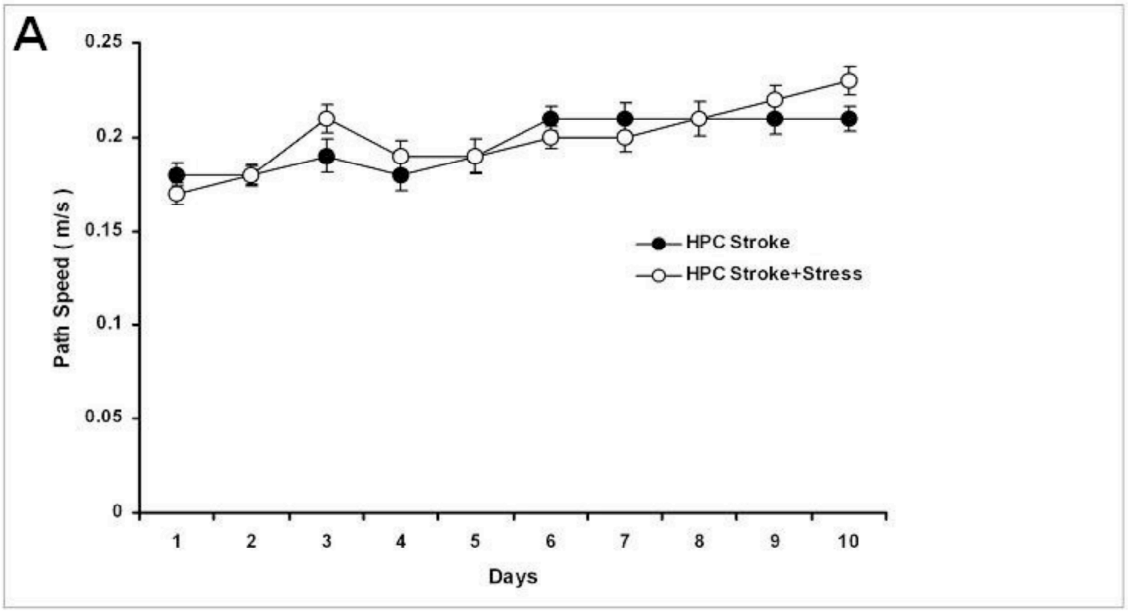


Figure 13. Testing in MWT. (A) Latency to find the hidden platform. Over 10 days of training, latency to reach the hidden platform significantly decreased in HPC stroke + stress. (B) Average latency for both HPC stroke-only and HPC stroke + stress groups on the different- and same-platform days. Error bars denote average ± SEM for each group. Asterisks indicate significance: * $p < 0.05$; ANOVA. L: Location of platform.



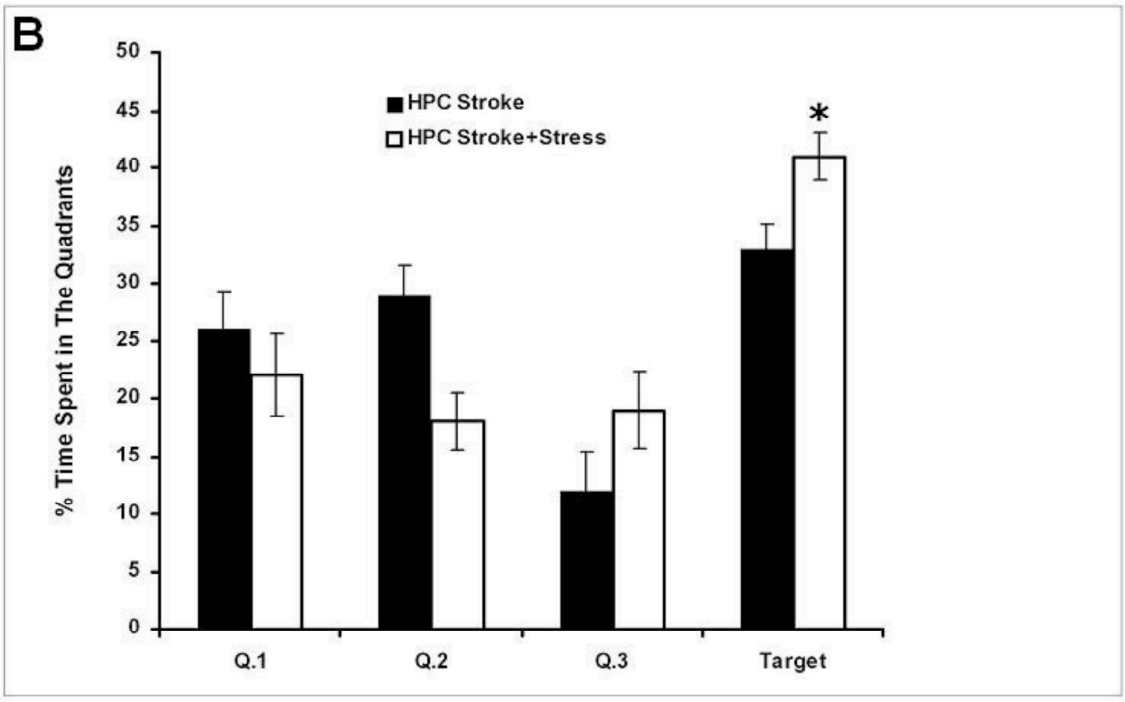


Figure 14. (A) Mean path speed averaged across 10 days of testing in MWT. No significant difference was found between groups in path speed during the spatial navigation. (B) The mean percentage of time spent in the four quadrants of MWT during the 60 s of the probe trial conducted on day 11. The HPC stroke + stress group spent significantly more time searching the platform in the target quadrant relative to HPC stroke-only group. * $p < 0.05$; Independent samples *t*-test. Error bars show ± SEM.

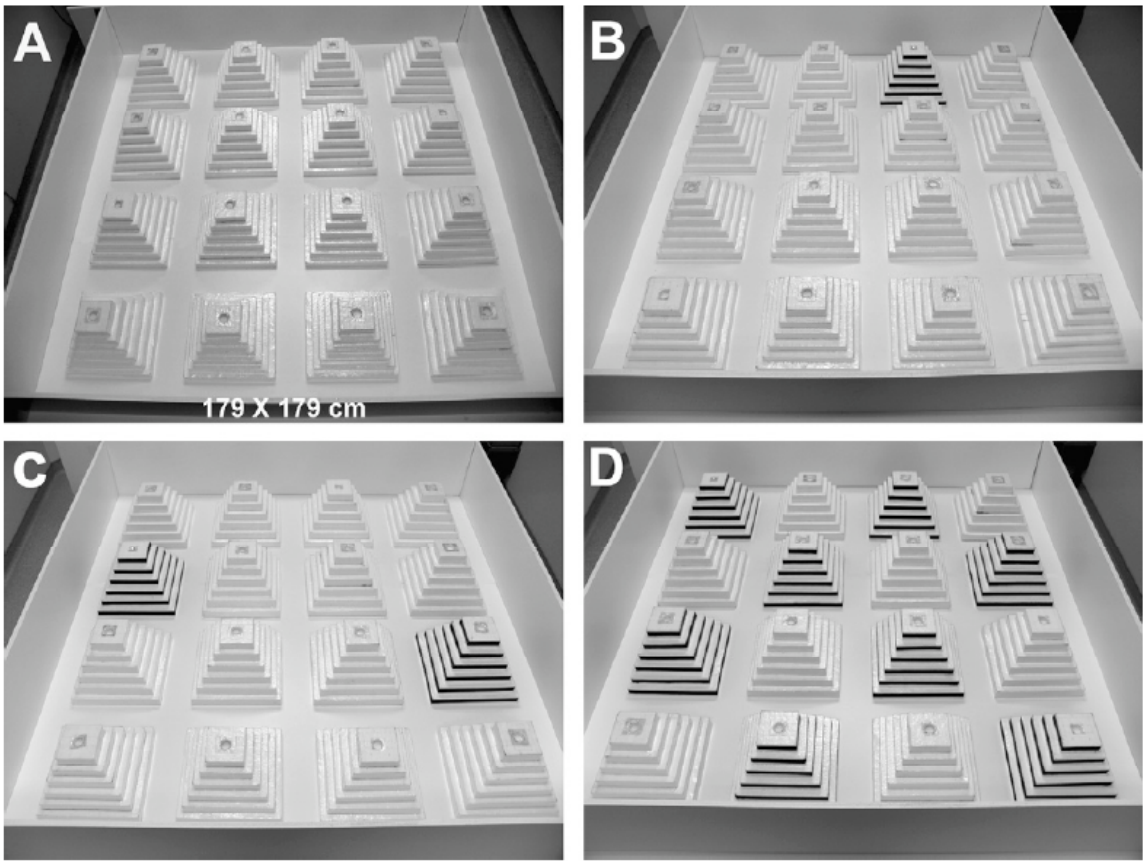


Figure 15. The ziggurat task (ZT). The task requires rats to learn and remember that the top of 1 out of 16 ziggurats in the open field is baited with a food reward. (A) Standard or non-cued version of ZT for spatial learning which is subjected to the present study. In this environment animals must use spatial cues to navigate to the goal ziggurat. (B) Cued version of ZT for non-spatial learning. In the cued version of ZT, rats learn that only the black ziggurat has food. (C) Single foil or matching to sample version of ZT. Rats in this environment are required to learn only one black ziggurat is a true goal, and thus has food. The second black ziggurat is not baited. (D) Multi-foil version of ZT in which animals must learn and remember that only one black or white ziggurat has food.

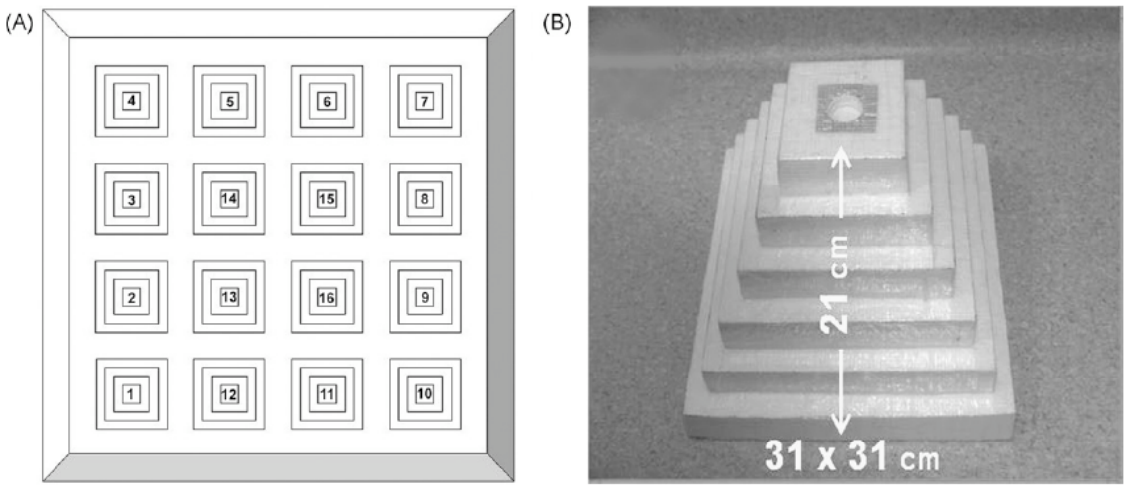


Figure 16. (A) A vertical-view graph of the ziggurat task containing sixteen pyramidal ziggurats, arranged in a four-by-four matrix. (B) A photograph of an individual ziggurat, 31 × 31 cm in base, and 21 cm in height.

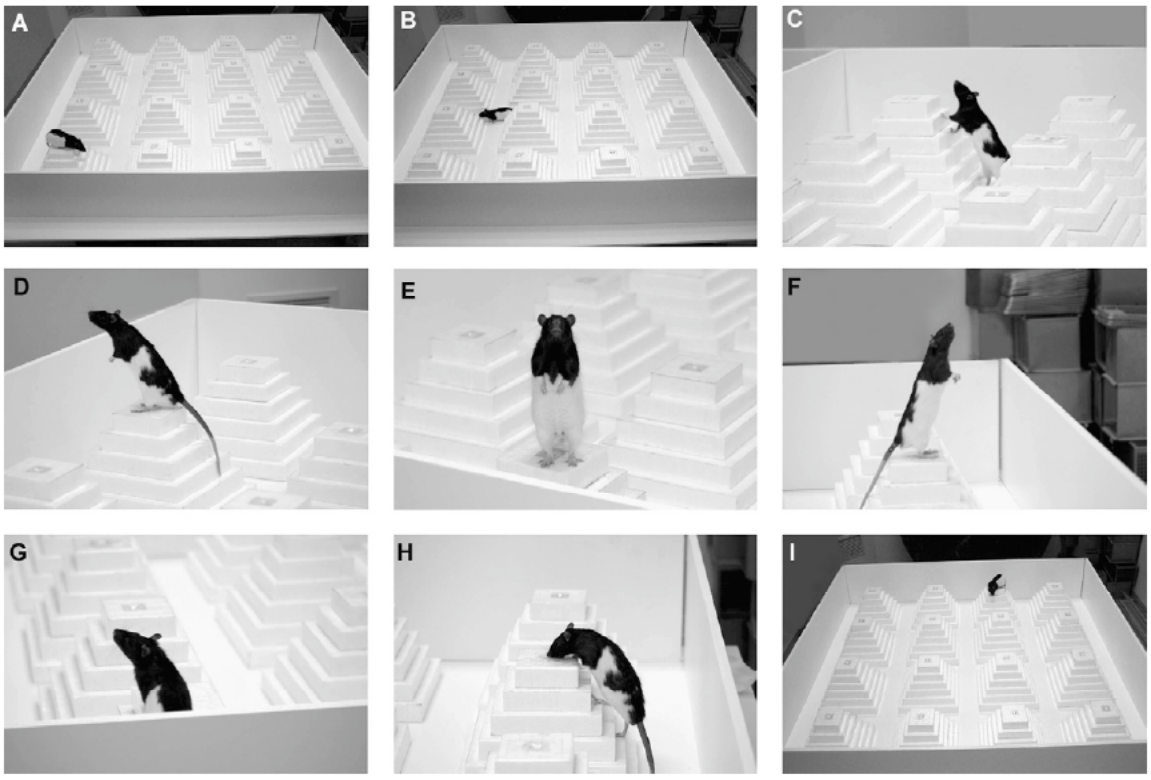


Figure 17. Photograph of a typical rat (A) released on starting point 1, (B-H) searching for the goal ziggurat and food, and (I) after finding the goal ziggurat having completed a trial on the ziggurat task. In this version of ZT the animal is released at random starting points in each corner and is required to navigate to the goal ziggurat defined by extra task cues.

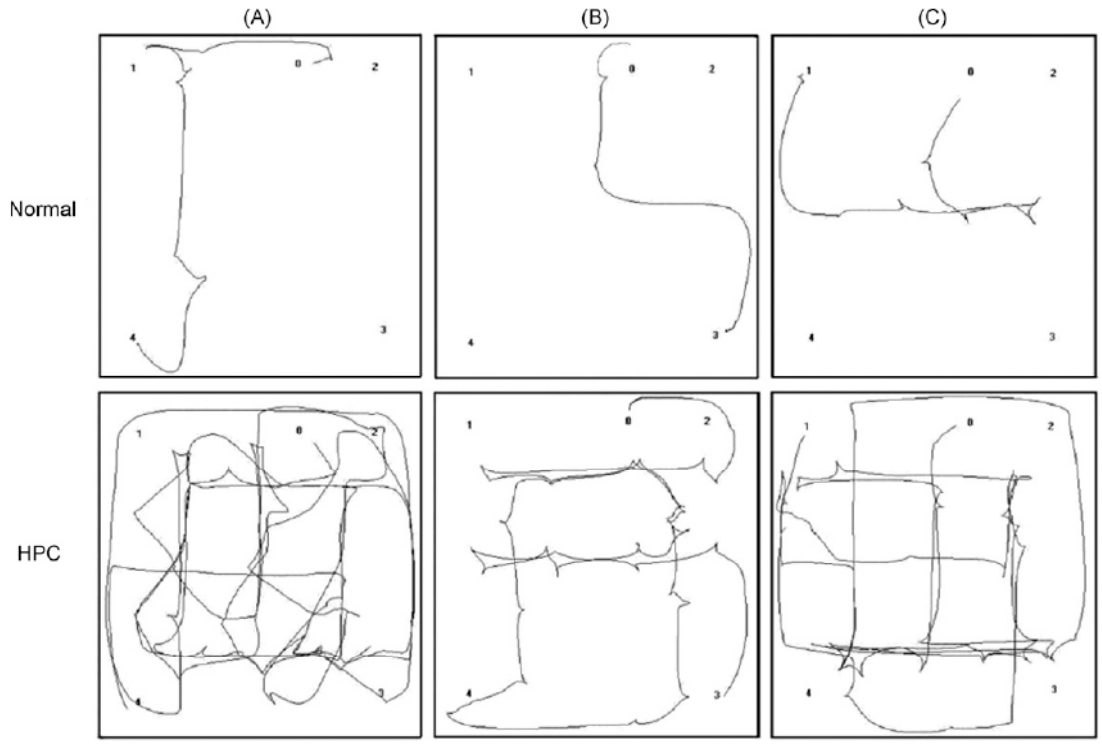


Figure 18. Paths taken on trials 6(A), 7(B), and 8(C) by a control and hippocampal rat from different starting points to the goal ziggurat (number 0) in the ziggurat task. Note initial localized searching in the central ways taken by the HPC rat showing its inability to switch from the former goal towards a new peripheral goal prior to finding the goal ziggurat in the peripheral way.

