THE ROLE OF THE NMDA RECEPTOR IN THE HIPPOCAMPUS IN CERTAIN FORMS OF LEARNING

by

Sabrina Davis
Department of Pharmacology

Submitted to the University of Edinburgh for the Degree of Doctor of Philosophy

June, 1990

In accordance with the requirements of the University of Edinburgh regulation 3.4.7 this thesis has been composed by myself and the work presented herein is my own.

To K.A., for your unquestioning support.
I am indebted.

ACKNOWLEDGEMENTS

The work described in this thesis was supported by a grant from the Wellcome Trust. I am grateful to Professor Kelly for the facilities provided by the Department of Pharmacology.

Many people have been instrumental in helping me through this very important part of my career. First and foremost I should like to express very special thanks to my supervisor, Dr. Richard Morris for allowing me to work on an extremely exciting project and for all his support and advise in both the running of the experiments and in the final writing of the thesis.

I should secondly like to thank Dr. Steven Butcher, for essentially converting me from a psychologist to a neuroscientist by teaching and guiding me through the pharmacological aspects of the thesis. We have spent many a happy hour discussing the implications of the research.

I would like to extend my thanks to those that have been instrumental in filling in the gaps of my knowledge both within and outside the department. I am grateful to the technical assistance of Miss Elma Forrest in the histology and Miss Caroline Stewart for her help on the more mundane aspects of running animals in the behavioural task. I am also grateful to Miss Audrey Kerr for converting my attempts at typing this thesis into a "proper document".

Finally, to all of my friends and colleagues in the department who have cajoled, bullied, coerced and supported me through this "apprenticeship" and helped me maintain my sanity....Thank you.

Activation of the N-Methyl-D-aspartate (NMDA) receptor is crucial for the induction of hippocampal Long-term potentiation (LTP), a form of synaptic plasticity that has come to be regarded by many neuroscientists as a neural substrate underlying the processing of information during learning. The hippocampus is also a structure that has long been implicated in spatial reference and/or working memory. The aim of this thesis is to test more rigorously, the hypothesis that hippocampal NMDA receptors are involved in "hippocampal learning" through induction of LTP, by investigation the dose-response effect of the NMDA receptor antagonist, AP5 on (i) induction of LTP and spatial learning, tested in individual animals, and (ii) it's effect on working memory.

In the first experiment a range of concentrations (5mM, 13mM, 20mM, 30mM, 40mM) of (D-AP5) were chronically infused (icv) into rats, at a rate of 0.5μl a day for 14 days. Control animals consisted of either sham or unoperated rats, or rats infused with aCSF. During the 14 day experimental period rats were tested on a spatial reference memory task in the open field water maze for 6 days and then an attempt to evoke LTP in each rat was made. At the end of the experiment micro dialysed samples of ecf were taken from the left hippocampus for 2 hours. Finally, tissue samples from 5 brain areas, including the right and left hippocampus were dissected out and the exact content of AP5 in the brain during the experimental period was measured using HPLC. The animals were regrouped according to the whole tissue concentration of AP5 in the hippocampus. The results showed a dose dependent impairment of spatial reference memory that correlated with the dose dependent blockade of LTP. The amount of AP5 recovered from the ecf of the hippocampus was estimated to be compatible with binding studies showing percentage receptor occupancy and electrophysiological studies showing the amount of AP5 required to block LTP in the hippocampal slice.

In the second experiment, a single concentration known to impair spatial reference memory and block LTP (30mM D-AP5) was used to test animals ability to learn a spatial working memory task in the open field water maze. AP5 caused no impairment in working memory when the delay between trials was short (30s). When the delay was extended to 2 hours or 5 hours, however, animals infused with AP5 showed a significant impairment. Also at the 5 hour delay, control animals showed a trend towards an impairment in the task.

The results from this thesis suggest activation of the hippocampal NMDA receptors are necessary for the processing of spatial information. The high correlation between the learning impairment and the induction of LTP strengthens the hypotheses that the physiological activity underlying learning is a form of plasticity similar to that seen with LTP. Furthermore, from the working memory experiments it can be implied that this type of plasticity occurs when information is required over longer periods of time.

CONTENTS PAGE

ABBREVIATIONS

aCSF	artificial cerebrospinal fluid
Ala	alanine
AP4*	2-Amino-4-phosphonobutyrate
AP5*	2-Amino-5-phosphonopentanoate
AP6*	2-Amino-6-phosphonohexanoate
AP7*	2-Amino-7-phosphonoheptanoate
AP8*	2-Amino-8-phosphonooctanoate
APV	2-Amino-5-phosphonovalerate
Asp	aspartate
CGP 37849	(DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid
7-Cl-KYN	7-chlorokynurenic acid
CNQX	6-cyano-7-nitroquinoxaline-2,3-dione
CPP	3-((-)-2-carboxypiperazin-4-yl)propyl-1-phosphonate
DAA	D-α-aminoadipate
DAP	D-α-aminopimelate
DGG	γ-D-glutamylglycine
DNQX	6,7-dinitroquinoxaline-2,3-dione
EAA	Excitatory amino acid
ecf	extracellular fluid
EGTA	ethylene-bis(oxyethylenenitrate)tetra-acetic acid
EPSP	Excitatory postsynaptic potential
FP	Field potential
GABA	γ-Aminobutyrate
Glu	Glutamate
Gln	Glutamine
HPLC	High performance liquid chromatography
icv	intracerebroventricle
ip	intraperitoneal
IP	Inositol phosphate
ITI	inter-trial interval
K	Kainate
L-APB	L-2-Amino-5-phosphonobytrate
LTP	Long-term potentiation
MK-801	(+)-5-methyl-10,11-dihydro-5h-dibenzo[a,d]cyclohepten-5,10-imine
NMA	N-Methyl-aspartate
NMDA	N-Methyl-D-aspartate
OPA	o-Phthalaldehyde
PCP	Phencyclidine
PDA	*cis*-2,3-piperidine dicarboxylic acid
PND	Post natal day
PNW	Post natal week
PNM	Post natal month
PS	population spike
Q	Quisqualate
QX222	Trimethylamonium acet-2,3-xylidide
SK 10,047	n-allynormetazocine
Tau	Taurine
THF	Tetrahydrofuran
Val	Valine

* These compounds can be either the L-isomer, the D-isomer or the racemate (DL)

CHAPTER 1: INTRODUCTION

The NMDA receptor complex in the hippocampus is thought to be involved in the processing and storage of information through the same or similar mechanisms that are involved in the induction of Long-term potentiation (LTP). The theory is rooted in three independent areas of research: (i) the functional role of the hippocampus; (ii) the theoretical assumption that LTP reflects the activity of a neural substrate for learning; and (iii) the properties of the NMDA receptor-complex. Although the notion that the hippocampus was involved in learning (Scoville and Milner, 1956) and the possibility that specific changes in neural activity took place during learning (Hebb, 1949) had been established to a certain extent, it is only over the past 10 to 15 years that the theory has evolved. First came the discovery of LTP (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973), an artificially induced form of synaptic enhancement that has now come to be regarded by many neuroscientists as a mnemonic device. Second came the discovery that the NMDA receptor was involved in evoking LTP in the hippocampus (Collingridge, Kehl and McLennan, 1983; Harris, Ganong and Cotman, 1984) and thirdly, Morris and colleagues (Morris, Andersen, Lynch and Baudry, 1986) showed that the NMDA receptor was somehow involved in certain forms of learning attributed to the hippocampus. Ongoing research in each of the three areas has continued to lend support to the theory, but to date there has not been a fully comprehensive study to link all three areas, and so there are gaps that have had to be filled by logical assumptions.

To briefly describe the aim of this thesis (it is explained in more detail in last section of the introduction), it is to gain information from all three areas and test more rigorously the involvement of the NMDA receptor in the induction of LTP and how it relates to certain types of learning normally attributed to hippocampal function. Three sections in the introduction provide a detailed description of the research carried out in each area, providing the essential background information on which to base a hypothesis of NMDA receptor involvement in learning. Finally, the last section of the introduction draws the necessary information from these 3 areas together, to describe the experimental design and the underlying rationale for the experiments which test the hypothesis.

1.1. THE INVOLVEMENT OF THE HIPPOCAMPUS IN LEARNING

The hippocampus has long been associated with learning. It became apparent in the 1950's when a patient, H.M., was given a bilateral resection of the temporal lobes in an attempt to reduce the number of recurring attacks of epilepsy. Although the epilepsy was reduced, it left him in a state of profound anterograde amnesia (Scoville and Milner, 1957). Interest in the amnestic syndrome of patients with damage to the hippocampus (often caused by head injury, anoxia, and ischaemic brain damage) led to an explosion of animal studies designed to investigate the effect of lesions to the hippocampus on animals performance in learning tasks. These, in turn led to the development of a number of theories of hippocampal function (some of these are listed in table 1.1). The theories are many, and at the outset they seem to differ quite radically, but by and large they are a variation on a theme, and as Barnes points out: "While they differ in detail, they all agree that this structure is important for the acquisition of certain kinds of new information", (Barnes, 1988).

Table 1.1: THEORIES OF HIPPOCAMPAL FUNCTION

Spatial Navigation	O'Keefe and Nadel, 1978
Working Memory	Olton, Becker and Handleman, 1979
Memory Indexing Theory	Teyler and Discenna, 1986
Temporal Contiguity	Rawlins, 1985
Declarative Memory	Squires and Cohen, 1984
Contextual Information for Conditional Response	Gabriel, Sparenborg, and Stolar, 1987
Configural Associational Theory	Sutherland and Ruddy, 1989

The two most striking of these theories are the spatial mapping theory (O'Keefe and Nadel, 1978) and the working memory theory (Olton et al, 1979). The spatial mapping theory suggests a specific type of information is processed by the hippocampus; the working memory theory attributes a type of memory processing to the hippocampus, regardless of the type of information. The other theories are less specific and could easily accommodate either or both theories. A long standing debate between the proponents of the spatial mapping and working memory theories has evolved, because at the procedural level it is difficult to dissociate the two

explanations and in some of the tasks used to test these theories, animals with lesions to the hippocampal show poor performance on both tasks. For this reason these theories have probably generated most of the current rodent research on hippocampal function.

1.1.i. Spatial Mapping Theory

O'Keefe and Nadel (1978) postulated a theory that the hippocampus, at least in the rat, was involved in cognitive mapping and spatial navigation. The theory is based largely on data from recordings of cells in the hippocampus and from lesion studies. In describing their theory, O'Keefe and Nadel suggest that animals learn to find their way around their environment by laying down cognitive maps. The notion of cognitive mapping is rooted in the Kantian philosophy of absolute space and in the characteristics of a cognitive map that had been "vaguely" suggested by Tolman (1948). The neural mapping system is used to represent the environment, external to the animals relative body position within it. According to Kant, places do not exist in the physical world but are notions constructed by the brain, in order to organise sensory input (in O'Keefe and Nadel, 1978). Therefore, when a map is constructed, it is based on a unique arrangement of several stimuli in the environment. It is laid down as the animal explores the environment and is based on direction and distance between stimuli as the animal moves about. When a map has been laid down, an animal should be able to reach a particular goal from any direction, taking the most appropriate and direct route. This has been borne out by experiments with chimpanzees learning a "travelling salesman" problem (Menzel, 1978). The mechanisms for the mapping system are held to be in the hippocampus and are described as the "locale system". Included in this system is a mis-match mechanism, a means by which the animal can update an already existing map. If a new map is to be laid down, the mis-match mechanism signals completely novel sensory input.

As well as the "locale system", O'Keefe and Nadel propose the existence of a "taxon system", which operates egocentrically and computes coordinates relative to the animals body or eye axes. In this way, an animal may use environmental stimuli to orientate itself or direct itself towards a particular goal. Unlike the "locale system", which allows the animal to "step outside" and

"mentally" rotate the mapped environment, the stimuli encoded in the "taxon system" may only be meaningful from the original position. O'Keefe and Nadel suggests this system is located (diffusely?) outside the hippocampus. Sensory information about the environment is first represented somewhere outside the hippocampus (at least, partly in sensorimotor cortex) as single taxon representations. These then converge on the dentate gyrus of the hippocampus via the perforant path, bringing information from the entorhinal cortex, which in turn has received input from sub and cortical areas. Based on temporal contiguity of stimuli impinging on the animal at a specific time and location, a compound "place" representation (of several taxon stimuli) will be recorded in the dentate gyrus.

This information is then conveyed to the CA_3 and CA_1 areas of hippocampus via the mossy fibre system and the Schaeffer collaterals. The map and mis-match mechanisms are thought to be located in these areas. O'Keefe and Nadel acknowledged uncertainty about the actual location of the map, based on the lack of complete anatomical and physiological data at the time. They suggested if the map is laid down in CA_3, then the CA_1 area would deal with the mis-match system. The output from activation of the mapping system generates purposeful behaviour in familiar surroundings, such that an animal may go directly to a location where food or water may be found. Alternatively, if a map is being laid down or updated, search or exploratory behaviour may ensue. The physiological activity within the hippocampus that drives the laying down of these maps is based on (i) the firing patterns of cells in the dentate gyrus, CA_3 and CA_1; (ii) afferent input from the septum which generates theta rhythm in CA_1 and CA_3; and (iii) the effect of sustained dendritic activity (or LTP) which would activate the map based on sensory information entering the hippocampus from only a subset of stimuli, originally used to lay down the map.

Much of the data on place cells comes from unit recordings of single cells in animals moving freely about a maze. Cells fire when an animal is in a particular orientation (O'Keefe and Dostrovsky, 1971) and when manipulation of environmental have been used to show selective firing pattern of cells called place fields (O'Keefe, 1976; O'Keefe and Conway, 1978).

Two major cell types are required for the mapping system. These are place units and displace units (O'Keefe, 1976). Place units are located in the dentate gyrus, CA_3 and CA_1). They are characterised by a firing pattern of complex-spiking (or Ranck's "complex spike cells" - Fox and Ranck, 1981) and correspond to the pyramidal cells. They fire only when the animal is in a specific location. Displace units (or Ranck's "theta cells" - Fox and Ranck, 1981) are located in the same hippocampal subfields as place cells, but are not present in the dentate gyrus. They fire in synchrony with theta rhythm and correspond to the basket cells of Cajal. They fire when the animal is engaged in some sort of movement or behavioural activity, regardless of its position (see O'Keefe and Nadel, 1978).

Theta rhythm generated in the dentate gyrus is correlated with behavioural activity (Vanderwolf, 1969, 1975) and is thought to contribute to the mapping system in the following manner: Afferents from the septum, gate the activity of the granule cells in the dentate gyrus such that these cells fire only at one phase of the theta cycle and increase the overall cellular activity (Rudell, Fox & Ranck, 1980). This activity occurs when the animal moves about its environment and is related to the animal's intended movements (Vanderwolf 1975, Morris & Hagan, 1983). Its function in the mapping system would be to shift the focus from one set of place representations to another.

Thus, the essential factor of the mapping system is that the hippocampus is involved exclusively in spatial information processing. This theory is also extrapolated to humans and it has been suggested the right hippocampus is involved in spatio-temporal information, whereas the left hippocampus is involved in linguistic processing (Smith & Milner 1981).

From lesion studies, in which animals have damage to the hippocampus or to one of its major input-output systems (fimbria-fornix), O'Keefe and Nadel (1978) have pointed out that the type of behaviour that is impaired would be necessary for spatial navigation. For example, exploratory behaviour, response to novel environments, failure to alternate responses in a spontaneous alternation task, performance in both simple T-mazes and more complex mazes.

In their own lesion study, O'Keefe et al (O'Keefe, Nadel, Keightley and Kill, 1975) showed that animals with lesions to the fornix were impaired in a spatial learning task tested on a circular track. Animals had to learn the location of a fixed reward by using extramaze cues around the testing room. Compared with controls, animals with fornix lesions showed poor performance in learning the task and made more errors. When they were cue assisted on the same task however, their performance was significantly better.

1.1.ii. Working Memory

Olton et al (1979) suggested the hippocampus was involved in a dynamic form of short term information processing; the type of processing that can be tested in a matching-to-sample tasks (Honig, 1978). Examples of working memory are: the retention of a telephone number whilst dialling (Olton, 1978); or remembering a list of items required for a particular situation (Olton, 1983). In fact, Olton (1983) suggests any situation where a generalised set of rules and procedures occur, change in the variation of the items that these rules apply to, is coded by working memory. The essential point of the working memory theory involves a description of the processing dynamics and not the memory content *per se*.

Experimentally, a procedure designed to test working memory requires the animal to retain information for one trial only but not for subsequent trials (Honig, 1978). The working memory concept evolved out of an operational distinction made between working memory and reference memory procedures in animals trained in simple mazes, and from their own experiments using an 8 arm radial maze (Olton & Samuelson, 1976). From these experiments, Olton determined a number of properties characterising working memory: it has a limited capacity; there is little if any serial learning; it is robust up to several minutes, after which it begins to decay; no generalisation processing could be attributed to the errors; and interference between choices increased subject to the number of choices made available (Olton, 1978).

The simplest demonstration of working memory in the radial arm maze is when all arms are baited with food on each trial (Olton and Samuelson, 1976). The optimal strategy is for the animal to visit each arm once and retrieve the food reward. In this procedure, the animal has to remember where it has been within the trial so as not

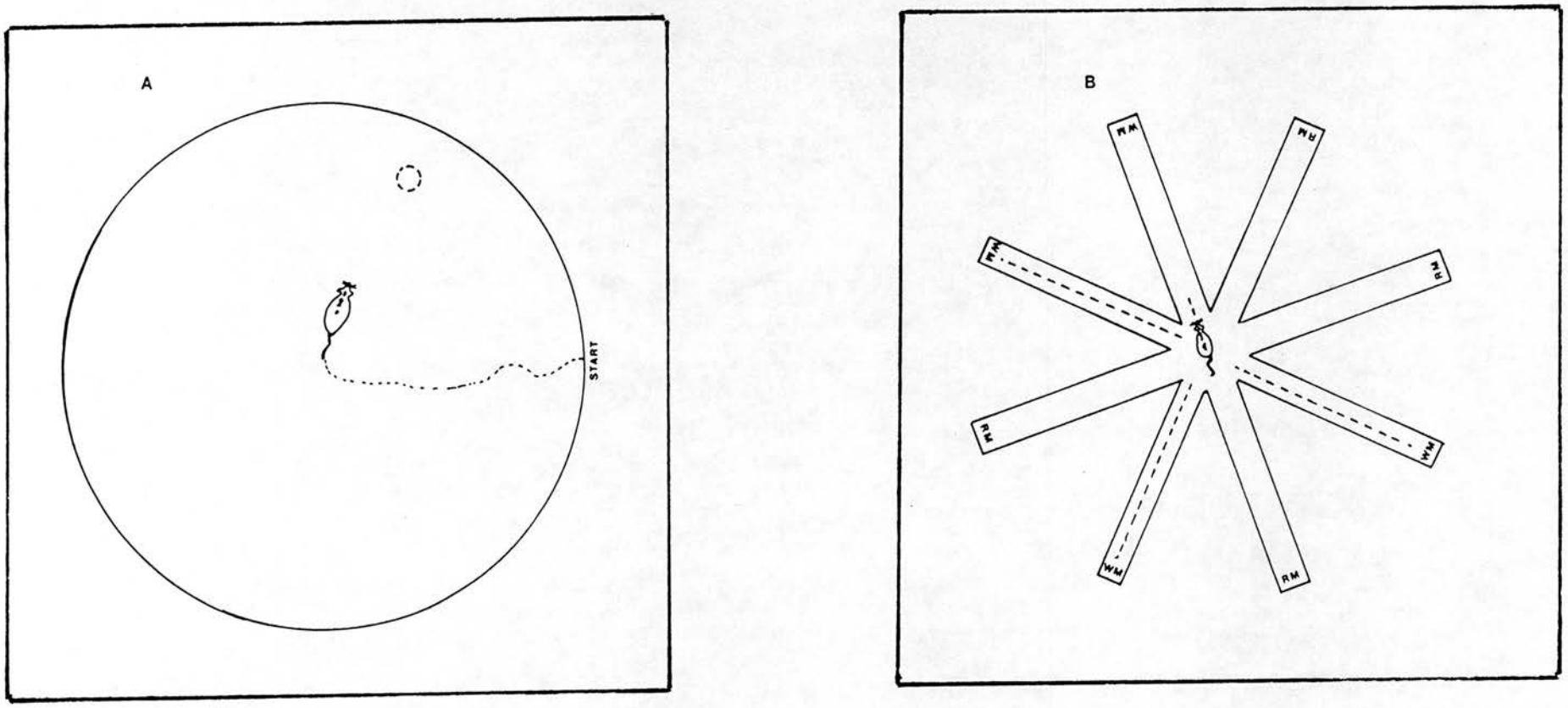


Fig 1.1:
(A) The open field water maze (B) Radial arm maze

to revisit an arm. Remembering information from a previous trial does not help because the animal has to visit each arm on every trial. There are, however, some reference memory elements to the task; for example, the animal must learn and remember throughout the entire test period what the nature of the task is, that there is a reward in each arm, and the optimal strategy is to visit each arm only once. The experiment designed by Olton and Papas (1979) to dissociate working memory from reference memory was tested in a 17 arm radial maze. This was achieved by never baiting some of the arms (reference memory) and baiting the others only once during each trial (working memory). The overall results showed that animals with large lesions were impaired in working memory but not reference memory (Olton and Papas, 1979).

1.1.iii. Hippocampal Lesions and their effect on Learning

Following on from these initial studies, others have been carried out which lend support for either or both theories. Spatial reference memory has been tested mainly using the radial arm maze and the open field water maze (see Fig. 1.1). Typically, the testing procedure in the radial arm maze and the cross maze is to bait only some of the arms repeatedly over several trials. Animals use extramaze cues to learn the spatial location of the rewarded arms. The open field watermaze consists of a large tank of opaque water with a hidden but fixed escape platform. Animals use extramaze cues to learn the location of the platform over several trials and eventually swim directly to it, regardless of the position in which they are placed in the water. Jarrard (1983, 1986; Jarrard, Okaichi, Steward, and Goldschmidt, 1984) and Nadel and McDonald (1980) used a more complex task where performance was also tested under cue assisted conditions. The 16-hole board used by Oades (1981) consisted of a matrix of holes in which some of the holes were baited with food, in much the same way as the radial arm maze.

