CEREBRAL ISCHEMIA, SPATIAL MEMORY AND LOCOMOTOR ACTIVITY

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by

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A thesis submitted to the School of Graduate Studies
in partial fulfillment of the requirements for the degree of
Master of Science

Division of Basic Medical Sciences
Faculty of Medicine
Memorial University of Newfoundland
September 6 th, 1990

St. John's Newfoundland

ISBN 0-315-65320-5

To My Mother, Father and Sisters.

ACKNOWLEDGEMENTS

I would like to express my thanks to Dr. D. Corbett for providing me with the opportunity to work on this project and for his encouragement, guidance and support through all stages of this research. I would also like to thank Dr. R. Neuman for his wise council and encouragement, Dr. P. Moody-Corbett for her support and helpful comments, and Ms. S. Evans for her technical support.

I am grateful for the financial support provided by the fellowship from the School of Graduate Studies and the bursary from the Faculty of Medicine, without this support it would not have been possible for me to study here.

ABSTRACT

Stroke is among one of the leading neurological disorders in clinics. The purpose of this study was to investigate the functional deficits of the experimental "stroke" animal. In order to get a better understanding of the real mechanisms behind these deficits, a systematic observation of the behavioural and pathological changes associated with cerebral ischemia was carried out in three experiments in Mongolian gerbils.

In Experiment 1, an animal model using delayed, repetitive cerebral ischemia was used to determine the behavioural and neuropathological changes following multi-episodes of cerebral ischemia.

In Experiment 2, animals were pre-exposed to the test environment before ischemia and an attempt was made to determine whether the post-ischemic hyperactivity resulted from a simple change in motor function or a deficit in spatial mapping ability.

In Experiment 3, the neuropathological and locomotor activity changes resulting from different carotid artery occlusion durations (5, 10 and 15 minutes) were investigated. An attempt was made to determine whether the graded ischemia resulting from the different ischemic durations resulted in graded increases in locomotor activity.

From these three experiments it is concluded that the basis for the increased locomotor activity following an episode of cerebral ischemia is an alteration of spatial learning ability.

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LIST OF ABBREVIATIONS

ANOVA -- Analysis of Variance

AMPA ---- ∂-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

AP4 ----- 2-amino-4-phosphonobutyrate

AP5 ----- 2-amino-5-phosphono-valeric acid

AP7 ----- 2-amino-7-phosphonoheptanoic acid

CGS ----- cis-4-phosphonomethyl-2-piperidine carboxylic acid

CPP ------ 3-(2-carboxyl-piperazin-4-yl)propyl-1-phosphonic acid

EAA ----- Excitatory Amino acid

LTP ----- Long-term potentiation

MK-801-- (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5,10-imine maleate

NMDA - -- N-methyl-D-aspartic acid

MCID ---- Microcomputer Image Devices

PCP ----- 1-(1-phenylcyclohexyl)-piperidine

GENERAL INTRODUCTION

1. Cerebral ischemia

Clinical features of stroke

The goal of the present experiments was to investigate the relationship between cerebral ischemia, hippocampal pathology and functional deficits in an animal model. Before getting to the animal model of cerebral ischemia, a brief review of clinical stroke is presented.

Stroke was recognized as a group of neurological syndromes called " Zhongfeng" in a traditional Chinese medicine book " Huang Di Nei Jing" (Canon of Medicine) about 500 B. C. This book describes the neurological syndromes caused by "the imbalance of the blood in the body as a result of catching a cold wind". In Europe, Soranus of Esphesus (A.D. 98-138) observed the "hemiplegic paralysis" most often occurring in the elderly during winter (Porkert, 1974; Fields and Lemak, 1989).

Nowadays, stroke is one of the most common life-threatening diseases and is the third leading cause of death in the United States after heart disease and cancer (Wolf et al., 1986). The American Heart Association (1983) reported that there are about 500,000 stroke victims each year. The types of ischemic stroke can be identified by different clinical features. Data from the population of Rochester, Minnesota (Garraway et al., 1983), give us an idea about mortality rates among different stroke types. In this report, the average annual incidence rates per 100,000 population for specific stroke types from

1975-1979 are cerebral infarction (75), intracerebral haemorrhage (13), subarachnoid haemorrhage (11), stroke of uncertain types (4), and all types (103). In addition, many medical disorders can cause strokes. These include, anoxic/ischemic damage of the brain following: cardiac arrest (Caronna and Finklestein, 1978); cardiac surgery (Branthwaite, 1972); trauma and dissection of cervico-cerebral arteries (Oragon et al., 1981). From the above it can be seen that stroke has a high incidence, high mortality rate and many causal factors. In the search for treatments of this complicated disease, many different "stroke" models have been examined in the last 150 years.

Models of Cerebral Ischemia :

1). Completely cutting the blood supply to the brain - This method is represented by decapitation. Brown-Sequard made the first observations of this kind in 1858. He observed changes of respiratory movement in decapitated dogs (Weinberger et al.,1940). Later Hayman and Barrier reported that automatic reflexes were gone after 12 minutes of cerebral ischemia (Weinberger et al., 1940). All of the functional observations in the decapitated animals were focused on the ischemic effects on the nerve reflexes, respiratory, cardiac regulatory and vasomotor centres (Heymans et al., 1937).

2). General circulatory collapse- This method is represented by stopping the general circulation prior to reviving the animals. Different methods have been used: chloroform intoxication and resuscitation by intraarterial injection of epinephrine and cardiac massage (Crile and Dolly, 1908); electrical shock to produce ventricular fibrillation and cardiac massage to restore the normal heart beat; and haemorrhage or asphyxia (Weinberger et al., 1940).

3). Occlusion of the arterial supply - Usually the carotid or vertebral arteries or both are occluded . This method was first introduced by Cooper in 1836, in which he ligated both carotid and vertebral arteries in the dog. Since then a great deal of work has been done using this ischemic model including establishment in other animals (Gildea and Cobb, 1930).

In recent years, the study of cerebral ischemia has tended to use rodent models (Ginsberg and Busto, 1989). However, unilateral or bilateral occlusion of the carotid arteries does not produce ischemic brain damage in the rat due to an efficient collateral circulation (Payan et al., 1965; Eklof and Siesjo, 1972). In order to produce cerebral ischemia in this animal, systemic hypoxia or hypotension is required (Smith et al., 1984; Nordstrom and Rehncrona, 1977). In 1979, Pulsinelli and Brierley introduced a four-vessel occlusion method in the rat by electrocauterizing the vertebral artery through the alar foramina of the first cervical vertebra and reversible clamping of both carotid arteries.

4). Focal ischemia - All of the ischemic models mentioned above involve global ischemia. Recently, a method for occluding the middle cerebral artery has been introduced in which the artery is occluded by a subtemporal craniectomy (Tamura et al., 1981; Shigeno et al., 1985; Bederson et al., 1986). This model relates more directly to the regional ischemia commonly observed clinically.

The last two models mentioned above have some advantages in different studies, but they are not the most ideal model in functional studies due to surgical complications: such as damage of the vertebral arteries in the four-vessel occlusion model and open-skull surgery in the focal ischemia model. Recently a gerbil model (described below) of cerebral ischemia has been used quite productively in research on stroke.

Mongolian gerbils, Meriones unguiculatus, are rodents of the family Cricetidae. They are widely distributed in the regions of Mongolia and northeastern parts of China (Rich, 1968). In 1966, Levine and Payan observed that gerbils were susceptible to cerebral ischemia produced by occlusion of the carotid arteries (two-vessel occlusion). This is because of the special cerebral-vascular circulation in the gerbil. Generally, the brain is supplied by two pairs of arteries, the internal carotid and the vertebral. The latter fuse into the basilar arteries at the base of the brain. The internal carotid artery divides into posterior, middle, and anterior cerebral arteries. The two anterior cerebral arteries join rostral to the optic chiasm. The basilar artery bifurcates into the two superior cerebellar arteries. In humans and most mammals, there are a pair of arteries, the posterior communicating arteries, which connect the posterior cerebral and the basilar arteries to form a "communicating" blood supply at the base of the brain called "the circle of Willis" (Carpenter and Sutin, 1983; Yamori et al., 1976). However, there are no efficient connecting arteries between the carotid and the vertebrobasilar arteries in the circle of Willis in gerbils (Levine and Sohn, 1969; Kahn, 1972; Harrison et al., 1973). This makes it possible to produce forebrain ischemia in gerbils by occluding the carotid arteries. The gerbil model has features of simple surgery, fewer post-surgical complications and effective ischemic damage compared with some of the ischemic models mentioned before.

From the animal models of ischemia it has been possible to classify the brain damage observed into 2 types:

1). Selective neuronal vulnerability. The most extensive brain damage following ischemia is in the hippocampus. Specifically, neurons in CA1 area are most sensitive to ischemic insult (Ito et al., 1975; Kirino, 1982). Since the

present experiments focus on the post-ischemic functional deficits closely related to the function of the hippocampus, a brief review of the anatomy and neurotransmitters of the hippocampus is presented below.

2). Pan-necrosis. In this type the damage not only effects neurons but also the glial and vascular cells. Prolonging the duration of ischemia or irreversible occlusion of the blood supply will transform selective neuronal damage into pan-necrosis (Hossman and Kleihues, 1973; Kirino and Sano, 1984). To study more specific functional deficits which is only related to certain brain structures this type of damage is avoided.

2. Neuroanatomy of Hippocampus

The hippocampus in mammals consists primarily of pyramidal neurons and associated interneurons. These neurons are packed together in one layer of a three-layered structure, compared to the six-layered structure in the cortex (O'Keefe and Nadel, 1978). The hippocampus (Figure 1) is divided into two major parts, the fascia dentata (dentate gyrus) and hippocampus proper (cornus ammonis). The hippocampus proper has been further divided into four subfields Cornus Ammonis (CA)1-4 (Lorente de No, 1934). CA1 refers to the area "regio superior" which is located near the distal end of the dense plexus. CA2 and CA3 are mainly included in the area called "regio inferior" which are located at the dentate end. CA4 stands for the pyramidal cells and interneurons scattered inside the hilus of the fascia dentata. There are many hypotheses about neurotransmission between the different regions within the hippocampus (Frotscher et al., 1988). In order to get a clear view about this neurotransmission, a concise description of the input,

transmission and output of the hippocampus (Zola-Morgan et al., 1986) is given here. As shown in Figure 1, this neural circuitry finishes as a closed loop in the entorhinal cortex. Among the pathways mentioned above, there are three pathways using excitatory amino acids (EAA) : 1. The perforant pathway forms the main glutamate/aspartate - containing afferents in the hippocampus (Fonnum, 1984); 2. The mossy fibres terminating in the stratum lucidum (White et al., 1977); 3. The commissural fibres (contralateral) and Schaffer collaterals (ipsilateral) from CA3 which terminate in CA1 and CA3 (Cotman and Nadler, 1981).

Besides the glutamate system there are many hypotheses about neurotransmission in the hippocampus, e.g., cholinergic system and 5-HT system etc. (Frotscher et al., 1988). Because it has been found that the glutamate system has a dual role in memory and ischemic neuronal death, the role of the excitatory amino acid is emphasised here.