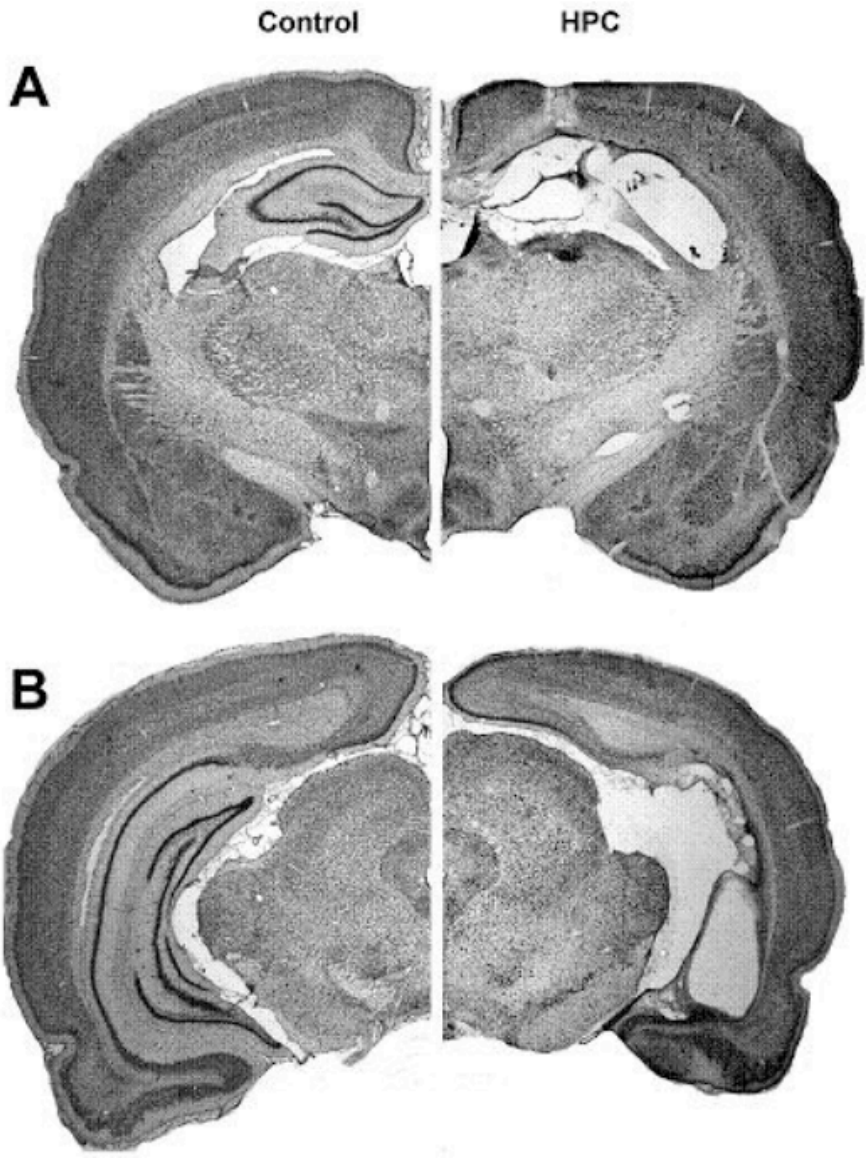


Figure 19. The extent of cell death in the dorsal and ventral hippocampus of a HPC rat (right panel) compared to a control rat (left panel).

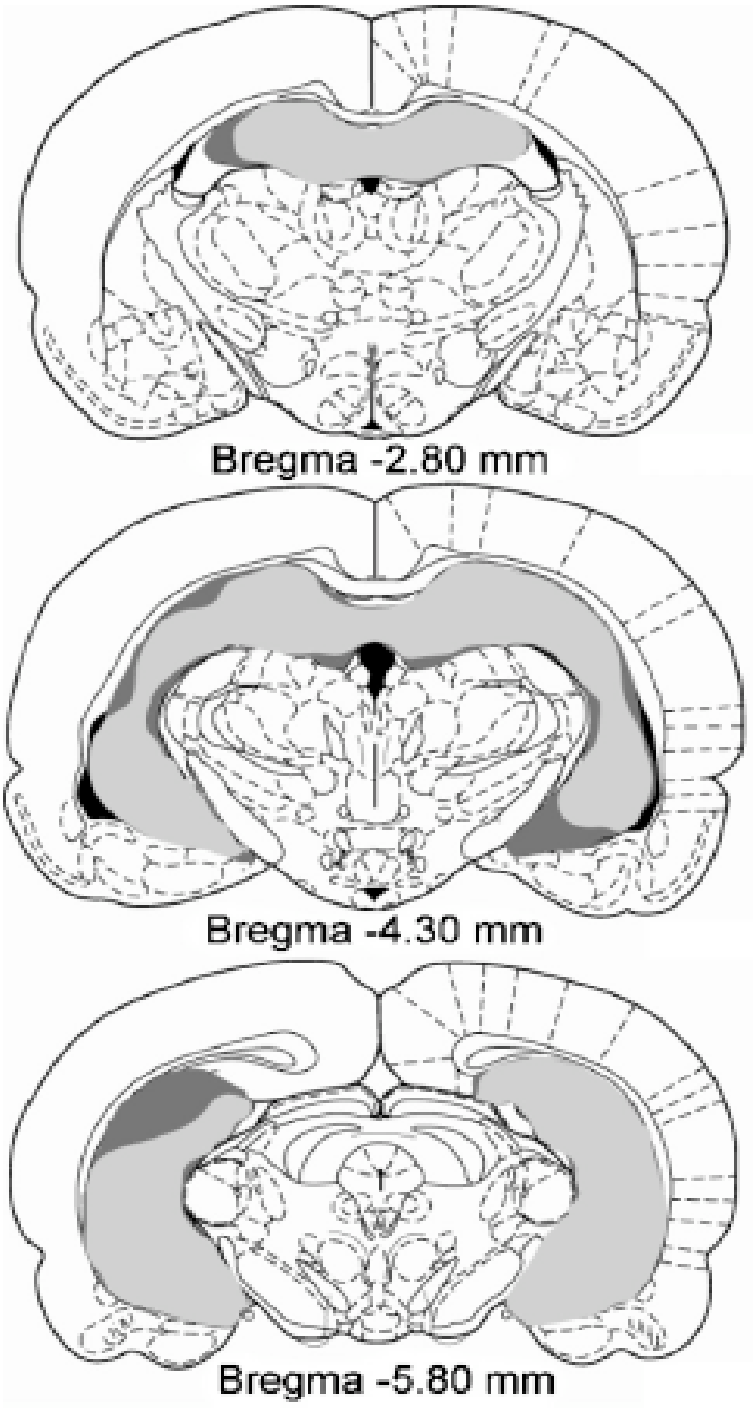


Figure 20. Illustrations of the smallest (light grey) and largest (dark gray) lesion observed bilaterally through the rostral and caudal extent of the HPC. Atlas plates are from Paxinos and Watson (1997).

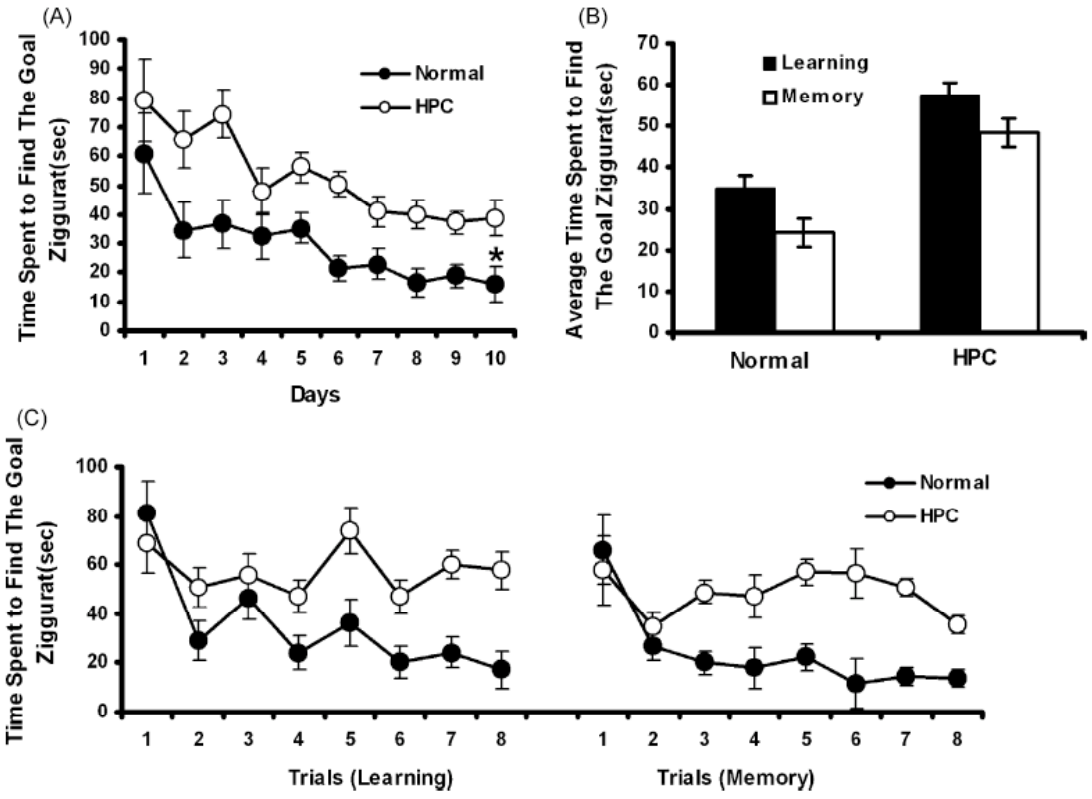


Figure 21. Testing in ziggurat task. (A) Latency to find the goal ziggurat during 10 days of testing. (B) Average latency for both control and hippocampal groups in the different- and same-platform days (learning and memory) . (C) Latency to find the goal ziggurat along 8 trials on different- and same-platform days. Error bars denote average ± SEM for each group. Asterisks indicate significance: * $p <$ 0.05; ANOVA.

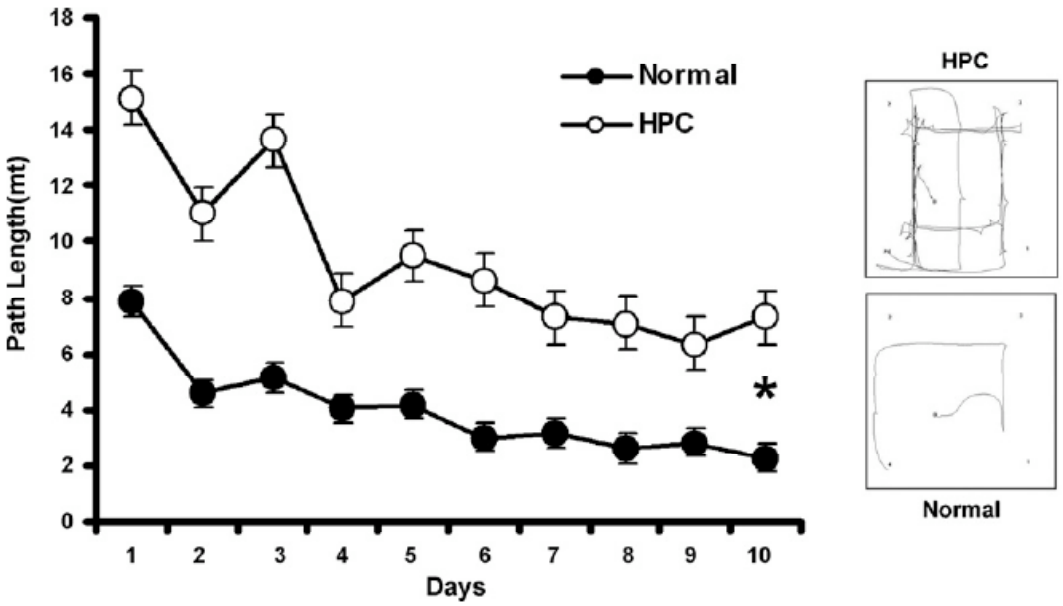


Figure 22. Mean distance traveled (path length) to locate the goal ziggurat during each day of testing. Error bars show ± SEM. Asterisk indicates significance: * $p < 0.05$; ANOVA.

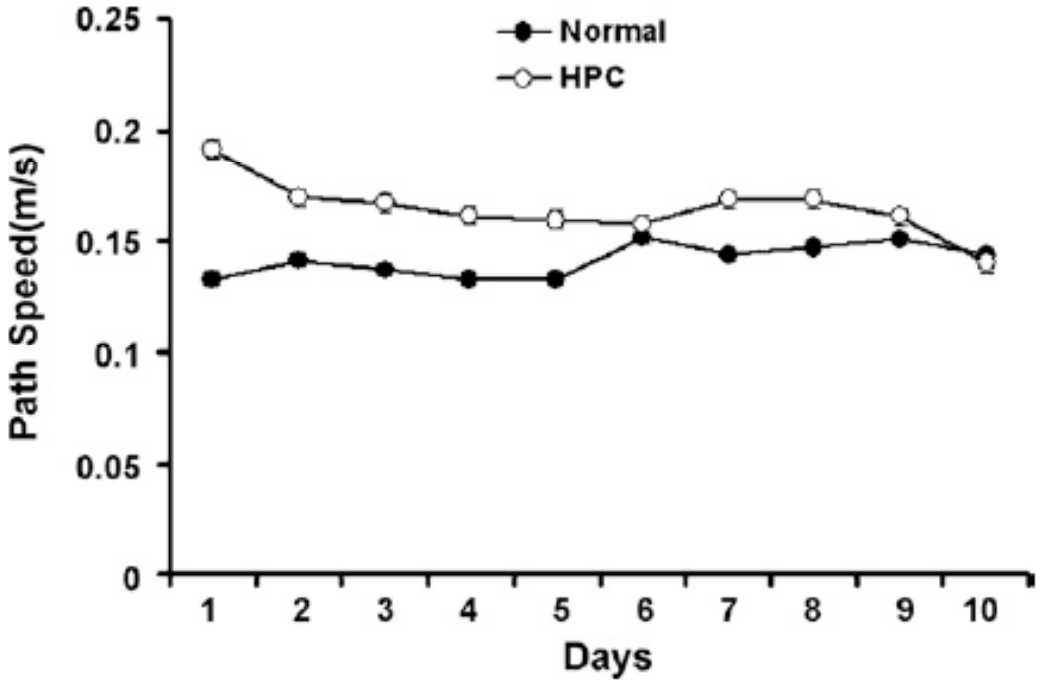


Figure 23. Mean path speed averaged across 10 days of testing in the ziggurat task. Rats with hippocampal damage moved consistently faster than rats in control groups except on days 6 and 10. Error bars show ± SEM.