Evidence supporting the spatial mapping theory has shown animals are unable to carry out a spatial reference memory task when they have complete hippocampal lesions (Nadel and McDonald, 1980; Jarrard, 1983, 1986; Jarrard et al, 1984; Oades, 1981; Morris, Garrud, Rawlins and O'Keefe, 1982; Sutherland, Whishaw and Kolb, 1983; Gage, 1985) or selective lesions to hippocampal subfields

(Jarrard, 1983; Sutherland et al, 1983). These include the dentate gyrus (Jarrard et al, 1983; Sutherland et al, 1983) and the subiculum (Jarrard et al, 1984; Schenk and Morris, 1985). Damage to the major input-output paths, fimbria-fornix (O'Keefe and Conway, 1980; Sutherland et al, 1983) or the entorhinal cortex (Jarrard et al, 1984; Schenk and Morris, 1985; Rasmussen, Barnes and McNaughton, 1989) also impairs spatial reference memory. Furthermore, when the cues were "distributed" rats with fimbria-fornix lesions were worse than when the cues were "clustered" (O'Keefe and Conway, 1980). The effect of lesions on animals performance have been tested in the radial arm maze (Jarrard, 1983; 1986, Jarrard et al, 1984; Nadel and McDonald, 1980; Gage, 1985), a cross maze (O'Keefe and Conway, 1980), a 16 hole board (Oades, 1981), and the open field water maze (Morris et al, 1982; Morris, 1983; Sutherland et al, 1983, 1985; Schenk and Morris, 1985).

Some of the groups that have tested the effect of lesions to the hippocampus on spatial reference memory have also shown an impairment in animals' performance in spatial working memory (Nadel and McDonald, 1980; Oades, 1981; Jarrard, 1983, 1986; Morris, 1983; Jarrard et al, 1984; Gage, 1985; Rasmussen et al, 1989). Other groups have shown impairment in the working memory task with whole hippocampal lesions (Aggleton, Hunt and Rawlins, 1986; Winocur, 1980), CA_1 lesions (Jarrard, 1978) or fimbria-fornix lesions (Olton, Walker and Gage, 1978; Olton and Papas, 1979).

When comparing these data it can be seen that there are some qualitative differences in the performance on both spatial reference and working memory tasks. These relate to the type and size of lesion and on whether training is given before the lesion and are described below.

In general, mechanical, electrolytic, aspiration or radio frequency lesions (Olton et al, 1978, Olton and Feustle, 1981; Jarrard, 1978, 1983, 1986; Nadel and MacDonald, 1980; Aggleton et al, 1986; O'Keefe and Conway, 1980; Winocur, 1980; Oades, 1981; Morris et al, 1982; Sutherland et al, 1983; Gage, 1985; Schenk and Morris, 1985) lesions cause greater impairment than neurotoxic lesions, on both working memory (Jarrard, 1983, 1986) and reference memory (Jarrard, 1986; Jarrard et al, 1984).

Lesioning a sub area of the hippocampal formation seems to have less effect than a complete lesion. For example, Jarrard found that kainic acid lesions to CA_3 (1983) or ibotenic acid lesions to the dentate gyrus (1984) had only a transient effect on performance on either task. Schenk and Morris (1985) also found that lesions to the entorhinal cortex made by radio frequency showed only a transient impairment.

When training is given before the lesion, the amount of impairment on both tasks is less severe than if the lesion is made first. Jarrard (1978) found that damage to CA_1 impaired working memory when no pretraining was given, but only a transient effect if it was given. Schenk and Morris found a similar effect of pretraining in place learning with radio frequency lesions to the entorhinal cortex (1985), and Gage (1985) found no impairment in both spatial working and reference memory with electrolytic lesions, when pretraining was given. If training was given before the lesion, animals showed poor performance in spatial working memory tasks (Jarrard, 1978; Winocur, 1980; Oades, 1981; Gage, 1985; Aggleton et al, 1986) and reference memory tasks (Sutherland et al, 1983; Sutherland, 1985; Oades, 1981; Morris et al, 1982; Schenk and Morris, 1985).

Finally, some groups (Jarrard, 1983, 1986; Jarrard et al, 1984; Nadel and MacDonald, 1980; Gage, 1985; Oades, 1981; Sutherland et al, 1983) have attempted to dissociate spatial working memory from spatial reference memory by testing animals on both tasks, following the results of Olton and Papas (1979). However, none of these groups were clearly able to show that animals with hippocampal lesions were not impaired in reference memory but were in working memory. There was a slight indication that the place task showed a transient impairment, leaving working memory intact (Jarrard, 1986; Jarrard et al, 1984).

Based on these results it is not surprising that, as, Rasmussen et al (1989) point out...."each theory has found a measure of experimental support". This group (Rasmussen et al, 1989) did, in fact attempt to overcome these problems by systematically testing animals' performance with one type of lesion (to the entorhinal cortex), in the same apparatus, but using both spatial learning and working memory paradigms. In addition they tested the effect of

massed, distributed and cued trials, and found that in general animals were more impaired in spatial navigation than working memory. Moreover, the massed trials condition was more difficult.

Despite the confusion, several global statements can be made about the effect of lesions on animal's performance. First of all, complete hippocampal lesions, and lesions made electrolytically or by radio frequency, aspiration, or mechanical damage cause more severe impairment than neurotoxic lesions. This is not surprising, as Jarrard (1986) points out neurotoxin lesions spare fibres of passage and adjacent areas that are obviously damage by the other types of lesion. In addition, all lesions can vary in the amount of damage or cell loss they cause.

Secondly, damage to certain areas, such as the subiculum fimbria-fornix and entorhinal cortex, seem to cause more impairment than others, such as CA_3 or the dentate gyrus (see Jarrard, 1986). It is interesting to note that the subiculum, fimbria-fornix and entorhinal cortex constitute major inputs and outputs for the hippocampus. One would expect that suppression of sensory information into the hippocampus via the entorhinal cortex would have a detrimental effect on performance. Similarly the suppression of information going out of the hippocampus via the subiculum and fimbria-fornix to other sensory and motor areas would effect performance. The sparing of performance with selective hippocampal lesion could be explained in the light of more recent anatomical data (Amaral and Witter, 1989) which suggests the trisynaptic loop is neither completely sequential, nor lamellarly organised as was originally suggested by Andersen, Bliss and Skrede (1971a). Damage to a single subfield in the hippocampus need not disrupt the flow of information.

Thirdly, if animals have training on the task before the lesion they show less or no impairment in both tasks. This is in keeping with the suggestion that the hippocampus may be involved in acquisition and processing of information but permanent memories are stored elsewhere (Squire and Cohen, 1984; McNaughton, Barnes, Rao, Baldwin and Rasmussen, 1986). It does, however, present difficulty for the working memory theory because one of the fundamental requirements for a working memory task is to dissociate reference memory from it in the experimental procedure, and show that place learning remains intact, while impairing working memory. The need

to dissociate the two types of memory is important, because animals require some reference information before they can begin to manipulate lists of places they use in a working memory. The most obvious way of showing this is by pretraining animals so they are familiar with the spatial aspect. In this way, any impairment after the lesion can only be attributed to working memory. If no pretraining is given, then one is never free from the argument that the effect could be due to a reference memory impairment, and this has been repeatedly pointed out (Morris, 1983; Olton, 1983; Jarrard, 1986).

In an attempt to avoid the ambiguity of the effect of reference memory on performance, Olton and Feustle (1981) tested animals with fimbria-fornix lesions in a nonspatial working memory task. Animals were required to use intra rather than extramaze cues and their performance was impaired. However, Aggleton et al (1986) found aspiration lesions to the hippocampus did not impair animals performance on a nonspatial matching-to-sample task in a Y-maze. Morris (1983) suggests, the results from Olton and Feustle (1981) may have been due an increased level of difficulty in the task, because control animals took more trials to reach criteria than they do in a spatial working memory task using extramaze cues. Rasmussen et al (1989), who compared the performance of animals with entorhinal lesions in both spatial and nonspatial working memory tasks found animals were more impaired on the spatial version rather than the nonspatial version. In the nonspatial version, entorhinal animals showed no significant difference in performance than control; in the spatial version entorhinal animals were not able to reach criterion.

The data supporting the working memory hypothesis suffer under the ambiguity of a behavioural paradigm that is not clear cut, and shows variability under different conditions. Although, in general, the data lend more support to the spatial mapping theory, the controversy over the function of the hippocampus remains unsolved. In one sense, there is no reason not to suggest that the hippocampus is involved more specifically in a form of associative learning of which spatial navigation and working memory are examples.

In this thesis the effect of AP5 is tested on both spatial reference memory and working memory, and the ambiguity surrounding the lesion data has two different effects on the rationale underlying the experiments. On one level, the emphasis is placed on investigating a cellular mechanism that may underlie the processing of information that occurs during spatial learning. Here the discrepancy between the lesion data does not play such an important role, as does correlating a cellular activity with animals ability to learn a spatial task (be it interpreted as reference or working memory). On the second level where the effects of AP5 is compared with performance on both spatial reference and working memory the discrepancy in the lesion literature is very relevant. It creates a point of reference, from which a qualitative assessment can be made about the effect of NMDA receptor blockade in the hippocampus has on these forms of learning. The underlying rationale of these experiments are describe in more detail in section 4 of the introduction.

1.2 LONG-TERM POTENTIATION (LTP): IMPLICATIONS FOR A NEURAL SUBSTRATE OF LEARNING

Long-term potentiation (LTP) (Bliss and Lomo, 1973) is an activity-induced enhancement at the synapses, that can outlast the input stimulus for any time between 1 hour and several weeks, depending on the stimulating parameters used for its induction (Barnes, 1979; Barnes and McNaughton, 1980; Reyman, Malish, Schulzeck, Brodemann, Ott and Matthies, 1985; Douglas, 1983). It is not fully understood what synaptic changes occur, but some suggest that it may be an increase in the number of receptors on the dendritic shafts (Lee, Schottler, Oliver and Lynch, 1980; Chang and Greenough, 1984; Desmond and Levy, 1986), changes in the number of dendritic spines (Chang and Greenough, 1984) and also morphological changes of spinal configuration (Van Harrenveld and Fifkova, 1975; Chang and Greenough, 1984; Wenzel and Matthies, 1985). Although other forms of plasticity exist; for example some of these are reactive synaptogenesis (Liu and Chambers, 1958), facilitation (Andersen, 1960), augmentation (McNaughton, 1983), post-tetanic potentiation potentiation (McNaughton, 1983) and kindling (Goddard, McIntyre and Leech, 1969), the interest in LTP as a substrate for learning is based on a notion postulated by Hebb (1949). He suggested how changes underlying learning in a cell assembly might occur:

> "When an axon of cell A is near enough to excite a cell B and repeatedly or persistently takes part in firing it, some growth process or metabolic changes takes place in one or both cells such that A's efficiency as one of the cells firing B is increased." (Hebb, 1949)

LTP was first discovered by Lomo (1966) but reported in detail by Bliss and Lomo, (1973) and Bliss and Gardner-Medwin, (1973) in the *in vivo* rabbit. Since then, much research has been generated from it and, although it remains a completely artificial phenomenon, knowledge of the mechanisms for activation and about its characteristics have led to an understanding of how a similar type of activity may occur naturally.

1.2.i. Characteristics of LTP

LTP in the hippocampus is recognised as an enduring enhancement of synaptic activity, and can be shown as an increase in the size of the excitatory post synaptic potential (EPSP) of a population of cells (see Fig. 1.2). It occurs in response to brief, high frequency stimulation of a monosynaptic input (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973; Levy and Steward, 1979; Douglas and Goddard, 1975; Douglas, 1977; McNaughton, Douglas and Goddard, 1978). In the hippocampal slice spiking in the soma, measured intracellularly, is also an indication of the enhancement, and it correlates with an increase in the extracellular EPSP, measured simultaneously (Schwartzkroin and Wester, 1975; Andersen, Sundberg, Sveen and Wigstrom, 1977; Andersen, Sundberg, Sveen, Swann and Wigstrom, 1980).

Two principle components of the field potential can be measured to show LTP. These are a slow, positive going EPSP, which shows synaptic activity (Lomo, 1971) and a more rapidly occurring, negative population spike (PS) that is superimposed on the EPSP and reflects the synchronous firing of the cells (Andersen, Bliss and Skrede, 1971b). The extracellular current flow, measured by the EPSP, is a more important measure of LTP as it measures dendritic activity. The PS is not such a reliable indicator as it is a reflection of the increased synchrony of cells firing. The height of the PS can be used as an indicator of the number of cells being discharged and the size of the afferent volley (Andersen et al, 1971b). However, this effect could be a combined expression of both excitatory and inhibitory circuits, as shown in the dentate gyrus see Bliss and Lynch, 1988). Preventing the PS from occurring, by blocking sodium spikes with the lidocaine derivative, QX222 (Kelso, Ganong and Brown, 1986) does not block the induction of LTP.

After the original demonstration of LTP in the rabbit (Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973), subsequent studies showed that LTP could be evoked in all subfields of the hippocampus (Schwartzkroin and Wester, 1975; Alger and Teyler, 1976; Buzsaki, 1980; Bliss, Lancaster and Wheal, 1983; Doller and Weight, 1985; Racine, Milgram and Hafner, 1983; McNaughton and Miller, 1986); in cortical areas (Komatsu, Toyama, Maeda and Sakaguchi, 1981; Lee, 1982; Sakimoto, Porter and Asanuma, 1986; Bindman, Meyer and Pockett, 1987; Artola and Singer, 1987), pyriform cortex (Stripling

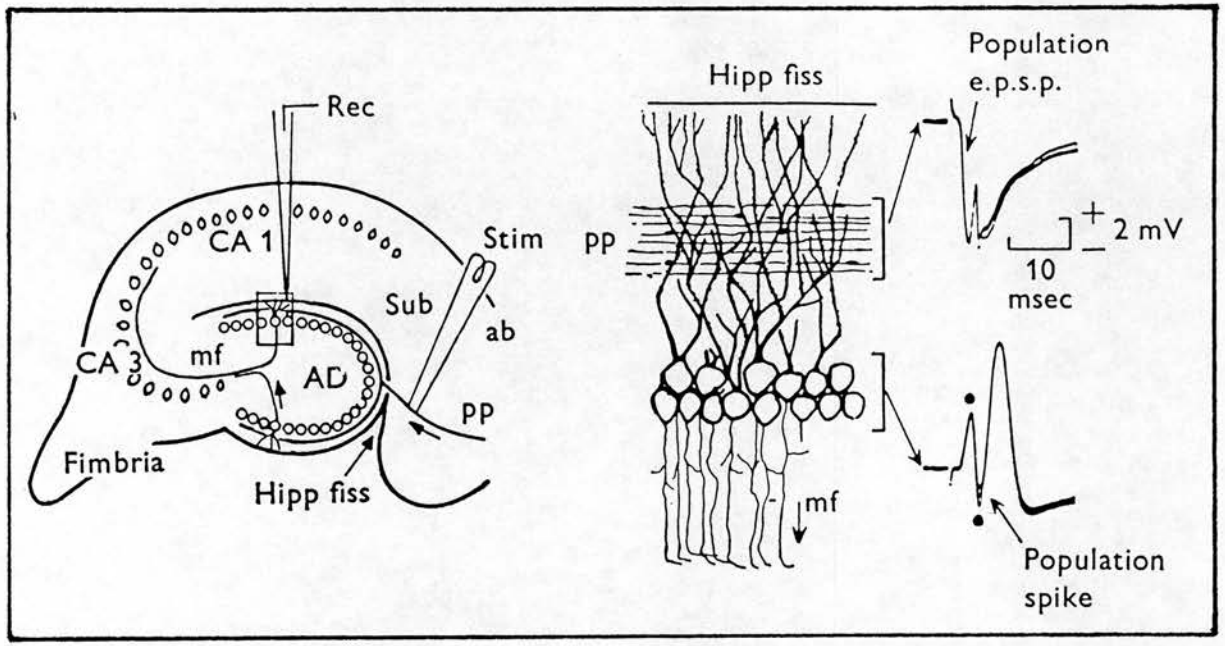


Fig. 1.2:
Schematic representation of the the induction of LTP, showing (A) stimulation of the perforant pathway and recording in the hilus of the dentate gyrus, and (B) the field potentials measured in the dendrites (top part) and the cell bodies (bottom part).

and Patneau, 1985), medial geniculate nucleus (Gerren and Weinberger, 1983) and the amygdala (Racine et al, 1983). It has also been shown in the non mammalian brain (see Bliss and Lynch, 1988; Teyler and Discenna, 1987 for reviews).

High frequency stimulation is the most commonly used method for evoking LTP, particularly in the whole animal preparation. Bliss and colleagues (Bliss and Lomo, 1973; Bliss and Gardner-Medwin,1973) evoked LTP in the rabbit, using trains of pulses (between 10 and 20 per second for 10 to 15 seconds) to stimulate the perforant pathway. The build up of synaptic potentiation in the granule cells of the dentate gyrus was measured. Since then, Douglas and colleagues (Douglas and Goddard, 1975; Douglas, 1977, Douglas, 1983) have systematically studied the parameters for stimulating LTP, and found that an optimal number of pulses at a particular frequency could evoke a purely post synaptic event, without the interference from fibre potential or abnormal activity, such as epileptiform afterdischarge (Lomo, 1966; Andersen, Holmqvist and Voorhoeve, 1966). They found the optimal parameters were very short lasting, high frequency trains of pulses. However, there are other ways to manipulate the synapse and evoke LTP, particularly in the slice preparation (see table 1.2). These reflect both the different characteristics of LTP, and the number of ways in which the single mechanism thought to underlying the induction of LTP, may be invoked in a more natural event.

1.2.ii. Mechanisms For the Induction of LTP

The activity thought to trigger the induction of LTP is the influx of calcium into the postsynaptic membrane through an NMDA receptor activated channel. Experimentally, it has been shown that an enduring increase in CA_1 potentials occurs in the hippocampal slice when high concentrations of Ca^{++} were added to the bathing medium (Turner, Baimbridge and Miller, 1982; Reyman, Matthies, Frey and Vorobyev, 1986), although this has been debated by others (Auyeung, Goh, Li, May, Paletta and Sastry, 1986). Furthermore, LTP can be blocked by intracellular injections of the Ca^{++} chelator, EGTA into the post synaptic cell (Lynch, Larson, Kelso, Barrionuevo and Schotter, 1983). The binding of Ca^{++} to the post synaptic membrane has been shown to increase during the induction of LTP (Kuhnt, Mihaly and Joo, 1985), and there is a correlation between

Table 1.2:
VARIOUS WAYS OF EVOKING AND BLOCKING THE INDUCTION OF LTP

INDUCTION OF LTP		
1.	Pairing of a strong and a weak input.	Levy and Steward, 1979; Lee, 1983; Barrineuvo and Brown, 1983; Sastry et al, 1986.
2.	Patterned stimulation.	Larson and Lynch, 1986a; Larson and Lynch, 1986b; Rose and Dunwiddle, 1986; Staubli and Lynch, 1987; Larson and Lynch, 1988
3.	Pairing a presynaptic volley with depolarisation of the post-synaptic membrane.	Auyeung et al, 1986; Kelso et al, 1986; Nicholl et al, 1986; Sastry et al, 1986
4.	Pairing a single pulse with post-synaptic depolarisation.	Wigstrom and Gustafsson, 1986
5.	Increasing calcium (a) with a presynaptic volley (b) alone	 Dunwiddie and Lynch, 1979 Turner et al, 1982 (however, see Auyeung et al, 1986)
6.	Reduced pre-synaptic stimulation paired with GABA antagonists	Wigstrom and Gustafsson, 1983
7.	Pre-synaptic stimulation with reduced concentration of Mg^{++}	Huang et al, 1987
BLOCKADE OF LTP		
1.	Presynaptic volley with a post synaptic hyperpolarising current	Malinow and Miller, 1986
2.	Presynaptic volley and NMDA receptor antagonists: Competitive	Collingridge et al, 1983; Harris et al, 1984; Morris et al, 1986; Errington et al, 1988; Abraham and Mason, 1988
	Non-competitive	Abraham and Mason, 1988
3.	Presynaptic volley with a voltage clamped post-synaptic membrane	Kelso et al, 1986
4.	Increased Mg^{++} concentration	Dunwiddie and Lynch, 1979

the intensity of the tetanus required to evoke LTP and a decrease in the amount of extracellular Ca^{++} (Melchers, Pennartz, Wadman and Lopez da Silva, 1988). Thus, an influx of Ca^{++} through the postsynaptic membrane is one crucial underlying event for triggering LTP.

Although Ca^{++} is able to enter the postsynaptic membrane through its own voltage sensitive channels (L-type, N-type or T-type: Miller, 1987; Hoffman, Nastainczyk, Rohrkasten, Schneider and Sieber, 1987), LTP is induced by the passage of Ca^{++} through the NMDA-receptor associated channel. In mouse spinal cord cultures, Mayer, Westbrook and Guthrie (1984) showed that the selective Ca^{++} channel blocker, cadmium chloride, had much less effect on blocking the influx of Ca^{++} than Mg^{++} (which normally blocks the NMDA receptor, until the postsynaptic membrane is depolarised). MacDermott, Mayer, Westbrook, Smith and Barker (1986) later showed unambiguously that the NMDA receptor was permeable to Ca^{++}. They clamped the post synaptic membrane at -60 mV and showed an intracellular increase in Ca^{++}, using the Ca^{++} indicator dye, arsenazo III. The significance of clamping the membrane at -60 is that Ca^{++} cannot pass through its own voltage-gated channel.