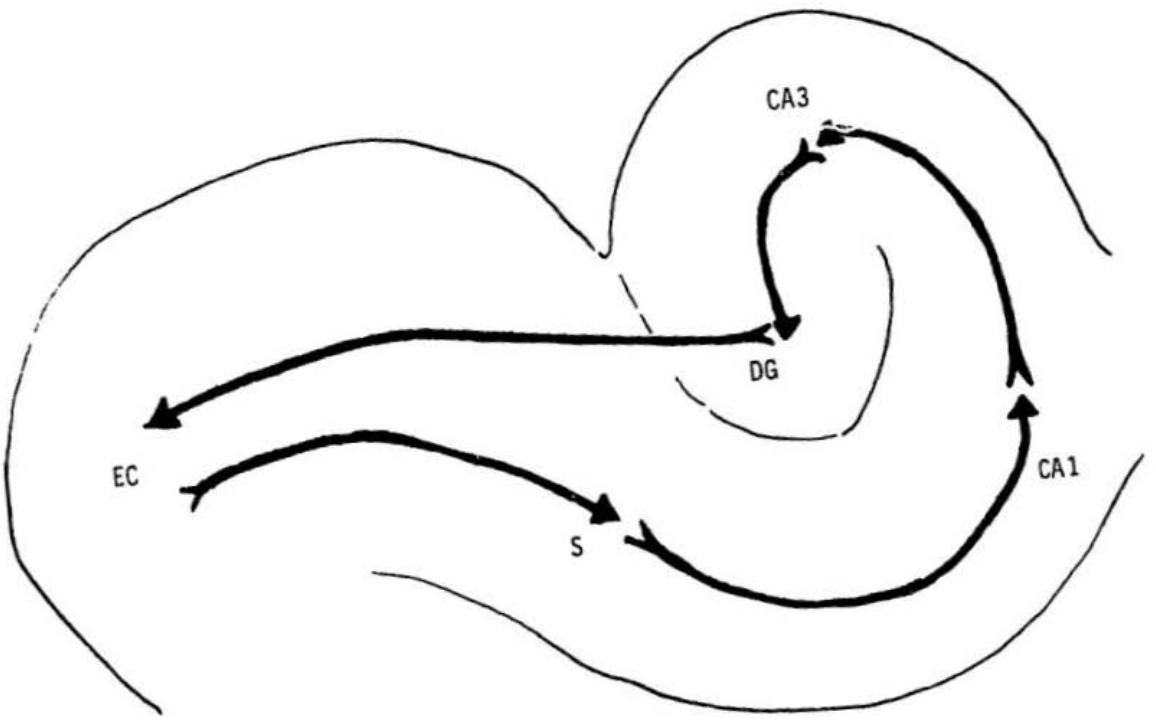
3. Excitatory amino acids

In the early 1960's, glutamate was first proposed as a neuroexcitatory agent by Curtis and Watkins (1963). Recently EAA have been suggested to be implicated in learning, memory and other brain functions. In addition to their role as neurotransmitters the EAA are also neurotoxic at high concentrations (Harris et al., 1984; Clineschmidt et al., 1982; Schwarcz et al., 1984; Cotman and Iversen, 1987; Rothman and Olney, 1987; Monaghan et al., 1989). Within the last 10 years it has become clear that there are multiple EAA receptors, not just the glutamate receptor as originally thought.

Figure 1. Schematic drawing of the hippocampus.

The lines show the unidirectional pathway (adapted from Zola-Morgan et al., 1986).

(1). Entorhinal cortex (EC) (major input)
| (perforant pathway)
(2). Dentate gyrus (DG)
| (mossy fibers; excitatory)
(3). CA3
| (Schaffer collaterals)
(4). CA1
|
(5). Subicular cortex (S)
|
Entorhinal cortex.


CA3
DG
EC
S
CA1

The existence of EAA receptor subtypes was deduced by the relative actions of selective agonists and antagonists (Cotman and Iversen, 1987). Based primarily on agonist response the EAA receptors have been classified into five types (Watkins et al., 1990): 1) N-methyl-D-aspartate (NMDA); 2) ∂-amino-3-hydroxy-5-methyl- 4-isoxazolepropionic acid (AMPA); 3) kainate (K); 4) 2-amino-4- phosphonobutyrate (AP4); 5) metabotropic receptors.

NMDA, a glutamate analogue, is a selective agonist which has received considerable attention as several functions have been attributed to the receptor activated by this agonist (see below). Radioligand autoradiography shows that the highest level of NMDA binding sites in the brain is in the hippocampus (Monaghan et al., 1983; Monaghan and Cotman, 1985; Mayer and Westbrook, 1987). Within the hippocampus, the CA1 region shows the highest level of NMDA binding sites, whereas moderate levels are found in CA3 and dentate gyrus (Geddes et al., 1986; Monaghan and Cotman, 1986).

AMPA , a structural analogue of quisqualic acid, is more specific in binding studies than quisqualate (the original agonist used to define this receptor) (Honore et al, 1982; Monaghan et al., 1989). The distribution of AMPA binding sites in the central nervous system is parallel to NMDA binding sites. The function of the AMPA receptor involves generation of fast EPSPs in many central EAA pathways (Monaghan et al., 1989; Watkins et al., 1990).

Kainate is not as specific an agonist as NMDA and AMPA, and some response to kainate may be mediated by AMPA receptors (Monaghan et al., 1989; Honore et al, 1982). However, the finding of a pure population of kainate receptors occurring on mammalian C-fibers in the absence of AMPA and NMDA receptors provides the evidence for discrete existence of kainate receptors (Agrawal and Evans, 1986; Watkins et al., 1990).

The last two receptors, AP4 and metabotropic are not as clearly understood as the former three receptors, and work is still required to characterize these receptors (Monaghan et al., 1989; Watkins et al., 1990).

NMDA receptors and learning and memory

One of the most exciting findings about EAA is the role of the NMDA receptor in the induction of long-term potentiation (LTP). LTP refers to a phenomenon in which a short burst of high frequency stimulation results in potentiation of the evoked population spike. This potentiation may last for hours or days and is widely regarded as a cellular model of learning and memory (Bliss and Lomo, 1973; Alger and Teyler, 1976; Collingridge and Bliss, 1987; Wroblewski and Danysz, 1989).

The role of NMDA receptors in the induction of LTP was first demonstrated in the CA1 region of hippocampal slice. LTP in the Schaffer/commissural pathway is reversibly prevented by iontophoretic administration of 2-amino-5-phosphono-valeric acid (AP5), a competitive NMDA antagonist, into the synaptic region (Collingridge et al., 1983; Harris et al., 1984; Collingridge and Bliss,1987). Also, phencyclidine (PCP) and ketamine, both non-competitive antagonists of the NMDA receptor prevent the induction of LTP in the CA1 region (Stringer and Guyenet, 1983). Morris et al. (1986) found that chronic intraventricular injection of AP5 could cause a selective impairment of place learning in rats, and AP5 treatment also suppressed LTP in vivo. There are a number of other studies implicating NMDA in learning and memory. For example, other NMDA antagonists, e.g., 3-(2-carboxy-,piperazin- 4-yl)propyl-1-phosphonic acid (CPP), cis-4-phosphonomethyl-2- piperidine carboxylic acid

(CGS19755) and PCP, have been found to impair performance on passive avoidance tests (Benvenga et al., 1989). (+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cyclohepten-5, 10- imine maleate (MK-801), a non-competitive NMDA receptor antagonist, can produce impairment on the radial arm maze and increase locomotor activity in the open field test (Shapiro and Caramanos, 1989; Heale and Harley, 1990). It seems NMDA plays an important role in learning and memory. Unfortunately the same cellular action of NMDA receptors which appear important for learning and memory, can lead to neuronal toxicity when the receptor is excessively activated.

Ischemia induced neuronal death

Many hypotheses have been put forth to explain the mechanism of neuronal death following ischemic episodes. Neurotoxicity of excitatory amino acids provides one of the most popular concepts about ischemia induced neuronal death. The neurotoxicity of EAA was first shown by Lucas and Newhouse in 1957, who found that systemic injection of glutamate destroys the inner neural layers of the immature mouse retina. Since then many studies on the neurotoxicity of the EAA have been carried out (Olney et al., 1971; Olney, 1978, Monaghan et al., 1989). These studies show that systemic administration of glutamate destroys neurons of the brain in newborn mice, rats or monkeys in brain regions which lack a blood brain barrier.

The selective loss of neurons is seen in regions characterized by extensive NMDA binding, and glutamate levels are increased in the hippocampus during ischemia (Jorgensen and Diemer, 1982; Cotman and Iversen, 1987). Some EAA antagonists can protect against ischemic brain damage. For example, local

administration of a selective NMDA antagonist 2-amino-7-phosphonoheptanoic acid (2-APH) into hippocampus effectively prevents ischemic neuronal damage (Simon et al., 1984); Systemic administration of other NMDA antagonists, e. g., MK-801, CGS-19755 and riluzole have also been reported to protect against ischemic brain damage, giving further support to the proposed theory that excessive NMDA activation may trigger ischemic brain damage (Gill et al., 1987; Malgouris et al., 1989).

4. Functional Studies

From the above, it might be concluded that stroke is one of the most life-threatening diseases. To study this phenomenon many animal models of cerebral ischemia have been established. In these models most research has focused on the hippocampus because it was the most vulnerable to ischemic damage. Because the hippocampus plays an important role in learning and memory, it is important to understand the functional deficits resulting from hippocampal damage.

Scoville first reported a case of severe anterograde amnesia following a bilateral medial temporal lobe resection (damage to the anterior 2/3 of hippocampus and medial temporal cortex) in a patient (H. M.) who suffered from intractable seizures in 1954. Although H. M. was extensively studied (Scoville, 1954; Scoville and Milner, 1957) and continues to be studied, his damage was clearly not confined to the hippocampus. The conclusion that damage limited to hippocampus can cause amnesia was not certain until the case report of R. B. by Zola-Morgan et al. in 1986. Patient R. B. suffered severe learning and memory impairments following an ischemic episode resulting from coronary

bypass surgery. Systematic memory tests performed during the five years preceding his death showed that R.B. exhibited severe anterograde amnesia, almost no retrograde amnesia and no significant change in his personality and intelligence. After he died, whole brain sections were made, and the most prominent lesions involved the CA1 region of the hippocampus. Damage of the CA1 region is the only lesion that could be found to explain R. B.'s amnesia.

In animal studies, a variety of tests have been used to measure the animals' spatial learning and memory (Barnes, 1988). Many recent studies have been performed to determine the relationship between functional deficits (e.g., learning and memory) and the hippocampus in ischemic models. An overview of the tests which have been used in testing learning and memory in ischemic models are described below :

1). Morris Water Maze - This test was first introduced by Morris et al. in 1982, the basic principle of the test is that there is an escape platform which is hidden beneath the surface of water, made opaque with milk, in a fixed location relative to visible environmental cues outside the pool. Animals are released at random locations, and after a few trials the normal animals learn to swim directly to the hidden platform, whereas animals with hippocampal lesions take a significantly longer time to find the platform. In this test animals are thought to use their reference memory of the environment to locate the platform (Morris et al., 1982). Some studies have shown that ischemic damage limited to half of the CA1 sector of the hippocampus can be severe enough to impair the animals' performance on this test (Auer et al., 1989). This finding gives further evidence for an important role of the CA1 sector in place learning and memory.

2). Radial Arm Maze - With this experimental paradigm, animals obtain food pellets in one of several arms, usually there are 8 to 10 arms. In order to get the

food the animals have to use memory of the "environment" to locate the correct arm (Nagni et al. 1979). Two types of memory are distinguished in the radial arm test: reference memory and working memory. Reference memory relies on the location of fixed external stimuli (e.g., location of a window) relative to each test arm. These cues do not change from test day to test day. Working memory refers to trial by trial changes in that the animal must remember which arms have been entered on a particular trial. It has been shown that animals with hippocampal lesions take longer to locate the right arm than control animals (Olton 1978; Barnes 1988). Peeler and Smith (1990) showed that 5-7 minute bilateral carotid occlusion in gerbil which produced severe bilateral loss of CA1 cells led to significantly more re-entries in the radial arm maze.

3). Passive Avoidance Test - Even though this test is not a specific spatial memory test, some studies have shown that gerbils with ischemic damage of hippocampus show poor performance on this test (Malgouris et al., 1989; Tominaga and Ohnishi, S. T., 1989).

Different from all the above learning and memory tests, Chandler et al. (1985) introduced open field tests in ischemic studies. Chandler et al's study showed that the most prominent behavioural change following 5 minutes of forebrain ischemia in the gerbil is a large increase in locomotor activity, evident at 24 hrs postocclusion and gradually diminishing to normal after about 5 days. In a more systematic study, Gerhardt and Boast (1988) examined the relationship between locomotor activity and degree of ischemic damage of the hippocampus in gerbils. In their study ischemic damage of the hippocampus was induced by bilateral occlusion of both carotid arteries for 20 minutes. Locomotor activity was recorded on the first and fourth days after occlusion. Histological assessment revealed neuronal damage in the CA1 area of the

hippocampus. The mean distance that the ischemic animals travelled (as a index of locomotor activity) during Day 1 and Day 4 was approximately three-fold compared to the control animals. It was found that there was a positive correlation between the postischemic hyperactivity and the degree of neuronal degeneration in the hippocampus.