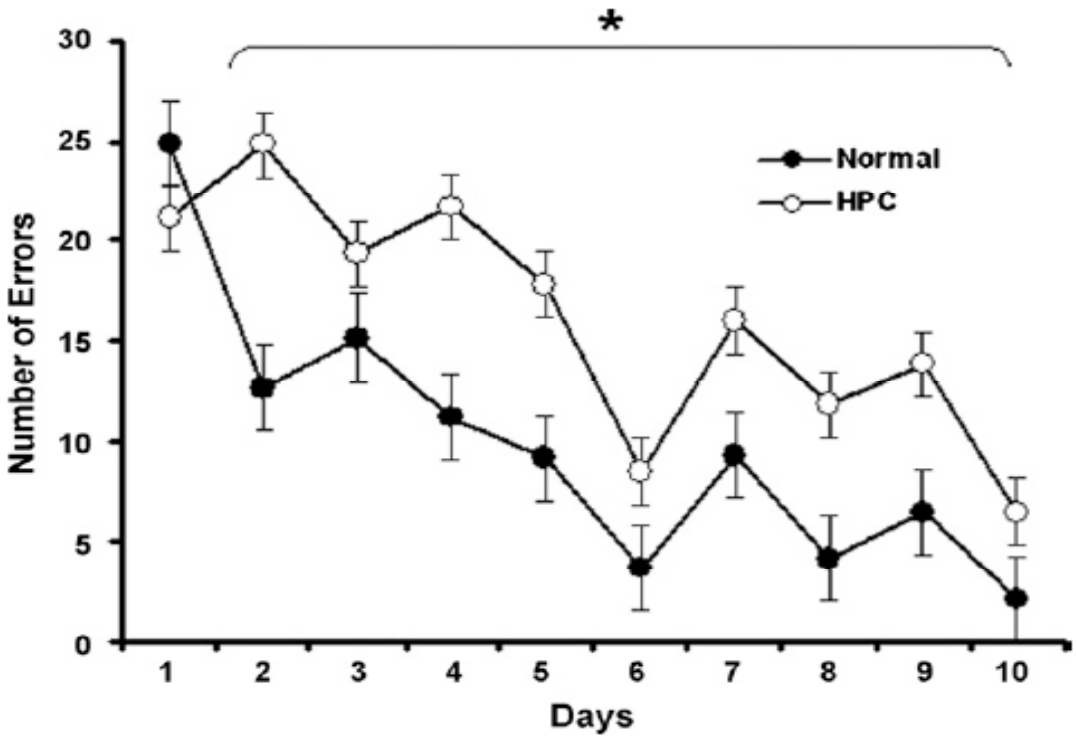


Figure 24. Averaged number of errors in the ziggurat task during 10 days of testing. Rats with hippocampal damage produced more errors on the testing days than did normal rats except on the first day. Asterisk indicates significance: * $p < 0.05$; ANOVA.

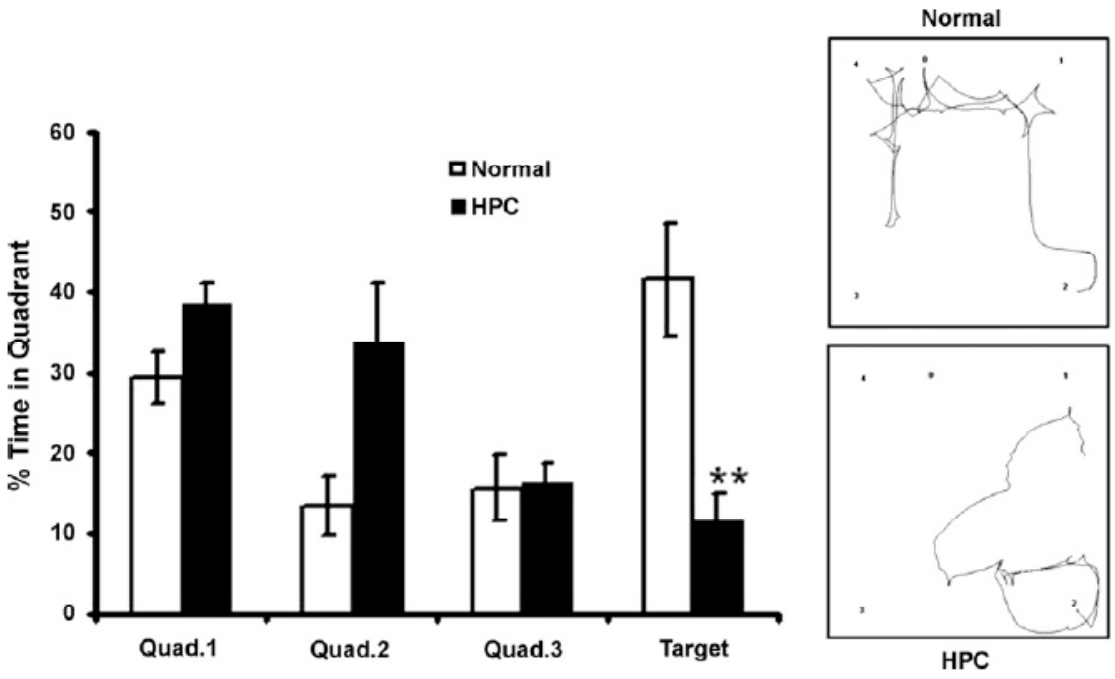


Figure 25. The mean percentage of time spent in the four quadrants of the ziggurat task during the 70 s of the second probe trial conducted on day 11. The goal ziggurat had previously been located in the first trial and the training quadrant during acquisition but no food might be found on it. Representative paths are included. Normal rats presented active memory for the training goal ziggurat (target). Error bars show ± SEM. Asterisks indicate significance: ** $p < 0.01$; unpaired *t*-test.

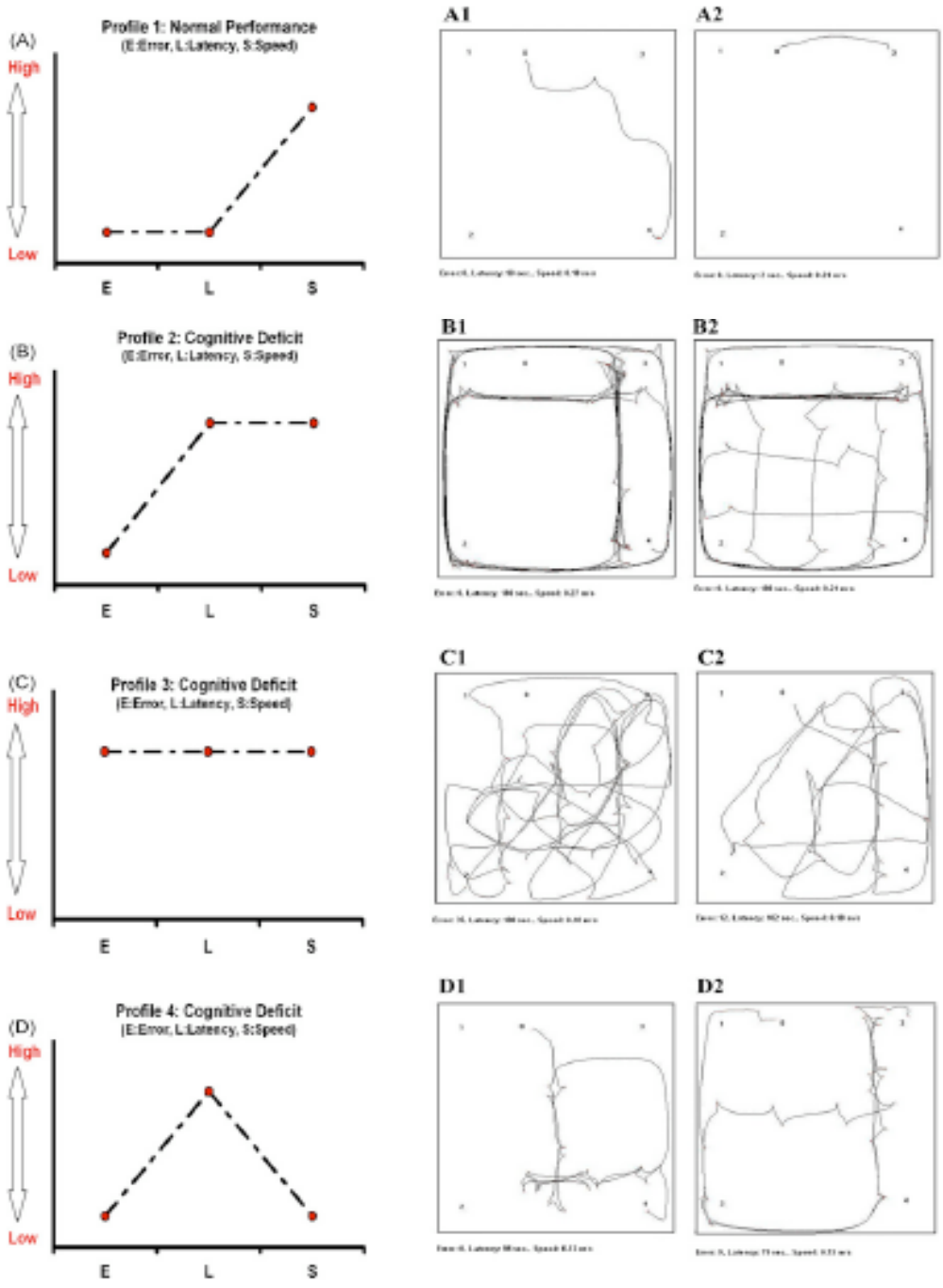


Figure 26. ELS scale and different cognitive profiles of rats in the ziggurat task. (A) Profile 1 which is usually a normal profile characterized by low error, low latency and high path speed, (B) profile 2 for cognitive deficit and is associated with low error, high latency and path speed, (C) profile 3 is characterized with high error, high latency, high speed and a clearly non-focused navigation, and (D) profile 4 with low error, high latency and low speed is specified for a type of cognitive disturbance associated with a motivational decline to navigate in the ziggurat task. E: error, L: latency, S: speed.

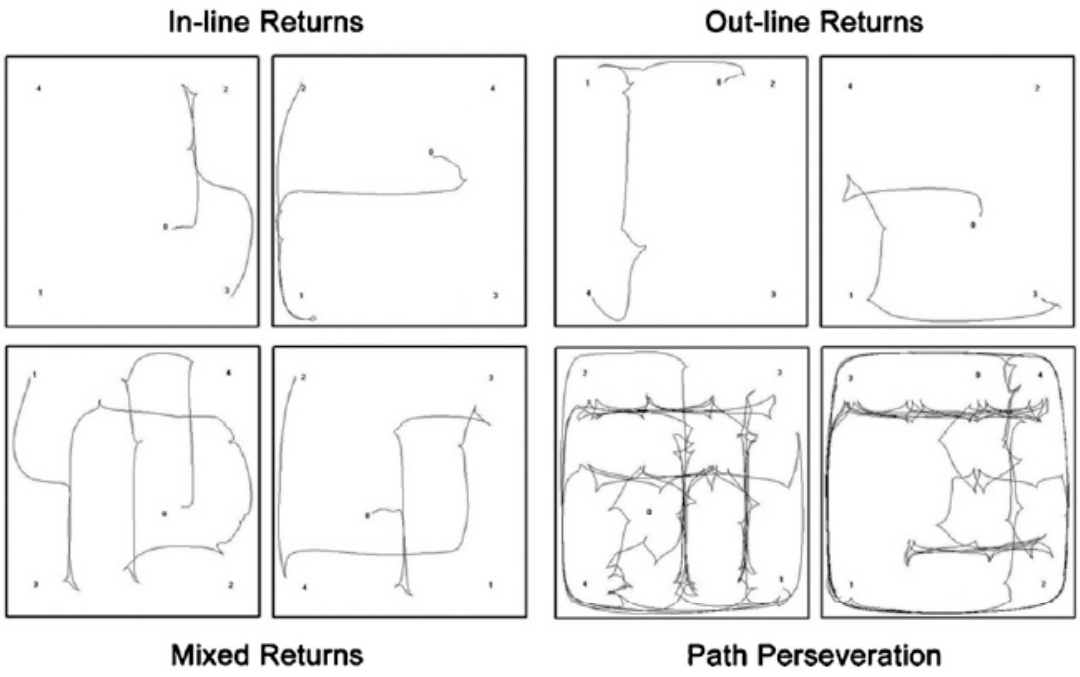


Figure 27. Paths taken to the goal ziggurat (number 0) by the rats in control and HPC groups from different starting points. In an in-line return, animals show returns to the pervious pathway, whereas in an out-line return they choose a new pathway in order to locate the spatial goal. Returns generally demonstrate that animals are actively involving in correction of their direction during the goal-based navigation in the ZT. Mixed returns reflect both in-line and out-line returns in a given trial. Path perseveration, however, refers to a situation in which widespread hippocampal-damaged rats show an uncontrollable repetition of particular returns in given direction(s).

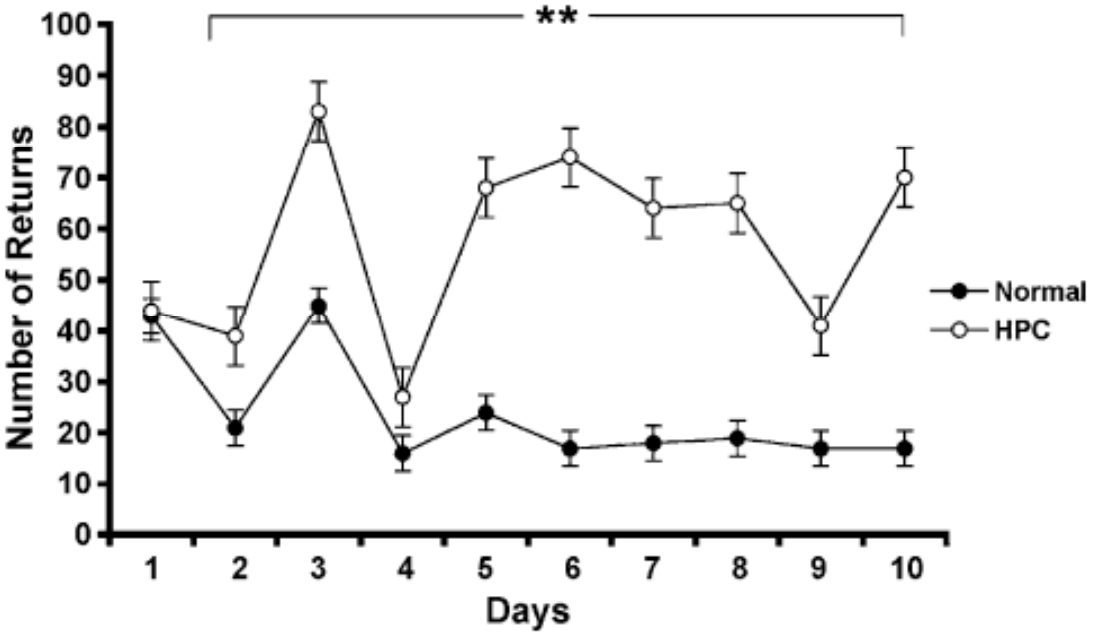


Figure 28. The number of returns in the ZT for both groups. HPC rats showed significantly more returns than controls rats over 10 testing days. Error bars denote average ± SEM for each group. Asterisks indicate significances: ***p* < 0.01, ANOVA compared to control values.