The influx of Ca^{++} through the NMDA receptor can only occur, when the post synaptic membrane has become depolarised. Under normal resting conditions, activation of the NMDA receptor remains ineffective because the channel is blocked by Mg^{++}. Only when the membrane becomes depolarised is the Mg^{++} released from the channel (see Chapter 1.3.ii for details of the Mg^{++} block). The receptor, which is activated by the neurotransmitter can open the channel and allow an influx of Ca^{++} to flow through. From here, Ca^{++} triggers a cascade of events involving second messengers, to mediate the maintenance and expression of LTP. Further explanation of the NMDA receptor-channel complex involvement in the induction of LTP is explained in chapter 1.3.

The requirement for depolarisation of the post synaptic membrane and the influx of Ca^{++} is the underlying reason why LTP can be evoked in so many ways (see table 1.2) and also helps to clarify the nature of the characteristics of LTP, and the parameters for evoking it, as described below.

1.2.iii. Input Specificity

Only those synapses that would ordinarily be activated by the pathway being stimulated are eligible to support LTP (Lynch, Dunwiddie and Gribbkoff, 1977; Dunwiddie and Lynch, 1978; Andersen et al, 1980; Barrineuvo and Brown, 1983). This has been demonstrated *in vitro* by stimulating more than one input to different synapses on the same cell, and recording from either a single, or two of the synaptic regions. For example, in the hippocampal slice, when the fibres of stratum oriens and radiatum of the Schaeffer collaterals are stimulated separately, and independent recordings of their en passant synaptic connections on the apical and basal dendrites are made, then tetanising one input has no potentiating effect on the other. The potentiation is also confirmed by the recording of a population spike at the pyramidal layer (Andersen at al, 1977; Lynch at al, 1977; Barrioneuvo and Brown, 1983). Some laboratories, however, have shown that hetrosynaptic potentiation can be evoked from different inputs to CA_1 (Yamamoto and Chujo, 1978; Misgeld, Sarvey and Klee, 1979; Yamamoto, Matsumoto and Takagi, 1980; Higashima and Yamamoto, 1985; Bradler and Barrioneuvo, 1989). Input to CA_3, however, comes from both the commissural fibres (Gotlieb and Cowan, 1972; Segal and Landis, 1974) and the mossy fibres (Blackstadt, Brink, Hem and Jeune, 1970; Swanson, Wyss and Cowan, 1978). The mossy fibres appear to be a special case, because there is a low concentration of NMDA receptors here (Monaghan and Cotman, 1985), and tetanic stimulation of them is resistant to NMDA blockade with the antagonist, AP5 (Harris and Cotman, 1986; Kauer and Nicholl, 1988). It has been suggested that CA_3 pyramidal cells express two different types of synaptic plasticity; an associative, NMDA-receptor type mediated via the commissural fibres and a non associative, NMDA receptor resistant form mediated via the mossy fibres (Kauer and Nicholl, 1988).

1.2.iv. Co-operativity

Increasing the stimulus intensity results in a greater degree of LTP (Schwartzkroin and Wester, 1975; McNaughton et al, 1978; Lee, 1983). In fact, there is an intensity threshold, where LTP can only be evoked if stimulation is given above a certain level. As the intensity of the stimulus increases, more fibres within a single

pathway are activated and potentiation occurs. McNaughton et al (1978) showed that separate stimulation of the lateral and medial perforant path produced a small amount of potentiation, but simultaneous stimulation of both pathways increased the LTP to more than double the amount. In the hippocampal slice preparation, co-operation has been shown by combined orthodromic and antidromic stimulation of Schaeffer collaterals (Schwartzkroin and Wester, 1975; Lee, 1983) and CA_1 cells (Yamamoto and Sawada, 1981). The explanation for the threshold effect, is that recruitment of a minimal number of fibres are required to evoke enough activity at the synapse, to depolarise the post synaptic membrane, and allow the activation of the NMDA receptor, from the ensuing transmitter release.

1.2.v. Associativity

Bliss and Lynch (1988) suggest that associativity is closely related to co-operativity. They describe the fundamental difference between the two in the following way: co-operativity describes stimulation of a single pathway, recorded with a single electrode; associativity describes two independently stimulated pathways that converge on to a single synaptic region (see section ii, this chapter). *In vitro* experiments often use recording electrodes at each stimulus input and sometimes one at the cell body level or intracellularly (Andersen et al, 1977; Schwartzkroin and Wester, 1975; Andersen et al, 1980; Barrioneuvo and Brown, 1983).

The classic demonstration of associativity is the pairing of a weak input with a strong input (Levy and Steward, 1979). Normally, the weak input alone is not enough to potentiate LTP, but when it coincides with a strong input, it does. Levy and Steward (1979) used the crossed entorhinal input to the dentate gyrus to show this. Tetanisation of the strong ipsilateral perforant path input to the dentate gyrus evoked LTP. Tetanisation of the contralateral dentate gyrus, which receives only sparse innervation from the crossed fibres, however, was too weak to evoke LTP. When the weak input was paired with the strong input, the weak input then became potentiated. This associative effect has also been shown in the hippocampal slice (Barrioneuvo and Brown, 1983, Sastry, Goh and Auyeung, 1986; Kelso et al, 1986).

Although the weak input can be potentiated when paired with a strong input, there are constraints on the timing of the stimulation, and in what order the stimuli are delivered. The timing of paired stimuli is crucial; a narrow window, with a maximum upper limit of 100 msecs exists, in which time LTP can be evoked. If the second input comes any time after that, LTP will not be supported by the weak input (Levy and Steward, 1983; Kelso et al, 1986; Robinson, 1986). Even within the time limit, LTP can only be evoked if the weak input precedes the strong input, or if they are delivered simultaneously. LTP will not be evoked if the order of delivery is reversed (Levy and Steward, 1983; Gustafsson and Wigstrom, 1986, Sastry et al, 1986). The importance of the temporal constraints of the two converging inputs and the order that they are delivered, again relate to the requirement for the post synaptic membrane to be depolarised.

1.2.vi. Pre and Post synaptic activation

Simultaneous activity pre and post synaptically, is a requirement for the induction of LTP, and if these conditions are not met, LTP can not be evoked. Unlike the original criteria suggested by Hebb (1949), that the post synaptic cell has to be firing, the post synaptic side needs only to be depolarised. If the postsynaptic membrane has been depolarised, then LTP can be evoked with low frequency stimulation that would other wise be ineffective (Gustafsson and Wigstrom, 1986). The postsynaptic membrane can be manipulated to induce and block LTP in a number of ways. Low frequency stimulation can be paired with (i) an injection of a depolarising current to the post synapse (Sastry et al, 1986; Kelso et al, 1986; Wigstrom, Gustafsson, Huang and Abraham, 1986); (ii) blockade of the inhibitory transmitter receptor, GABA, with picrotoxin and other GABAergic antagonists (Wigstrom and Gustafsson, 1983; 1985a; 1985b); (iii) raising the extracellular concentration of Ca^{++} (Turner et al, 1982) or (iv) reducing the levels of extracellular Mg^{++} (Huang, Wigstrom and Gustafsson, 1987). Similarly, LTP can be blocked by preventing the post synaptic membrane from being depolarised, either by (v) injecting a hyperpolarising current into the post synapse (Manilow and Miller, 1986); (vi) voltage clamping the postsynaptic membrane so it cannot become depolarised (Kelso et al, 1986); (vii) increasing the

concentration of Mg^{++} or reducing the concentration of Ca^{++} (Dunwiddie and Lynch, 1979); (viii) by abolishing intracellular Ca^{++} (Lynch et al, 1983), or (ix) by blocking the NMDA receptor with competitive (Collingridge et al, 1983; Harris et al, 1984; Morris et al, 1986; Errington, Lynch and Bliss, 1987; Abraham and Mason, 1988) and noncompetitive (Abraham and Mason, 1988) antagonists. It must be mentioned here, that noncompetitive antagonists block the channel associated with the NMDA receptor and in a use-dependent manner. The use-dependent effect and its functional consequence is described in more detail in the following section, on the NMDA receptor.

Depolarisation of the post synaptic membrane alone, however, is not sufficient to evoke LTP; there has to be presynaptic activation as well. Hvalby, Lacaille, Andersen and Hu (1987) depolarised the post synaptic membrane without activating the presynapse, by iontophoresing glutamate on the dendritic region of CA_1 cells. They found that LTP could only be evoked if a low frequency stimulation was applied in conjunction with the depolarisation; iontophoretic application of glutamate alone did not evoke LTP.

Combined pre and post synaptic activation creates the appropriate conditions for evoking LTP, without having to tetanise a pathway. High frequency stimulation merely allows the post synaptic membrane to be depolarised rapidly by activating non-NMDA receptors. The depolarisation removes Mg^{++} from the channel associated with the NMDA receptor, and then transmitter interaction at the receptor allows an influx of Ca^{++} through the channel. If the post synaptic membrane is already depolarised, then only low frequency stimulation is required to allow the inflow of Ca^{++}.

Patterned stimulation has also been used to create the right synaptic conditions, so that a lower frequency stimulation can be used to evoke LTP. Lynch and colleagues (Larson and Lynch, 1986; Rose and Dunwiddie, 1986; Staubli and Lynch, 1987; Larson and Lynch, 1988) showed that LTP could be evoked by mimicking the bursting of certain types of neurons in the hippocampus at the theta frequency (5-10 Hz). This has important implications because theta rhythm has been associated with spatial learning (Winson, 1978).

1.2.vii. Implications for the involvement of LTP in Learning

Scientists have searched for a type of physiological activity that may underlie learning and memory for many years (Cajal, 1911, Hebb, 1949, Lashley, 1950). LTP is an attractive candidate for a mnemonic device for many reasons: On a purely physiological level, the characteristic of LTP, suggest that synaptic enhancement may be achieved by the activity of normal functioning cells, rather than the abnormally high frequency stimulation used in an experimental procedure.

From a theoretical point, some of the characteristics of LTP that make it an attractive candidate for a mnemonic device are that it requires both pre and post synaptic activity which conforms to the Hebbian rules for a cell assembly to become capable of representing learning at a cellular level. It can be evoked in the hippocampus, a brain structure associated with learning and memory. Co-activation of (at least) two pathways to evoke LTP is reminiscent of Pavlovian conditioning, whereby a strong or conditioned input can signal a weak or unconditioned input. These parameters are also suitable for explaining mechanisms of learning using neural network theories.

The question of LTP's involvement in learning has been addressed experimentally by a relatively small number of research groups, who have essentially used two means of investigating the effect: (i) Manipulating LTP and testing animals in various learning tasks, and (ii) Measuring changes in field potentials, in a learning or exploratory situation and comparing these changes with those seen with LTP.

Two groups have evoked LTP in the same pathway and measured its effect on animals' ability to learn a task susceptible to hippocampal function. The results from each group, however, have been conflicting. Berger (1984) has shown that the classical conditioning response in the rabbit nictitating membrane is improved when the dentate gyrus cells were potentiated by stimulation of the perforant path. McNaughton, Barnes, Rao, Baldwin and Rasmussen (1986) on the other hand, found that animals performance in a spatial learning task was impaired when the same pathway was potentiated. They argued that the impairment was a consequence of LTP saturating the level of excitability in cells, causing a ceiling effect in their potential plasticity. Furthermore, this group has

shown that after saturation, improvement on a spatial reference memory task correlates with the rate of decay of LTP to baseline (Castro, Silbert, McNaughton and Barnes, 1990).

Manipulating LTP in another way, Morris et al (1986) found that chronic infusion of AP5, an NMDA receptor antagonist, impaired performance on a spatial learning task at a concentration sufficient to block the induction of LTP. Laroche, Doyere and Bloch (1989) manipulated LTP to establish a correlation between the magnitude of potentiation in the dentate gyrus (by stimulation of the perforant path) with acquisition of a classical conditioning task (pairing perforant path stimulation (CS) with footshock (US) in a lever pressing food reward paradigm). If LTP was saturated or blocked with AP5, there was a learning deficit; if perforant path stimulation was given between these two extremes, there was a linear relationship between learning, and the size of the field potentials evoked.

The other means of investigating the link between LTP and learning, has looked at the increase in potentials in animals exposed to novel or enriched environments, or when they are engaged in learning tasks. Long term exposure of rats to an enriched environment, has shown an increase in the population spike (Sharp, McNaughton and Barnes, 1985; Sharp, Barnes and McNaughton, 1987; Green and Greenough, 1986) and an increase in the EPSP (Sharp et al, 1985; Green and Greenough, 1986) in the dentate gyrus. The increase in population spike decays more rapidly in aged rats (Sharp et al, 1987), and this seems to parallel similar rates of decay of LTP and forgetting in the aged rats (Barnes and McNaughton, 1985). Animals exposed to a novel environment for brief periods of time (15 minutes) show an increase in the EPSP that is related to the amount of exploration, and the animals familiarity with the environment (Sharp, McNaughton and Barnes, 1989). This change in potentials does not seem to be due to movement related behaviour, *per se* (Green, McNaughton, Keith and Barnes, 1989).

In learning paradigms using footshock motivated brightness discrimination (Rutherich, Matthies and Ott, 1982) or appetitively motivated operant discrimination tasks (Skelton, Scarth, Willkie, Miller and Phillips, 1987), an increase in the population spike was measured. Skelton et al (1987) found that the increase in population spike lasted for 10 days after the end of training. In

Rutherich et al's (1982) experiment, the increase did not occur before 4 hours after training and from this they inferred that the physiological change underlay the consolidation of learning. The increase was also confined mainly to those animals that were good learners and a similar result has been reported by Ramirez, Orsingher and Carrer (1988) who found the threshold for evoking LTP was lower in good learners compared with bad learners. This group (Ramirez and Carrer, 1989), also showed a similar negative correlation between the threshold for evoking LTP and the number of correct responses in a shuttle box avoidance task.

The evidence which suggests LTP may be a phenomenon underlying learning is circumstantial. This is mainly because it is correlational and does not show a causal link between the two events. The evidence relies heavily on coupling the phenomenon LTP to a theory that learning is subserved by a form of sustained cellular activity postulated some 40 years ago by Hebb (1949). However, manipulation of LTP has extended our knowledge of its characteristics and the mechanisms of its induction, and has led to an understanding of what changes may occur during learning, and how they may be measured. It has been shown in those experiments measuring "naturally" occurring changes in the potentials that they do share some of the same changes seen in LTP. Focusing this information on a type of neural mechanism underlying learning, places LTP into a more realistic framework and suggests the possibility that LTP may be a "cruder" measure of the type of cellular activity thought to occur during learning.

1.3 THE N METHYL-D-ASPARTATE RECEPTOR (NMDA): CHARACTERISTICS AND FUNCTIONAL SIGNIFICANCE

The NMDA receptor is a subtype of excitatory amino acid (EAA) receptor at which the putative neurotransmitters, Glutamate (Glu) and Aspartate (Asp) act. It is associated with a channel that carries a Ca^{++} current and is blocked by Mg^{++} in a voltage dependent manner. Only when the postsynaptic membrane becomes depolarised to -30mV can the receptor be activated. Although widely distributed throughout the brain, the highest concentrations of these receptors is found in the hippocampus. Its activation is required for the induction of LTP, and based on the theoretical grounds that LTP is thought to be a putative model for a neural substrate of learning, it has been implicated in learning. More recently, its involvement in learning has been tested directly by administering NMDA antagonists to animals which are tested in learning paradigms.

There are five known receptors at which Glu and Asp act: Kainate (K), Quisqualate (Q) and the N-methyl D aspartate (NMDA) receptors are located postsynaptically (see Watkins and Evans, 1981; Foster and Fagg, 1984). A fourth receptor is the L-2-Amino-phosphonobutyrate (L-APB) sensitive site, thought to be located presynaptically (Monaghan, Bridges and Cotman, 1989; Young and Fagg, 1990; Watkins, Krogsgaard-Larsen and Honare, 1990) and a fifth candidate has been suggested, the Metabotropic site (Sugiyama, Ito and Hirono, 1987) thought to be coupled with Inositol phosphate (IP) turnover.

The three most well established receptors, NMDA, K, and Q were originally subtyped according to their exogenous prototypic agonists (see Watkins and Evans, 1981; Foster and Fagg, 1984; McLennan, 1983 for reviews). The existence of these receptors were supported by electrophysiological studies (see Watkins and Evans, 1981) and the classification system was supported by ligand binding studies (see Foster and Fagg, 1984; Monaghan et al, 1989).

Until recently, the only selective antagonists available were NMDA receptor antagonists. Biscoe and colleagues (Biscoe, Evans, Francis, Martin and Watkins, 1977) and Evans and colleagues (Evans, Francis and Watkins, 1978) found that DL-(α)-aminoadipate could block the NMDA response with no effect on the K/Q responses.

Historically, further generations of the competitive antagonists were developed, by modifying the basic structure and these have proved more selective and more potent than their predecessors (Watkins and Evans, 1981; Watkins, 1989). The second generation antagonists, such as AP5 (Davies, Francis, Jones and Watkins, 1981; Evans, Francis, Jones, Smith and Watkins, 1982) and AP7 (Evans et al, 1982; Perkins, Collins and Stone, 1982) had longer chains and were found to be more selective and more potent than DαAA. Evans et al (1982) also found the D-isomer to be more potent than the L-isomer. Chemical modification of these agents led to the development of third generation antagonists such as CPP (Davies, Evans, Herrling, Jones, Olverman, Pook and Watkins, 1986; Harris, Ganong, Monaghan, Watkins and Cotman, 1986), and CGP 37849 (Fagg, Pozzo, Olpe, Baumann, Bittiger, Schmutz, Angst, Brundish, Allgeir, Heckendorn and Dingwall, 1989) which, in turn, were found to be more potent.

The classification of receptors changed to NMDA and non-NMDA receptors, grouping the K/Q receptors together. This resulted partly from the lack of selective antagonists for the K/Q receptors but, more importantly, it was discovered that the NMDA receptor and its associated channel had unique physiological properties for activation (MacDermott and Dale, 1987; Ascher and Nowak, 1987) suggesting that it had a specific functional significance in synaptic transmission (see Cotman, Monaghan and Ganong, 1988).

More recently a group of quinoxalinediones, CNQX and DNQX have been synthesised which are more potent and more selective to the K/Q sites (Honare, Davies, Drejer, Fletcher, Jacobsen, Lodge and Neilsen, 1988). Electrophysiological experiments show that CNQX and DNQX do have an effect at the NMDA receptor, but it is weak and thought to be mediated via the glycine site (Watkins et al, 1990). More importantly, however, CNQX has been implicated in the expression of LTP (see section 3.v., this chapter).

1.3.i. NMDA Receptor Recognition Sites

The receptor complex has several recognition sites, at which different compounds can exert their effects by varying mechanisms. (see Fig. 1.3). The main site is that for the agonist, which is located on the outside of the membrane. Both the neurotransmitter, Glu and the competitive antagonists interact at this site.

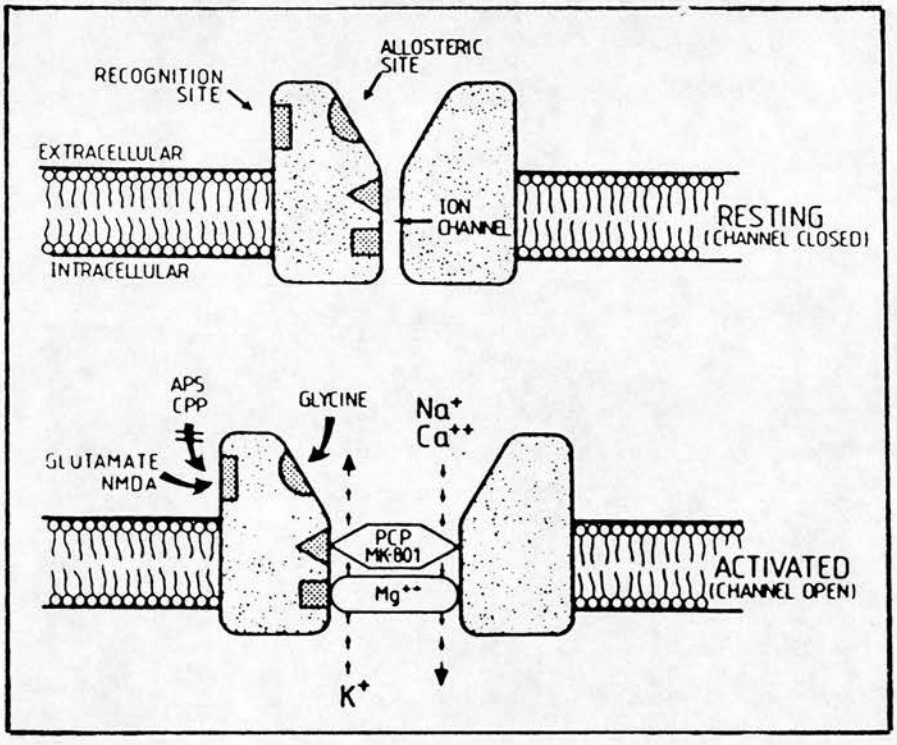


Fig. 1.3.
Schematic diagram of the NMDA receptor-channel complex, with the different recognition sites. Top diagram shows the channel closed and the bottom diagram shows the channel open. The recognition site for the agonist, glutamate and competitive antagonists, AP5 and CPP is external to the channel. The recognition site for the non-competative antagonists, PCP and MK-801 are located inside the channel, near the Mg^{++} site. Glycine interacts at a site on the outside of the channel. (Taken from Foster and Fagg, 1987)

Non-competitive NMDA receptor antagonists, such as MK-801, PCP, TCP, Ketamine and the sigma opiate, SKF 10,047 (Lodge, Aram, Church, Davies, Martin, Millar and Zeman, 1988) act at a site located inside the channel (Kemp, Foster and Wong, 1987; Lodge et al, 1988). They do not interact at all at the agonist site (Monaghan and Cotman, 1986) as these compounds do not inhibit the binding of [^{3}H] D-AP5. Non-competitive antagonists exert their effect by blocking the channel and preventing the influx of Ca^{++}. This means their antagonist effect is dependent on the receptor being agonist-bound and the channel open, so that they can reach their recognition site. Both Church, Davies, Lagnado, Lodge, Martin and Marshall (1987) and Kemp, Priestley and Woodruff (1986) have shown that MK-801 binding is enhanced in the presence of an agonist. The use-dependency of these antagonists has important implications for relating their effect on learning to the induction of LTP (see section 3.vi., this chapter).