Comparing the open field test to the other three tests mentioned above, it seems that open field test has the advantage of simplicity. However, it has not been established that the open field test is an effective functional test in cerebral ischemia experiments. Furthermore there has not been a clear indication of the mechanism underlying the post-ischemic increase in locomotor activity. Is the increase in locomotor activity a result of a simple motor hyperactivity or some other functional deficit, for example, spatial learning or memory deficit ? In the present experiments the relationship among cerebral ischemia, locomotor activity and spatial memory was studied.

EXPERIMENT ONE

INTRODUCTION

At present, most studies of cerebral ischemia in gerbils utilize a one-episode ischemia model. A few studies have involved repetitive cerebral ischemia in which the intervals between the episodes of cerebral ischemia were limited to a few hours (Tomida et al., 1987, Vass et al., 1988, Kato et al., 1989). However, these studies did not examine any of the behavioural deficits resulting from repetitive cerebral ischemia. Results of a recent study (Gerhardt and Boast, 1988) suggest that the degree of hippocampal damage is positively correlated with increased locomotor activity. Concerning the relationship between severity of the ischemia and the degree of heightened locomotor activation, would more severe ischemic damage, resulting from repeated ischemic episodes or longer carotid occlusion times, produce corresponding increases in locomotor activity? In the first experiment a model of repeated ischemia (Tomida et al., 1987; Vass et al., 1988) was used to address this question.

METHODS

Subjects

Adult female gerbils were provided by High Oak Ranch Ltd, Goodwood, Ontario, Canada, and weighed 45-70 g at the time of surgery. The gerbils were housed in plastic cages at a temperature of 22°C on a 12-hr day/night cycle and fed with commercial pellets and water ad libitum. All of the animals had been resident in their cages for about four weeks before the experiments began.

Surgery

Animals were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) following pretreatment with an intramuscular injection of atropine (0.1 mg/kg). They were fixed in a supine position and an incision about 1.5 cm in length was made in the anterior cervical midline. Both carotid arteries were carefully separated from the surrounding tissue. A chronic occluding device was implanted (Figure 2), such that the ends of the occluding and releasing threads were directed subcutaneously to the back of the neck where they extended 1 cm out of the skin. One week was allowed for the animals to recover from the surgery.

Prior to the occlusion, the animals were anesthetized in a plastic chamber with ether, and the anesthesia was maintained by an "ether mask" (the open end of a syringe which was filled with ether-soaked cotton). Occlusion was accomplished by bilateral tightening of the occluding threads and was terminated by bilateral tightening of the releasing threads (Figures 2 and 3). Animals were kept under a 60 w lamp (50 cm in height) from the beginning of the surgery until the animal recovered from the anesthesia.

Procedure

Twenty-three adult female gerbils were used in this experiment. In the ischemic group (n =12), daily 10 minute open-field tests were begun 24 hrs after the occlusion for 4 successive days in the first week. In the second week the same procedures of occlusion and open field tests were performed again.

Figure 2. Diagram of Occluding Device

The occluding device as described by Tomida et al. 1987. The implantation procedure was modified by threading the occluding and releasing threads through the device with the aid of a loop of #6.0 silk suture.

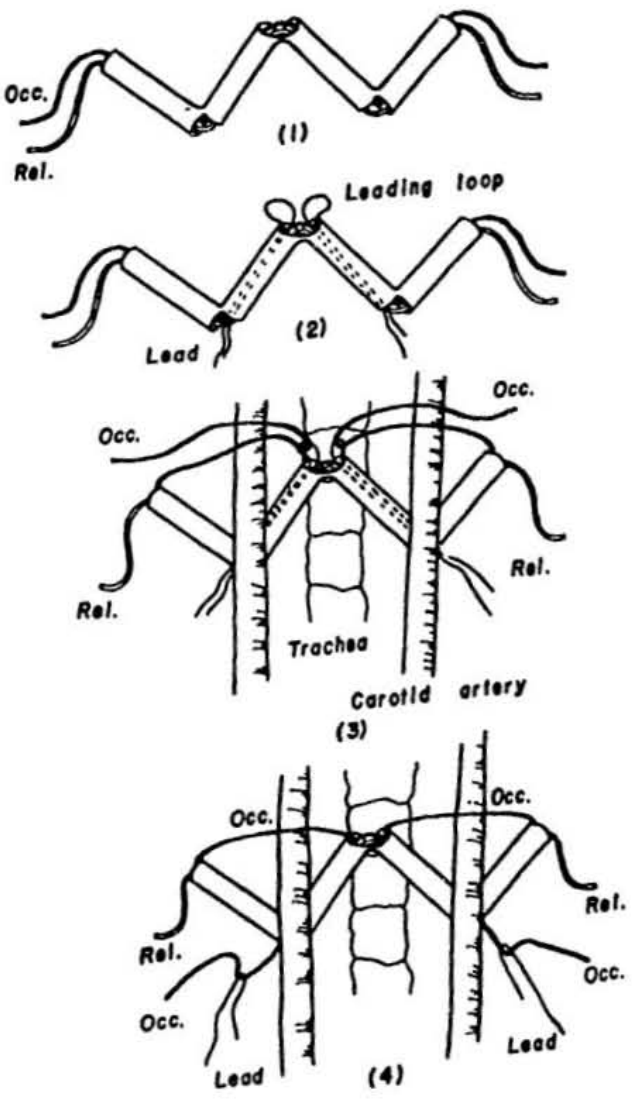
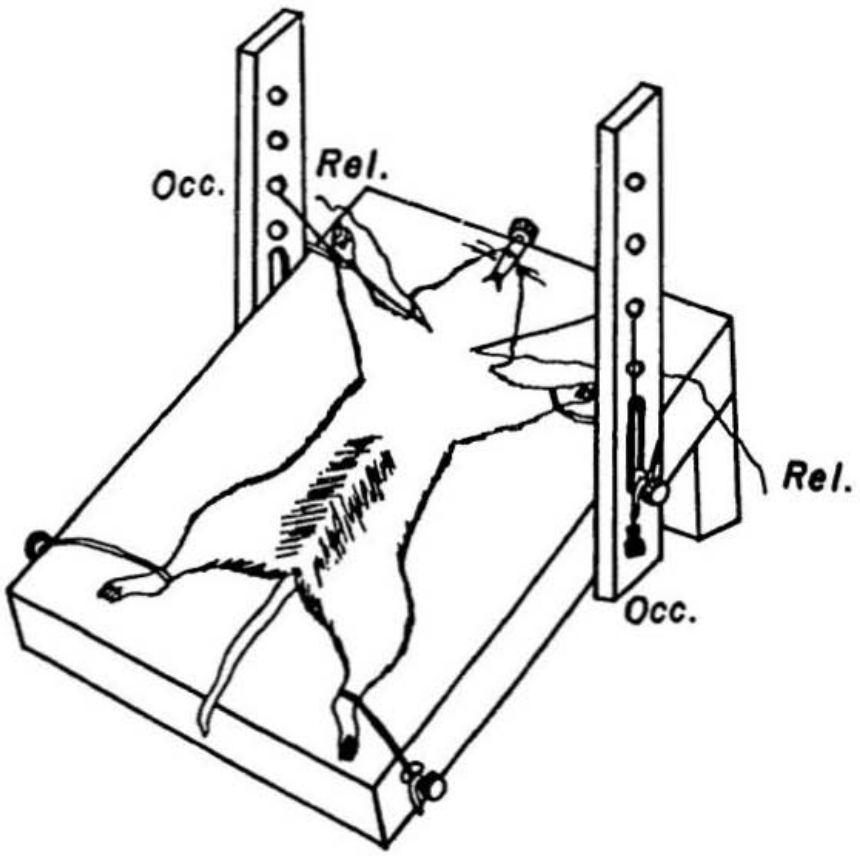

Occ.
Rel.
(1)
Leading loop
Lead
(2)
Occ.
Occ.
Rel.
Rel.
Trachea
Carotid artery
(3)
Occ.
Occ.
Rel.
Rel.
Occ.
Occ.
Lead
Lead
(4)

Figure 3. Diagram of Occluding Table

The occlusion was made by bilateral tightening of the occluding threads (Occ) maintained by two weights (about 80 grams) hung at each each end of the occluding threads. The occlusion was terminated by bilateral tightening of the releasing threads (Rel).


Occ.
Rel.
Rel.
Occ.

After finishing the second week of open field testing, four animals from this group were perfused for histology. A third occlusion was performed on the seven animals remaining in the group. These animals were perfused four days after open field testing.

In the control group (n =5), the same procedures of surgery were performed but without occlusion. The open field tests were carried on for three weeks (four test days per week).

A single occlusion (10 minutes) was performed in six animals. The animals were perfused five days after the occlusion without behavioural testing. This group was used to examine the histological changes after a single ischemic episode.

Open-field tests

Open-field tests were performed in a wooden box measuring 75 cm x 75 cm x 50 cm. The floor was divided into 25 equal squares of approximatly 15 cm x 15 cm. The box was illuminated by two 60 W lamps positioned about 1.5 meters above the floor. A video camera mounted over the box was used for recording the activity of the animals. The number of squares the animals entered in 10 minutes was counted as the measure of locomotor activity.

Histology

After the open field testing was finished, the animals were transcardially perfused with 20 ml of 0.9% heparinized saline followed by 30 ml of 10% buffered formalin. The brains were fixed in 10 % formalin at room temperature

overnight, they were then cut into 40 μm coronal sections at -23 ° C on a freezing microtome. Sections were taken from the most rostral tip of the hippocampus to 5.0 mm posterior to bregma. Sections were dehydrated with graded ethanols and stained with cresyl violet.

A Microcomputer Image Device (MCID; Imaging Research Inc. Brock University, St. Catharines, Ontario) was used to assess damage of the CA1 region of the hippocampus. The basic principle of MCID is to measure the area of a brain structure using the relative optical density. The relative optical density of the dentate gyrus was chosen as the target value (similar to normal CA1 relative optical density), and the relative optical density of the molecular layer ventral to CA1 was chosen as the background value. The threshold value was determined as follows:

Threshold value = (BV - TV)/0.4 + TV

BV---Background value

TV---Target value

The threshold value was critical in measuring the CA1 area, because only the relative optical density level above the threshold value could be detected. In this experiment, the sections for MCID analysis corresponded to a plane 1.6 mm posterior to bregma. The CA1 region from its medial to lateral boundary was delineated and the area exceeding the threshold value was measured. The mean area (mm^2) of both right and left hemispheres was combined to yield a final score.