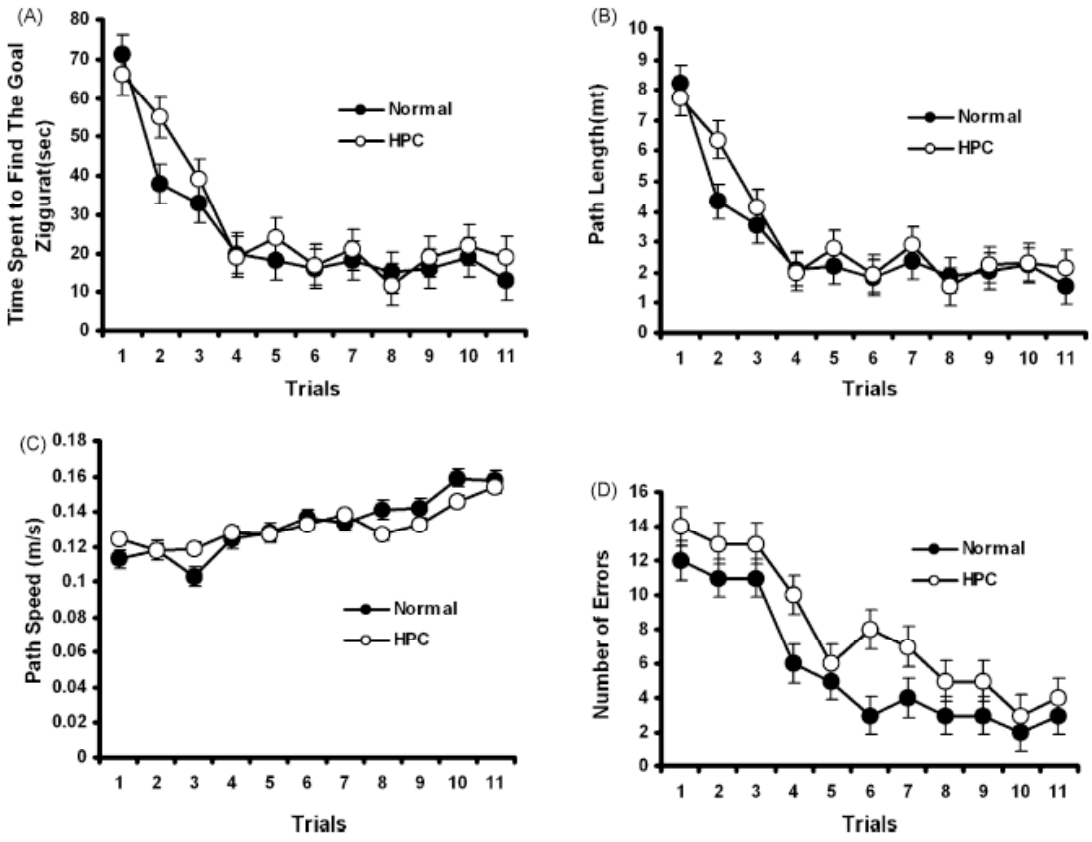


Figure 29. Non-spatial performance of control and HPC rats on an 11-trial block of ZT in which a black ziggurat was considered''goal ziggurat. (A) Latency or time spent to find the cued goal, (B) path length or mean distance traveled, (C) path speed, and (C) the number of errors to locate the cued-goal ziggurat. Groups did not differ significantly in the indices to locate the cued goal for the entire block.

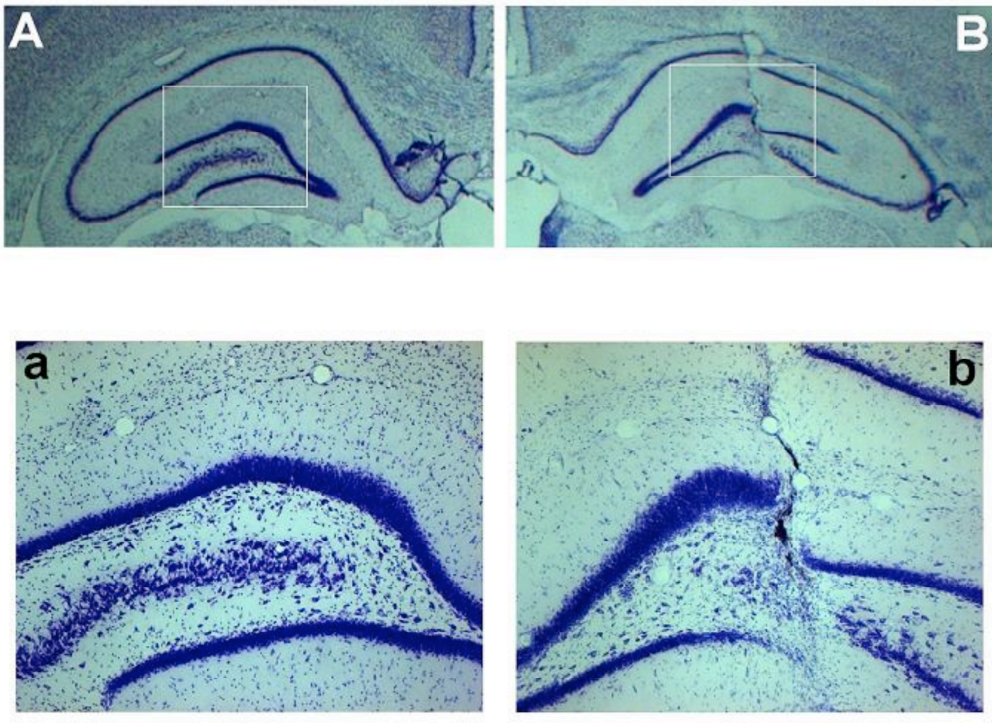


Figure 30. Photomicrograph of a coronal section of a dorsal region of the hippocampus (top panel, magnification 1×) for a control (A & a) and HPC stroke (B & b) rat. White squares are focusing to show the dentate gyrus (DG) area of the hippocampus for the control and HPC rats. Both low and higher magnifications 1× and 10× (top and below-B & b) of the DG show the hippocampal damage in the HPC stroke rat.

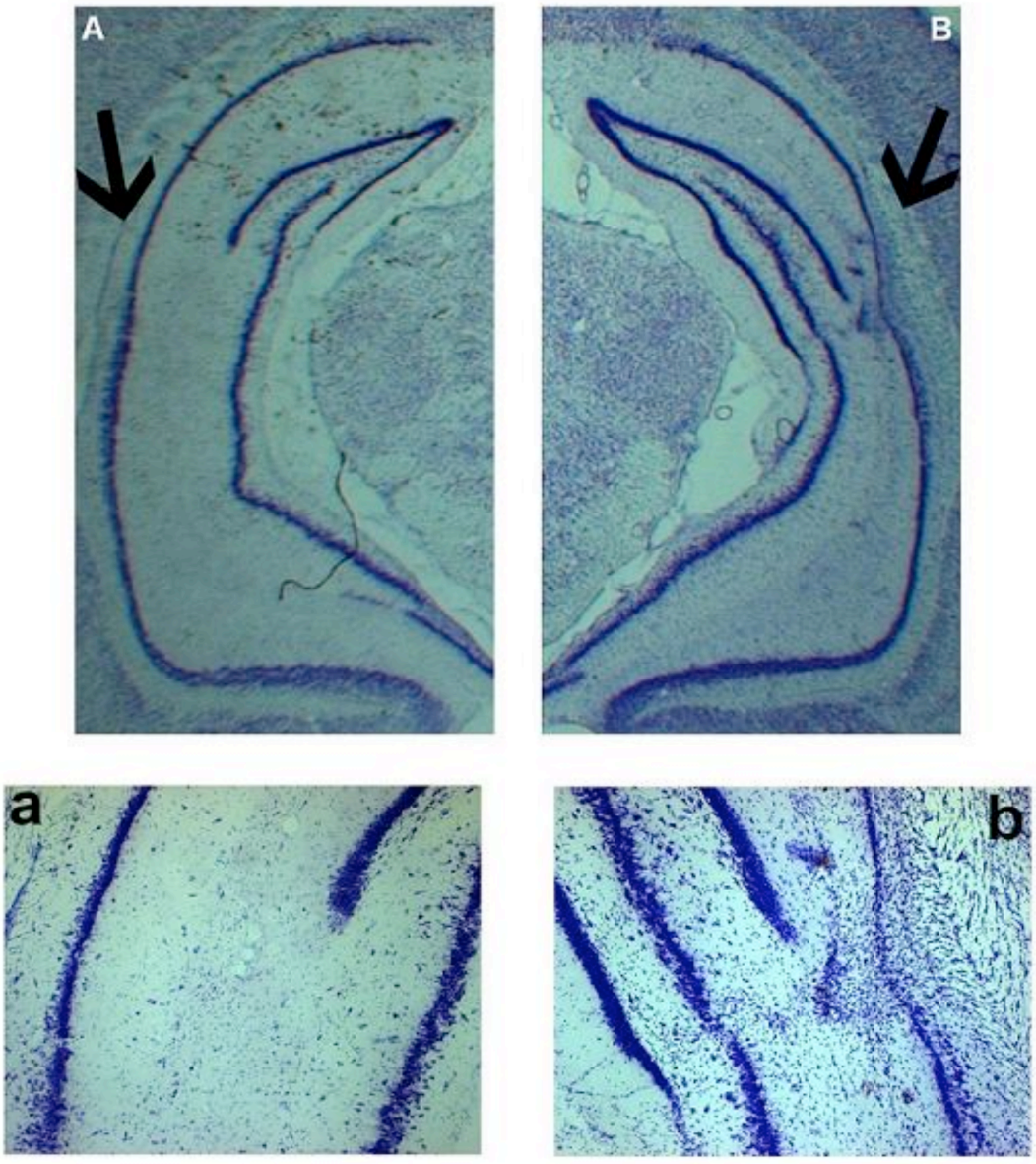


Figure 31. Photomicrograph of a coronal section of a ventral region of the hippocampus (top panel, magnification 1×) in a control (A & a) and HPC stroke (B & b) rat. Black arrows show the CA2 area of the hippocampus. Higher magnification (10×) of CA1 in panel below shows that all ischemic rats had cell death in the CA2 area resulting from ET-1 infusion.

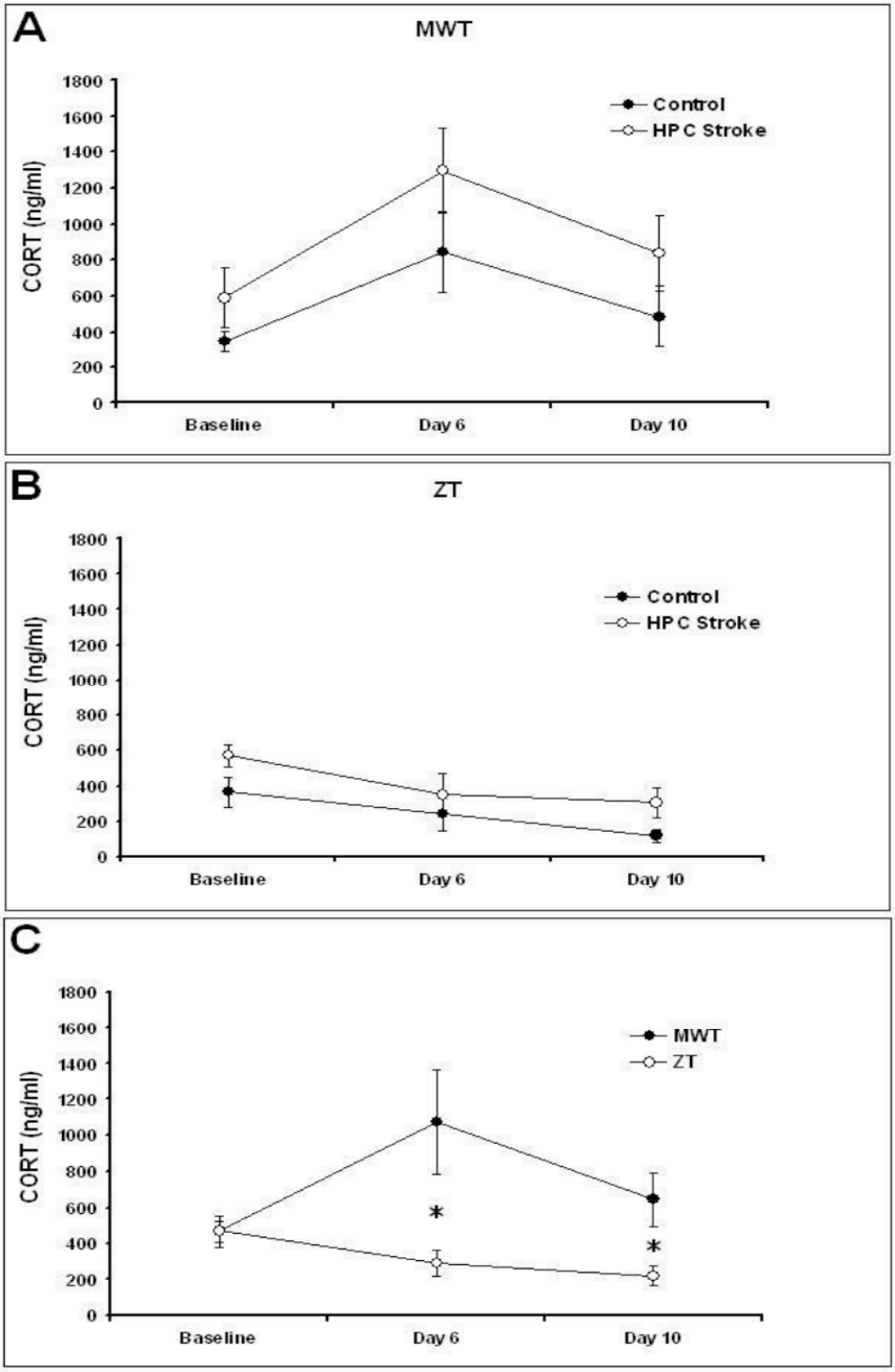


Figure 32. (A) Plasma CORT concentration in the MWT and (B) the ZT at different time points. (C) Circulating CORT levels in the both groups were significantly higher in the MWT relative to the ZT. * $p < 0.05$; independent samples *t*-test for between-subject comparison. Error bars show ± SEM.

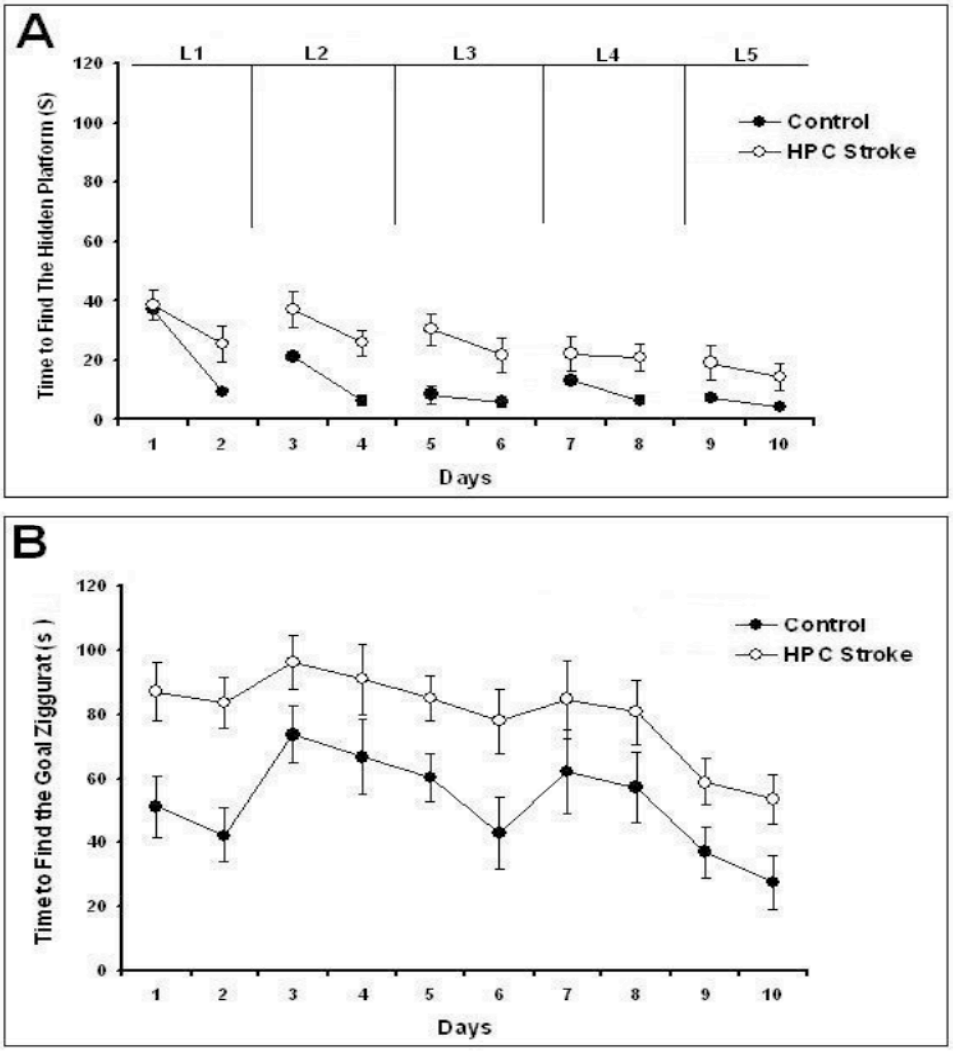


Figure 33. (A) Average latency to find the hidden platform on different- and same-platform days in MWT, and (B) the goal ziggurat in the ZT for all groups. In both environments, the HPC stroke rats showed significantly higher latency than the controls. Error bars denote average ± SEM for each group. L: Location of platform.