Also inside the channel is the Mg^{++} recognition site. Mg^{++} remains inside the channel, blocking it until the post synaptic membrane becomes depolarized (Nowak, Bregestovski and Ascher, 1984; Mayer et al, 1984), after which it is removed. This action is discussed more fully in the next section.

A glycine subunit exists near the agonist site (Johnson and Ascher, 1987) and it has been speculated that it exerts a modulatory effect over the activity at the agonist site. In fact, Kleckner and Dingledine (1988) have shown in xenopus oocytes that the NMDA channel can not be opened by NMDA in the complete absence of glycine. In the hippocampal slice it has been shown that glycine can potentiate NMDA receptor mediated responses (Minota et al, 1989) and the amplitude of the PS was depressed when an attempt to evoke LTP was made in the presence of the glycine antagonist, 7-chlorokynurenic acid (7-Cl-KYN: Bashir, Tam and Collingridge, 1990). It has also been suggested that the glycine site may modify the relative affinity for either agonists or antagonists at the glutamate recognition site (Monaghan and Cotman, 1989; Monaghan, Olverman, Nguyen and Cotman, 1988; Fadda, Danysz, Wroblewski and Costa, 1988).

1.3.ii. Voltage Dependant Magnesium Block

Under normal membrane resting conditions, the channel associated with the NMDA receptor is blocked in a dose-dependent manner by Mg^{++} ions, present in the ecf (Nowak et al, 1984; Mayer et al, 1984; MacDermott et al, 1986). The Mg^{++} has no discernable effect on the activation of the non-NMDA receptors, K/Q shown in isolated spinal cord preparations (Ault Evans, Francis, Oakes and Watkins, 1980). The first indication that the channel was voltage gated came from reports by Engberg, Flatman and Lambert (1979) and MacDonald, Porietis and Wojtowicz (1982), who showed a region of negative slope conductance at low voltage inputs, in a current-voltage relationship. The negative slope was strongest between -80 and -40 mV, but when the voltage input was increased above that level to about -30 mV, the current-voltage relationship resumed a normal linear function. The non-linear relationship between the voltage input and the recorded current output is an indication that the receptor channel is prevented from being open by some form of voltage-dependent mechanism.

Later studies (Nowak et al, 1984; Mayer et al, 1984; Ascher, Bregestovski and Nowak, 1988) showed that the voltage-gating was due to Mg^{++} blocking the channel. At -30 mV the membrane becomes depolarised enough to release Mg^{++} and allow the flow of Ca^{++} ions through the channel. In the absence of extracellular Mg^{++} there is no voltage dependency and the current flow through the channel changes linearly with the change in membrane potential (Nowak et al, 1984; Ascher et al, 1988). In the presence of Mg^{++} in concentrations between 10μM and 5mM the current flow through the channel is reduced as the membrane becomes more hyperpolarised (Nowak et al, 1984; Mayer et al, 1984).

1.3.iii. Calcium Permeability

Once the channel has been freed of Mg^{++}, it becomes permeable to Na^{+} and K^{+} in the same way as the channels associated with the non-NMDA receptors. Unlike the channels associated with the non-NMDA receptors, the NMDA channel is more permeable to Ca^{++} and less permeable to N^{+} and K^{+} at about a 9:1 ratio. This is shown by a shift in the reversal potential at the NMDA channel as a result of altering the extracellular concentration of Ca^{++}, which does not occur at the non-NMDA channels (Ascher and Nowak, 1986; MacDermott

et al, 1986). It is also shown by the fact that an increase in the concentration of Ca^{++} is seen inside the cell when the channel has been opened, measured using the Ca^{++} sensitive dye, arsenazo III (MacDermott et al, 1986) and a Ca^{++} sensitive electrode (Melchers et al, 1988). The permeability of the NMDA receptor associated channel to Ca^{++} has already been discussed in section 1.2.i: Mechanisms for the induction of LTP.

1.3.iv. Distribution

NMDA receptors are distributed throughout the entire cns with varying degrees of density. The original autoradiographic studies (Monaghan, Holets, Toy and Cotman, 1983; Monaghan and Cotman, 1985) used AP5 displacement of L-[^{3}H] Glutamate to map the distribution of NMDA receptors because it had the highest affinity for the NMDA receptor (Olverman, Jones and Watkins, 1984). The regions of the brain showing the highest density were the hippocampus, the cortex and striatum. In the hippocampus, CA_1 was particularly high, then to a lesser extent, the dentate gyrus and all strata in CA_3, the exception being stratum lucidium, which was quite low. In the basal ganglia the nucleus accumbens showed the highest density, followed by the striatum. In the cortex, the frontal, insular, pyriform, perirhinal areas and anterior cingulate showed the highest binding. Other brain areas with afferent or efferent connections to the hippocampus showing a high concentration of NMDA receptor binding, include the dorsal lateral septum, amygdala, thalamus and, to a lesser extent, the hypothalamus. As mentioned earlier (Chapter 1.2.ii) one of the areas that shows the lowest density of binding is the mossy fibre connection between the dentate gyrus and CA_3.

More recent mapping was carried out comparing the effect the binding of the competitive antagonist [^{3}H]-CPP with CPP or AP5 displacement of [^{3}H]-Glu which showed a slightly different distribution between the agonist and antagonists sites (Monaghan, Olverman et al, 1988). Dense binding was found in cortical areas, with some overlap and some difference between agonist and antagonist preferring areas. In the hippocampus there were similar sites in CA_1, but CA_3 showed a higher antagonist binding. There was a similar dissociation of distribution between agonist and antagonist

binding in the striatum and the thalamus. (Monaghan and Cotman, 1989). On the whole, however, the hippocampus did not show any selective distribution.

1.3.v. NMDA Receptors and LTP

The first indication that the NMDA receptor was involved in LTP was shown by Collingridge et al (1983) when they iontophoretically applied the selective NMDA receptor antagonist, APV onto CA_1 cells in the hippocampal slice. They showed that APV had no effect on the normal EPSP's but did block the induction of Schaeffer collateral evoked LTP. In contrast, the non-selective EAA receptor antagonist, γ-D-glutamylglycine (DGG), iontophoretically applied to the same area blocked normal transmission.

The AP5 blockade of LTP in the hippocampal slice was found to be reversible, such that when AP5 was washed out, LTP could be subsequently (Collingridge, Coan, Herron and Lester, 1987). Also in the slice (Harris et al, (1984) showed the blockade of LTP to be dose-dependent. At concentrations of 50μM, APV caused a near complete block of LTP but as the concentration decreased so the amount of LTP increased. At the lowest concentration (0.5μM) only 20% LTP was blocked. Furthermore, Harris et al (1984) showed that the (i) APV homologues (AP7, AP8, AP6, AP4) blocked LTP based on their potency with NMDA receptor and (ii) only the D isomer of AP5 blocked LTP, the L-isomer being ineffective.

Subsequent studies have shown that AP5 blocks the induction of LTP in the perforant path to dentate gyrus *in vivo* (Morris et al, 1986; Morris, 1889; Errington et al, 1987). Morris et al (1986) also showed the L-isomer to be ineffective *in vivo*. APV has also been shown to block the commissural innervated LTP in CA_3 but not the mossy fibre input (Harris and Cotman, 1986; Kauer and Nicholl, 1988). LTP evoked by the NMDA receptor can also be reduced by antagonists that interact with other sites on the complex, such as the glycine antagonist, 7-Cl-KYN (see section 3.i., this chapter) and Mg^{++} (see Chapter 1.2.v)

LTP has also been shown to be blocked by the non-competitive antagonists PCP, (Stringer, Greenfield, Hackett and Guyenet, 1983) and MK-801 (Abraham and Mason, 1988) but not by Halliwell and Morris (1987). The discrepancy between the results from Abraham and Mason (1988) and those of Halliwell and Morris (1987) relates to the use-

dependence of non-competitive antagonists. In an experimental condition, non-competitive antagonists are subject to a time delay; Abraham and Mason (1988) found that LTP could be blocked if the tetanus was given 150 minutes after injection of MK-801, but not 30 minutes after the injection. The time-delay is supported by Halliwell and Morris, who found LTP could still be induced 30 minutes after a 1mg/Kg injection of MK-801.

In the experiment carried out by Stringer et al (1983) the measure for the amount of LTP evoked was the percentage increase in the amplitude of the population spike, whereas Abraham and Mason (1988) used the more reliable measure of the initial rise time of the slope of the EPSP (see Chapter 1.2). In addition Abraham and Mason (1988) also measured the amplitude of the population spike and found that during the time course following injection of MK-801 the EPSP did not change after 30 minutes but there were large dose-dependent decreases in the amplitude of the population spike.

The NMDA receptor is only involved in the initiation of LTP and not the maintenance (Errington et al, 1987; Davies, Lester, Reyman and Collingridge, 1989; Collingridge and Davies, 1989). Errington et al (1987) showed that if animals were perfused with D-AP5 after LTP had been established there was no suppression of the enhanced field potentials in CA_1 or the dentate gyrus. The maintenance of LTP is thought to be mediated at least in part via the Q receptor (Collingridge and Davies, 1989). Davies et al (1989) showed that, once potentiated, the NMDA response was not effected by a concentration of ketamine that would normally block activation of the NMDA receptor. The selective Q antagonist, CNQX, reduced the potentiated response to the pre potentiated level. This experiment gives clear evidence that the NMDA receptor is only involved in the induction of LTP and not required for expression or the maintenance of LTP, a position supported by other groups (Muller, Joly and Lynch, 1988; Kauer, Malenka and Nicholl, 1988)

1.3.vi. NMDA Receptor Antagonist effect on Learning and Behaviour

It is only recently that the effects of NMDA receptor antagonists have been tested on learning and behaviour. Morris et al (1986) showed that APV, impaired animals performance in a spatial learning task that was similar to animals with lesions to the hippocampus, but not quite so severe. Following this, subsequent

studies emerged in which the effects of NMDA antagonists were investigated behaviourally, particularly in spatial learning and working memory. Most of the studies however, have been carried out using the non-competitive antagonists MK-801 and PCP. They cross the blood brain barrier readily and can be administered systemically whereas the competitive antagonists do not and must be administered directly into the cns or the brain tissue. However, as mentioned before, there are theoretical problems associated with their use-dependent action, which will be discussed later.

Both types of antagonist impair animals performance on established learning tasks, but they also both cause stereotyped behaviour and sensorimotor impairment at high concentrations. The non-competitive antagonists are more potent than the competitive antagonists. This presents the problem of interpreting the effects and dissociating the learning impairment from effects on performance. Below the experiments showing the effects of antagonists on both learning and behaviour are described briefly.

Learning Impairment

The non-competitive antagonists, PCP and MK-801 have impaired animals performance on several hippocampally-sensitive tasks: (i) spatial learning, tested in the T-maze (Handelmann, Contreras and O'Donohue, 1987 [PCP]), the open-field water maze (Mondadori, Weiskrantz, Buerki, Petschke and Fagg, 1989 [MK-801]; Robinson, Crooks, Shinkman and Gallagher, 1989 [MK-801]; Whishaw and Auer, 1989 [MK-801]) and in the radial arm maze (Shapiro and Caramanos, 1989 [MK-801]). (ii) Working memory, tested in the radial arm maze (Kesner, Hardy and Novak, 1983 [PCP]; Danysz, Wroblewski and Costa, 1988 [PCP]; Butelman, 1990 [MK-801]; Shapiro and Caramanos, 1989 [MK-801]; Wozniak, Olney, Kettinger, Price and Miller, 1990). (iii) brightness discrimination (Tang and Ho, 1988 [PCP]) and dark avoidance (Mondadori et al, 1989[MK-901]).

The competitive antagonists, impaired animals performance in a spatial learning task, tested in the water maze (Morris et al, 1986; Morris, 1989 [AP5]); in working memory, tested in the radial arm maze (Danysz et al, 1988 [AP5]; Ward, Mason and Abraham, 1990 [CPP]) and response on a DRL schedule (Tonkiss, Morris and Rawlins, 1988 [AP5]). In addition to impairment on hippocampally sensitive tasks, AP5 has been shown to impair olfactory discrimination (Staubli, Thibault, DiLorenzo and Lynch, 1989 [AP5]); aversive avoidance

(Danysz et al, 1988 [AP5]) and a conditioning task using perforant path stimulation as the stimulus to an associated footshock (Laroche et al, 1989 [AP5]: see Chapter 1.2.vi). The Mondadori group also found that post trial administration of MK-801 and/or AP7 had no effect in some tasks (spatial learning, dark avoidance) but facilitated performance on others (dark and stepdown avoidance).

Behavioural Impairment

The major non-learning effects antagonist have on animals performance are stereotypy, sensorimotor impairment and mild sedation. In general, these effects are seen to be less severe with the competitive antagonists and localized injections have shown the effects to be mediated via brain areas other than the hippocampus.

The stereotyped behaviour is characterised by head weaving, repetitious sniffing and grooming, nondirectional movements and can be induced by (PCP) in a dose-dependent manner (Compton, Contreras, O'Donahue and Monahan, 1987; Koek, Woods and Ornstein, 1987; Tricklebank, Singh, Oles, Preston and Iversen, 1989). The same effects are also induced by MK-801 (Contreras, Contreras, O'Donahue and Lair, 1988; Tricklebank et al, 1989); CPP (Tricklebank et al, 1989) AP5 (Koek et al, 1987; Tricklebank et al, 1989); and AP7 (Compton et al, 1987). They rank in the following order of potency:

MK-801> PCP> CPP> AP7> AP5.

The sensory impairment is characterised by lack of response to somatosensory or painful stimulation. Salt and colleagues (Salt, 1986; Salt, 1987, Eaton and Salt, 1989) found that cells in the ventrobasal nucleus of the thalamus, which normally respond to stimulation of the vibrissae were inhibited in their rates of firing during iontophoretic application of AP5 and CPP. They also found the non-NMDA antagonist CNQX inhibited the cells response to the same stimulation (Eaton and Salt, 1989), indicating that both the NMDA and the non-NMDA receptors were involved in mediating the sensory response.

Localised intracathecal injections of AP5 but not AP4 to the lumbar region of the spinal cord caused an analgesic effect in response to heat and pinch to the paw and tail, which was similar to that caused by morphine (Cahusac, Evans, Hill, Rodriquez and Smith, 1984). They also found that the AP5 caused paralysis of the

hindquarters but not the forquarters. They suggested the NMDA receptors in that region of the spinal cord may be involved in control of posture, movement and nociceptive information.

The movement disorders and ataxia seen in animals administered either type of antagonist is characterised by loss of balance, such as swaying, falling side to side, slow righting reflex, (Compton et al, 1987; Contreras et al, 1986, 1988; Tricklebank et al, 1989, Koek et al, 1987; Vincent, Kartalovski, Geneste, Kamenka and Lazdunski, 1979), jerky movements (Compton et al, 1987; Contreras, Rice, Jacobsen and O'Donohue, 1986; Contreras et al, 1988), abnormal gait, supporting weight on the stomach and holding it close to the ground during movement (Compton et al, 1987; Contreras et al, 1986, 1988; Tricklebank et al, 1989; Leung and Desborough, 1989).

Turski et al (Turski, Schwartz, Turski, Klockgether, Sontag and Collins, 1985a, Turski, Schwartz, Klockgether and Sontag, 1985b, Turski, Klockgether, Sontag, Herrling and Watkins, 1987) showed more specifically that the competitive antagonists CPP, AP7 and to a lesser extent AP5 caused muscle relaxation, which they tested in the gastrocnemius-soleus muscle of genetically spastic (Han-Wistar) rats. Injections of established muscle relaxants (baclofen, midazolam, muscimol) and AP7 into the substantia nigra showed this might be the site of action (Turski et al, 1985b). Furthermore, Dawborn and Pycock (1981) found AP5 caused reduction in motor activity and sedation when injected into the pars reticulata of the substantia nigra, but increased motor activity when injected into the pars compacta.

Local injections of AP7 into the ventromedial thalamic nucleus, caused catelepsy in a dose-dependent manner that could be reversed by injections of the agonist, NMDA (Klockgether, Schwartz, Turski and Sontag, 1986). Animals were unable to induce phasic activation of the gastrocnemius set of muscles, which resulted in an akinetic-rigid syndrome, characterised by tonic muscular action.

Finally, both competitive (Bennet and Amrick, 1986; Dunn, Corbett and Fielding, 1989) and non-competitive antagonists (Dunn et al, 1989) have been shown to produce anxiolytic effects similar to those caused by diazepam and other centrally acting muscle relaxants and anxiolytic compounds. Furthermore, AP7 has been seen to

generalise to diazepam (Bennet and Amrick, 1986), implying that the impairment seen in the learning may be caused, at least in part, by lack of motivation or sedative effects.

From the data described above, it could be implied that the effect of the antagonists on the ability to learn is inextricably linked with sensorimotor and sedative effects. In fact, one could go so far as to suggest that the learning impairment may be secondary to the sensorimotor or sedative effect (Keith and Rudy, in press).

Several factors in the data, however, indicate that the learning effect can be dissociated from the behavioural impairment, but they tend to favour the use of competitive antagonists rather than the non-competitive antagonists.

Firstly, the sensorimotor impairments and stereotypy occur at high concentrations of the antagonists and in most of learning experiments a range of concentrations have been used, where researchers report that a learning impairment occurs at concentrations below that which causes the sensorimotor impairment and stereotyped behaviour (eg. Mondadori et al, 1989; Robinson et al, 1989; Butelman, 1990; Ward et al ,1990).

Secondly, localised injections of competitive antagonists, at least show the sensorimotor effects may be mediated outside the hippocampus, such as the spinal cord (Cahusac et al, 1984) and the thalamus (Salt, 1986, 1987; Eaton and Salt, 1989; Klockgether et al, 1986) and the substantia nigra (Turski et al, 1985b; Dawborn and Pycock, 1981); areas already shown to be involved in motor control.

The third point to raise is perhaps the most important: the difference between the effects on behaviour evoked by the competitive and the non-competitive antagonists. Most of the stereotyped and sensorimotor effects are more potent when evoked by non-competitive antagonists. Morris (1989) found in his experiment the occurrence of sensorimotor impairment in animals infused chronically (icv) with AP5 was infrequent and sporadic. Furthermore, he found that familiarisation with the apparatus and the task before the infusion, either prevented or reduced the sensorimotor impairment.

Although it has been suggested that expression of the behavioural effects are mediated through a mechanism common to both antagonists, because MK-801 and the competitive antagonists

generalise to PCP (Koek et al, 1986; Willetts and Balster, 1988; Tricklebank, Singh, Oles, Wong and Iversen, 1987), Willetts and Balster (1988) point out that the generalisation of the competitive antagonists is only partial. furthermore, in an NMDA discrimination task they showed the response was antagonised by competitive antagonists but not by non-competitive antagonists seen at concentrations that impair the behavioural effect (Willetts and Balster, 1989).

In the MK-801 studies there is inconsistency between groups with the concentration that can impair learning without causing sensorimotor impairment. For example, Robinson et al (1989) and Shapiro and Camaranos (1989) showed that animals performance was disrupted, without sensorimotor impairment in the watermaze and the radial arm maze (respectively) at approximately 0.05mg/kg. However, Mondadori et al, (1989) found only a slight impairment at 0.1mg/kg and a significant impairment at 0.3mg/kg in performance in the watermaze, and Ward et al (1989) found no impairment in a working memory task in the radial arm maze at 0.33mg/kg. They did find an impairment in general maze performance at 0.1mg/kg but this was only after two hours post injection. These discrepancies indicate the difficulties in interpreting exactly how, and where in the brain these effects by MK-801 are elicited.

These points help to emphasise that a difference between the effect evoked by competitive and non-competitive antagonists may exist, and that it is not necessarily confined to a difference in potency. This difference is further emphasised by the mode of action. Whereas the competitive antagonist, which acts at the agonist site can exert its effect unconditionally, the non-competitive antagonist are dependent on the channel being open. This causes a time delay for evoking LTP with high frequency stimulation, and it might be expected, in the conscious animal their effect may be subject to the level of cell excitability at the time.

In attempting to relate the mechanism required for the induction of LTP to learning, the time delay for the induction of LTP, using non-competitive antagonists is again raised. In most of the experiments which showed an effect of MK-801 or PCP on learning (Kesner et al, 1983; Handelmann et al, 1987; Mondadori et al, 1989; Robinson et al, 1989; Shapiro and Caramanos et al, 1989) the testing started between 10 and 60 minutes post injection. Although,

Stringer et al (1983) had shown LTP in CA1 to be blocked *in vivo* by iv injected PCP, tested between 10 and 45 minutes post injection, Abraham and Mason (1988) found that 1.0 mg/kg MK-801, injected ip could only block LTP in the dentate gyrus if the perforant path was tetanised 150 minutes post injection (see section 3.v., this chapter).

With due consideration to the time delay required for the block of LTP at the non-competitive site in an experimental situation and the ambiguity that surrounds the PCP block of LTP, it is difficult to attribute, with any degree of confidence, the impairment in animals performance caused by MK-801 and PCP to a blockade of the induction of LTP. One must consider these data with caution and reservation.

1.4 THE NMDA RECEPTOR'S INVOLVEMENT IN LEARNING VIA SIMILAR MECHANISMS FOR THE INDUCTION OF LTP

The research described in the three areas reviewed has laid the groundwork and supplied the necessary background information for a framework in which to test a hypothesis implicating the NMDA receptor in learning through the same mechanisms in which it is involved in the induction of LTP. Theoretically, there are three main lines of evidence that support this general notion: (i) It is generally agreed that the hippocampus is somehow involved in the processing of information during learning (see O'Keefe, 1983; Barnes, 1988). In particular, spatial reference and spatial working memory are examples of the type of information that is processed. (ii) LTP is regarded as a nmemonic device because its characteristics and the conditions under which it can be evoked conforms to theoretical notions thought to represent the type of cellular activity that may occur during learning (see Hebb, 1949 and section 1.2.vii). In addition, it can be reliably evoked in the hippocampus. (iii) The mechanisms that underlie the activation of the NMDA receptor-channel complex (ie. the voltage-gating) provide an explanation of how LTP may be evoked in other ways than the abnormally high frequency tetanus, mainly used in the whole animal preparation. It also explains some of the conditions and restraints in the induction of LTP, such as convergence and associativity.