RESULTS

General observations

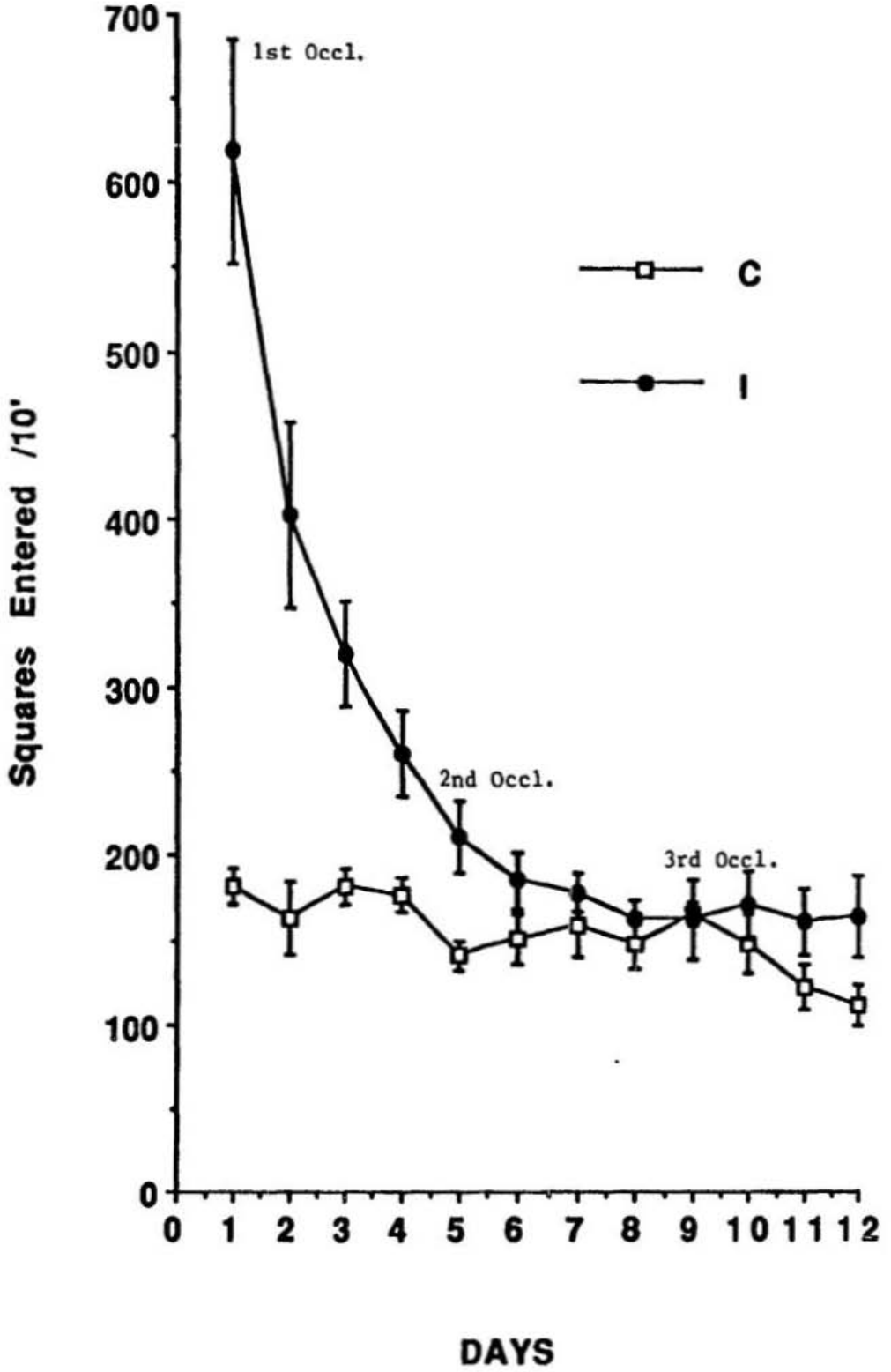
Abnormal behaviour was not evident post-ischemically, and the animals' food and water intake appeared normal (no weight loss in post-ischemic animals). No adverse symptoms were observed in animals with the chronic occlusion device during the 3 week testing period. An autopsy performed on one animal at the end of the third testing week showed regenerative tissue surrounding the device and no infection was observed. The device was situated in the original position and worked perfectly.

Locomotor activity

As shown in Figure 4, locomotor activity of the ischemia group increased after the first episode of ischemia [$F(1,15) = 12.0$, $P<0.01$, ANOVA], and was significantly elevated relative to the control group for the first three days ($p < 0.01$, Dunnett's Test). The second and the third occlusions failed to increase locomotor activity above control values ($P > 0.05$, Dunnett's Test). Locomotor activity was maximal 24 hours after the first ischemia, and was significant compared to the 2nd, 3rd and the following test days ($P<0.01$, Dunnett's Test). The level of locomotor activity of the control group remained relatively constant over testing days while the locomotor activity of the ischemic group dropped sharply, especially after the first two test days. This behavioural pattern yielded a significant Day effect [$F(3, 45) = 28.4$, $p < .001$] and a Treatment X Days interaction [$F(3,45) = 11.6$, $P < 0.01$].

Figure 4. Open Field Activity Following Repeated Ischemia

Open field activity expressed as squares entered per 10 min test session for the Control (C) and the Ischemia (I) animals. Days 1-4 represent the mean activity scores $\pm$ SEM for the I x 1 animals. Days 5-8 for the I x 2 animals and Days 9-12 for the I x 3 animals.


700
600
500
400
300
200
100
0
Squares Entered /10'
1st Occl.
2nd Occl.
3rd Occl.
C
I
0 1 2 3 4 5 6 7 8 9 10 11 12
DAYS

Histology

Previous studies have shown the CA1 sector to be mainly responsible for post-ischemic hyperactivity and spatial memory deficits (Gerhardt and Boast, 1988; Auer et al., 1989). Therefore, in this experiment and the other two experiments, only the CA1 sector was examined. It appears that CA1 region was progressively damaged after each ischemic episode (Figure 5).

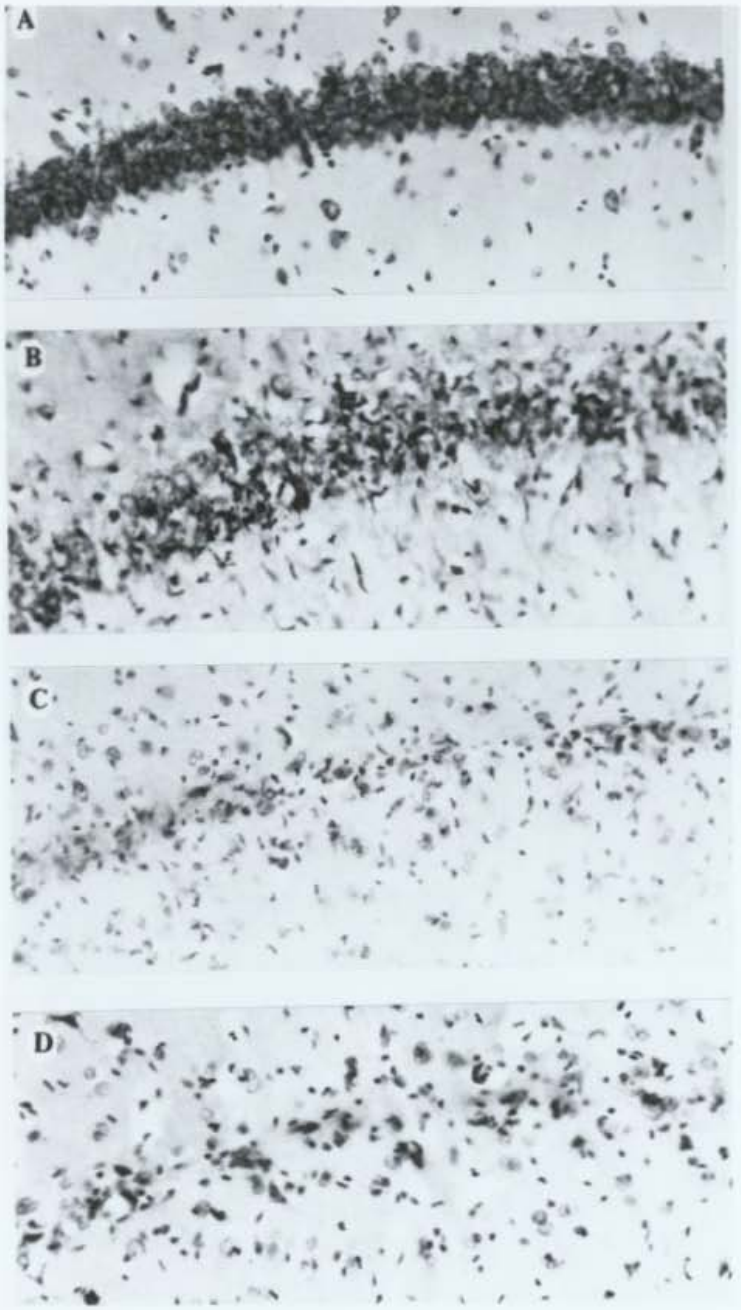
Figure 6 illustrates the computer-generated measurement of CA1 cell loss in each of the experimental groups compared to the control animals ($F(3,18) = 5.86$, $P < 0.01$). From MCID results, some comparisons of CA1 area (mm2) were performed. CA1 areas in the two time (I x2) and three time (I x3) ischemic groups were severely damaged compared to the control group (Control Vs. I x2: $p < 0.05$; Control Vs. I x3: $p < 0.01$; Dunnett's Test). However, CA1 sector was less damaged in the 1x I group, and did not reach statistical significance ($P > 0.05$, Dunnett's test). There was no statistically significant difference in damage among I x3 Vs. I x2 and I x2 Vs. I x1 ($P > 0.05$). Except I x3 Vs. I x1 was statistically significant (Fisher PLSD, $P < 0.05$).

DISCUSSION

In this experiment, locomotor activity was transiently increased after the first ischemic insult, which is consistent with earlier observations (Chandler et al., 1985; Gerhardt and Boast, 1988). However, additional ischemic damage resulting from the second and third occlusions did not further increase the activity level, which is not consistent with the conclusion that there is a positive relationship between locomotor activity and CA1 damage (Gerhardt and Boast,

Figure 5. Photomicrographs of CA1 areas

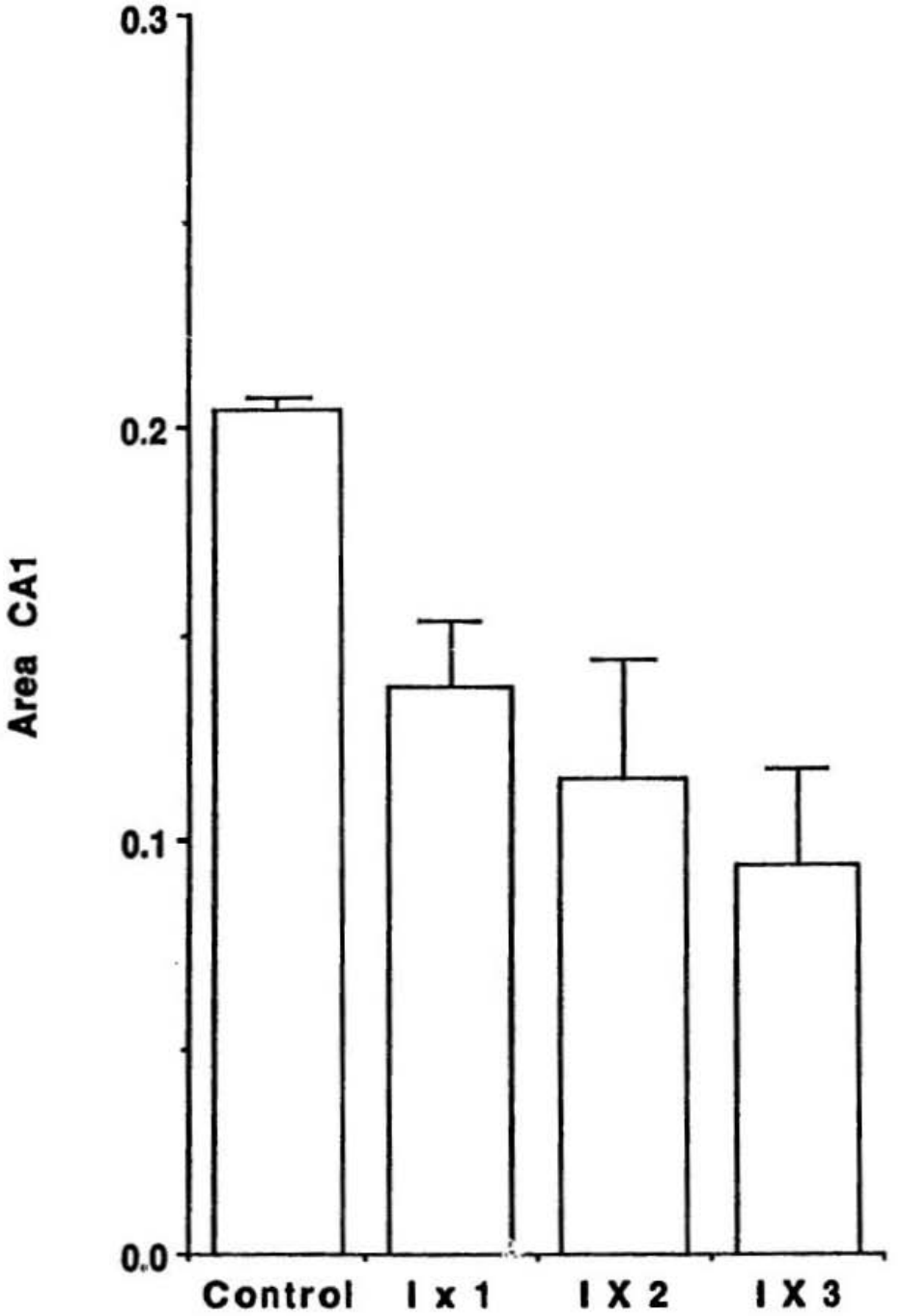
Representive photomicrographs taken from Control (A), I x 1(B), I x 2 (c) and I x 3 (D) animals. Note the progressive loss of pyramidal cells with each subsequent occlusion (B-D).