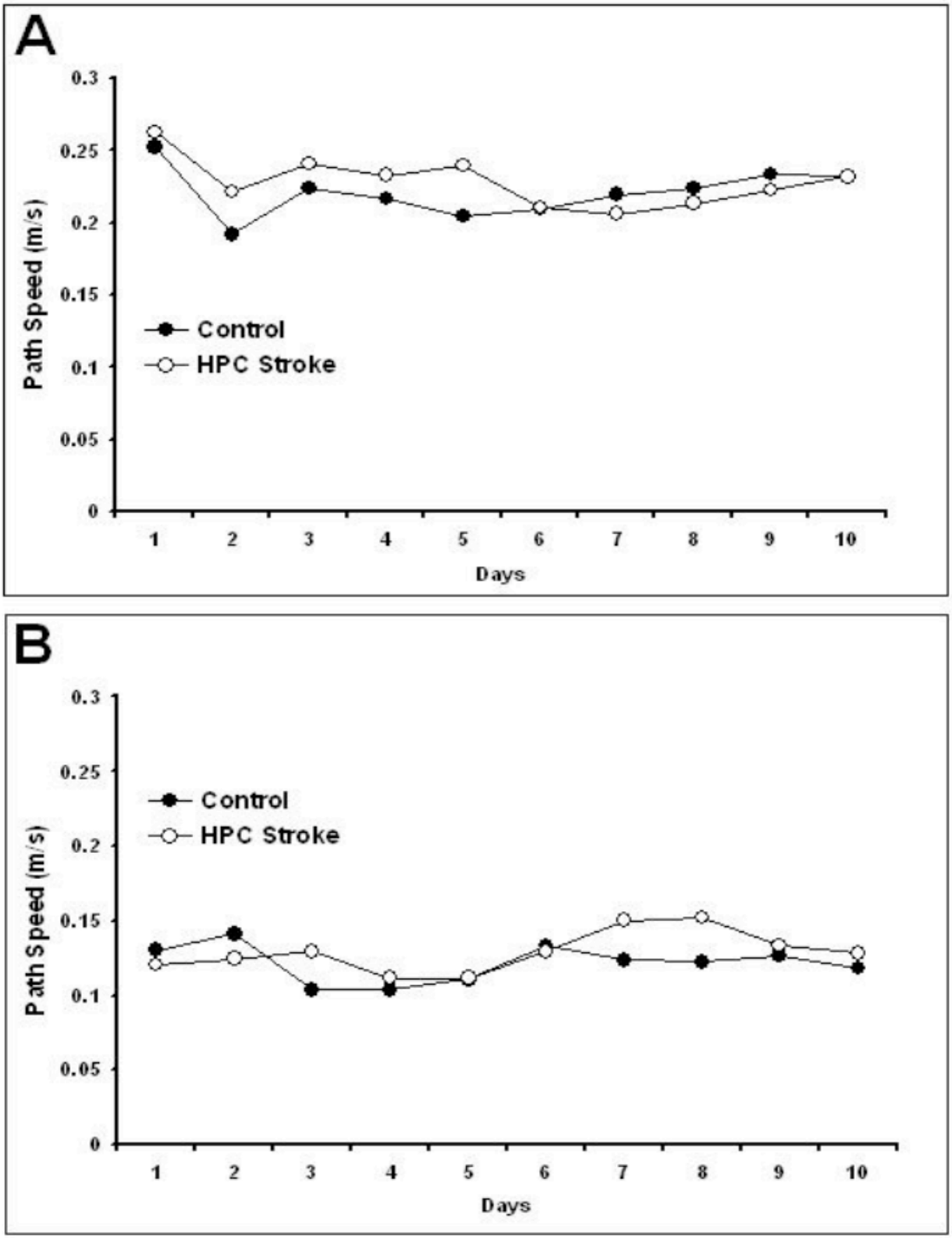


Figure 34. Path speed averaged across 10 days of testing in (A) MWT and (B) the ZT. Both groups showed relatively constant speeds across the 10 testing days in MWT and the ZT. No significant difference was found between groups. Error bars show ± SEM.

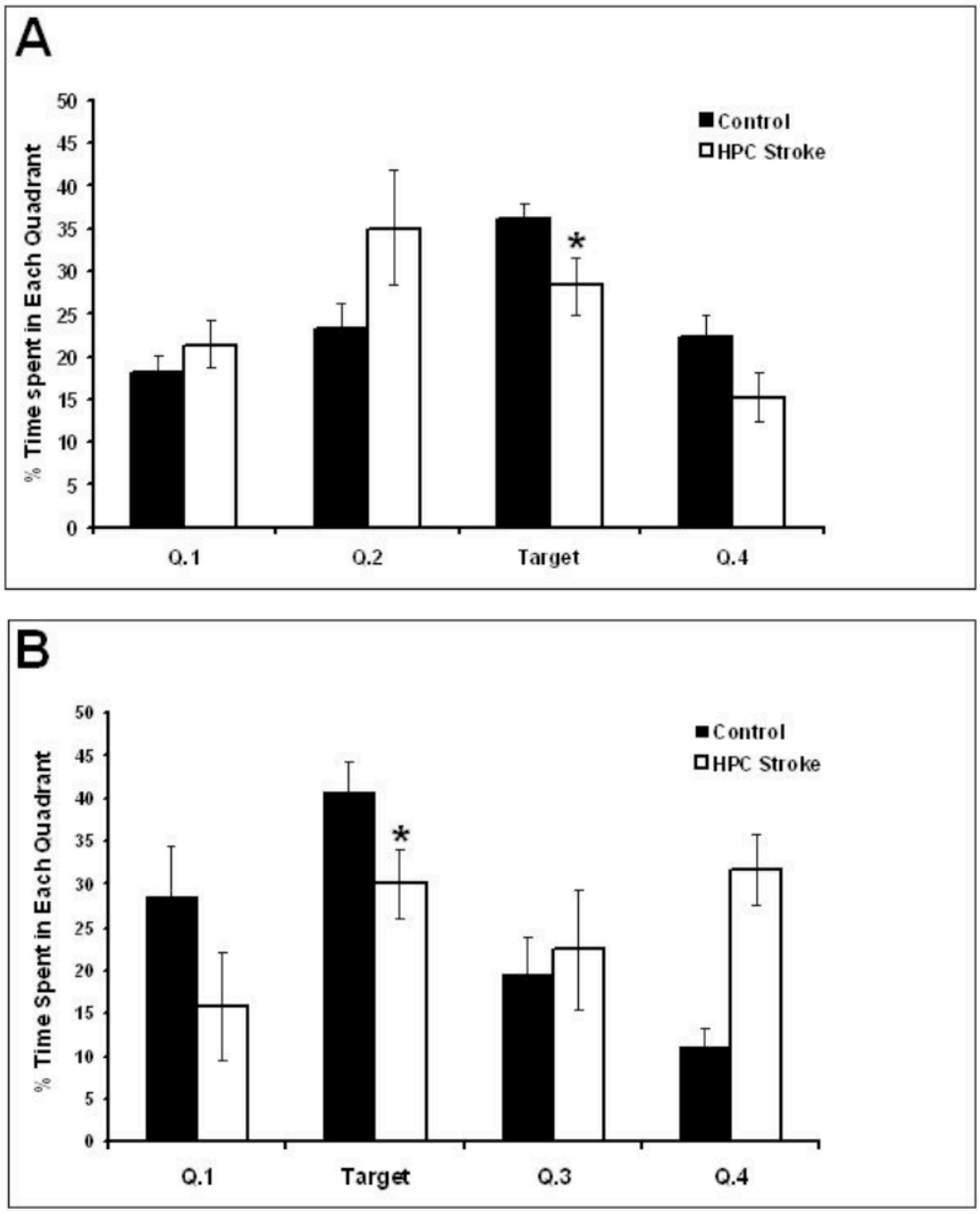


Figure 35. (A) The mean percentage of time spent in the four quadrants of MWT and (B) the ZT during the probe trial conducted on day 9. Controls spent significantly more time searching for the platform and goal ziggurat in the target quadrant relative to the HPC stroke group. * $p < 0.05$; Independent samples *t*-test. Error bars show ± SEM.

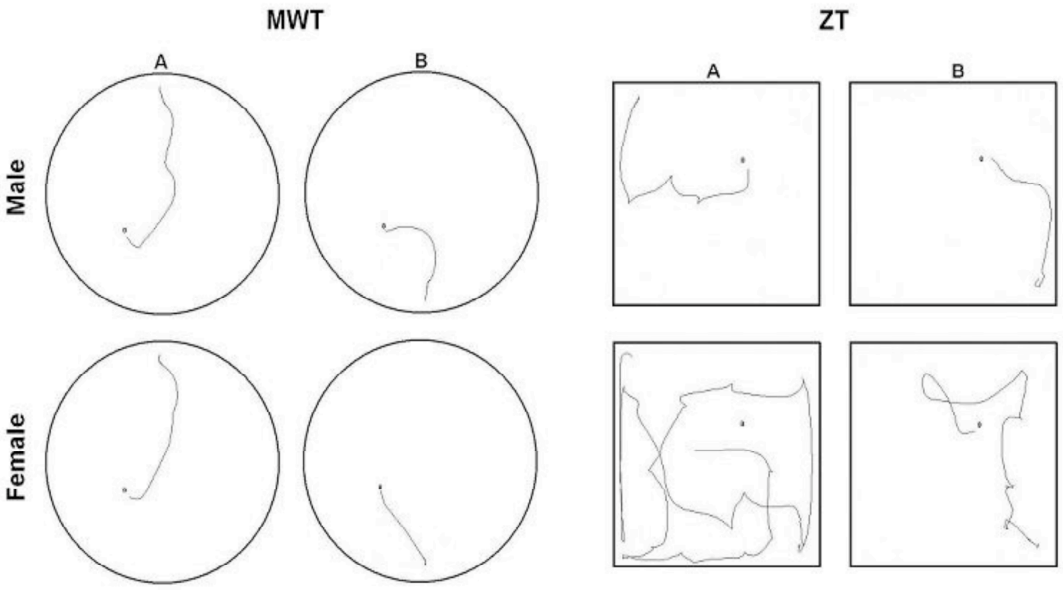


Figure 36. Path taken on trials 3 (A) and 8 (B) in MWT and ZT by a typical male and female rat. The tracks show the rats' trajectory from one starting point to the hidden platform or the goal ziggurat (number 0). Note the length of path taken by the female rat in the ZT that obviously is longer than that of the male rat even in the last trial.

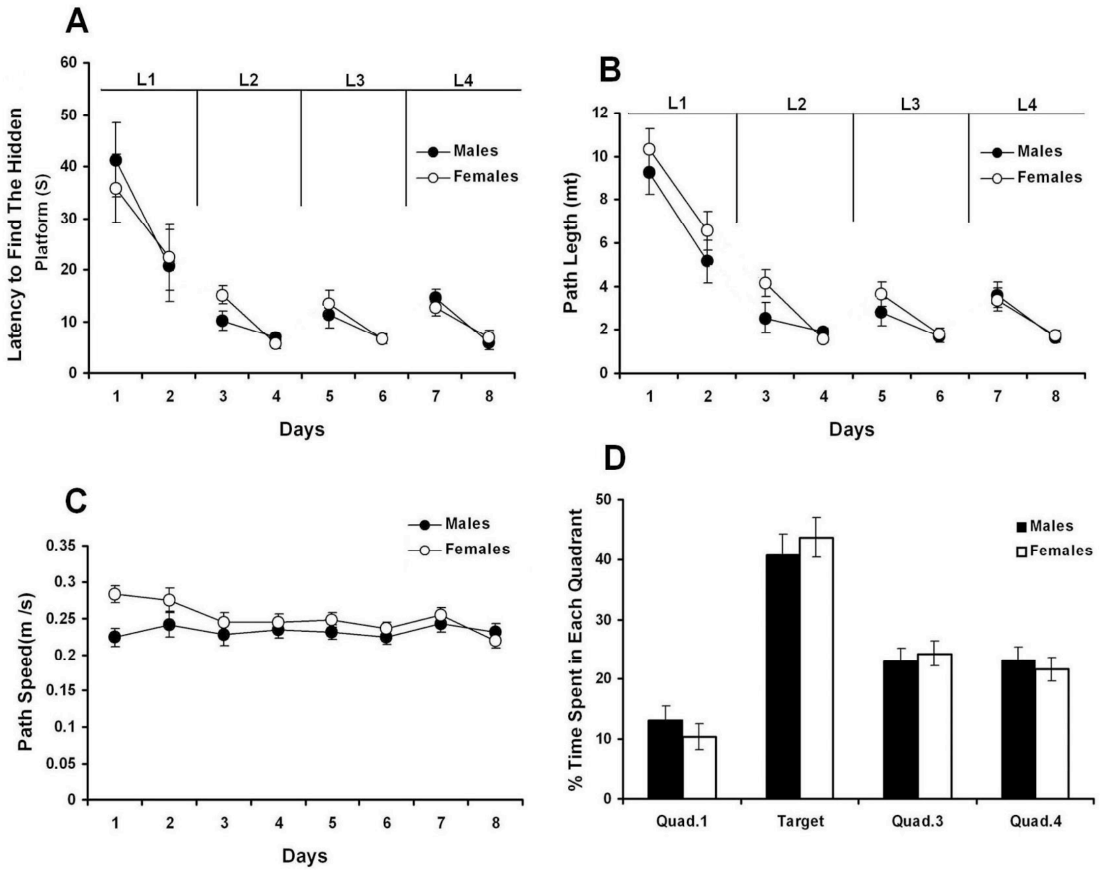


Figure 37. Testing in MWT. (A) Latency to find the hidden platform during 8 days of testing. (B) Mean distance traveled (path length) to locate the hidden platform during each day of testing. (C) Mean path speed averaged across 8 days of testing, and (D) the mean percentage of time spent in the four quadrants of MWT during the first half of the probe trial (30-s duration) conducted on day 9. No significant difference between groups was found in the behavioural measures of the task. L: Location of platform.

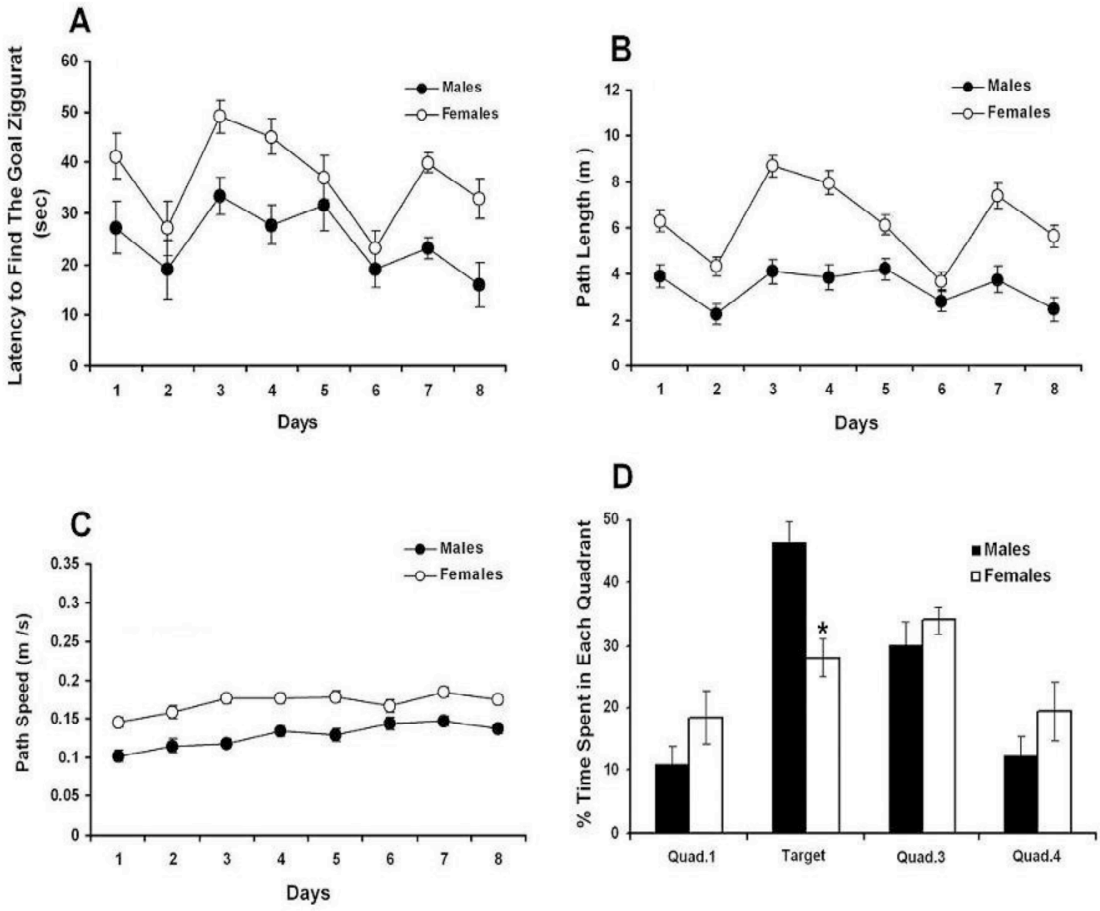


Figure 38. Testing in the standard or non-cued version of the ZT for spatial learning. In this environment animals must use spatial cues to navigate to the goal ziggurat. (A) Latency to find the goal ziggurat during 8 days of testing. (B) Mean distance traveled (path length) to locate the goal ziggurat during each day of testing. (C) Mean path speed averaged across 8 days of testing, and (D) the mean percentage of time spent in the four quadrants of the ZT during the first half of the probe trial (30-s duration) conducted on day 9. Female rats moved consistently faster than male rats. In all spatial measures of the task, they were significantly different. Error bars denote average ± SEM for each group. Asterisks indicate significance: * $p < 0.05$; ANOVA and independent sample *t*-test.