Experimentally, the evidence which has supported the NMDA receptor hypothesis has done so by strengthening one of the three theoretical issues by linking it to one of the others. For example, the NMDA receptor shows it highest density in the hippocampus (Monaghan et al, 1983), LTP can be blocked by NMDA antagonists in those areas of the hippocampus that show the highest concentration (Collingridge et al, 1983; Harris et al, 1984; Errington et al, 1987) but not in the mossy fibres where there are no NMDA receptors (Harris and Cotman, 1986; Kauer and Nicholl, 1988). LTP decays at a similar rate to forgetting (Barnes and McNaughton, 1985); saturation of LTP impairs animals performance in spatial learning (McNaughton et al, 1986); and, after saturation, an improvement in a learning task occurs in correlation with the decay rate of LTP (Castro et al,

1990). Also, changes in potentials similar to LTP have been seen in animals engaged in learning tasks of exploratory behaviour (see section 1.2.vi).

To date, the most direct link that has been made between the NMDA receptor and learning via the mechanisms of LTP has been shown by Morris and colleagues (Morris et al, 1986; Morris, 1989). They showed that chronic infusion of 40mM DL-AP5 (icv) impaired animals ability to learn a hippocampally sensitive task. At the same concentration, they showed that LTP could be blocked in the dentate gyrus.

In their experiments, however, they used two separate groups of animals to test the learning task and the induction of LTP. Furthermore, they used only a single concentration of DL-AP5 which they found could adequately block the induction of LTP. To infer a causal link between the activation of the NMDA receptor, the induction of LTP and processing spatial information, one has to cross several levels of explanation: a physiological activity at the molecular level is used to describe a cognitive processing involved in higher learning. The fact that blockade of the NMDA receptor results in the block of LTP in one group of animals and impairs learning in another, however, represents weak grounds for making this causal link; too many assumptions must be made. They are, in fact, two independent variables that are both effected by AP5. In the original experiment, Morris et al (1986) were not able to link these two effects on the strength of a single concentration of AP5. From their experiment they were unable to know whether all the NMDA receptors in the hippocampus had been saturated, whether the concentration was greatly in excess of that required to block LTP, whether reducing the concentration would fail to cause a behavioural impairment, or in fact whether AP5 was causing a neurophysiological effect in the hippocampus other than the blockade of LTP. Thus, they have laid the ground work for a series of experiments to test the NMDA receptor hypothesis more rigorously. Two major experiments have been carried out in this thesis to address these questions.

In the first experiment, the central question addressed was whether the NMDA receptor is involved in "hippocampal" learning through the same mechanisms as LTP. This was achieved by chronically infusing a range of concentrations of the NMDA receptor antagonist, D-AP5 into rats and testing them on a learning task, and

then attempting to evoke LTP in them. In this way, if one could establish a dose-response effect of AP5 on the blockade of LTP *in vivo* (as shown in the hippocampal slice: see Harris et al, 1984), and a similar dose-dependent effect in learning, in each animal, then one is in a much stronger position to infer a causal link between the activation of the NMDA receptor, the induction of LTP and learning. In addition to testing learning and inducing LTP in each animal, the actual amount of AP5 was measured in the hippocampal tissue and the extracellular space of the hippocampus. This gives concrete evidence on which to base the effects of AP5 and it allows the data to be interpreted with reference to established experiments which have shown AP5's affinity to the NMDA receptor in the hippocampus and the concentrations required to block LTP *in vitro*. The learning task used in this experiment was spatial navigation, tested in the open field water maze and served to maintain consistency with the early study from Morris et al (1986). Although there are discrepancies in the lesion literature about the type of information the hippocampus processes it does not present difficulty in this experiment because the emphasis was placed on a correlation between a learning impairment and the amount of LTP that could be evoked. Providing a learning task that had been shown to be sensitive to hippocampal function was used, the aim of this experiment could be met. A series of four further experiments were carried out, in an attempt to control for some of the findings in the dose-response study.

The second set of experiments did address the question of learning, and the lesion data was relevant. These experiments looked at the effect of AP5 on working memory at different time intervals. Basic information obtained from the dose-response study about the concentrations of AP5 which impaired performance and block the induction of LTP and the type of effect AP5 on spatial learning were considered. In this way, a single concentration of AP5 could be used in this experiment and the results from the two experiments could be considered together, such that one may be in a position to dissociate the type of information processing required for spatial reference memory from that required for spatial working memory based on cellular activity.

In essence, the most important underlying aspect of this thesis is: whatever the effects AP5 has on spatial learning and LTP, the exact concentrations in the hippocampus are known and this allows an explanation of the results to go beyond a "black box" approach by attempting to explain the neural mechanisms that may be involved in a cognitive process.

CHAPTER 2: DOSE-RESPONSE STUDY

2.1 METHODS

2.1.i. General Procedure

The experiment was run in replicates, lasting approximately 17 days where 12 animals were used per replicate. Animals were first given 3 days of pretraining, allowing them to become accustomed to the non-spatial aspects of the task. Following pretraining, surgery to implant osmotic mini pumps was carried out. The mini pumps contained concentrations of D-AP5 ranging from 5mM, 13mM, 20mM, 30mM, 40mM and 50mM. The control animals consisted of animals infused with aCSF, sham-operated and unoperated animals. Several, but not all concentrations were used in each replicate. During surgery to implant mini pumps, positions for both the electrodes required for invoking LTP and the microdialysis probe were marked on the skull surface. Two days of post operative recovery then followed. The first day of spatial training was 3 days after mini pump implantation and this training lasted for 5 days (6 trials/day). During this time the animals were tested for their ability to learn the location of a fixed but hidden platform in an open field water maze.

Immediately following the behavioural testing, the electrophysiological phase of the experiment commenced. Here, an attempt to evoke LTP in each animal was made. A stimulating electrode was placed in the angular bundle of the perforant pathway and field potentials (FPs) were recorded in the hilus of the dentate gyrus. After a 10 minute low frequency baseline, a brief high frequency tetanus was given. Stimulation was then returned to baseline frequency and the FPs were recorded for a further 50 minutes.

After the attempted induction of LTP, microdialysate samples of extracellular fluid were taken from the hippocampus for later analysis of AP5 and amino acid content using High Performance Liquid Chromatography (HPLC). On completion of the entire experimental procedure, the animals were sacrificed and their brains removed. Tissue was taken from various brain areas for additional assessment of brain AP5 and amino acid content.

Finally, brain sections stained with fast cresyl violet provided histological verification of the cannula position and allowed assessment of any morphological changes incurred by chronic implantation of the cannula and drug infusion.

2.1.ii. Subjects

Male Lister-hooded rats, weighing between 250-350 grams taken from breeding stock in the Department of Pharmacology were used in all experiments. They were housed individually and had free access to food and water at all times except for 12 hours prior to surgery. They were maintained on a light dark cycle of 14-10 hours with lights on at 0800 hrs. The animals weights were recorded from the day of surgery until the end of the experiment.

2.1.iii. Drugs

Artificial cerebrospinal fluid (aCSF)

Modified aCSF was made up using the methodology specified by the manufacturers of the osmotic mini pumps (Alza). The final ion concentration in mM/L was: Na, 150.0; K, 3.0; Ca, 1.4; Mg, 0.8; P, 1.0; Cl, 155.0 (pH: 7.3 ± 0.1).

D-2-amino phosphonopentanoate (D-AP5)

A range of doses of D-AP5 was used: 5mM; 13mM; 20mM; 30mM; 40mM; 50mM. An equivalent stock concentration of 100mM D-AP5 was made from the acid by dissolving the D-AP5 into 100mM NaOH. This was kept as frozen aliquots and the concentrations required for the dose range were made up at the start of each replicate by diluting the stock with aCSF. Each dose was "spiked" with NaOH (100mM) until it reached a pH of 7.4.

Tribromoethanol (Avertin)

Tribromoethanol was used as a recoverable anaesthetic during the surgery to implant minipumps (0.29gm/kg). A stock concentration was kept at 4°C, in a dark container to avoid light degradation. A dilution of 1 in 55 was made up in absolute alcohol and saline (0.9%), 12 hours prior to surgery. The initial injecting dose was 10ml/kg body weight, supplemented by 0.5ml injections as required throughout the surgery.

Urethane Carbamate

Urethane Carbamate (1.5gm/kg) was used as a non-recoverable anaesthetic during the electrophysiological phase of the experiment and during sampling of hippocampal extracellular fluid. A single injection was given at the start of the electrophysiological testing.

Osmotic mini pumps

Osmotic mini pumps, supplied by Alza (model 2002) were used for chronic delivery of the D-AP5 concentrations and aCSF into the right lateral ventricle. The pump contained approx 250μl of drug which was pumped into the ventricle at a rate of 0.5μl/hour over a 14 day period.

2.1.iv. Surgical Procedure

Animals were water deprived 12 hours prior to surgery to avoid respiratory congestion during surgery and the immediate post operative recovery period. Mini pumps were loaded with drug and prepared prior to implantation. An L-shaped cannula made from a 23 gauge syringe needle was placed in one of the stereotaxic manipulators. A length of silastic tubing (4.0cm) was placed on one end of the cannula which was then flushed through with 0.1ml of the dose of drug used in the mini pump. The pump was then attached to the other end of the silastic tubing via a flow modulator. Animals were anaesthetized with tribromoethanol (0.29ml/kg) and placed in a Kopf stereotaxic device. A midline incision along the scalp was made to expose the skull surface. This was scraped clear of connective tissue. The co-ordinates to place the cannula in the right lateral ventricle were measured relative to Bregma (Paxinos and Watson, 1982). AP: -0.9mm, ML: -1.3mm, DV: skull surface - 4.5mm. At the same time, the positions for (i) the stimulating electrode (AP: -8.0mm, ML: -4.1mm) (ii) the recording electrode (AP: -3.5mm, ML: -2.0mm) and (iii) the microdialysis probe (AP: -5.8mm, ML: -5.2mm) were marked on the skull (see Fig. 2.1). This was done because Bregma was often obscured by the dental acrylic used to secure the cannula in place.

Holes were drilled in the skull for the cannula and 3 stainless steel watchmakers screws which were used as anchors for dental acrylic to grip onto. The screws were positioned (2 anterior, 1

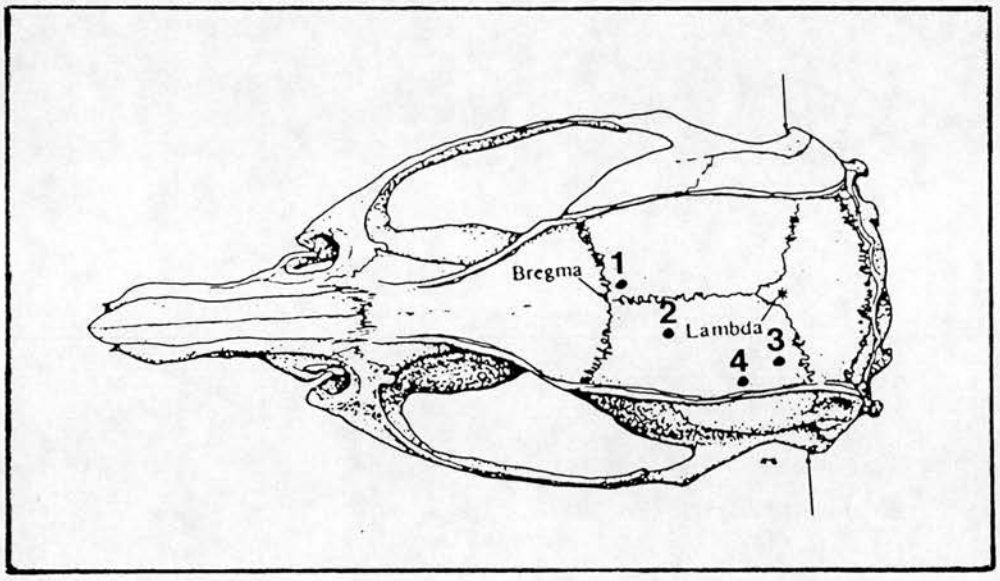


Fig. 2.1:
DOSE-RESPONSE STUDY
Positions on the skull surface are marked, relative to Bregma, for (1) cannula, (2) recording electrode, (3) stimulating electrode, and (4) microdialysis probe.

posterior) and the cannula was lowered into the ventricle with the aid of the vertical manipulator. Dental acrylic was placed over the cannula and screws to keep it in place. Using a bone curette, a subcutaneous pocket was made to place the pump intrascapularly. It extended from the caudal end of the incision. The scalp incision was closed with discontinuous suture and the animal was then placed in a post operative recovery box and monitored during the immediate recovery period.

2.1.v. Behavioural Equipment.

Spatial learning was tested in an open field water maze, developed by Morris (1981, 1984). The maze consisted of a large circular tank of opaque water. Animals were placed in the water and allowed to swim in order to locate a hidden but fixed escape platform, using extramaze cues.

The pool was 2.0m in diameter and 0.6m in height. The structure was made from glass fibre and placed on a large wooden platform, 0.6m off the floor, in the centre of the testing room. The pool was plumbed into the water system in the laboratory and could be automatically filled and drained daily. The filling and draining of the pool was controlled by a time switch which operated the opening and closing of a valve system. The water level in the tank was 0.28m high, 1.0cm above the height of the platform (see Fig. 2.2). In order to conceal the platform, the water in the tank was made opaque by adding milk powder.

The escape platform was constructed out of a length of plexiglass tubing, 10cm in diameter. A square wooden base was attached to the tubing with glass fibre strips. The tubing was filled with stones to weigh it down and the top was sealed with a glass fibre mesh. A piece of scouring material was placed on top of the platform to allow a rough surface for the animals to grip on to when climbing upon the platform. To prevent visibility in the pool the platform was painted white.

The animals swimming behaviour was monitored by a video tracking system. A camera was fixed directly above the pool, in a position where it could view the entire surface area. Appropriate room lighting was created, using 4 x 500 watt halogen flood lights angled into the corners of the ceiling. This level of lighting allowed enough contrast sensitivity between the black head of the

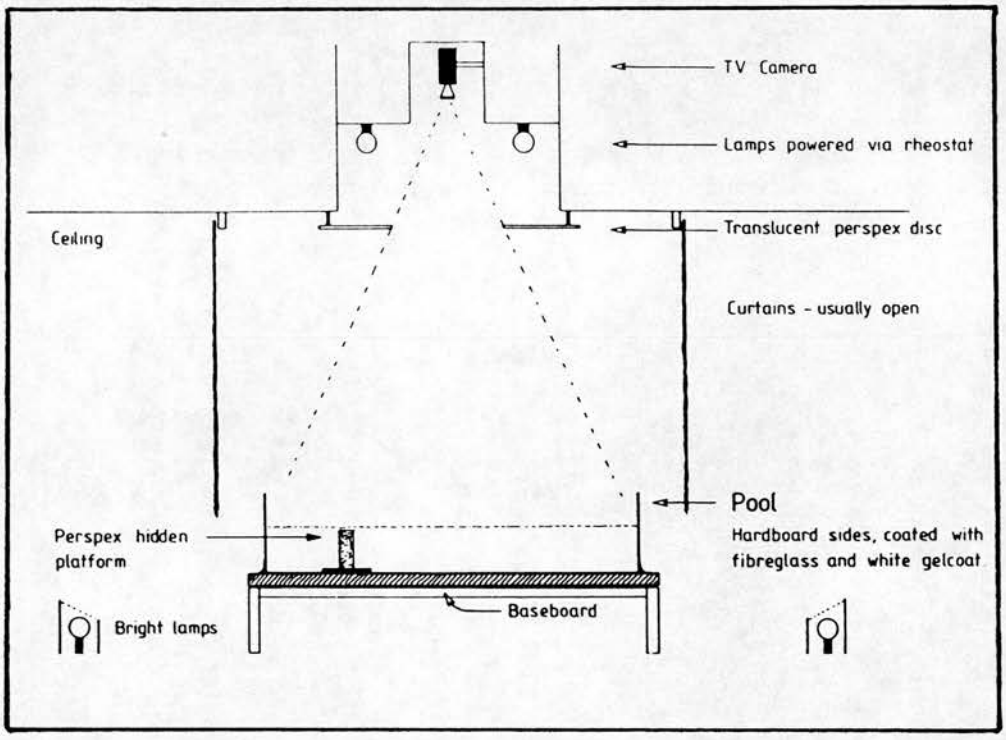


Fig. 2.2:
DOSE-RESPONSE STUDY
Cross section of the watermaze with camera positioned for recording swim paths. (Taken from Morris, 1984).

rat and the white of the pool for an image analyser (HVS, model 112) to track the rat's swim path through the video camera. Signals from the image analyser of the rats position in the pool were sampled at a rate of 10Hz and relayed to a BBC microcomputer, where they were stored on disc as x,y co-ordinates. A video-recorder (Betamax stereo BNR from Sony) was connected between the camera and image analyser which allowed trials to be recorded on video tape and analysed off line if necessary.

A computer program converted the x,y co-ordinates stored on disc into: (i) a swim path, (ii) latency to find the platform, (iii) pathlength, and (iv) time spent in each quadrant. These analyses were based on a smoothing algorithm which plotted the path taken by the animal. It compared adjacent co-ordinates for probability, and eliminated false co-ordinates caused by the image analyser defaulting to its base or to another object. The missing co-ordinates were replaced by a mean average of those co-ordinates preceding and proceeding it. All programs were written in BBC Basic Software.

There were four start positions in the pool: north, south, east, west. Switches placed on the outside of the pool at each of these positions were used to start the tracking system. This was relayed through a Campden circuit panel to the computer. Four termination switches operating via the Campden circuit were located in the room to stop tracking the animal once it had reached the platform.

2.1.vi. Behavioural Procedure

Non spatial pretraining

Prior to surgery, all animals were given 12 trials of pretraining over 3 days (4 trials/day) in the watermaze with curtains drawn around it. This allowed animals to become accustomed to the water and to learn the non spatial elements of the task, which included, swimming away from the sidewalls and learning that there was a platform to escape to. A maximum swim time per trial of 120 seconds was allowed with a 30 second inter trial interval (ITI) on the platform. Curtains were drawn around the pool to occlude extramaze cues and the platform position was moved to a new location with each trial. Latencies during pretraining were recorded using a stop watch.

Spatial learning

The spatial learning task consisted of a learning or acquisition phase and a probe trial to test retention of the platform location. During the acquisition phase animals were trained to learn the location of a fixed platform position by using the extramaze cues in the room. Six trials a day were given for 5 days. There was a maximum swim-time of 120 seconds per trial with a 30 second ITI on the platform. If the animal had not located the platform in that time, it was placed upon it by the experimenter. Each start position was used equally in a totally random order and the animals were always placed in the water facing the side walls. All 4 possible quadrant positions for the platform locations were equally used amongst all of the groups.

In the probe trial, the platform was removed from the pool and animals were forced to swim for 60 seconds. Typically a normal animal would spend most of its time searching for the platform in the training quadrant. This trial was given immediately following the last trial of acquisition on day 5.

All trials were recorded on video and computer for subsequent analysis of (i) latencies of all trials, (ii) path length and swim speed of 6 trials on day 2 of acquisition, and (iii) time spent in each quadrant during the probe trial. Testing took place between 0800 and 1800 hours each day.

2.1.vii. Electrophysiological Equipment

Electrodes consisted of a bipolar stimulator and a single wire strand recording electrode made from 110μ diameter, teflon coated wire (55-3R from Clark Electromedical instruments). Both electrodes were cut transversely so that the tips were level and the teflon coating lay flush to the end of the tips. With the bipolar stimulating electrode the wire was twisted and the two ends were 1.5mm apart. At the connector end of the electrodes, gold amphenol pins were soldered to the wires. Epoxy resin was placed around this connection to give rigidity to the electrode and to isolate the current circuit in the stimulating electrode.

Stimulation to the perforant path consisted of square-wave pulses generated at 400 Hz with a Neurolog system (Digitimer). Each pulse was 10.0 msecs wide and 7 volts amplitude. The stimulus generated by the Neurolog was stepped down to 0.7mA by a stimulus

isolator (Neurolog NL-800 by Digitimer) before being delivered to the stimulating electrode. For low frequency stimulation, the pulse generated at 400Hz was gated to allow 1 pulse every 10 seconds (0.1 Hz). The high frequency tetanus consisted of 3 trains of 33 pulses generated at 400 Hz and the duration of each train was 82 msecs.

The potentials recorded were amplified via a Grass EEG amplifier and were filtered between 1Hz and 10kHz. The signals were converted from analog to digital in a Minc-11/23 computer (Digital) and the data stored on disc. Potentials were displayed on a Tectronix storage oscilloscope and on a Grass polygraph pen recorder; they were sampled by the computer every 10 seconds. The measure used to assess the amount of LTP was the early-rising slope of the FP (mV/msec) calculated by linear regression, (see Fig. 2.3). This measure is a more reliable indicator of LTP than the increase in the population spike (see section 1.2. Long-term potentiation).