100 μm

Figure 6. Degree of CA1 Cell Loss

CA1 area (mm2) in the control and ischemic groups. Each bar represents the mean area ±SEM pooled from both hemispheres.


0.3
0.2
0.1
0.0
Area CA1
Control
I x 1
I X 2
I X 3

1988). However, one main difference between the present experiment and Gerhardt and Boast's study is that the hippocampal damage in the present experiment was produced by repeated ischemia episodes, and in their study was produced after a single occlusion.

Several questions were raised by this experiment :

1). Why did the first 10 minute occlusion not result in significant CA1 damage, and why were there some variations in the degree of damage of CA1 even in the same ischemic group?

2). Why did locomotor activity increase only after the first ischemic episode and not after repeated ischemic episodes when hippocampal damage was more extensive?

3). What is the mechanism underlying these changes?

To partly answer question 1, one has to go back to the methods employed. This experiment was designed before it was appreciated that hypothermia had a protective effect against cerebral ischemia. Thus, in this experiment little attention was paid to body temperature and the "cooling effect" from the ether anesthesia. This could account for the relatively minor loss of CA1 after a single occlusion and the variation in the degree of damage of hippocampus, because other experiments have shown that lowering the body temperature one or two degrees can attenuate ischemic damage (Busto et al., 1987; Corbett et al., 1990).

For question 2, hypothermia might also explain the significant drop in locomotor activity over the first three post ischemic days and the failure of the second and third occlusion to increase it. The first period of ischemia caused incomplete damage of CA1 due to hypothermia. Thus, the many functioning CA1 neurons left after the first ischemic insult may have allowed the animals to

form a spatial map of the open field environment. This is further supported by the fact that the animals gradually decreased their locomotor activity over the first few post ischemic test days and dropped to the control level after four days. Subsequent ischemic episodes which caused more severe ischemic damage of the hippocampus would not be expected to alter the locomotor activity because the animal's memory or map had already been formed (see General Discussion). The map once formed is thought not to reside within the hippocampus (Squire, 1986; Zola-Morgan et al., 1986.).

The above results suggest that the increase in locomotor activity after cerebral ischemia (Chandler et al., 1985; Gerhardt and Boast, 1988; Present results) may be due to some disruption of spatial mapping ability. In order to further test this hypothesis, a second experiment was designed in which the animals were pre-exposed to the test environment prior to the ischemia.

EXPERIMENT TWO

INTRODUCTION

As noted in the first experiment, repeated ischemic episodes did not further increase locomotor activity, perhaps because the animal had formed a spatial map of the test environment If so, then pre-exposure to the open field prior to ischemic insult should block or attenuate increased locomotor activity since a "spatial map" should have already been formed.

In the previous study it was found that 10 minute occlusion with the occluding device was insufficient in producing effective CA1 damage, so in this experiment the procedure was modified in two ways: 1) In order to avoid protective effects of hypothermia all surgical procedures were carried out at 37.5 ° C; 2) Since only a single occlusion was to be performed, small microvascular clamps were used instead of the polyethylene occluding device that had been used in Experiment 1.

METHODS

Subjects

Forty adult female gerbils were randomly divided into three groups: ischemia (Group I, n =10), pre-exposure ischemia (Group FIN, n =10), pre-exposure surgery control (Group FSN, n =10), and surgery control (Group S, n =10).

Surgery and occlusion

Anesthesia was induced in a small plastic chamber with a mixture of oxygen :

nitrogen : halothane at a flow rate 30% : 70% : 2.5% (ml/min.). The anesthetic was delivered through a Fluotec Anesthetic Machine (Foregger Co., Inc. Roslyn Height, New York, U. S. A). The animals were then fixed in a supine position and halothane flow rate was reduced to 1.5 %.

A two cm anterior midline cervical incision was made, and both carotid arteries were carefully separated from the surrounding tissue and the sympathetic nerves. Five minute occlusion of the carotid arteries was made by clamping the vessels with Schwartz micro-serrefines (Fine Science Tools Inc., North Vancouver, B.C). After the occlusion the micro-serrefines were removed simultaneously from both carotid arteries. After making sure of good reflow in both arteries, the animals' incision was sutured and the animals were placed under a 60 w lamp in their cages. The body temperature of the animals was maintained at 37.5 ° C by a temperature-controlled blanket (Homeothermic Control Unit 482, Harvard Apparatus Ltd, Edenbride, Kent, U. K) throughout surgery.

Open-field test

Apparatus

Open-field testing was performed in the same testing apparatus as in Experiment 1. An image tracking system (VP112 scanning unit, HVS IMAGE, Hampton, England) coupled to a Tatung-7000 computer was used to count the number of squares that the animals entered during the testing period.

Testing

In Group I and Group S, daily 10 minute open-field tests were carried out 24 hrs after occlusion/surgery and continued for 10 successive days.

In the Group FIN and Group FSN, the daily 10 minute open-field tests were started five days before the surgery. After five days of testing, the animals in the FIN group were subjected to a 5 minute bilateral occlusion of the carotid arteries. For the FSN group, the carotid arteries were isolated but not occluded. Open field tests were begun 24 hrs after occlusion/surgery and continued for five days. After this five day test period, the activity of the pre-exposed groups in a semi-novel environment was examined. The test environment was altered by inserting an elliptical white plastic liner into the open field thereby obscuring the white and natural wood walls of the open field test box . The floor was covered with a piece of styrofoam. Only one light was used and its position relative to the test apparatus was changed. Pictures and other objects were hung on the walls of the testing room, the ceiling was covered by coloured pictures and the video camera was wrapped in a white coat. In addition, noise provided by a large fan was present on novel test days. Animals of the FIN and FSN groups were then tested in this "novel" test environment for five additional days.

Histology

Animals in the four groups were sacrificed about three weeks after occlusion. The procedures of perfusion and histological preparation and MCID analysis were the same as in Experiment 1.

RESULTS

Locomotor activity

The four experimental groups (Figure 7) consisted of two ischemia groups (I and FIN groups) and two control groups (S and FSN groups).

As expected, 5 minutes of ischemia produced a large increase in locomotor activity as illustrated by the performance of Group I animals. Locomotor activity of the I group was highly increased after the episode of ischemia relative to the S group [A two-factor ANOVA with repeated measures: $F(1,16) = 75.1$, $P < 0.001$]. Locomotor activity of the I group animals dropped to a lower level by the fifth day, but the level of locomotor activity was significantly higher than that of the S group even ten days after the occlusion [From Day 5 to Day 10, I Vs. S, $P < 0.01$, Dunnett's Test]. The level of locomotor activity of the S group remained relatively constant compared to the gradual reduction in locomotor activity of the I group (over the first five testing days, there was a significant Group X Day interaction: $F(9,144) = 11.9$, $P < 0.001$].

The locomotor activity of the I group gradually decreased over the first five testing days, which resulted in a significant Day effect [$F(9, 144) = 16.0$, $P < 0.001$]. Pre-exposure to the open-field prior to ischemia (Group FIN) virtually blocked the post-ischemic increase in locomotor activity. Although the I and FIN groups underwent the same ischemic insult, the locomotor activity of the I group was significantly higher than that of the FIN group in the familiar environment [$F(1,16) = 39.6$, $P < 0.001$]. Again there was a significant interaction [$F(4,64) = 6.2$, $P < 0.001$] and significant Day effect [$F(4,64) = 15.2$, $P < 0.001$]. Locomotor activity of the I group was highly increased compared to that of the FIN group on

Figure 7. Open Field Activity in Familiar and Novel Testing Environment

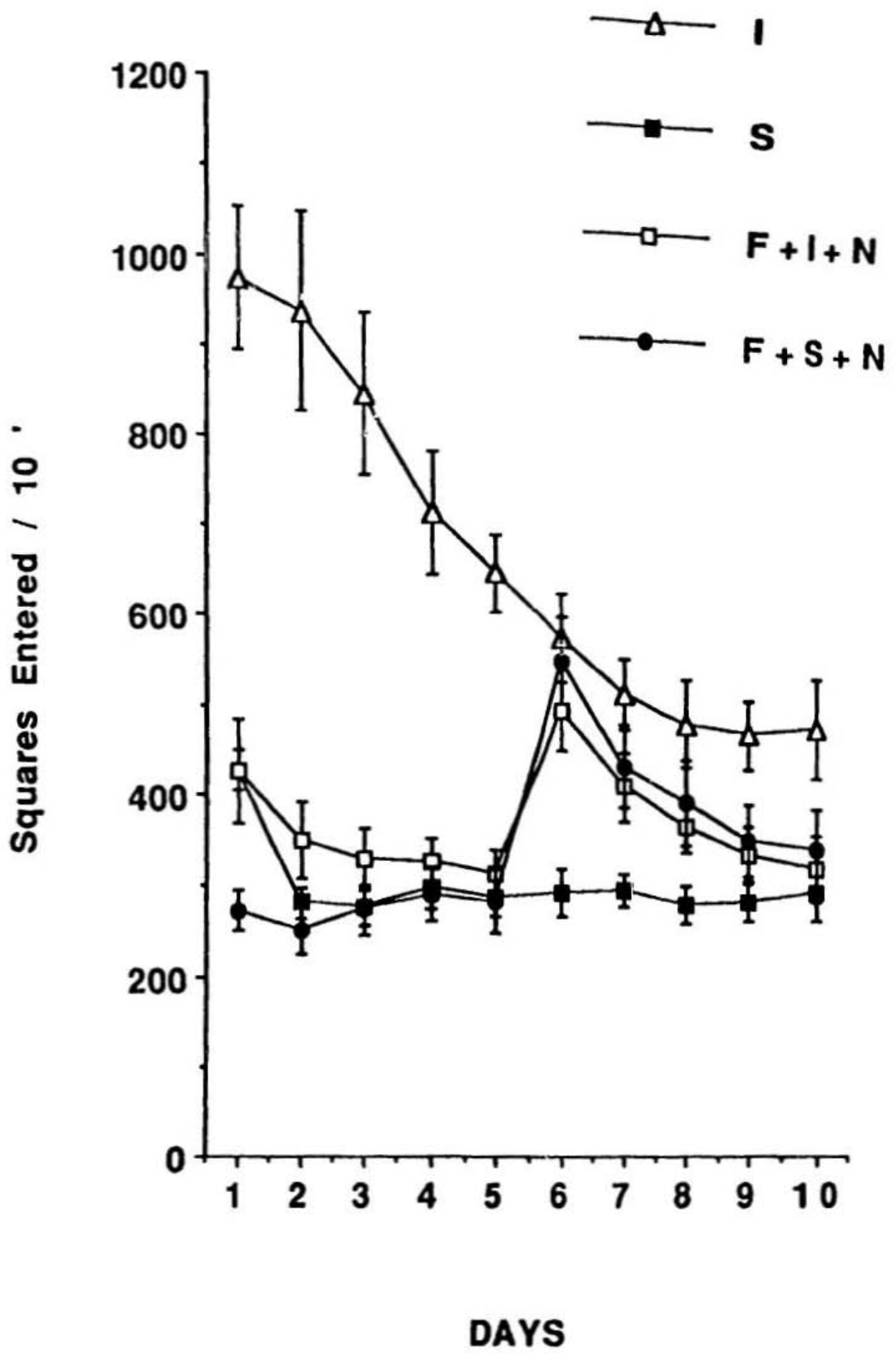
Daily mean ±. Locomotor scores of :

1). The ischemic animals (Group I).