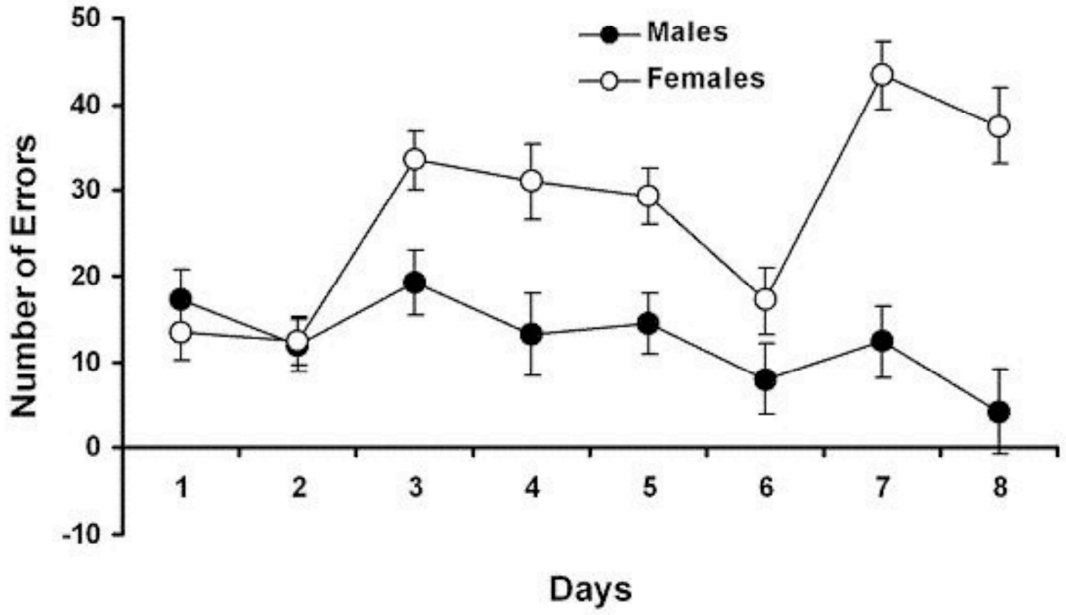


Figure 39. Averaged number of errors in the ziggurat task during 8 days of testing. Female rats produced more errors on the testing days than did male rats except on the first and second days. Error bars denote average ± SEM for each group.

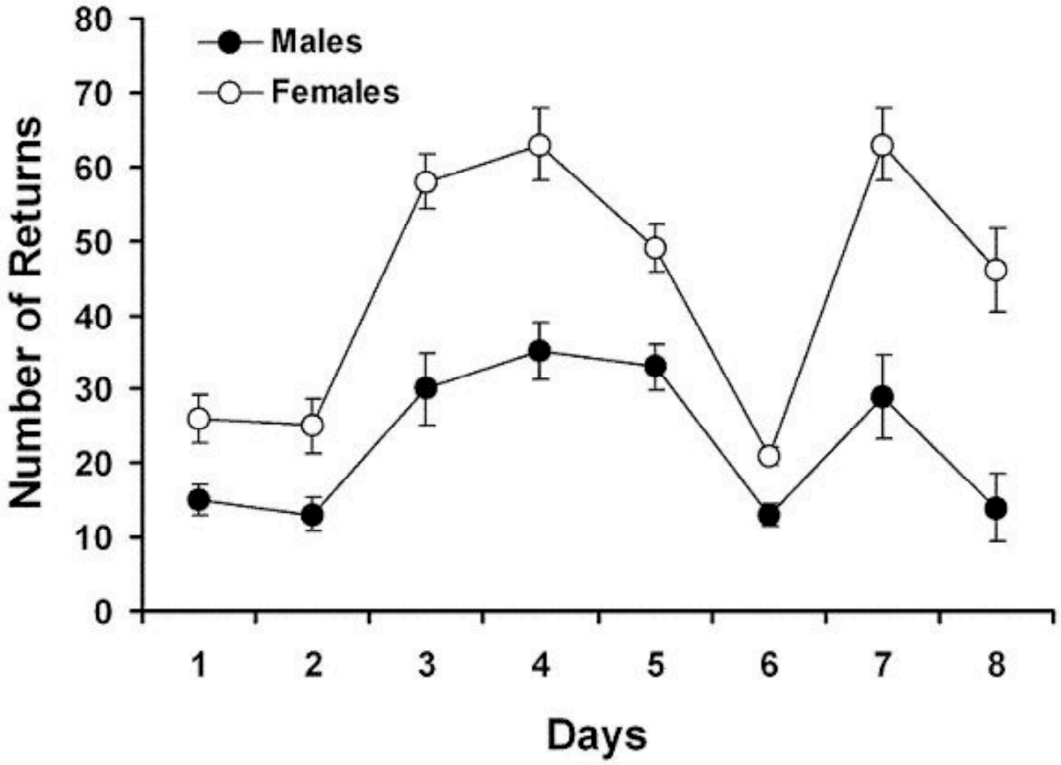


Figure 40. The number of returns in the ZT for both groups. Females showed significantly more returns than males over 8 testing days. Error bars denote average ± SEM for each group.

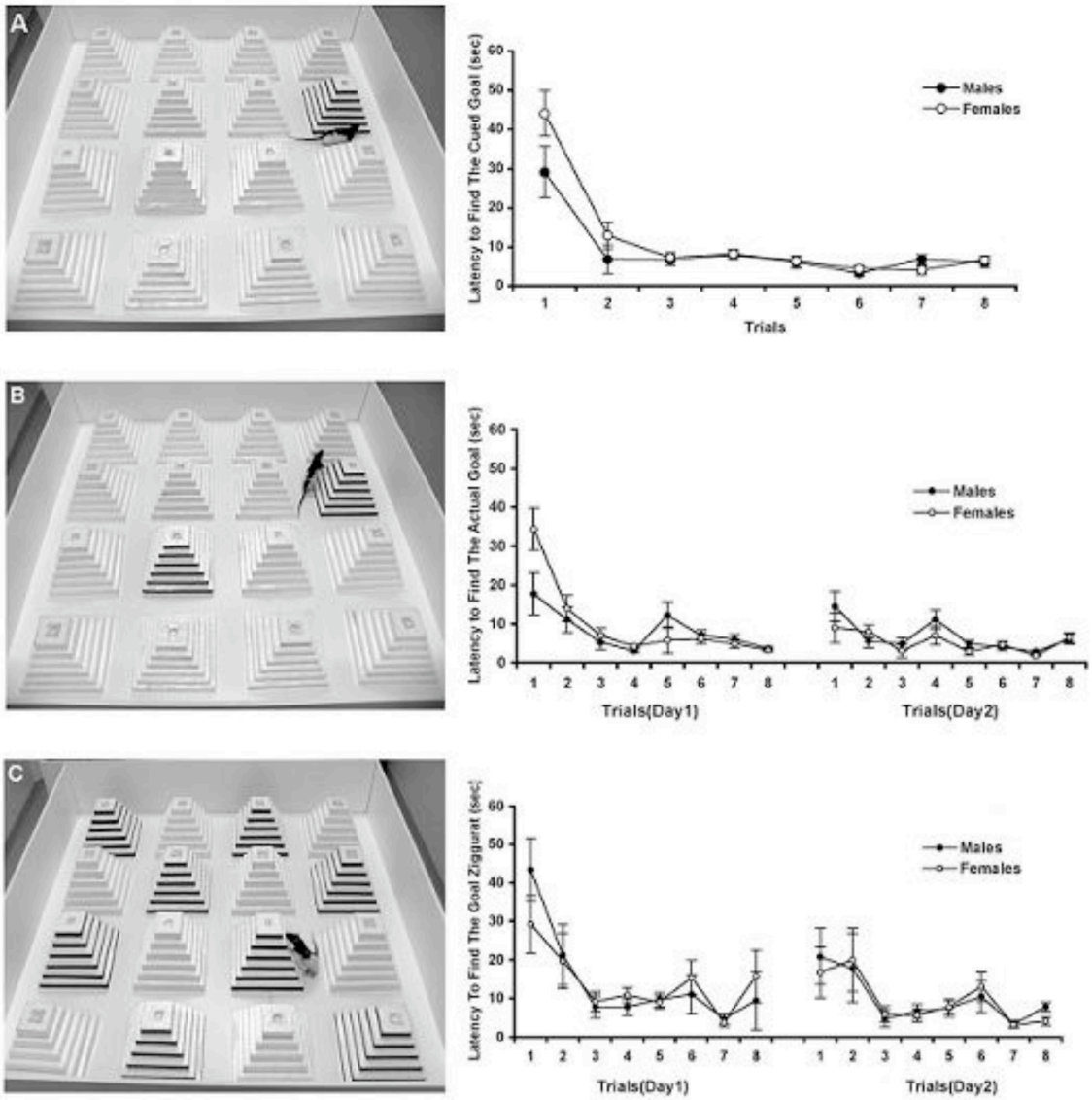


Figure 41. Testing in the different non-spatial variations of the ZT. (A) Cued version of the ZT for non-spatial learning, and latency to find the cued goal. In the cued version of the ZT, rats learn that only the black ziggurat has food. (B) Single foil or matching to sample version of the ZT, and latency to locate the actual goal. Rats in this environment are required to learn only that one black ziggurat is a true goal, and thus has food. The second black ziggurat is not baited. (C) Multi-foil version of the ZT in which animals must learn and remember that only one black or white ziggurat has food. As it can be seen, rats in the both groups did not show significant differences in terms of latency and other behavioural measures (data not shown).

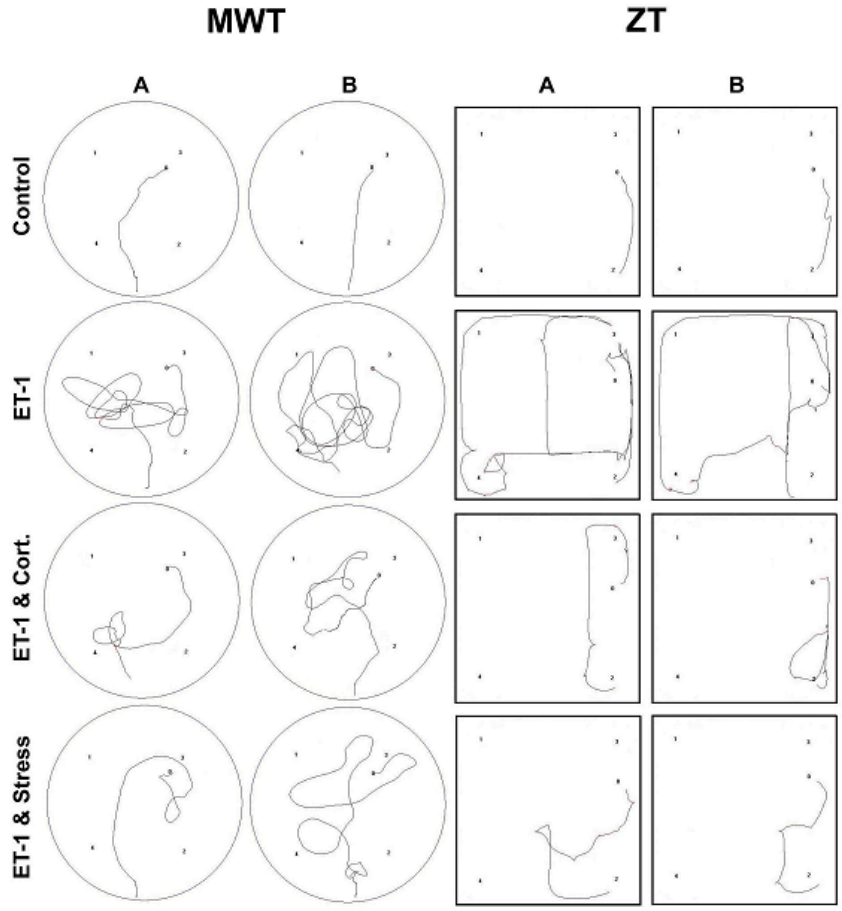


Figure 42. Path taken on trial 6 in MWT and on trial 8 in ZT by two rats (A & B) of each group from one starting point to the hidden platform or the goal ziggurat (number 0). Note the length of path taken by ET-1-only rats that obviously is longer than that of the other ischemic rats and controls within both tasks.

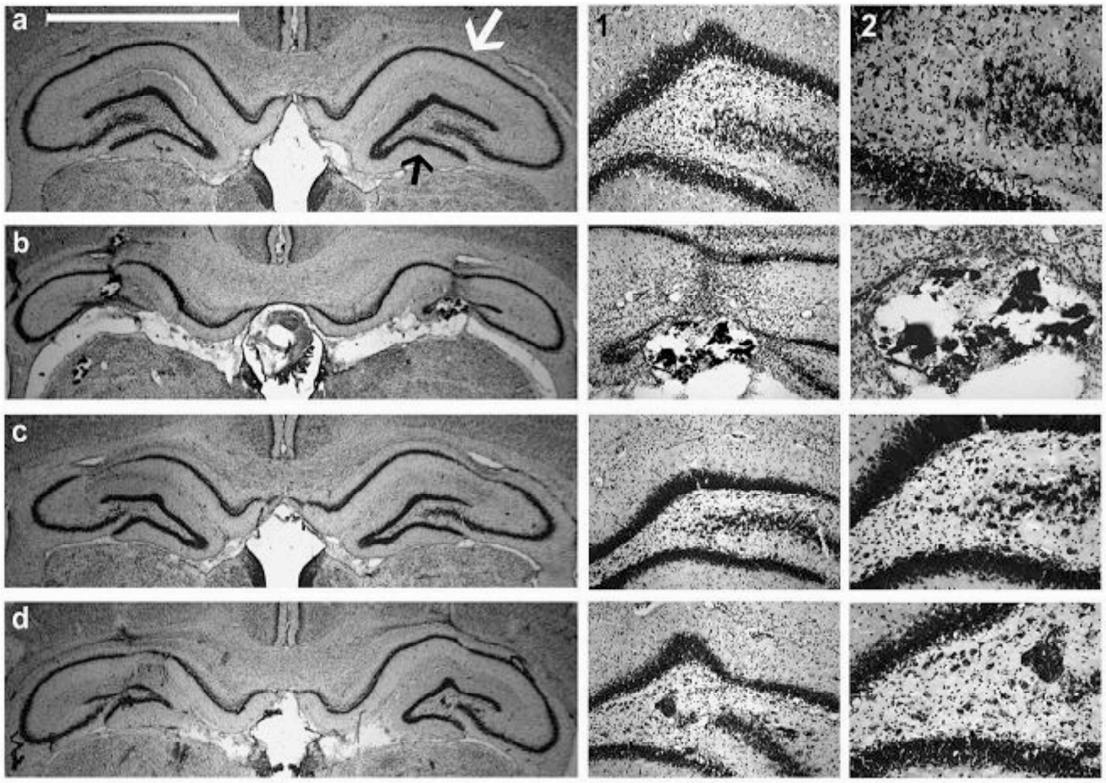


Figure 43. Photomicrograph of a coronal section of a dorsal region of the hippocampus (left panel, magnification 1×) for a control (a), ET-1 (b), ET-1+ corticosterone (c) and an ET-1 + stress (d) rat. White calibration bar measures 2 mm. Black arrow shows the dentate gyrus (DG) area and white arrow indicates the CA1 region of the hippocampus for the control rat. Both low and higher magnifications 10× and 20× (middle and right panels) of the DG show the extensive hippocampal damage in the ET-1-only rat and the post-stroke recovery following corticosterone treatment and stress in the same area.