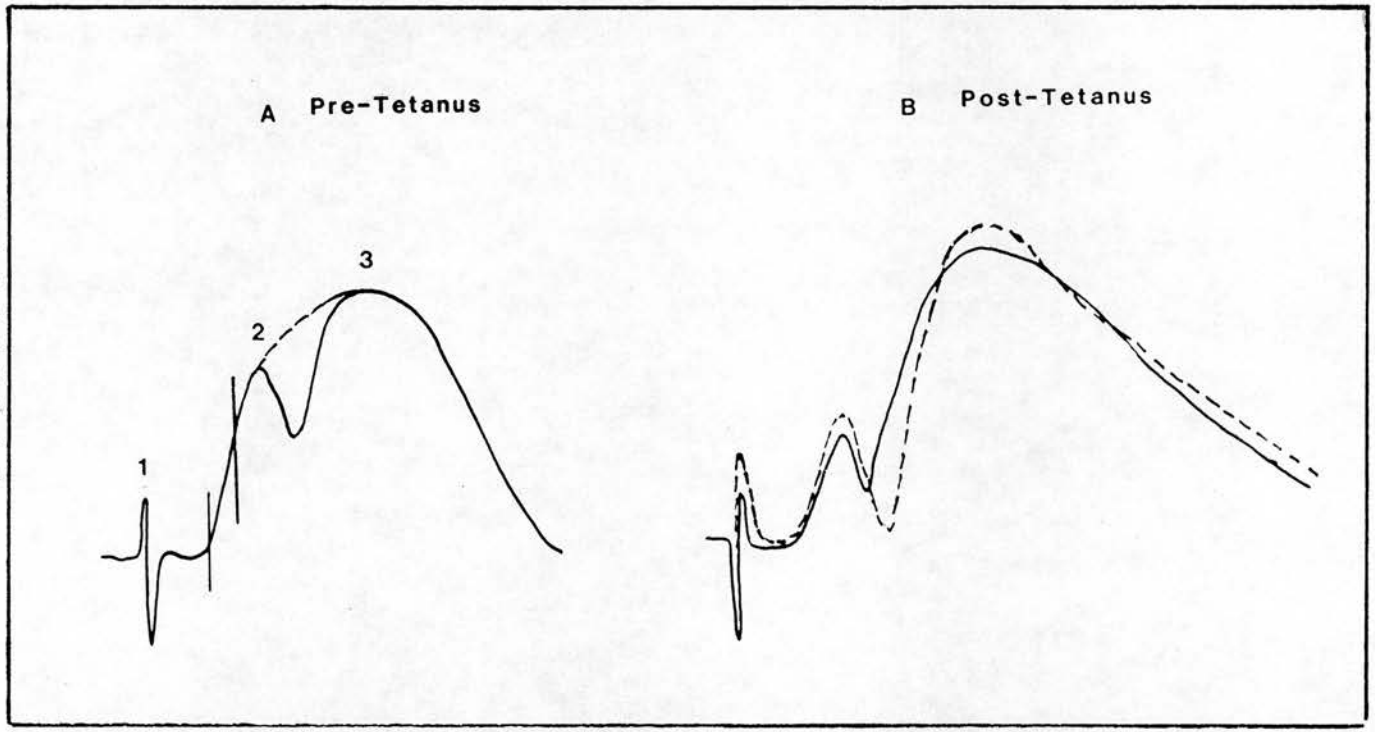
2.1.viii Electrophysiological Procedure

Animals were anaesthetised with urethane carbamate (1.5gm/Kg) and placed in a Kopf stereotaxic device. The scalp was opened to locate the electrode positions on the skull that had been previously marked. The holes for both electrodes, the dialysis probe and an earth pin were drilled out.

The stimulating electrode was lowered into the angular bundle of the perforant path to a depth of 2.7mm ±0.2mm. The recording electrode, placed to record potentials from the hilus of the dentate gyrus, was first lowered to a depth of 1.5mm. This positioned it in or near the CA1 region of the hippocampus and it was subsequently stepped down by 0.2mm. Potentials were checked on the oscilloscope at each step down for correct positioning. The final depth of the recording electrode was approximately 3.1±0.2mm.

After optimum adjustment of the electrodes, a period of 30 minutes was allowed for the tissue to settle after penetration. Low frequency stimulation was delivered during this period to measure the stability of the base line. If, after this time, a stable baseline had been attained, the main series of testing was started. First, low frequency stimulation (0.1 Hz) was delivered for 10 minutes as a pre-tetanus baseline. A high frequency tetanus was then given, after which the stimulation was returned to low

Fig 2.3:
DOSE-RESPONSE STUDY
A field potential (A) before and (B) after high frequency stimulation. (A) shows the components of the potential, 1: The stimulus artifact, 2: The positive- going EPSP and, 3: The negative-going population spike which is superimposed on the EPSP. Part A shows the field potential before enhancement and the lines show the slope value that is measured. Part B shows the potential after synaptic enhancement, the enhancement is shown by the broken line.



frequency again with potentials recorded for a further 50 minutes. Data from the resulting 360 potentials were recorded and stored on computer disc. These comprised 60 potentials recorded before the tetanus and 300 recorded post tetanus.

2.1.ix. Techniques for Pharmacological Analysis

In order to measure AP5 levels, dialysate samples from the hippocampus and whole-tissue taken from 5 brain areas (including the hippocampus) were analysed with HPLC. The principles of microdialysis and the theory of chromatography are described below to explain the theory underlying the quantification of the levels of AP5 acting at the receptor.

Microdialysis Theory

Microdialysis is a technique used for sampling the fluid in the interstitial space of brain tissue. It is based on the same principles of dialysis, whereby small molecules can diffuse through a semi permeable membrane. This allows for a more accurate measurement of the activity of substances in the extracellular fluid. A microdialysis probe consists of an input and an output capillary encased in the semi permeable membrane. When placed *in situ* molecules and water in the extracellular fluid pass through the porous wall of the membrane. The molecules that are allowed to penetrate the membrane are determined by the size of the pores in the membrane. By pumping a compound through the probe (eg. aCSF) a pressure is created on that side of the membrane and the process of ultrafiltration takes place. Various molecules and water are transported across the membrane in both directions. Those that pass from the extracellular fluid into the probe are collected at the output capillary (Ungerstedt, 1984).

The dialysis probe, has developed, historically, out of the push-pull cannula (Gaddum, 1961) and the "hollow fibre" tube (Ungerstedt and Pycock, 1974). These techniques recovered very small quantities of the endogenous chemicals and were below the levels required for accurate measurement at the time. Recent development of highly sensitive HPLC allows measurement of much smaller quantities and both techniques are widely used. The main difference between the microdialysis probe and the push-pull cannula is that the push-pull cannula is not encased in the semipermeable

membrane as the microdialysis probe. There are several advantages that the micro dialysis probe has over the push-pull cannula: First, it is a closed system so (a) there is less chance of introducing molecules that may alter the environment of the ecf and (b) the perfusate is not pumped directly into the brain as it is in the push-pull cannula and so it is less likely to cause pressure damage. Second, the probe can be calibrated by placing it in a medium containing a known quantity of test compound. This *in vitro* test determines the percentage recovery of the compound across the membrane of the probe and subsequent calculations of the amount of compound in the ecf can be made (Ungerstedt, 1984; Hamberger, 1985). Benveniste (1989) however, points out that the *in vitro* recovery may lead to a calculation of the content of the compound in the extracellular fluid that may be ambiguously low. They argue that the molecules in a beaker of solution may could pass more easily across the membrane of the probe than the molecules in the interstitial space of the brain tissue. One must be aware, therefore when calculating the concentration of the compound in the ecf that it is only an estimate and the actual concentration is likely to be higher.

Chromatographic Theory

Liquid chromatography is a method used to separate individual components of a chemical mixture by passing a mobile phase through a stationary phase. The separation is based on both the equilibrium distribution of the components between the two phases and the rate of retention of each individual chemical component in the stationary phase. Different aspects of the molecular characteristics of the components in the chemical mixture, such as the ionic charge or the degree of hydrophobicity, can be used to determine what mode of separation to use and whether any chemical modification is required. In HPLC the mobile phase, which contains the chemical mixture to be separated is pumped through a narrow bore column that is packed with the stationary or bulk phase. When a sample is injected onto a column the individual components of the mixture are adsorbed onto the surface of the stationary phase for varying lengths of time, depending on the degree of interaction that each individual component has to the two phases. The components therefore will elute from the column at different time intervals. These can be

measured by one of several forms of detection (electrochemical, ultraviolet, fluorescence, or refractive index), and the signals, in turn can be sent to either a pen recorder which produces a chromatogram or to a storage computer. The amount of each chemical component present in the sample is determined by the size of the peak traced out on the chromatogram, with reference to a sample of a known concentration of the compound being measured that is injected beforehand, (see chromatogram of an AP5 and amino acid standard in Fig. 2.4.).

Amino acids range widely in their classification based on their reaction to an aqueous solution (ie acidic, basic, neutral, aromatic). It is therefore impossible to separate all amino acids from one mixture using a single mode of chromatography. Furthermore, AP5 is very similar to aspartate and to prevent them eluting together, standard chromatographic conditions have to be modified to allow separation. A combination of two modes of separation, based on the degree of hydrophobicity and the difference in ionic charge have been used to make a complete separation of AP5 and the primary amino acids.

Separation based on the degree of hydrophobicity of the components in the solute is achieved with reverse phase chromatography. Here a non-polar packing material in the stationary phase is reacted with a polar mobile phase. The packing material in the stationary phase consists of a slurry of micro silica particles (either 3, 5 or 10μ in diameter). The surface of these particles are reacted with hydrocarbon chains, such as octodecyl. These produce a hydrophobic surface layer onto which compounds in the mobile phase adsorb for a specific time. Generally speaking, the more hydrophobic a molecule is the longer it will be retained within the column. However, the exact mechanism of separation using bonded hydrocarbon stationary phases is not fully understood.

The degree of hydrophobicity alone however is not enough to separate all amino acids. Further separation of components based on their ionic charge can be achieved with a gradient eluent system with the addition of modifiers. A gradient eluent system uses two buffers and gradually increases the amount of a secondary buffer in relation to the other. For amino acid separation, an aqueous phosphate buffer is modified with an organic buffer, methanol. As the methanol to phosphate ratio increases along the gradient further

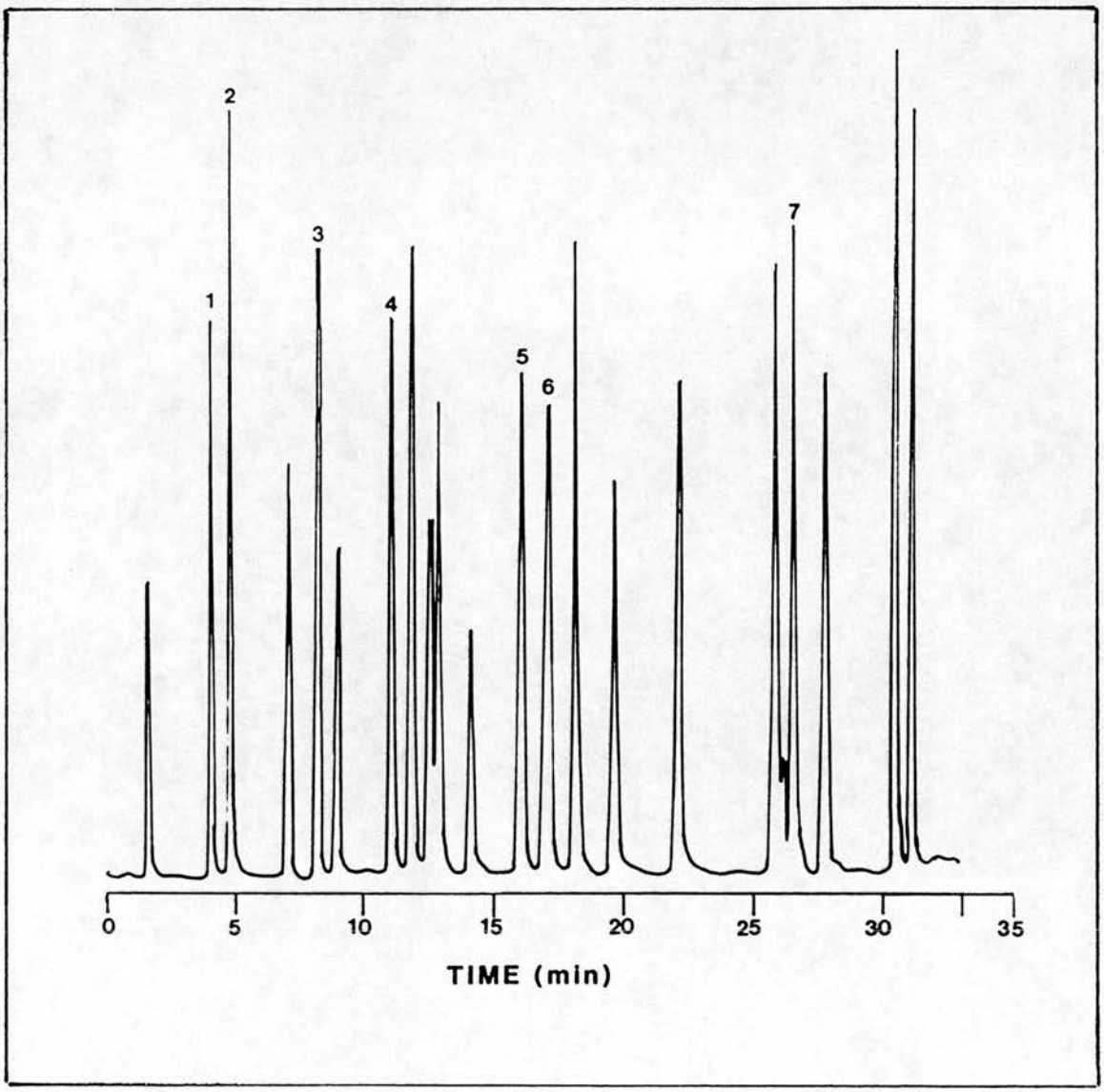


Fig. 2.4:
DOSE-RESPONSE STUDY
Chromatogram shows the elution pattern of AP5 and the primary amino acid separation, using a gradient buffer of methanol and phosphate. AP5 and the amino acid markers are numbered: (1) AP5; (2) aspartate; (3) glutamate; (4) glutamine; (5) taurine; (6) alanine; (7) valine.

separation of amino acids can be achieved. Tetrohydrofuran (THF) is included as an organic modifier in both buffers to permit separation of AP5 and aspartate.

Fluorescent detection of the amino acids and AP5 is achieved by precolumn derivitisation with o-Phthaldialdehyde (OPA). Before injecting a sample onto the column OPA is reacted with the amino acids and AP5 in the presence of the reducing agent 2-mercapto ethanol to make them highly fluorescent, (see Fig. 2.5).

2.1.x. Pharmacological Equipment

Micro dialysis probes

Probes for sampling ecf in the anaesthetised rat were made in the laboratory prior to the experiment. They consisted of two lengths of plastic coated vitreous silica (VS 170/110, from Scientific Glass Engineering) inserted into a dialysis tube (Cuprophan, B4 AH, Cobol Medical, Lafeyette, USA) that has a molecular weight cut off, of 5000D, (to prevent transfer of proteins across the membrane). The probes were assembled with the aid of a low-powered binocular microscope. A length of microdialysis tubing (5mm) was placed in a droplet of water to aid construction. The two pieces of silica tubing were inserted in the dialysis membrane staggered to a distance of 3.0mm apart. Artificial CSF was pumped into the probe via the shorter length of tubing and was collected from the outlet of the longer piece of tubing. The 3.0mm staggered area between the 2 pieces of silica tubing allowed space for molecules to pass across the dialysis membrane and be transported out for collection. A length of teflon coated electrode wire (WT3, Clark Electromedical Instruments) was inserted the full length inside the dialysis membrane to give rigidity to the probe. Both ends of the probe were sealed with cyano-acrylate adhesive and then the probe was placed in a metal casing (21g stainless steel tubing) 10mm above the dialysis membrane (see Fig. 2.6). The casing allowed the probe to be held in a stereotaxic manipulator, for insertion into the brain, without causing damage to the probe. Narrow bore polyethylene tubing was glued around the inlet silica capillary and was connected to a syringe mounted in a constant drive microinfusion pump (Carnegie Medicine, CMA 100).

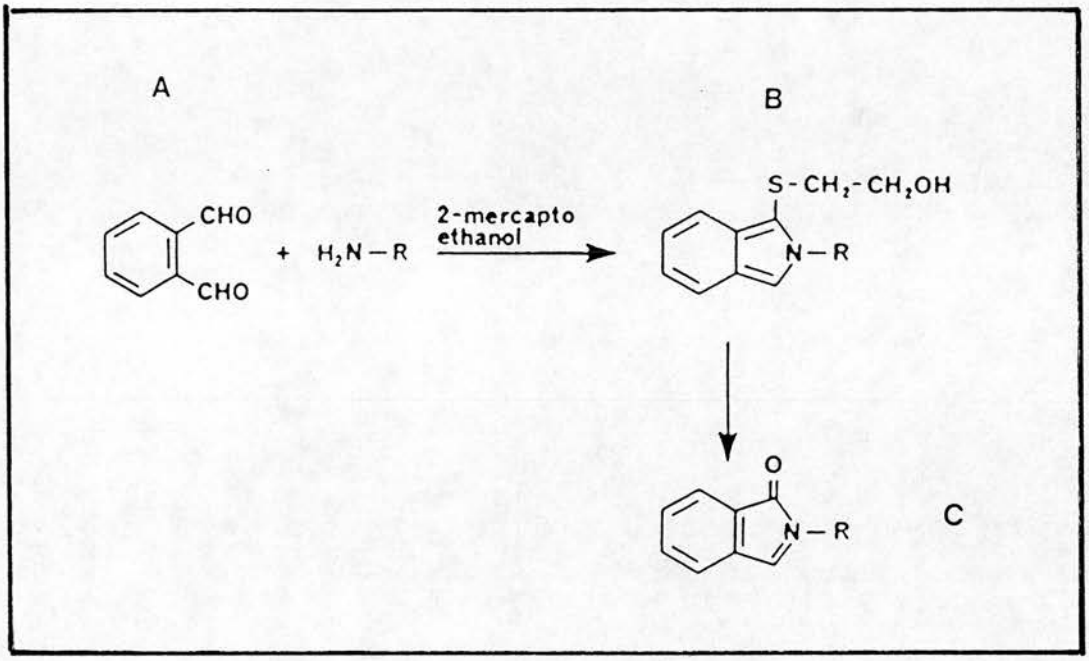


Fig. 2.5:
DOSE-RESPONSE STUDY
(A) OPA plus any of the primary amines, (and AP5) are reacted together with the reagent mecaptoethanol. They first bond together in one form (B) and then the configuration changes to take on a more stable form (C). (Taken from Lindthrop et al, 1986).

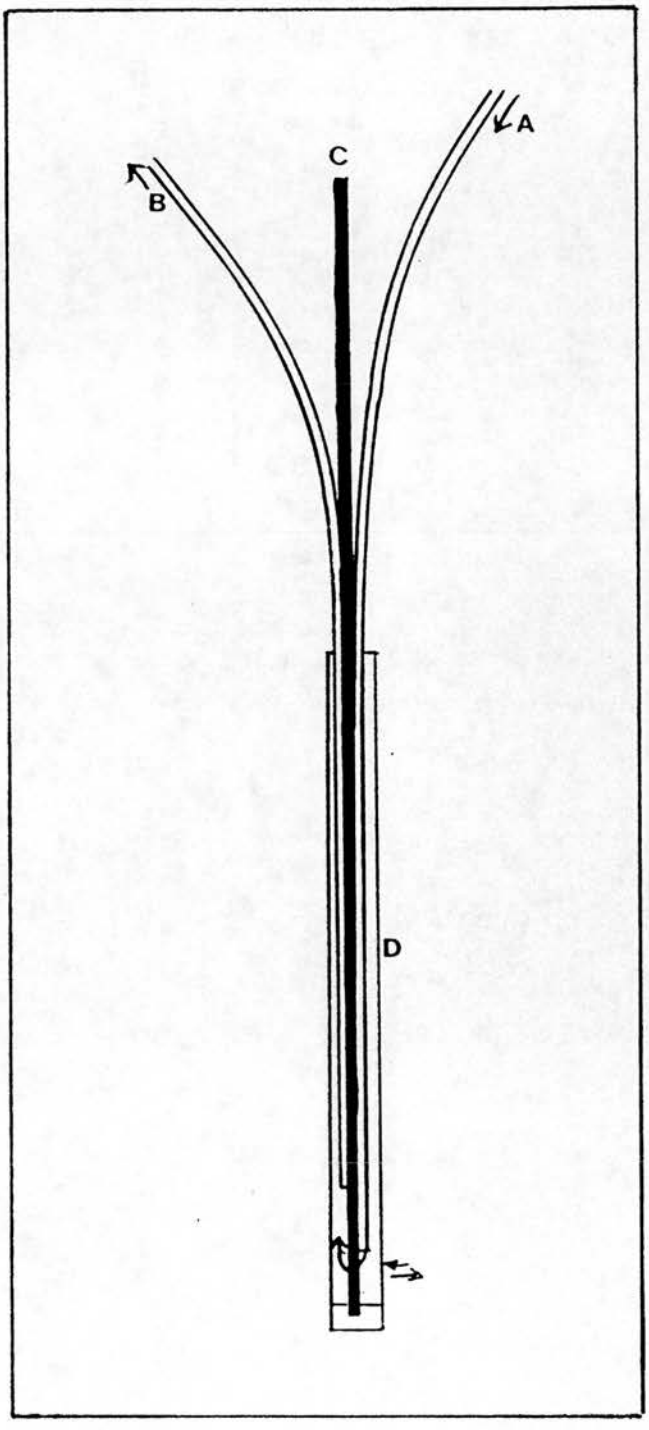


Fig. 2.6:
DOSE-RESPONSE STUDY.
Microdialysis probe, showing (A) the input capillary; (B) output capillary; (C) tungsten wire support; (d) dialysis membrane. The flow of fluid from the input capillary across the membrane into the interstitial space is indicated by the arrows.

High Performance Liquid Chromatography (HPLC)

The HPLC system consisted of a Vista 5500 pumping system (from Varian), a 9090 automatic column injector from Varian and a 5μm Nucleosil column (250±4.6mM) packed with a C18 hydrocarbon bonded stationary phase. As derivatives of the amino acids and AP5 elute from the column they were detected with an ABS Programmable fluorescent detector (from Applied Biosystems) set at an excitation wavelength of 230mM and an emission wavelength of 430mM.