2). The surgery control animals (Group S).

3). The pre-exposed ischemic animals (Group FIN).
(Familiar Environment +Ischemia + Novel Environment)

4). The pre-exposed surgery control animals (Group FSN).
(Familiar Environment + Sugery + Novel Environment)


I
S
F + I + N
F + S + N
1200
1000
800
600
400
200
0
Squares Entered / 10 '
1
2
3
4
5
6
7
8
9
10
DAYS

each of the five test days in the postischemic familiar environment (from Day1 to Day 5, $P < 0.01$, Dunnett's Test).

There was no significant difference between the FIN and FSN groups over the first five testing days [$F(1,16) = 0.3$, $P > 0.5$] even though the FIN gerbils had been subjected to bilateral carotid artery occlusion. The locomotor activity of the FIN group increased slightly over the first few days after ischemia relative to the FSN group yielding a significant interaction [$F(9,144) = 3.7$, $P<0.001$]. The locomotor activity in the novel environment was initially increased which produced a significant Day effect [$F(9,144) = 16.5$, $P < 0.001$] .

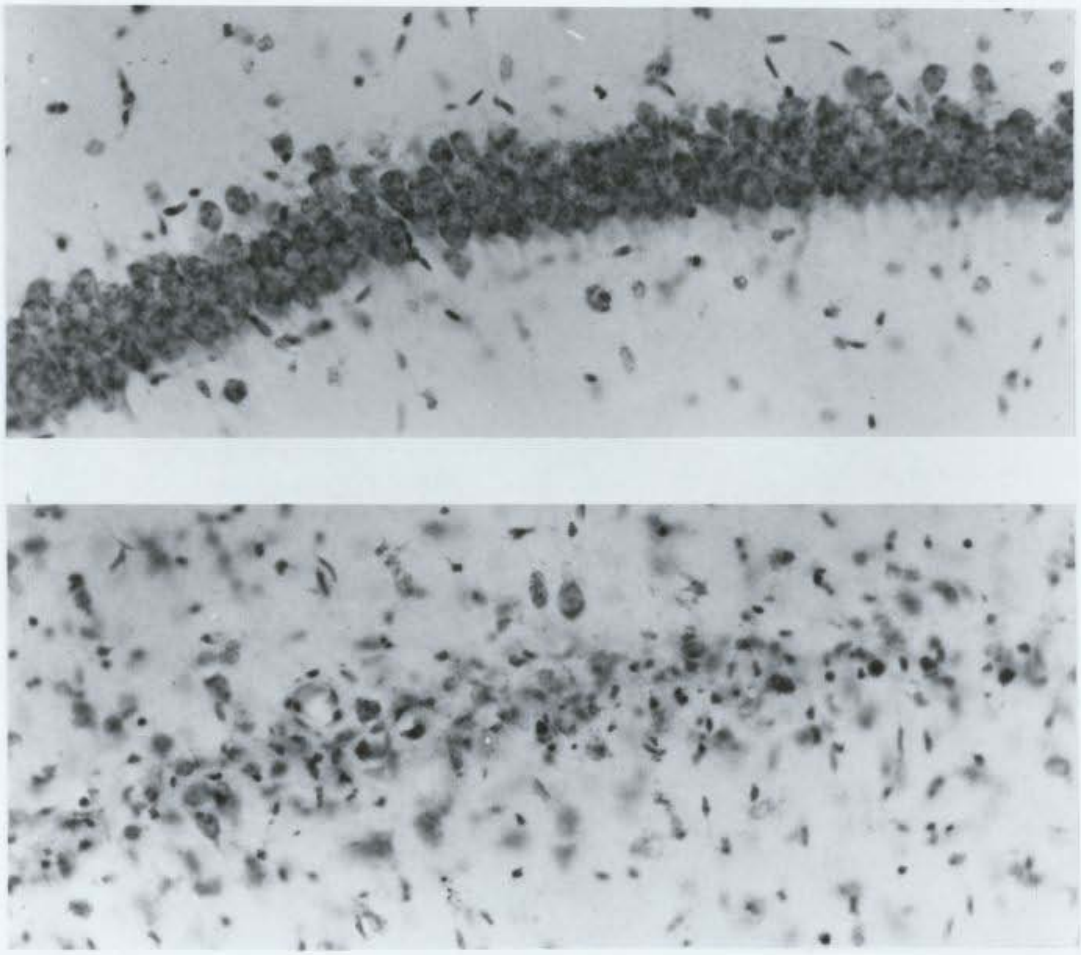
in the FIN group, comparison of the locomotor activity in the three testing periods (5 days pre-ischemia, 5 days post-ischemia in familiar environment, 5 days post-ischemia in novel environment) showed that there was no significant difference among these three periods [$F(2, 27) = 0.5$, n.s].

Histology

Severe neuronal necrosis and cell loss (Figure 8) could be seen in the CA1 area of hippocampus in both I and FIN groups. The CA1 sector was severely damaged in both ischemic groups [I and FIN Vs. S and FSN; $F(2, 27) = 20.8$, $P<.001$]. Individual comparisons between groups showed significant cell loss in the I and FIN groups relative to their respective controls ($P < 0.01$, Scheffe F-Test). There was no difference in damage to CA1 between the FIN and I groups (Figure 9).

Figure 8. Photomicrographs of CA1 Areas

Representive photomicrographs of the CA1 area from a surgery control (group S) animal (top) and from an ischemic (group I) animal (bottom). Magnification 160 X. Cresyl violet stain.

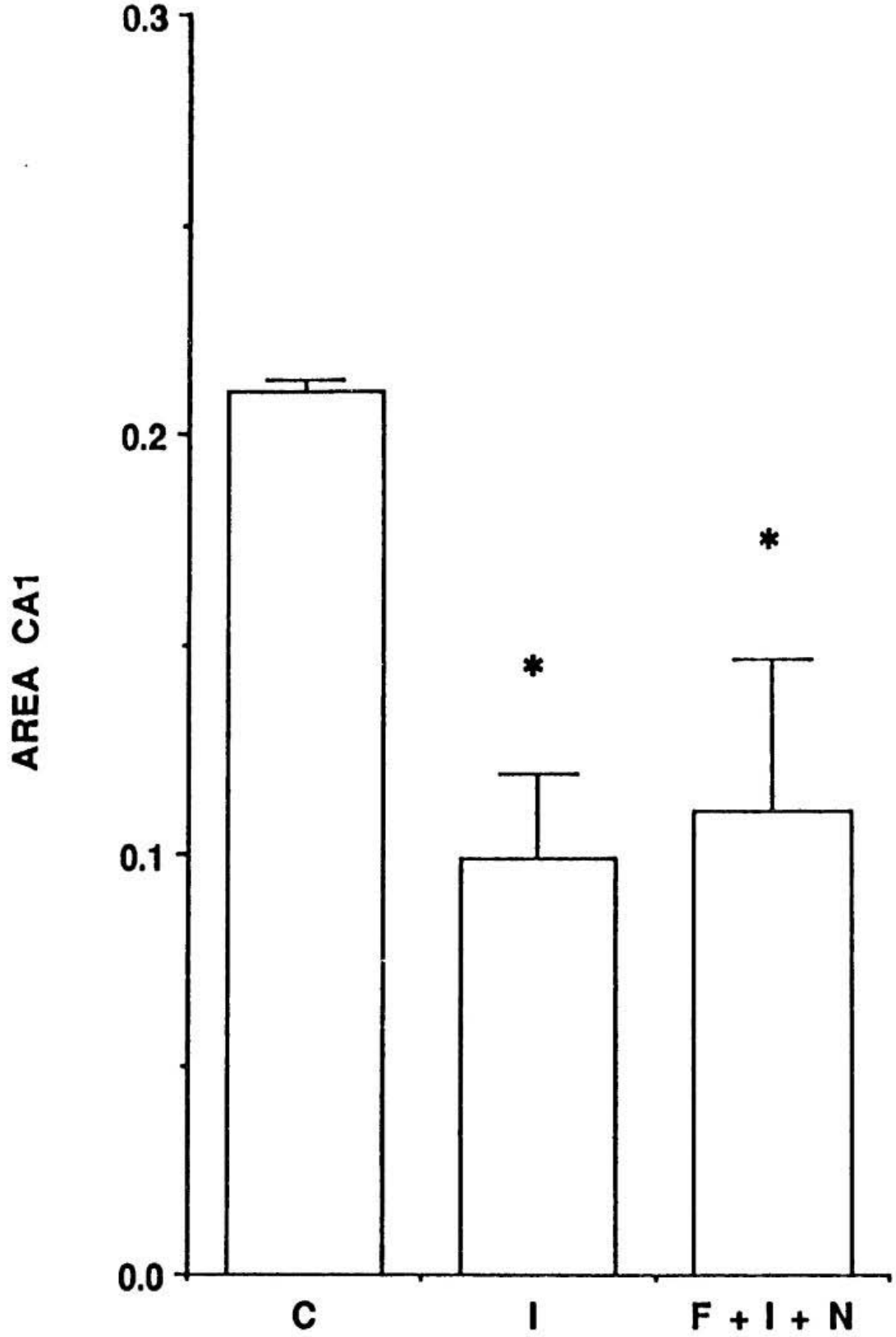


100 µm

Figure 9. Degree of CA1 Cell Loss

Mean area of CA1 (mm2) $\pm$ S. E. M. in the control (C) and the ischemic groups (group I and group FIN).

* P < 0.01, Scheffe Test compared to control.


0.3
0.2
0.1
0.0
AREA CA1
*
*
C
I
F + I + N

DISCUSSION

In the present experiment, the I and FIN groups underwent the same ischemic insult, however, the I group showed a significant increase in locomotor activity while the FIN group showed activity levels comparable to the unoperated control animals. Pre-exposure experience may thus have allowed the animals to form a spatial map of the environment. Thus, for the FIN group ischemic damage of the hippocampus would not have too much effect on the locomotor activity because of the pre-acquired spatial map. The hippocampus is thought to be a station for information-processing (Squire, 1986; Zola-Morgan et al., 1986) . The processed information (i.e., a spatial map of the open field) would be stored in other brain structures, such as the cortex (Squire, 1978; Zola-Morgan et al., 1986).

One would have expected the FIN group to show increased locomotor activity in the "novel" environment. However, there was no significant difference among the activity scores recorded during pre-ischemia, post-ischemia and "novel" environment, which may be due to the "novel" environment really being familiar to the animal. Although, a number of stimuli in the testing room were changed, a great many external cues (e.g., odour, lab shelves, video camera etc.) remained constant thereby decreasing the " novelty" of the novel environment.

One finding in this experiment that differed from previous studies (Gerhardt and Boast, 1988) and from Experiment 1 was that the level of locomotor activity of the I group never dropped to the control level, remaining significantly higher than that of the S group over all ten post-ischemic test days. This may be explained by two factors in this experiment which are different from Experiment

1: first, body temperature of the animals was maintained through-out the occluding procedures, so that there was no "hypothermia" protection; the second factor may be that the microvascular clips provided a more effective occlusion of the carotid arteries than did the occluding device used in Experiment 1. Thus, ischemic damage of CA1 was much more severe in this experiment than that in Experiment 1. In order to determine whether this increase in locomotor activity was proportional to the degree of ischemic damage, Experiment 3 was designed. In this experiment, a differing degree of damage to CA1 was produced by varying the duration of occlusion.

EXPERIMENT THREE

INTRODUCTION

As mentioned in the general introduction, in Gerhardt and Boast's study (1988), the analysis of the different degree of ischemic damage of the hippocampus was carried out in the same ischemic group (all ischemic animals underwent a 20 minute occlusion). Also in Experiment 1, the comparison of hippocampal damage was carried out using a model of "repeated ischemic episodes". In the present experiment, the relation between the degree of CA1 damage produced by varying the occlusion duration and locomotor activity was examined.

The purpose of this experiment then was to see whether more severe ischemic damage to CA1 would result in graded increases in locomotor activity.

METHODS

Subjects

Thirteen gerbils were randomly divided into two groups: 10 minute occlusion group (n = 8) and 15 minute occlusion group (n = 5). The data from S and I groups of Experiment 2 were added to the data collected from this experiment.