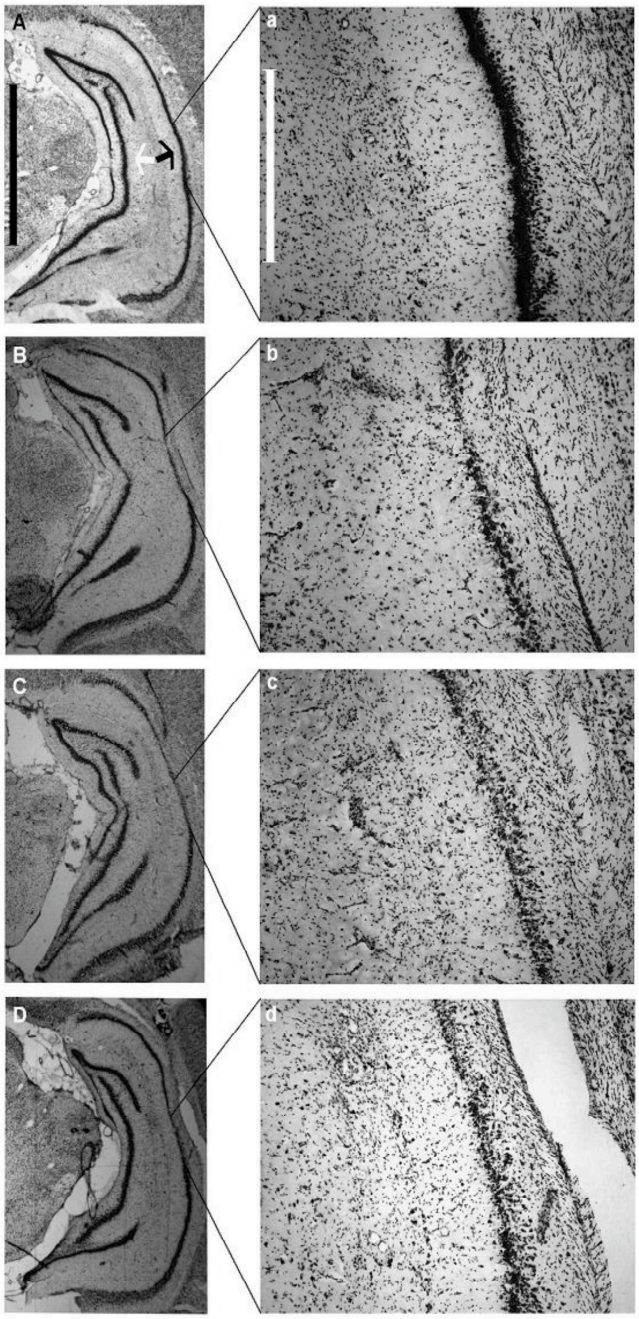


Figure 44. Photomicrograph of a coronal section of a ventral region of the hippocampus (left panel, magnification 1×) in a control (A), ET-1 (B), ET-1+corticosterone (C) and an ET-1+stress (D) rat. Black calibration bar measures 2 mm. Black arrow shows the CA1 area and white arrow indicates the CA3 region of the hippocampus for the control rat. Higher magnification (10×) of CA1 in the right panel shows that all ischemic rats (b, c, d) had an extensive cell death in the CA1 area. White calibration bar measures 200 μm. No recovery process was found in the ET-1 + corticosterone/stress (c & d respectively) groups.

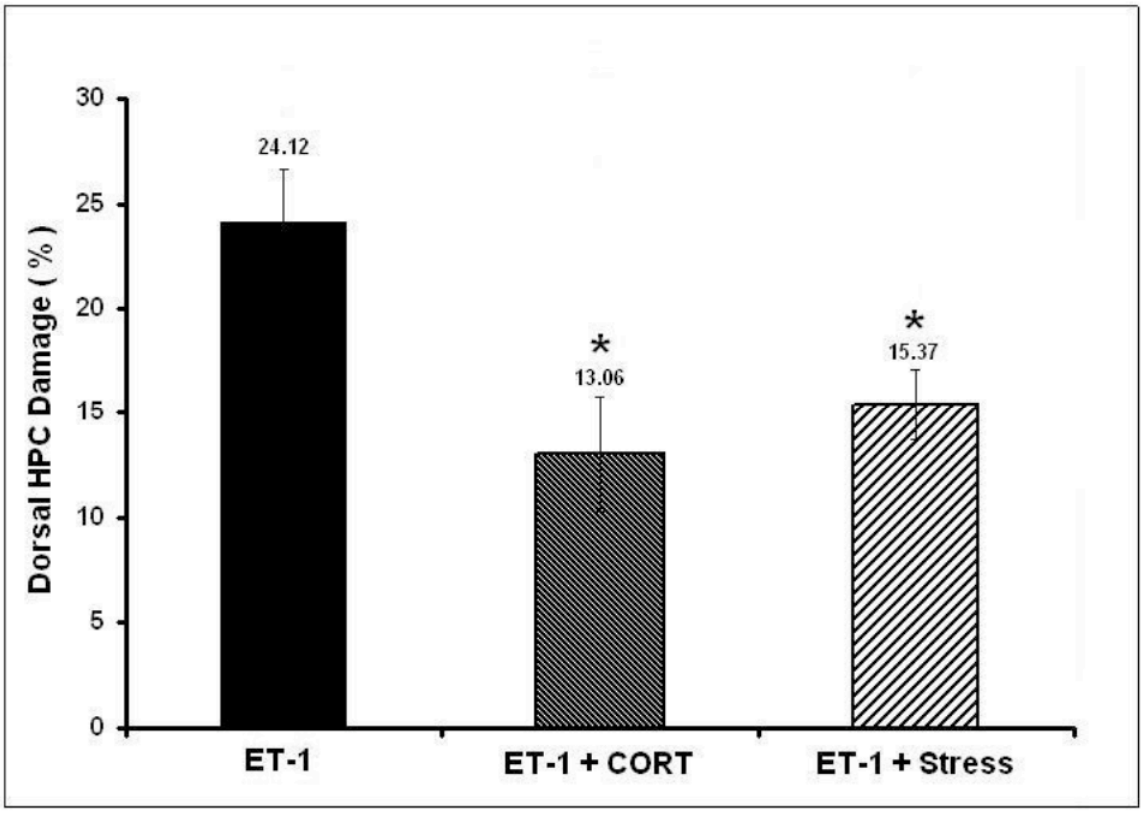


Figure 45. Dorsal hippocampal damage following stroke, CORT and stress treatments. Rats with stroke + CORT and stroke + stress showed significantly lesser tissue loss compared to stroke-only group. * $p < 0.05$; Error bars show ± SEM.

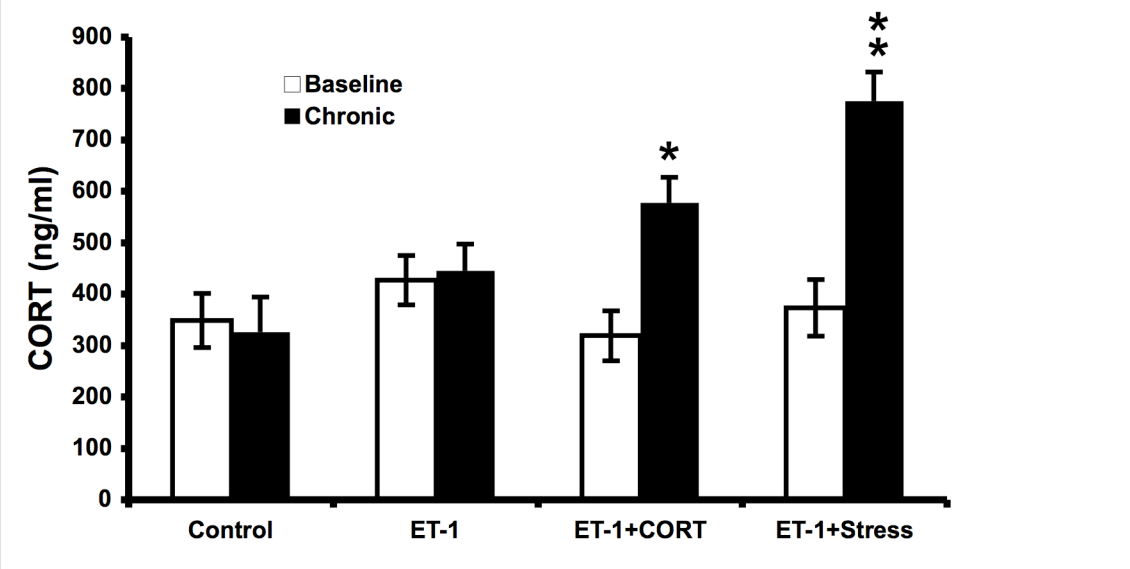


Figure 46. Plasma CORT concentration prior to (baseline) and at chronic levels (day 16 of daily treatment) of oral administration of corticosterone and daily immobilization stress. Circulating CORT levels were significantly higher after chronic administration of CORT in ET-1+CORT group and after restraint stress in the ET-1+Stress group. * $p < 0.05$ and ** $p < 0.01$; dependent samples *t*-test for within-subject comparison. Error bars show ± SEM.

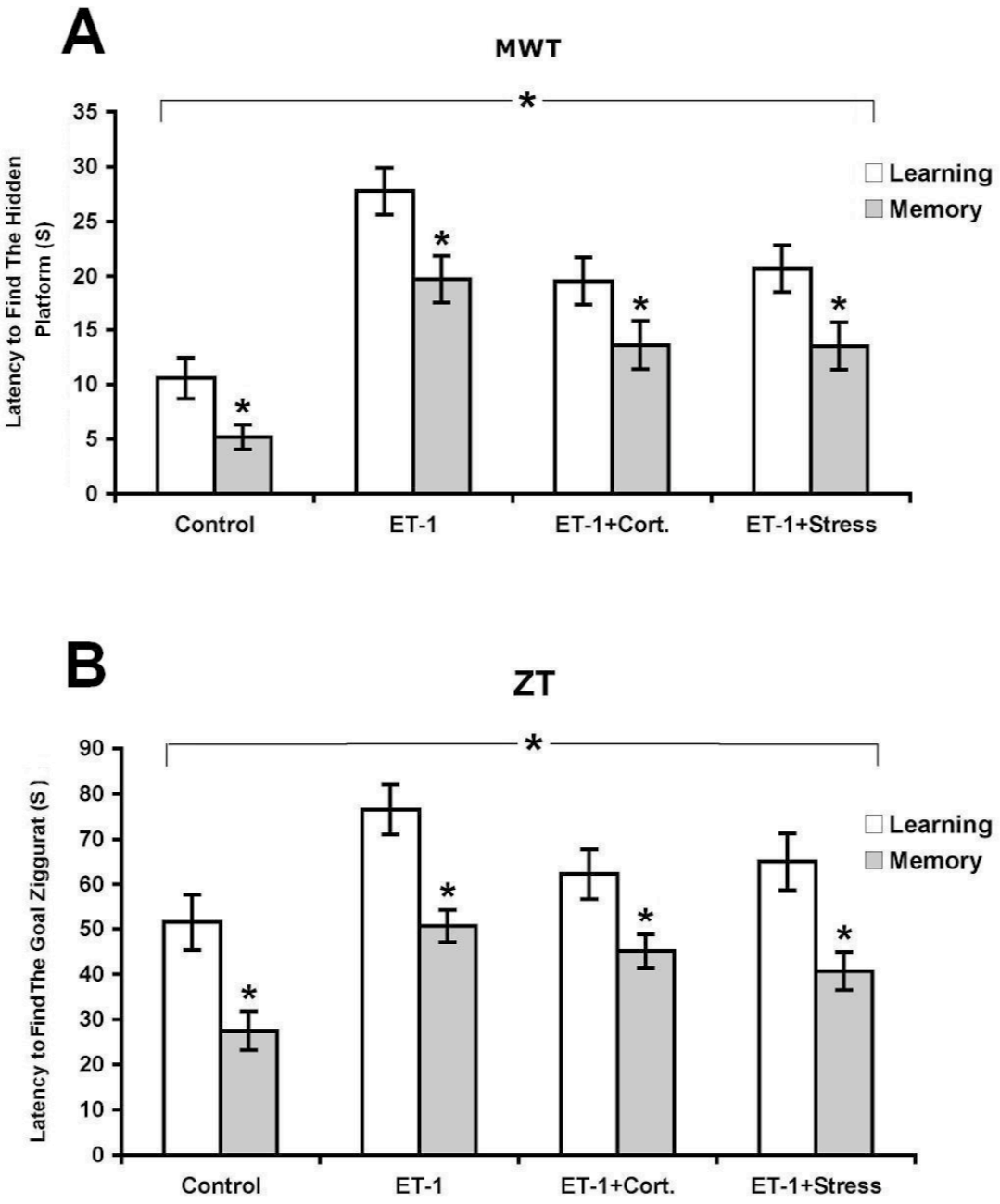


Figure 47. (A) Average latency to find the hidden platform on different- and same-platform days(learning and memory) in MWT, and (B) the goal ziggurat in the ZT for all groups. In both environments, the ET-1-only rats showed significantly higher latency than the other groups. Error bars denote average ± SEM for each group. * $p < 0.05$; repeated measure ANOVA and dependent samples *t*-test for within-subject comparison.

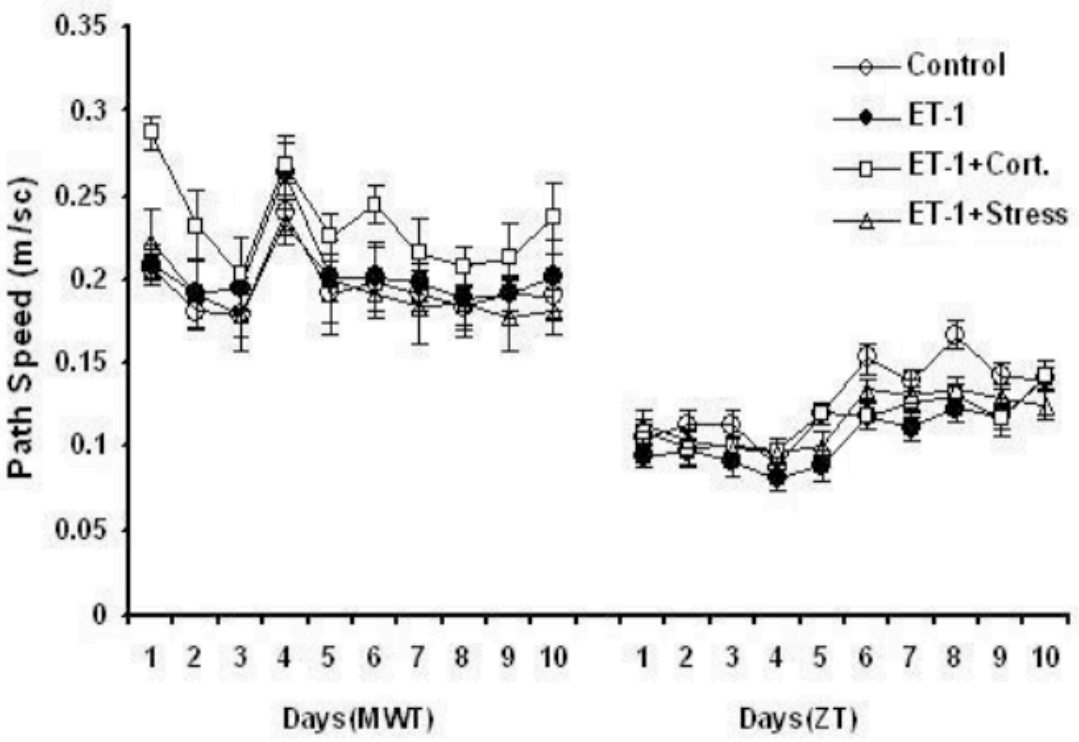


Figure 48. Path speed averaged across 10 days of testing in MWT and the ZT. Rats with ET-1-induced stroke and CORT swam consistently and significantly faster than rats in the other groups. This speed profile has not been found for the same group in the ZT. Error bars show ± SEM.