2.1.xi. Pharmacological Procedure

Probe Recovery

The microdialysis membrane is porous and is permeable to only those molecules with a weight of less than 50000. In order to estimate the amount of AP5 present in the extracellular fluid, the relative recovery of AP5 and the amino acids through the probe was calibrated in an *in vitro* experiment. Probes were placed in a beaker containing 5μM AP5 and two, 20 minute samples of fluid were taken at flow rates of 1.25μl/min and 2.5μl/min.

Sampling ecf from Hippocampus

The location for placement of the probe into the hippocampus had been previously measured (DV: skull surface - 7.6mm). Once placed in the hippocampus the probe was connected to the micro injection pump and aCSF (Alza method) was pumped through the probe at a rate of 1.25μl or 2.5μl per minute. Prior to connecting the probe to the pump,the tubing was flushed through with aCSF and air bubbles were removed. Over a two hour period, six 20 minute samples of ecf were collected from the interstitial space of the hippocampus and kept in frozen storage for later analysis.

Tissue Sampling

When the dialysis sampling was completed, animals were sacrificed and their brains removed on ice. Tissue from the right-and-left hippocampus, frontal and visual cortex were taken for comparative analysis of AP5 content (i) between the different brain areas and (ii) with the dialysates. Tissue was prepared for HPLC analysis by homogenising it in 1.0ml perchloric acid (0.6M) to precipitate tissue protein. The homogenate was kept at -4^{o}C overnight, and then centrifuged at 10000g for 2 minutes. The

supernatant was neutralised in potassium bicarbonate (2.0M) and centrifuged again at 10000g for 2 minutes. This preparation was diluted 1:10 with deionised water and injected onto the HPLC column.

High Performance Liquid Chromatography Analysis

Separation of amino acids was achieved with a gradient eluent using a phosphate buffer (Buffer A: 50mM at pH 5.12±0.2) with an organic modifier, methanol (Buffer B). The gradient profile for the phosphate buffer in minutes, where the pumping rate was 1ml/min was as follows: (Time, min; %B) 0,0; 5,0; 7,25; 15,50; 23,60; 25,90; 38,110; 32.100; 42,0. THF was added to the buffers (phosphate 2.5%; methanol, 1.25%) to modify the characteristics of the mobile phase and to elute the amino acid constituents from the column intact, such that on the chromatogram the peaks were sharper. The precolumn derivitising reagent, (OPA) was injected with each sample into the mobile phase to allow detection of primary amino acids by fluorescence: 20μl OPA was added to 20μl of the sample and 10μl was injected onto the column in the presence of 2-mecaptoethanol to form the bond between OPA and the amines. A standard containing known quantities of amino acids and AP5 were injected onto the column at the start and end of each daily session of analysis to calibrate retention time and peak area of each molecular component measured. Data was integrated and quantified using a microcomputer based integration package (Midas, Comas Instruments).

2.2 RESULTS

2.2.i. Data handling

Assignment of animals to groups

Animals were grouped according to the final whole-tissue concentration of AP5 in the hippocampus (measured in nmol/mg wet weight) rather than the amount of AP5 in the mini pumps. This was a more accurate means on which to assess the behavioural effect which was attributed to hippocampal function and the induction of LTP, which was also evoked in the hippocampus. Separation of animals into the groups was carried out prior to analysis of behavioural or electrophysiological data. The groups were:

Control	0.00 nmol/mg
Low concentration	0.01-0.10 nmol/mg
Mid concentration	0.11-0.20 nmol/mg
High concentration	0.21-0.30 nmol/mg
Very high concentration	> 0.31 nmol/mg

Under this grouping regimen, analysis of performance in the behavioural task, the amount of LTP that could be evoked, and the concentrations of AP5 and amino acids were carried out with analyses of variance using the statistical package, Alice (Alice Associates, Mass) on a mainframe at the University of Edinburgh. Correlations between measures of spatial learning, LTP and AP5 levels in the tissue and the dialysates were carried out using Pearsons Product Moment r on individual animal scores. Correlations were also performed using Alice.

Criteria for accepting data

Objective criteria for handling the large amount of data that was obtained throughout the experimental period were established. A total of 136 animals were run in 14 replicates (including 3 pilot studies). Of these 110 were potential contributors to behavioural, electrophysiological and pharmacological data. Not all animals completed the three phases of the experiment (because of faulty equipment, experimental error, or - the main reason for noncompletion - death of the animals during the final electrophysiological and dialysis phases). In addition, several

animals completed all phases of the experiment, but did not provide usable data for each phase (eg. unstable baselines in the electrophysiology or inadequate sampling during the dialysis). As shown in Table 2.1, there were a total of 58 animals that provided data for all three phases, and larger numbers contributed to at least two measures (except for dialysis which was only carried out on a subsection of controls).

The following criteria were adopted for selection of animals for analysis:

(a) General Health: Animals were only allowed to continue the experiment if they maintained a general level of health. This was checked every day during weighing and handling.

(b) Histology: Assessment of brain sections had to show the cannula placement to be in the right lateral ventricle and minimal damage to adjacent brain areas.

(c) Spatial Training: Animals had to demonstrate an ability to swim, get onto and remain on the platform. In practice, some of the high concentration AP5-treated animals became tired after 2 or 3 trials and were unable to complete the 6 trials each day. If this occurred on more than 2 consecutive days, the animal was removed from the experiment.

(d) Electrophysiology: For data to be accepted for analysis, the baseline series of the slope function had to be stable and of low variability.

(e) Dialysis: Data was accepted if a quantifiable volume was recovered to analyse with HPLC.

Numbers of animals in analyses

On the basis of the assignment to groups using tissue concentration and with regard to the criteria for acceptance of animals, various data sets were constructed as shown in Table 2.1. The "Main Series" consisted of 58 animals which contributed data from all three phases of testing. This data set was used in all correlational analyses and the analysis of all AP5 levels in the tissue and the dialysates. Restriction of the remaining analyses to this data set would however, have excluded a large amount of data. Accordingly, the decision was taken to conduct analyses of variance on the behavioural and electrophysiological phases using the

GROUP	"MAIN SERIES"	BEHAVIOURAL	ELECTRO-PHYSIOLOGY	DIALYSIS
CONTROL 0.00nmol/mg	13	13	13	4
LOW CONC 0.01-0.10nmol/mg	12	20	14	17
MID CONC 0.11-0.20nmol/mg	12	13	13	13
HIGH CONC 0.21-0.30nmol/mg	12	16	14	13
V.HIGH CONC >0.31nmol/mg	9	11	11	11
TOTALS	58	73	65	58

Table 2.1:
DOSE-RESPONSE STUDY
Analysis of variance was carried out on the behavioural, electrophysiological and pharmacological measures. The numbers of animals that contributed data in each group are presented. In the "main series" animals have contributed data to all three measures and are used in the correlation analysis.

sometimes larger data sets available for each phase. The logic for this is, given that tissue values were available in these additional animals, there seemed no good reason not to analyse behavioural data simply because electrophysiological data was missing.

2.2.ii. Histology.

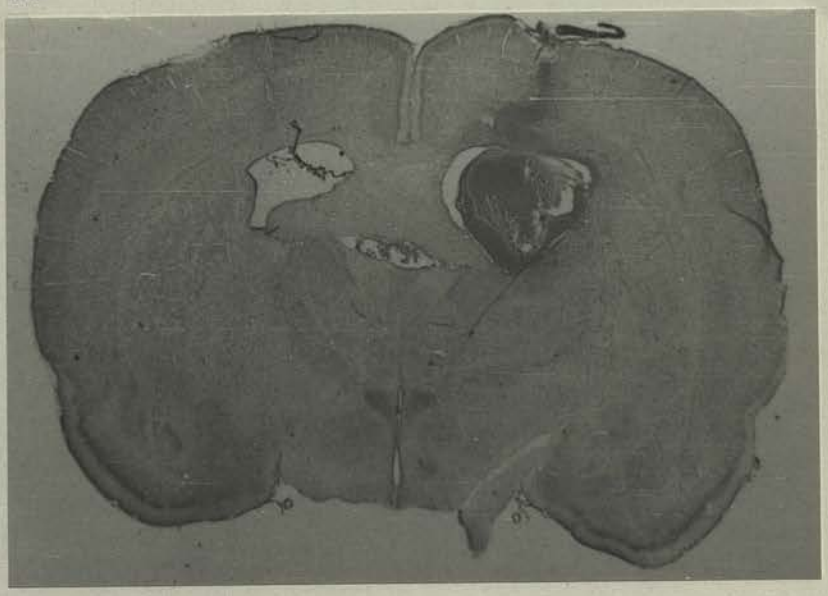
In general it was found that the cannula was correctly placed in the ventricle. The most common type of damage incurred was mechanical damage caused by the implantation of cannulae and in some cases an infection, indicated by the presence of polymorphs. This infection was surrounded and contained to a large extent by gliosis and was mainly around the tip of the cannula, but in some cases spread with varying degrees along the septo-fimbria pole. The ventricles into which the cannulae were placed were often found to be enlarged.

The amount of damage incurred in the brain varied between replicates. It also occurred in aCSF-infused controls, and this suggested that the infection was being introduced into the brains via a medium common to both the vehicle aCSF and AP5. A process of elimination focused the problem on the water used for making up solutions. By using pyrogen free water instead of millipore water, the amount of infection was greatly reduced, (see the microphotographs of 2.7 for comparison).

In general, the damage to the brains was evenly spread across all groups. A source of concern was that some of the behavioural impairments to be reported later might have been caused by this brain damage rather than by the AP5 infusion. However, this seemed unlikely as some animals in the low concentration group had quite extensive damage (eg 1633) but were not badly impaired in the learning task. Conversely, some animals in the mid concentration group were impaired in the learning task but had very little damage (eg 1657). Fig. 2.8 presents microphotographs showing the range of damage in these two animals.

The sham-operated animals and the controls implanted with minipumps containing aCSF showed comparable escape latencies throughout the experimental period ($F(1,11)=3.22$; $p>0.1$). Based on this, it was concluded that damage in the brains of the aCSF animals

A



B

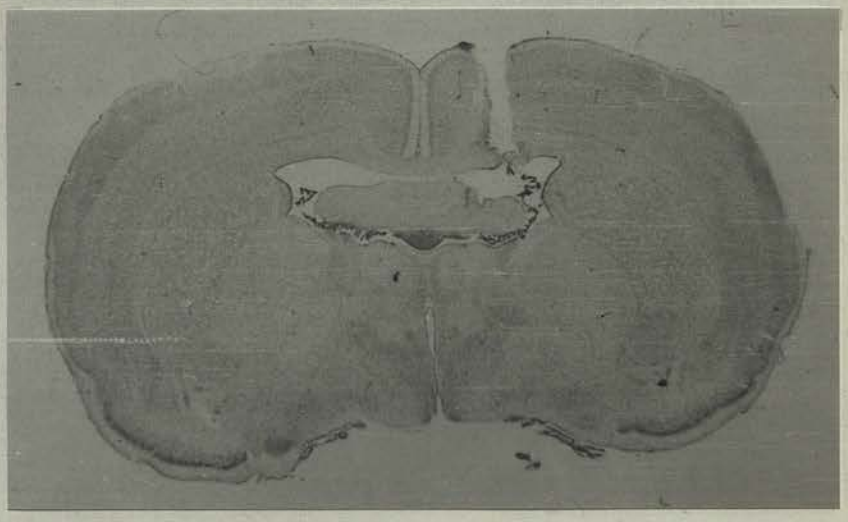
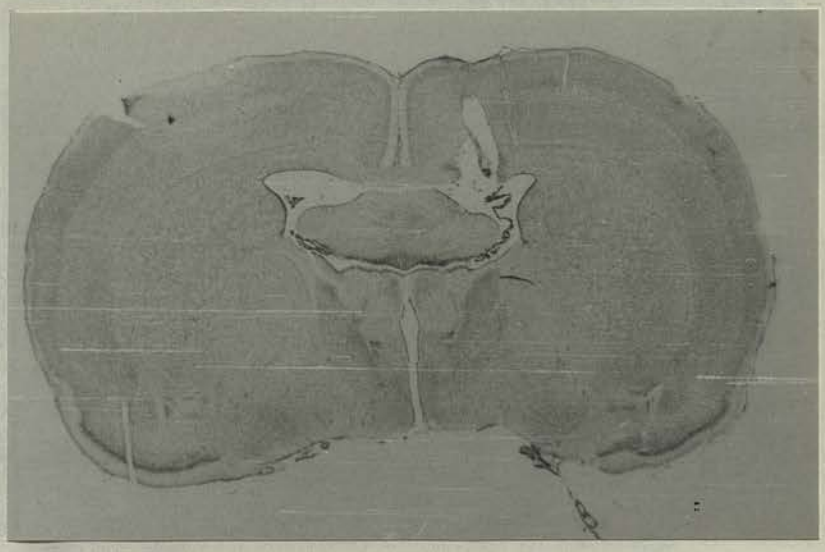


Fig. 2.7:
DOSE-RESPONSE STUDY
Microphotographs of 30μ coronal sections stained with fast cresyl violet, taken from the ventricular area of the brains of two control animals shows the difference in the amount of damage caused when solutes were made up in deionized water (A) and in pyrogen free water (B).

A



B

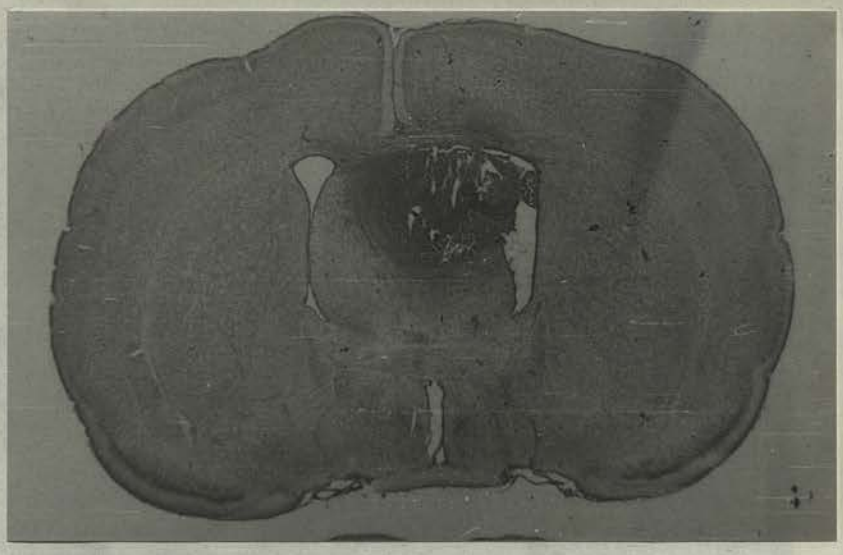
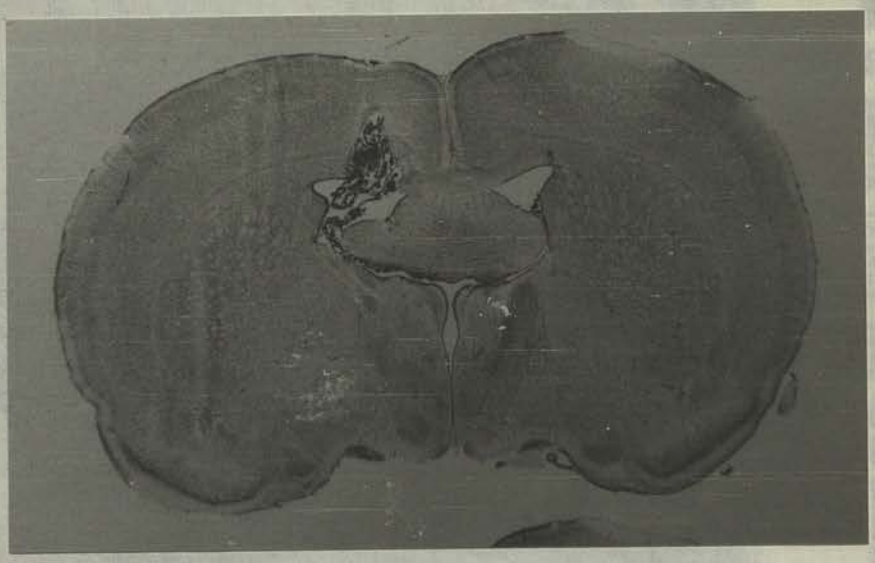


Fig. 2.8:
DOSE-RESPONSE STUDY.
Microphotographs of 30μ coronal sections stained with fast cresyl violet, taken from the ventricular area, show the range of mechanical damage caused by the cannula and the amount of infection.
(A) Rat 1657, a high concentration AP5-treated animal, (B) Rat 1633, a low concentration AP5-treated animal.

C



D

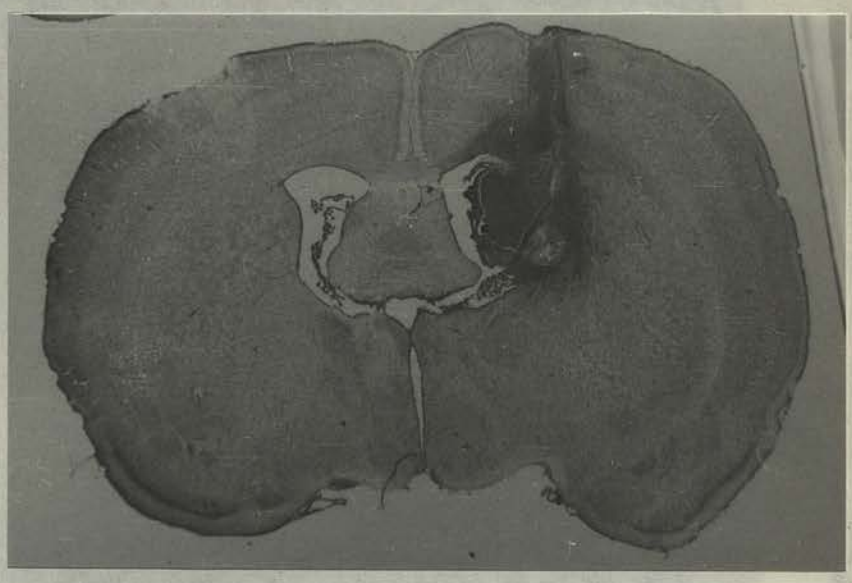


Fig. 2.8:
DOSE-RESPONSE STUDY.
Microphotographs of 30μ coronal sections stained with fast cresyl violet, taken from the ventricular area, of two aCSF treated animals (C and D) show the range of mechanical damage caused by the cannula and the amount of infection.

caused no detrimental effect on performance. A threshold for acceptable damage in the AP5 animals was set at the highest level found in aCSF animals.

2.2.iii. Weights

On the day following surgery all animals showed first an increase in weight and then a corresponding drop on the day after that. The initial weight gain after surgery was attributed to an increase in food and water intake after being water deprived the night before surgery. The corresponding decrease in weight was assumed to be a stabilising effect of food and water intake after the overshoot on the previous day. Thereafter, control animals continued to gain weight steadily over the rest of the experimental period. All animals infused with AP5, however, failed to gain weight over this period of time. An analysis of variance (N=68: data lost from 5 animals) showed that there was no overall difference in weight change between the groups ($F(4,63)=1.16$; $p>0.33$), but there was a group by day interaction ($F(24,378)=2.21$; $p<0.001$). Re-analysis of the data with controls subdivided into aCSF-infused animals and sham or unoperated animals showed that although there was still no significant difference between groups ($F(5,62)=1.98$; $p>0.05$) the groups by days interaction remained significant ($F(30,372)=1.67$; $p<0.03$). Importantly, the weights of the sham and unoperated controls showed a steady increase each day while the aCSF-infused animals showed a similar failure to gain weight as the AP5 groups (see Fig. 2.9; weight gain by sham and unoperated controls over 7 days = 25.6 gm, by aCSF controls = 6.0 gm, by low concentration group = 4.8 gm). This implies that the presence of the mini pumps and/or the pumping of a solution into the brain was causing or contributing to the weight loss, and not the AP5 *per se*.

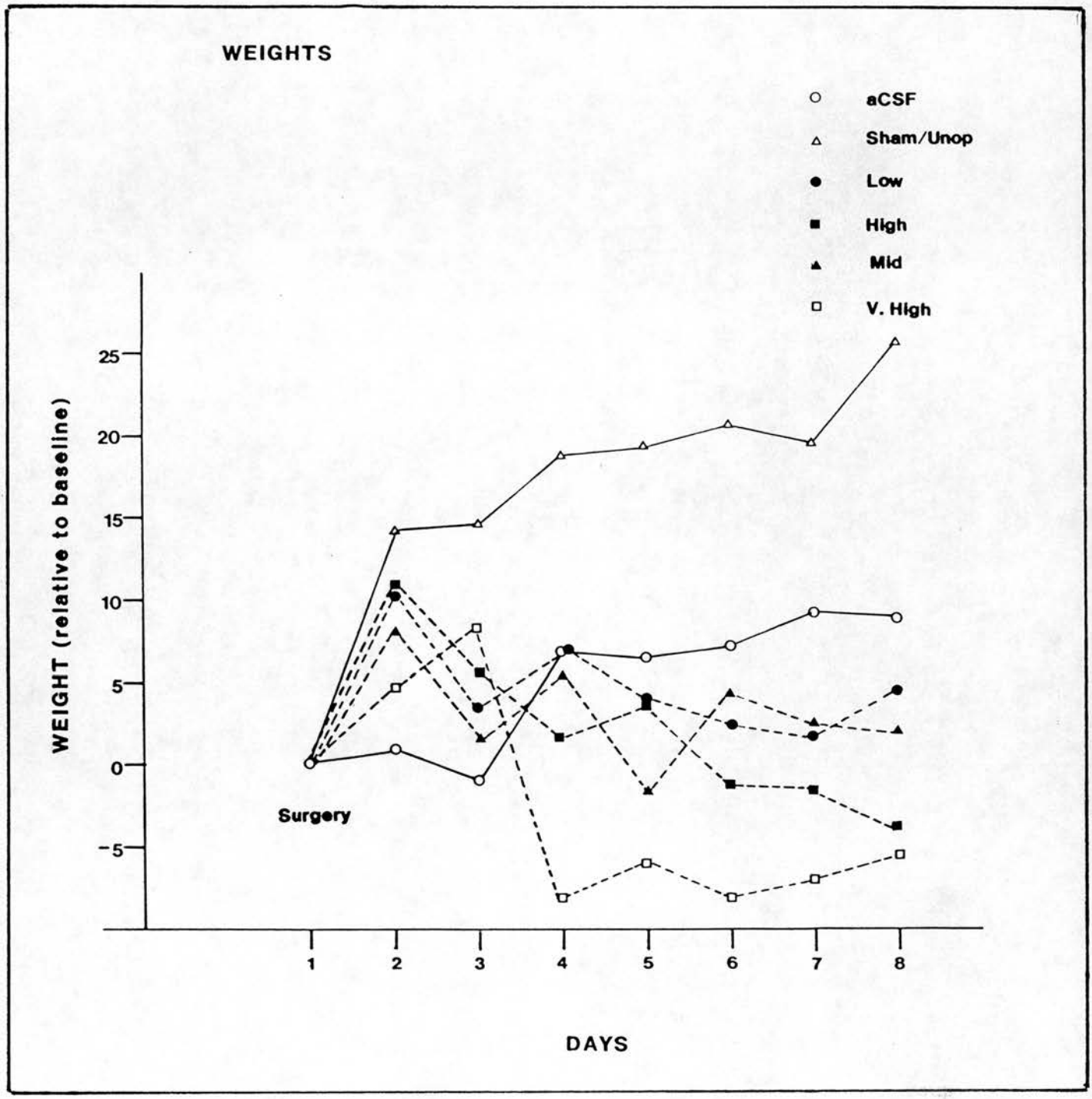


Fig. 2.9:
DOSE-RESPONSE STUDY
The mean weights (gm) for each group are plotted (days) as an increase or decrease against the baseline weight taken on the day of surgery.