Surgery and occlusion

The surgical and anesthetic procedures were the same as in the previous experiment, except for the occlusion times (10 minutes and 15 minutes).

Open-field test

Daily 10 minute open-field tests were performed for ten days for each group. The method of observation of locomotor activity was the same as in the previous experiment.

Histology

The preparation was the same as in the previous experiment.

RESULTS

Locomotor activity

As shown in Figure 10 and Table 1, Locomotor activity of the ischemia groups increased after occlusion [F (3,29) = 13. 8, P< 0.001]. Locomotor activity of the 5 minute occlusion group (I5) was significantly increased compared to the control group [Dunnet's Test: Day 1-Day 6, P < 0.01; Day 7-Day 10, P < 0.05]. The locomotor activity of the 10 minute occlusion group (I10) and 15 minute occlusion group (I 15) was significantly increased compared to the control group from Day 1 to Day 7 (P < 0.01) and Day 8 (P < 0.05) and from Day 1 to Day 8 (P<0.01) respectively. Among the ischemia groups locomotor activity of I15 group was increased significantly on 3 (Days 1, 4 and 5) of the first 5 test days [I 15 Vs. I5 and I 10, P < 0.01]. The locomotor activity of the ischemic groups dropped sharply, especially for the I15 group [a significant Treatment X Days interaction: F(3, 9) = 43.0, p < 0.001]. All four groups showed daily decreases

Figure 10. Open Field Activity of Graded Ischemia

Open field activity expressed as squares entered per 10 minute test session. The data of control and 5 min ischemia groups is from experiment 2.

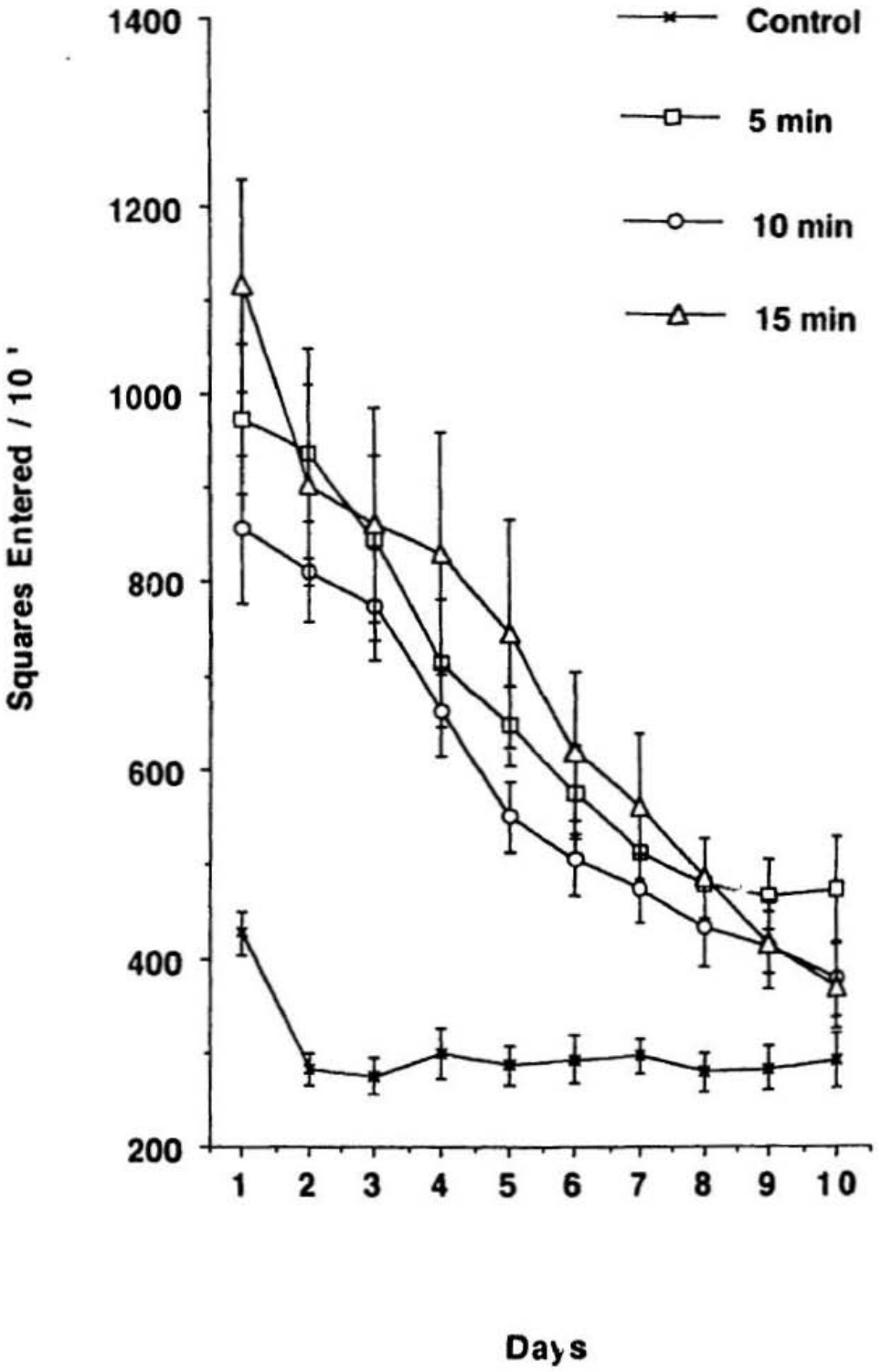

Control
5 min
10 min
15 min
1400
1200
1000
800
600
400
200
Squares Entered / 10 '
1
2
3
4
5
6
7
8
9
10
Days

Table 1. Comparison of locomotor activity

Control vs ischemic groups (I5, I10, I15), compared by Dunnett's Test.

	Day1--Day6	Day7	Day8	Day9	Day10
I5	**	*	*	*	n.s
I10	**	**	*	n.s	n.s
I15	**	**	**	n.s	n.s

Dunnett's Test:

** ----- P< 0.01
* ------ P< 0.05
n.s ----- not significant

in locomotor activity [a significant Day effect: $F(3, 27) = 5.2$, $P < 0.001$].

Histology

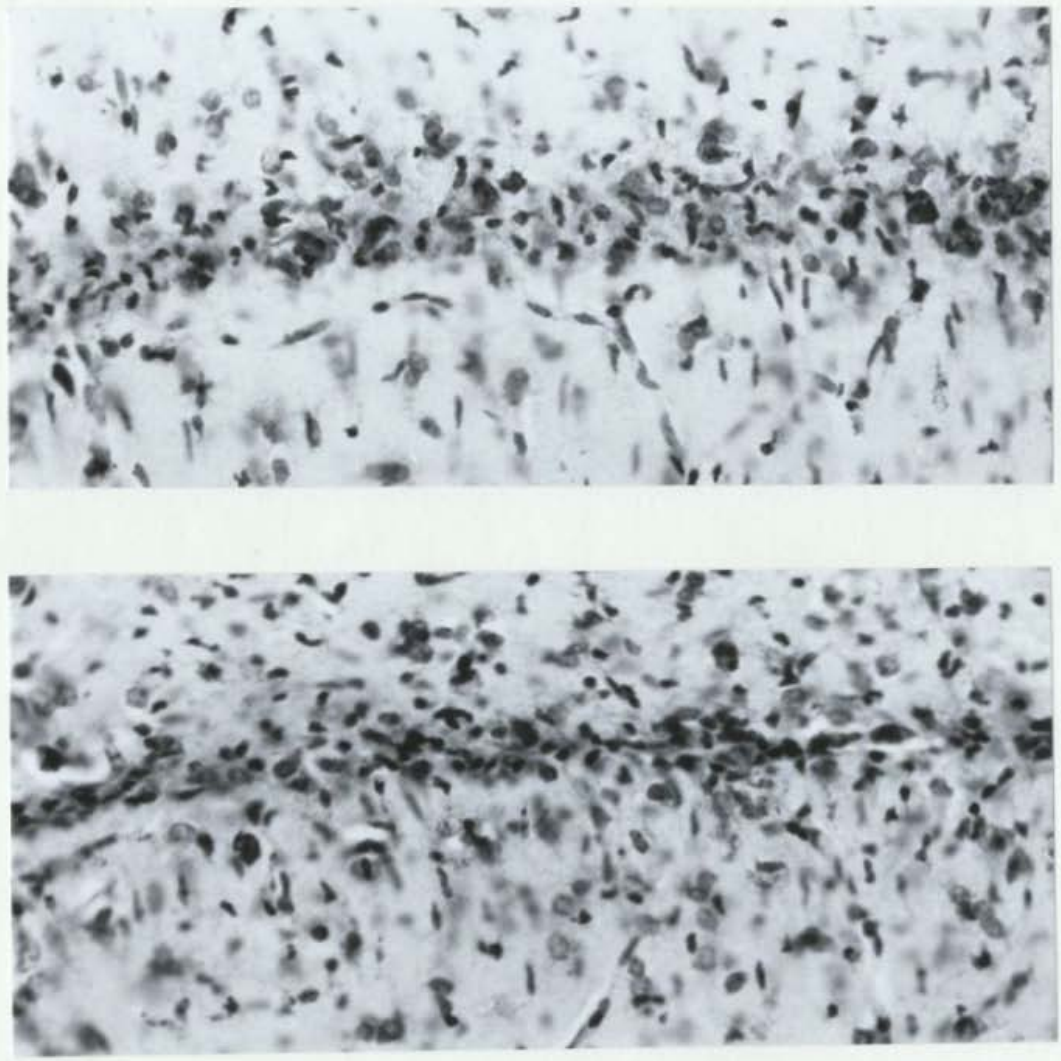
As shown in Figure 11 and 12, CA1 necrosis can be seen in all ischemic groups. A one way ANOVA showed significant CA1 cell loss [$F(3,29) = 28.7$, $P < 0.001$]. Subsequent comparison by Dunnett's Test revealed significant CA1 cell loss in each ischemic group compared to control ($p < 0.01$). However, there was no clear difference in severity of damage to CA1 among the three ischemic groups ($P > 0.05$) except I5 Vs. I15 (Fisher PLSD, $P < 0.05$).

DISCUSSION

The post-ischemic increase in locomotor activity after 10 and 15 minute occlusions was similar to that observed previously with a five minute occlusion. The locomotor activity of the I15 group was increased significantly compared to the I5 and I10 groups on Day 1, Day 4 and Day 5 but not on other test days, which may be due to the small number of the animals in the I 10 ($n = 8$) and I 15 ($n = 5$) groups. This may reflect the somewhat greater CA1 damage sustained by the I15 group. However, it would seem that changes in locomotor activity are near their maximal level after a five minute period of ischemia. The reason for this "limited increase" of locomotor activity may be due to "functional specialization" within CA1, e.g., rostral half of CA1 sector. Thus more extensive damage of CA1 sector will not have much further effect on locomotor activity. Another possibility is that the level of locomotor activity is near the maximal level for normal exploratory behaviour, in other words, a behavioural ceiling effect.