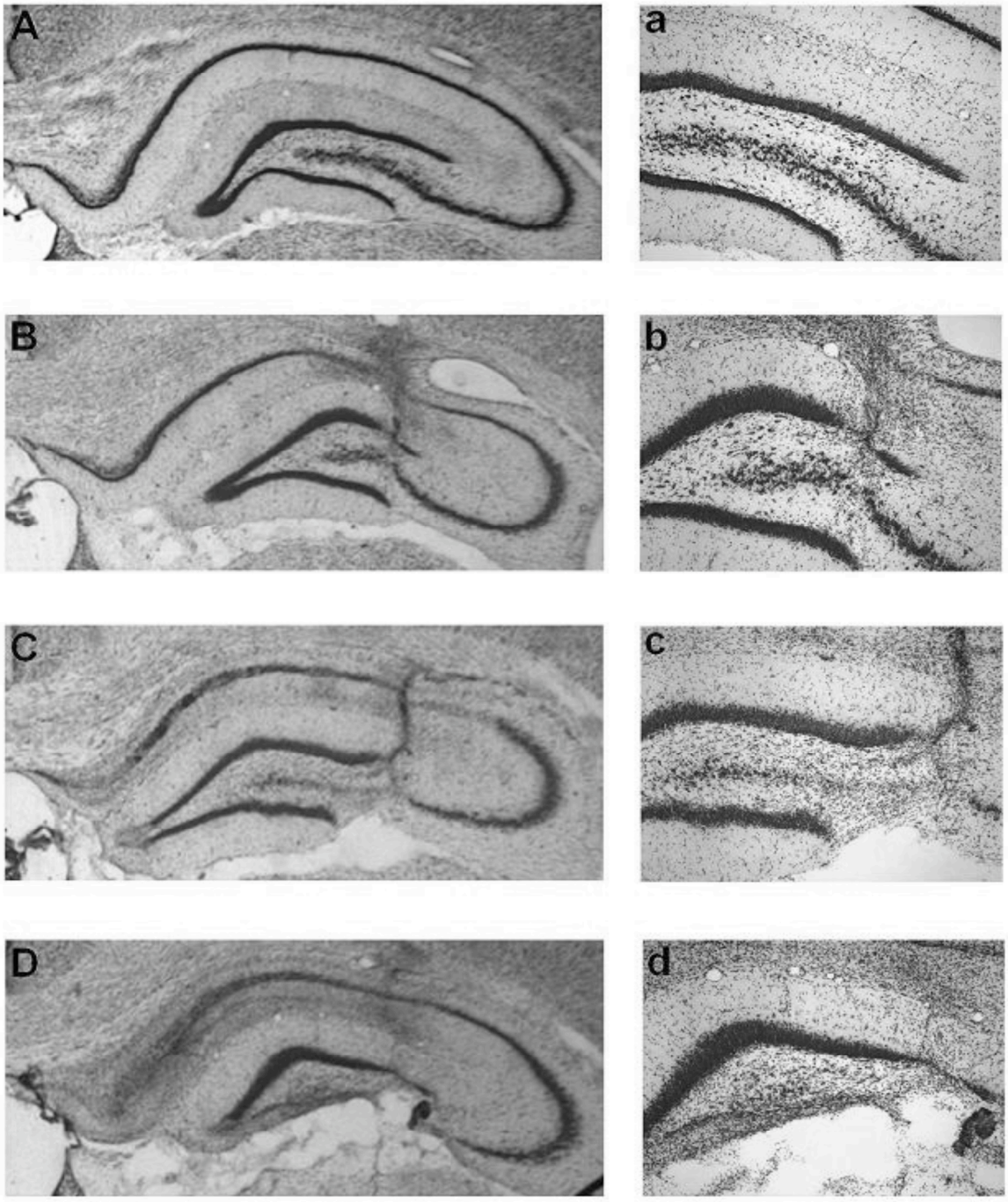


Figure 49. Photomicrograph of a coronal section of a dorsal region of the hippocampus (left panel, magnification 1×) for a control (A), HPC stroke (B), CORT + HPC stroke (C) and stress + HPC stroke (D) rat. Higher magnification (10×; right panels) of the CA1 and the DG shows the hippocampal damage in the HPC stroke (b) and CORT + HPC stroke (c) and stress+HPC stroke (d) rats. Stress-stroke combination was significantly associated with more cell loss than stroke-only and CORT + stroke groups.

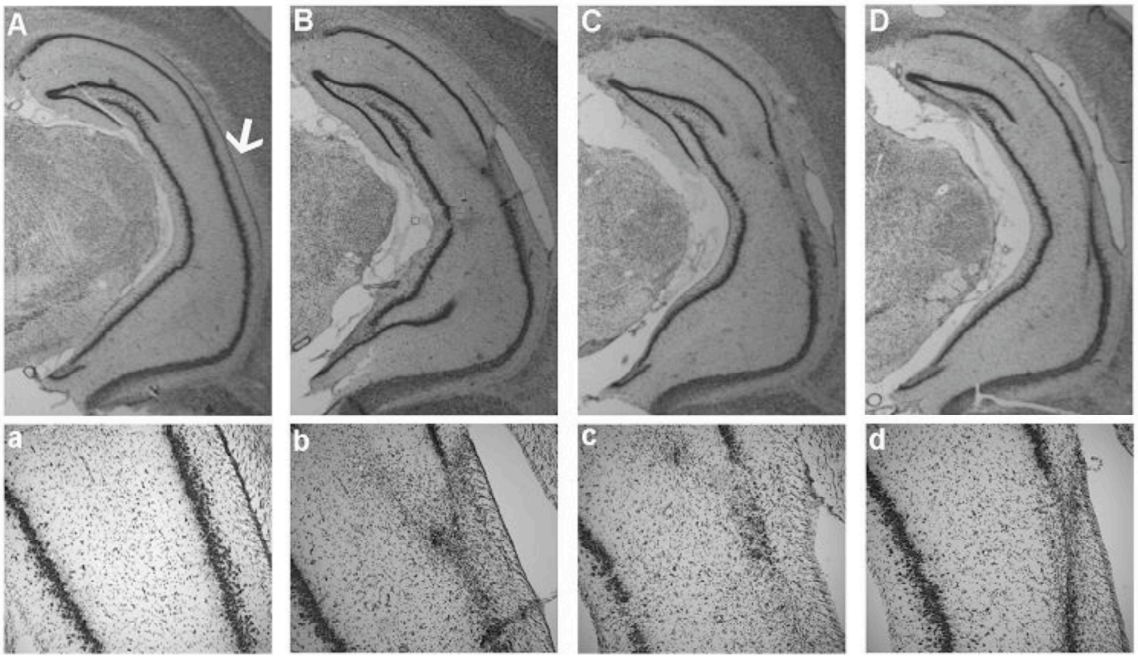


Figure 50. Photomicrograph of a coronal section of a ventral region of the hippocampus (top panel, magnification 1×) in a control (A), HPC stroke (B), CORT + HPC stroke (C) and stress + HPC stroke (D) rat. Black arrow shows the CA1 region of the hippocampus for the control rat. Higher magnification (10×; below) panels show that all ischemic rats (b, c, d) had an extensive cell death in the CA1 area. No destructive effects of CORT and stress were found in the CORT+ and stress + HPC stroke (c & d respectively) groups.

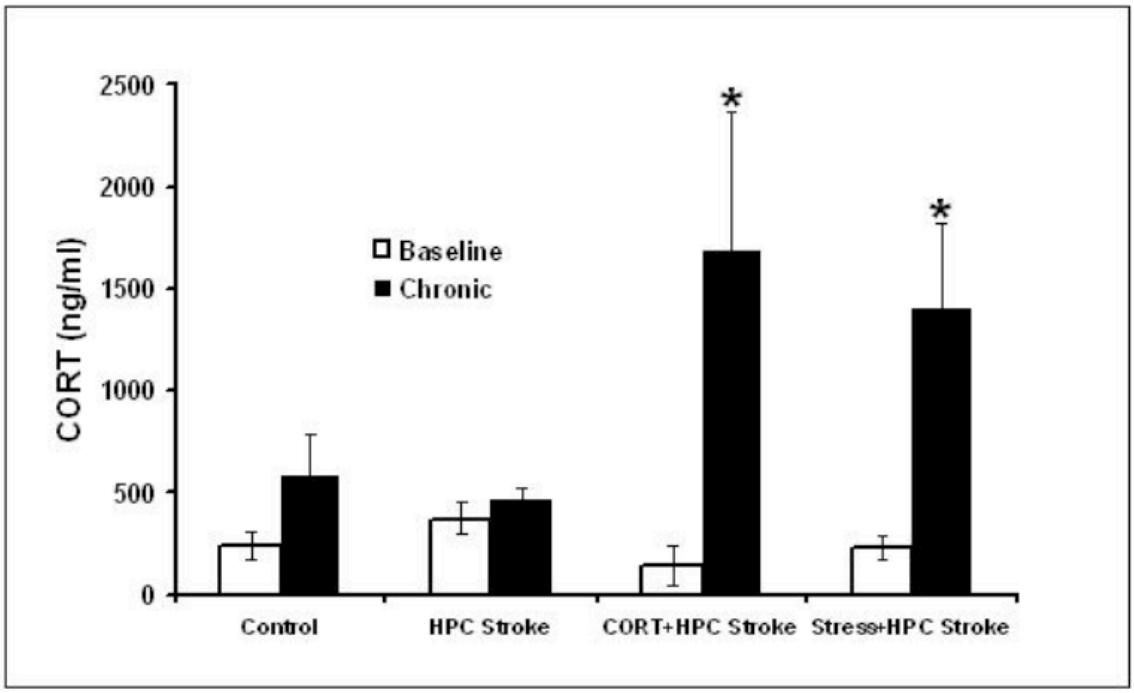


Figure 51. Plasma CORT concentration prior to (baseline) and at chronic levels (day 16) of daily CORT administration. Circulating CORT levels were significantly elevated after chronic CORT treatment in the CORT + HPC stroke group compared to controls and the HPC stroke group. * $p < 0.05$; dependent samples *t*-test for within-subject comparison. Error bars show ± SEM.

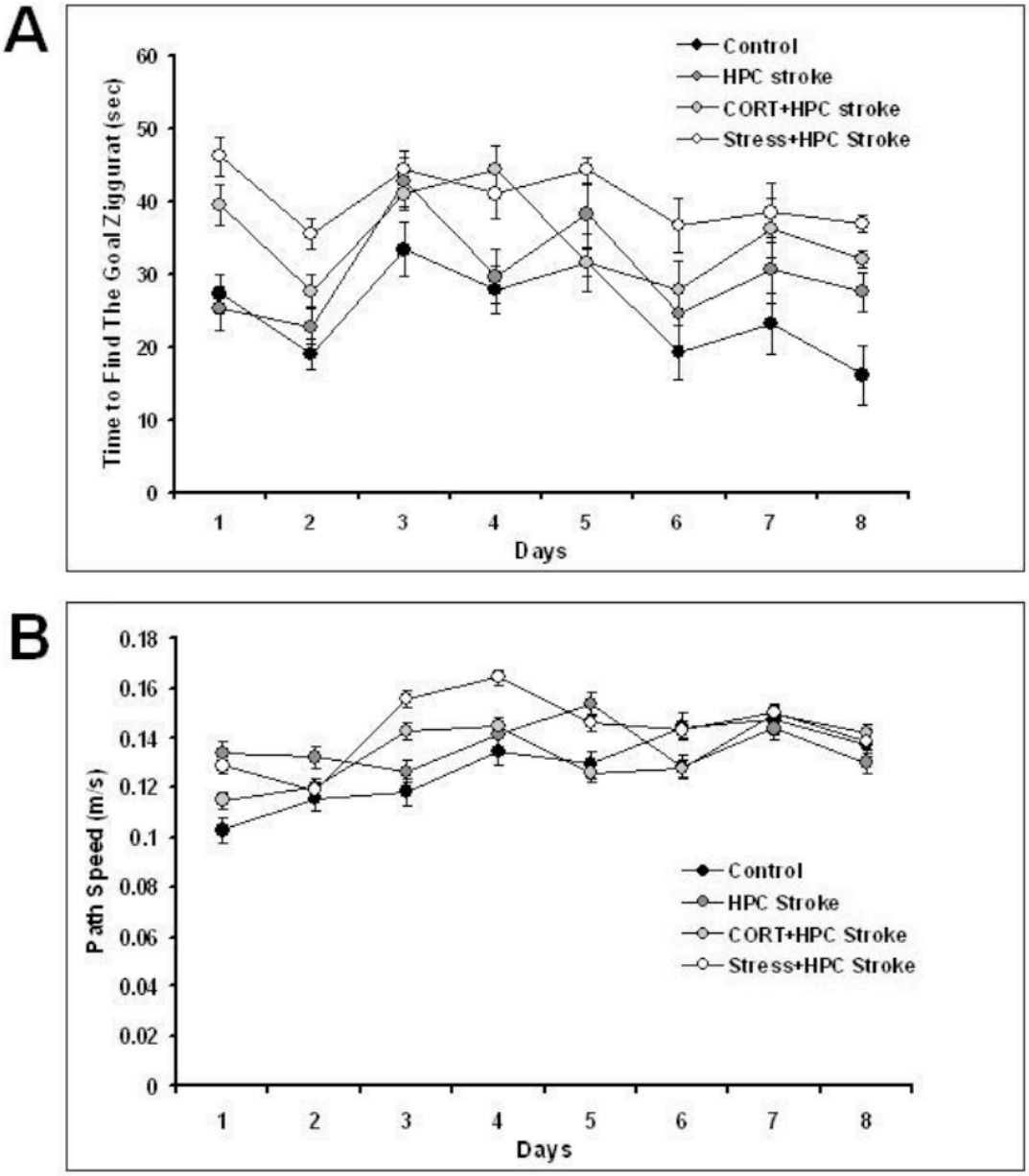


Figure 52. Testing in the non-cued version of the ZT for spatial learning. (A) Latency or time to find the goal ziggurat during 8 days of testing. (B) Mean path speed averaged across 8 days of testing. Error bars denote average ± SEM for each group.

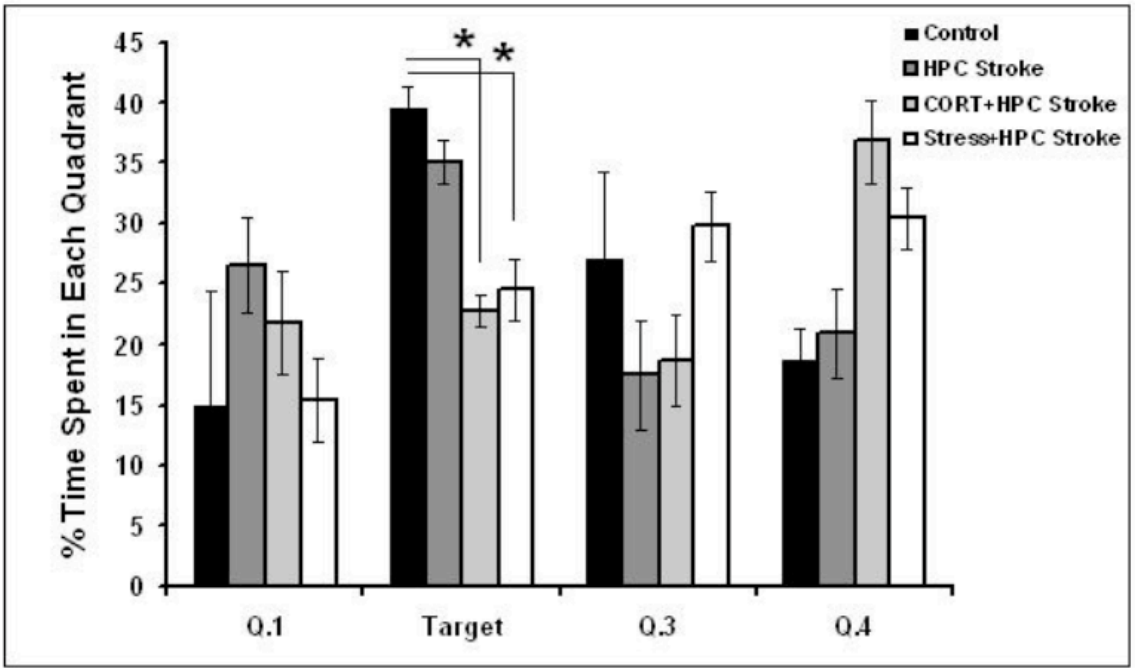


Figure 53. The mean percentage of time spent in four quadrants of the ZT during the 60 s of the probe trial conducted on day 9. Both CORT + HPC stroke and stress + HPC stroke groups spent significantly less time searching the goal ziggurat in the target quadrant relative to control and HPC stroke groups. Error bars show ± SEM.