Figure 11. Photomicrographs of CA1 Areas

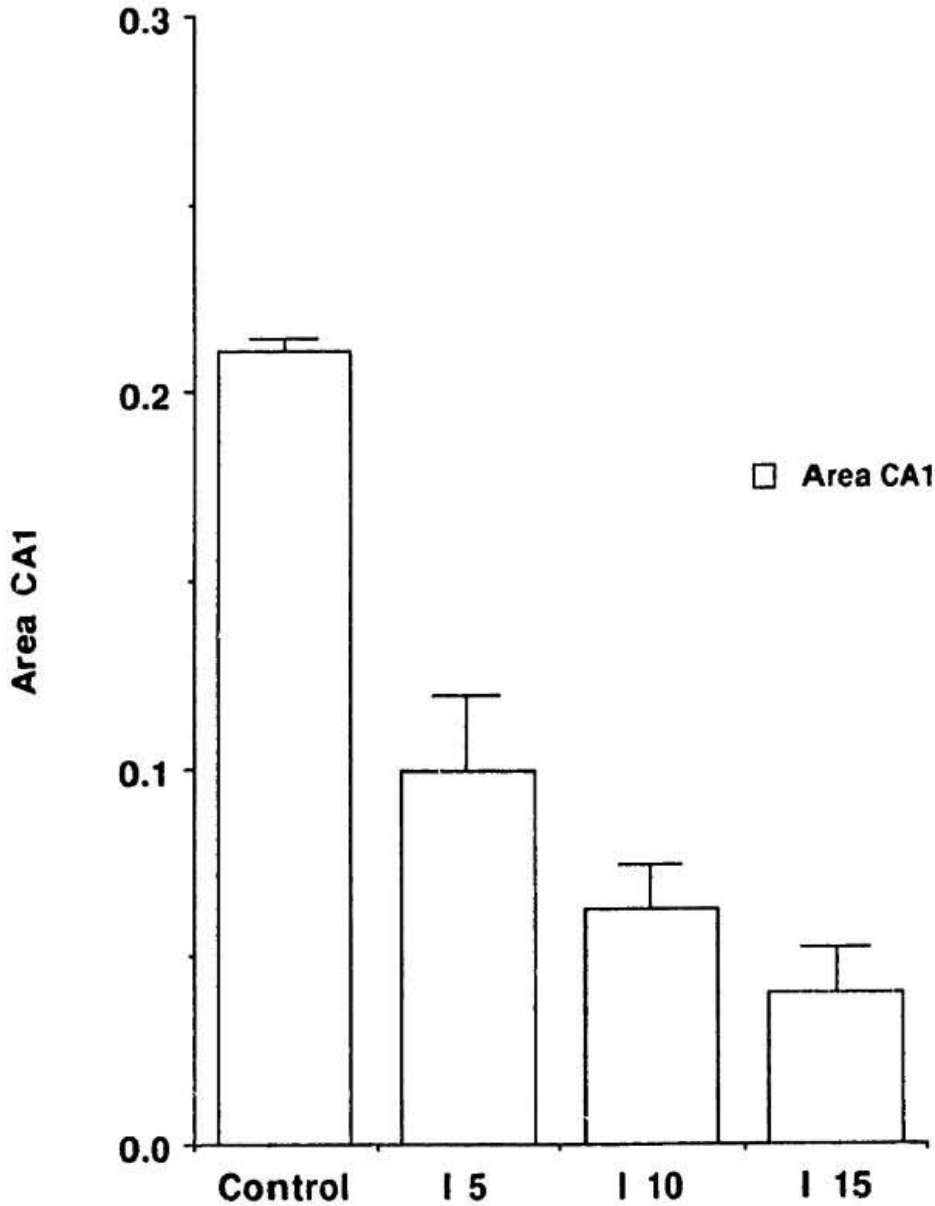
Representive photomicrographs of the CA1 area from a10 min occlusion animal (top) and a 15 min occlusion animal (botom).
Magnification 160 X. Cresyl violet stain.



100 µm

Figure 12. Degree of CA1 Cell Loss

Mean area of CA1 (mm2) $\pm$ S. E. M. in the control (C) and the ischemic groups (I5, I10 and I15). The data of control and I5 is from experiment 2.


0.3
0.2
0.1
0.0
Area CA1
Area CA1
Control
I 5
I 10
I 15

GENERAL DISCUSSION

Extensive damage of the CA1 region of the hippocampus was successfully achieved in the present studies. These results further confirm the two-vessel occlusion in the gerbil as an effective, convenient ischemia model for stroke research (Levine and Sohn., 1969; Kahn, 1972; Harrison et al., 1973; Ginsberg and Busto, 1989). In Experiment 1, animals subjected to repeated ischemic attacks did not show a graded increase in their locomotor activity. This was explained by the fact that repeated ischemic episodes would not be expected to change a preacquired spatial map formed after the first ischemic attack (not severe enough due to the protection by hypothermia). In Experiment 2, pre-exposure to the open field prevented the post-ischemic hyperactivity activity. This was a key observation since it suggested that the increased locomotor activity in the open field test was a spatial memory deficit rather than a motor hyperactivity. In Experiment 3, it was found that more severe ischemic damage of CA1 produced by longer occlusion durations (10 and 15 minutes) did not increase locomotor activity substantially above levels produced by standard 5 minute occlusion.

From all three experiments it was found that there was a sensitive relationship between the hippocampus, spatial memory, ischemia and locomotor activity. It should be interesting to see how these relate and affect each other. First, one question that should be clarified is whether spatial memory deficits represent a special functional deficit or a problem with general memory? In order to answer this question, the brain structures involved in spatial memory must be known. Since different features of spatial analysis are

performed by different cortical systems, the information must be brought together. The structure which encodes the relationship between the information and the cortical system is thought to be the hippocampus (Kolb and Whishaw, 1990). Thus, patients with only hippocampal damage do not pass information on to the cortical system, and should only show symptoms of topographical disorientation rather than general memory impairment (Zola-Morgan et al., 1986; Kolb and Whishaw, 1990). This may well be represented by the case of H. M. His IQ is above average (118 on the Wechsler Adult Intelligence Scale). He is quiet and well mannered socially. However, the following description is evidence of spatial deficits in H. M. --- After leaving the main highway, we asked him for help in locating his house. He promptly and courteously indicated to us several turns, until we arrived at a street which he said was quite familiar to him. At the same time, he admitted that we were not at the right address. A phone call to his mother revealed that we were on the street where he used to live before his operation --- (Milner et al., 1968).

It used to be thought that H. M's spatial problem resulted from his severe memory problems because he had combined lesions of the amygdala and hippocampus. However, as mentioned above, H. M. has good memory of the environment which he was familiar with before his surgery, and only shows severe anterograde amnesia. Therefore, H. M.'s anterograde amnesia is not the result of a general memory problem. Recently, studies on R. B. showed that ischemic damage to a small portion of hippocampus, the CA1 region, brought on severe anterograde amnesia (Zola-Morgan et al.,1986; Kolb and Whishaw, 1990).

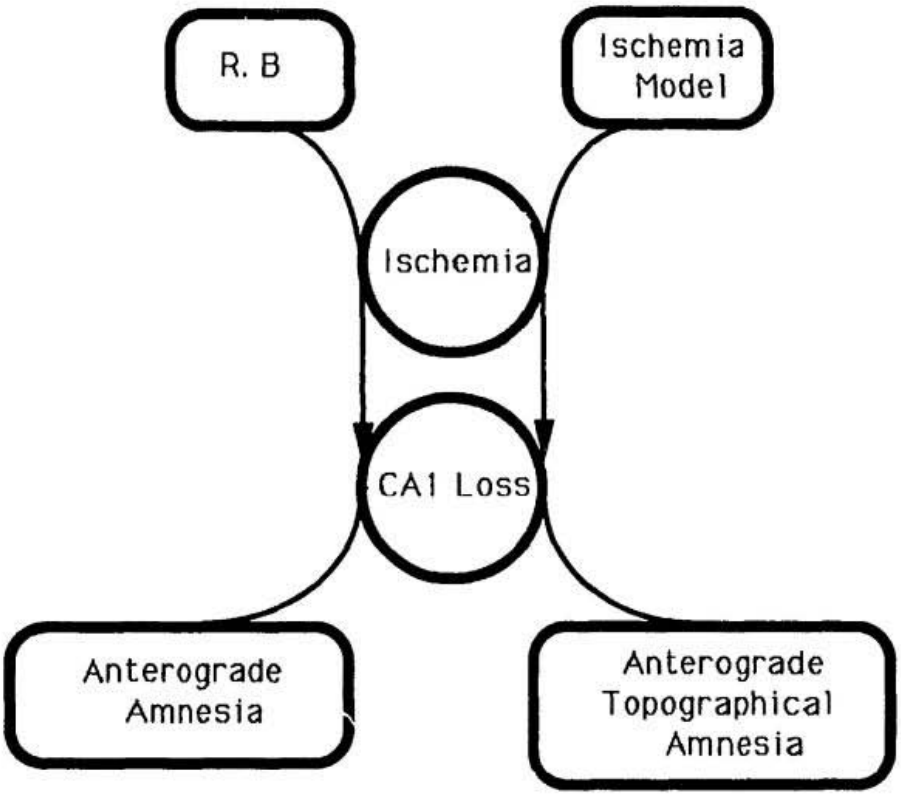
In the present experiments, animals did not increase locomotor activity in the environment which was familiar before the ischemic episode, but only showed

increased locomotor activity in a completely novel test environment (e.g., group I in Experiment 2), which may be also called an "anterograde topographical amnesia". Comparing this with the case of R.B (Figure 13), it can be seen that amnesia both in R. B. and the ischemia model has a similar mechanism : CA1 loss, and the CA1 loss can cause anterograde amnesia or anterograde topographical amnesia.

So far it is clear that ischemic damage of CA1 can cause anterograde topographical amnesia. In the present experiments, if locomotor activity is taken as an index of spatial memory, it is necessary to know how spatial memory and locomotor activity are related. In addition, it is also necessary to discuss the choice of the open-field test as opposed to a radial arm maze or Morris water maze, which are thought to be "purer" tests of spatial memory. In order to get a clear understanding, the concept of "environmental psychology" (Wohwill, 1970) has to be introduced here. The ongoing activity of any animal is a complex mixture of different activities, for example, walking, drinking, eating, sniffing, sleeping, grooming, etc. Each species of animal engages in a certain mixture of activities which make them differ from other species, and the reactions to particular external stimulation may vary from species to species. However, there are certain patterns of behaviour that remain constant across species. For example, in the situation of threat or stress most animals react by fleeing to safety (O'Keefe and Nadel, 1978). The reaction of the animals depends on the novelty of the external stimuli. Novelty elicits a state of anxiety, and exploration is motivated by this state. Spatial information obtained during exploration will reduce fear and habituation to the external stimulation will develop (Halliday, 1968; Groves and Thompson, 1970). In the open-field test,

Figure 13. Comparison of R. B and Ischemia Model

It can be seen that both anterograde amnesia in R. B. and anterograde topographical amnesia in ischemia model are caused by the same mechanism : CA1 loss.


R. B
Ischemia Model
Ischemia
CA1 Loss
Anterograde Amnesia
Anterograde Topographical Amnesia

the environment is novel to the animal. The animal explores the environment and exhibits increased locomotor activity until a spatial map is formed (habituation). Threat, stress, food, water and a sexual mate are thought to be in the same class of external stimuli (O'Keefe and Nadel, 1978). Animals tested in an open-field test (stress of novel environment), Morris water maze (stress of water) or radial arm maze (food deprivation) would undergo the same class of external stimulation. Therefore, a similar spatial mapping process should be utilized in all three tests . The open-field test should be considered as testing an aspect of spatial memory, and post-ischemic changes of locomotor activity could be used as a sensitive index to examine the animal's spatial memory.

The advantages of the open-field test are its simplicity, ease of observation and analysis compared to the water maze. Moreover, the animals do not have to be food deprived as in the radial arm maze test. It also allows early testing after the

ischemic insult. Previous open-field studies have not given a clear indication that measurement of locomotor activity could be used as a method to measure spatial mapping ability. The open-field test may be a useful method to determine the functional status of the hippocampus as a result of drug treatment, lesions, stimulation, etc.

Despite the usefulness of the open field test, more elaborate testing and recording procedures are necessary to evaluate ischemic damage and more particularly the effectiveness of therapy aimed at reducing the consequences of ischemic damage. Future studies should utilize multiple memory tests (e.g., radial arm maze); EEG recording and make the ischemic episodes similar to the clinical situation. For example, the occluding device used in Experiment 1 would be useful in establishing an animal model resembling the clinical multi-episode stroke (Kato et al., 1989). The occlusion period could be shortened

enough to resemble a transient ischemia attack (TIA). TIA result in temporary amnesia and may occur many times a day over a period of several weeks (Kato et al., 1989; Warlow and Morris, 1982). The occluding device would be ideal for such studies. Pharmacological prevention of ischemic brain damage has become one of the most exciting areas in neuroscience. However, most of the studies employ only histological criteria to assess the effectiveness of these drugs. It perhaps is more important to determine if neurons that appear normal histologically still function normally. The present experiments suggest that the open-field test would be a useful functional index in conjunction with other tests to assess new drugs for their ability to prevent or reduce ischemic damage.

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