2.2.iv. Tissue Analysis

Based on the regrouping of the animals according to the concentration of AP5 in the combined right and left hippocampal tissue, the mean concentration of AP5 in each group was:

Controls (N=13)	0.00nmol/mg wt.wt.
Low concentration (N=12)	0.08nmol/mg wt.wt
Mid concentration (N=12)	0.16nmol/mg wt.wt.
High concentration (N=12)	0.27nmol/mg wt.wt.
Very high concentration (N=09)	0.41nmol/mg wt.wt.

The amount of AP5 measured in the the 5 selected brain areas (right and left hippocampus, right and left visual cortex, and the frontal cortex) was unevenly distributed in the low concentration group ($F(4,44)=6.17$; $p<0.001$); the high concentration group ($F(4,44)=17.86$; $p<0.001$) and the very high concentration group ($F(4,32)=20.17$; $p<0.001$). In general the right side showed a higher concentration than the left side, (see Fig. 2.10).

The mean concentration of AP5 for the whole brain tissue measured in nmol/mg wet weight was:

Low concentration	0.06
Mid concentration	0.13
High concentration	0.22
Very high concentration	0.33

This concentration was compared with the amount of AP5 that was calculated to have been infused into the brain over the number of days for which the animals were tested (see Table 2.2A and calculation Nr. 1 in Table 2B). It was found that on average approximately 10% of the AP5 remained in the brain. In the low concentration group there was 7.52%; the mid concentration, 7.00%; the high concentration 13.02% and the very high concentration group 13.00% remained.

Finally, an analysis of variance was carried out on the 6 amino acids measured in the hippocampal tissue (N=55: 3 unoperated control animals did not contribute data). In general there was no difference in the levels of the amino acids; aspartate, glutamate, glutamine, taurine, alanine and valine ($F(4,50)=1.26$; $p>0.3$; see

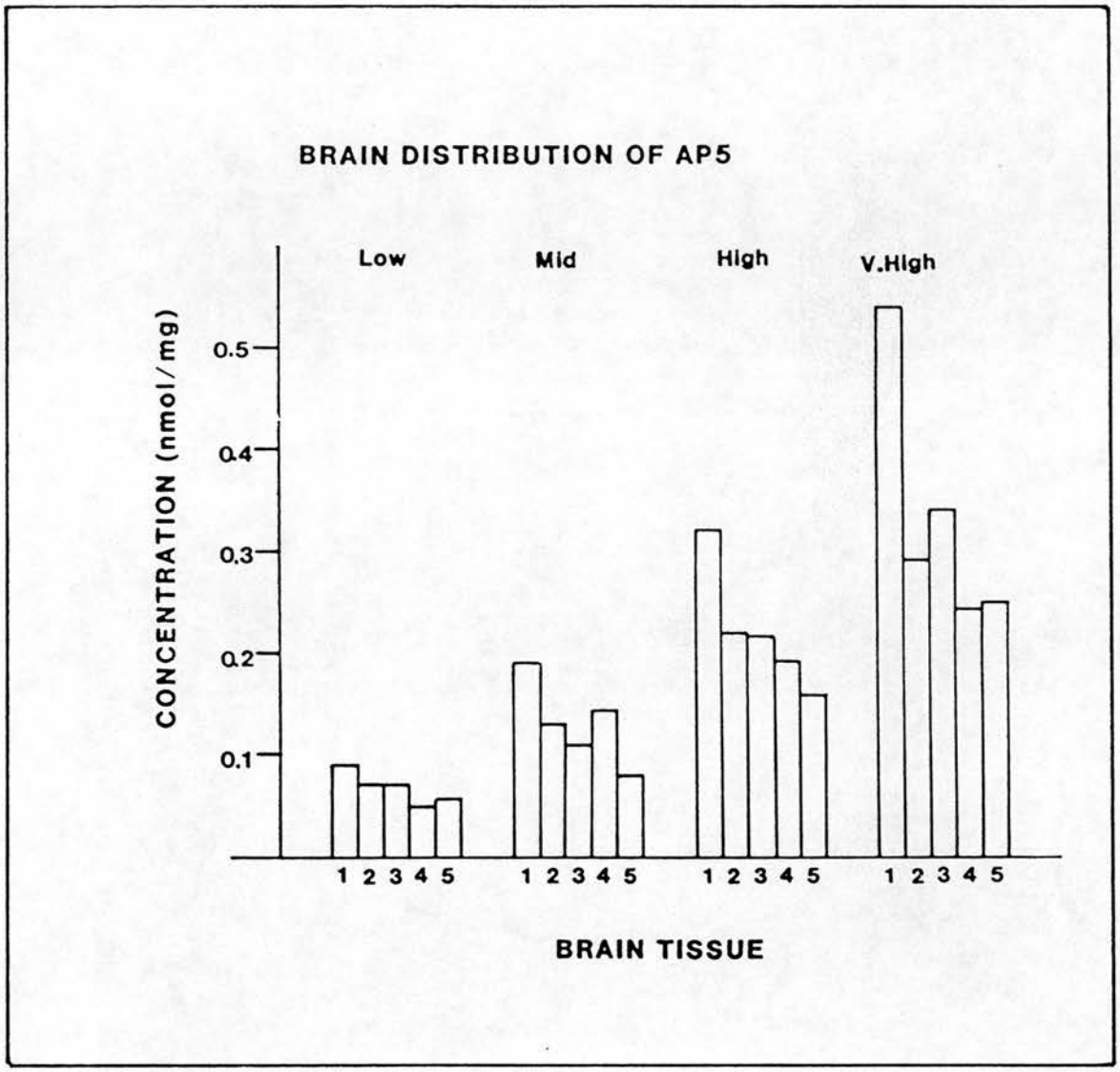


Fig. 2.10:
DOSE-RESPONSE STUDY
Histogram shows the concentration of AP5 (nmol/mg wet weight) for each group in 5 different brain regions: (1) right hippocampus; (2) left hippocampus; (3) right visual cortex; (4) left visual cortex and (5) frontal cortex.

AP5 CONCENTRATION							RATIO
GROUP	Amount in hippocampal tissue nm/mg wt.wt.	Amount infused into brain µmol	Whole brain Tissue	Amount remaining in brain %	Dialysate Concentrate µM	Hippocampal ECF Estimate µM	Whole brain Tissue: ECF
LOW 0.00-0.10	0.08	1.78(±1.16)	0.12(±0.05) µmol/1.5gm 77.30µM	7.5	0.10 (±0.10)	1.51(±1.55)	50
MID 0.11-0.20	0.16	2.70(±0.93)	0.18(±0.04) µmol/1.5gm 127.00µM	7.0	0.18(±0.16)	3.72(±2.60)	42
HIGH 0.21-0.30	0.27	3.02(±0.59)	0.37(±0.05) µmol/1.5gm 246.70µM	13.02	0.55(±0.27)	8.76(±4.69)	30
V.HIGH >0.31	0.41	4.17(±1.65)	0.50(±0.15) µmol/1.5gm 333.30µM	13.0	0.96(±0.44)	14.77(±5.90)	23

Table 2.2A:
DOSE-RESPONSE STUDY
Table showing the comparison between the levels of AP5 measured in the tissue, the dialysates and the amount of AP5 estimated to be in the extracellular fluid based on probe recovery. (See Table 2.2B for calculations.)

Table 2.2B:
DOSE-RESPONSE STUDY
Calculations used to derive the comparisons of AP5 levels displayed in table 2.2.9A.

1. AMOUNT INFUSED
Concentrations in pump x flow rate x number of days pump is active.

$= (x \times 10^{-3}) \times y \times 10^{-6} \times z \times 24$

where: x = concentration in pump (mM)
y = flow rate (0.5µl/hr)
z = number of days pump is active
24= number of hours each day

2. WHOLE BRAIN CONCENTRATIONS
Mean concentration of 5 brain areas x average weight of brain.

$= x \times 1.5$ = nmoles in whole brain

where: x = x mean concentration in all 5 brain areas (nmoles/mg)
1.5 = average weight of rat brain (gms)

3. PERCENTAGE AP5 REMAINING IN BRAIN
Concentration in whole brain divided by amount infused over experimental period.

$$\frac{x}{y} \times 100 = \%$$

where: x = whole brain tissue (µmoles)
y = amount infused (µmoles)

4. ESTIMATED AMOUNT OF AP5 IN EXTRACELLULAR FLUID
The dialysate concentration x reciprocal of % recovery rate of probe measured from in vitro study.

$$= x \times \frac{100}{6.2} = \mu M$$

where: x = dialysate concentration (µM)
6.2 = the % recovery rate from probe in vitro exp.

5. RATIO OF TISSUE CONCENTRATION OF AP5 TO EXTRACELLULAR CONCENTRATION
whole brain concentration converted to µM divided by concentration of estimated ECF.

$$= \frac{x\ \mu mol/1.5\ gm}{y\ \mu M} = \frac{x \times 10^{-6} / 1.5 \times 10^{-3}}{y\ \mu M}$$

where: x = concentration (µmol) of AP5 in whole brain
1.5 = average brain weight (gm)
y = estimated dialysate concentration (uM)

Fig.2.11). There was an overall difference between the levels measured in the right and left hippocampus ($F(1,50)=5.43$; $p<0.05$), but this difference was due mainly to the difference in levels on only some of the amino acids in the very high group ($F(5,40)=2.49$; $p<0.05$) and to an overall difference between all amino acids in the high concentration group ($F(1,11)=6.96$; $p<0.05$).

Discussion

The pharmacological assays of AP5 concentration in hippocampal tissue allow for certain assumptions to be made about the activity of AP5 in the brain with a reasonable amount of confidence. First of all, comparison of the amount of AP5 measured in the brain with the calculation of how much was infused, indicated that most of the AP5 was excreted or metabolised out of the brain; only 10% of the AP5 that was infused into the brain could be measured in the tissue at the end of the experiment. This raises the question of whether the levels of AP5 in the brain are at a steady-state throughout the experimental period. The minipumps are said to reach a steady output rate within 6 hours of implant (Alza). If, however, the rate of excretion from aCSF into the body varied, then the concentration of AP5 in the brain would also vary throughout the experiment and have a consequential effect on the behavioural testing and the correlated block of LTP.

Of the remaining 10%, its distribution between the 5 brain areas showed that in general, AP5 was unevenly distributed: the right hemisphere, which was the side into which the AP5 was infused had a higher level than the left hemisphere. Both the hippocampi and the visual cortex had a higher level of AP5 than the frontal cortex. This level of distribution complies with reports from Kasamatsu et al (1981) who showed that radiolabelled norepinephrine was always highest in the tissue adjacent to the cannula and declined exponentially the further away measurements were made from the cannula. This would explain why the right hippocampus had a slightly higher level than the left hippocampus and the frontal cortex, and even though the AP5 would be transported throughout the entire brain via the CSF, the frontal cortex may be too far from the site of infusion to receive very much. AP5 had no effect on the other amino acids in the tissue. It could be concluded, that whatever effect AP5 has on behaviour or on the induction of LTP it

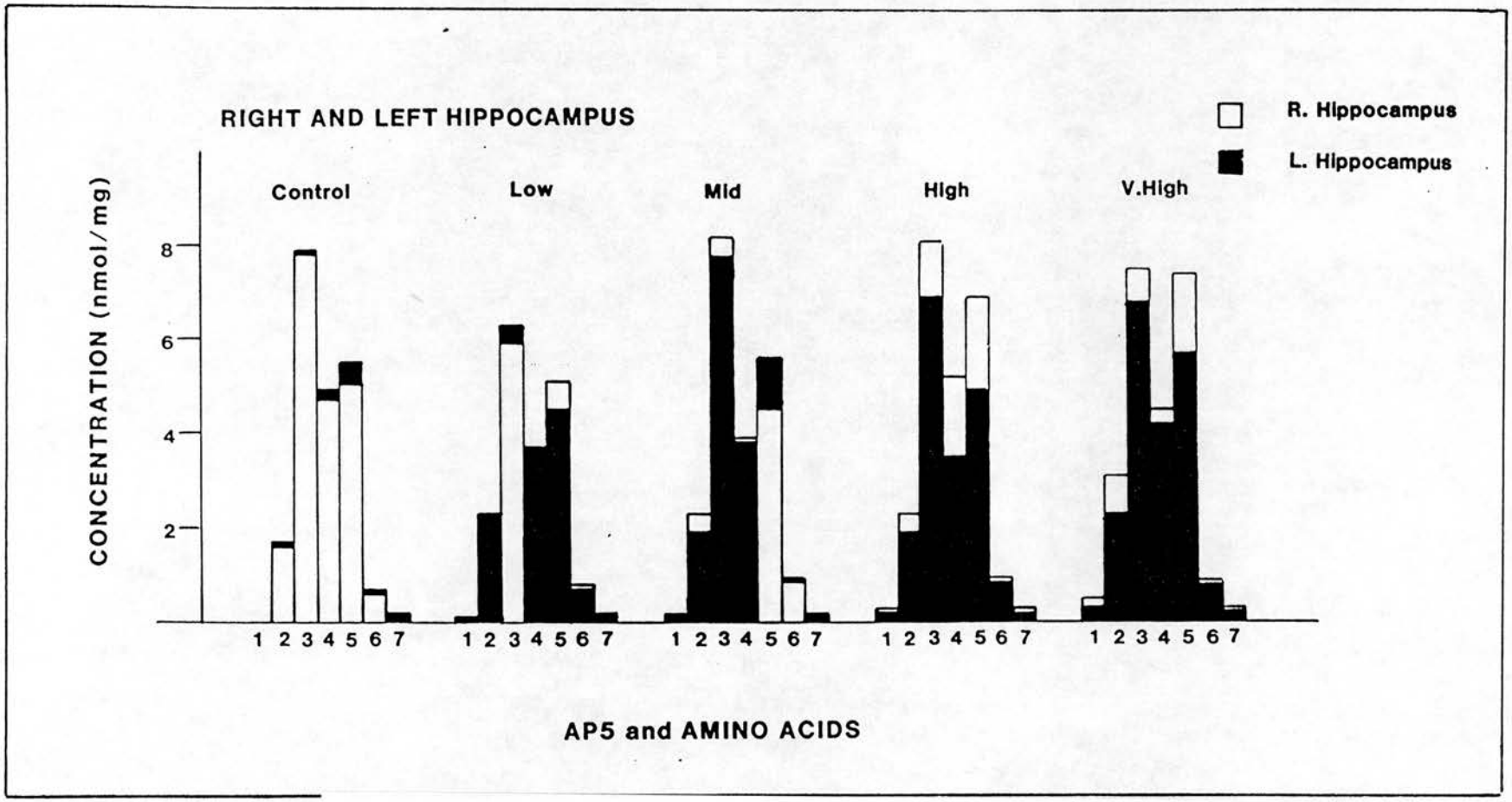


Fig. 2.11:
DOSE-RESPONSE STUDY
Histogram shows the concentration of the 6 amino acids (nmol/mg wet weight) in the right and left hippocampus: (1) AP5; (2) aspartate; (3) glutamate; (4) glutamine; (5) taurine; (6) alanine and (7) valine.

was likely to be elicited through action at the NMDA receptor and not through any modulatory effect on amino acid precursors or metabolites.

2.2.v. Behavioural Analysis

Pretraining

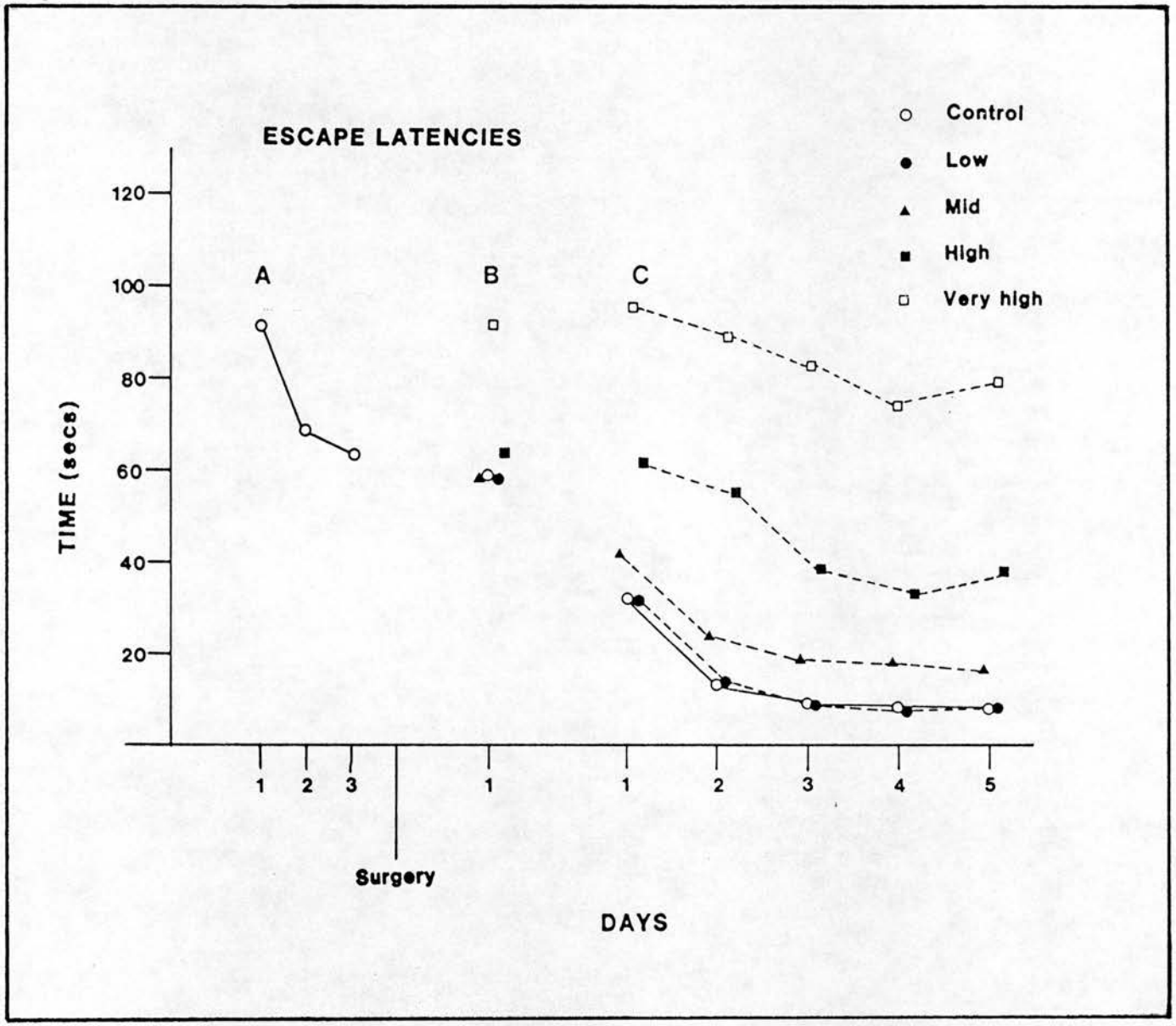
Animals learned the non-spatial aspects of the task such as swimming away from the side walls, learning to use the platform as a means of escape and staying still on the platform. Over the 12 trials all animals that completed the behavioural training (N=73) showed a significant decrease in latencies to escape the water ($F(11,792)=10.37$; $p<0.001$) to a mean level of 75.1(±5.1)s on the last trial, (see Fig. 2.12A).

Acquisition

On the first trial all animals, except those in the very high concentration group escaped the water in approximately 60s; the mean escape latency for the very high group was 92.7(±12.9)s (see Fig. 2.12B). An analysis of variance, however, showed that this difference was not significant ($F(4,68)=1.59$; $P>0.18$). The average escape latency on trial 1 for the controls (59.5±11.7s), low (59.9±3.9s), mid (58.7±12.1s) and high (62.4±11.6s) concentration groups was less than that taken by all animals on the last day of the nonspatial pretraining (75.1s), but the very high concentration group showed a higher latency.

Over the next 5 days of testing the control animals learned the location of the platform rapidly and escaped the water with decreasing latencies (see Fig. 2.12C). By the end of the behavioural testing period, they were escaping the water in 8.1 (±1.2)s; The AP5 animals showed a dose-dependent impairment, compared with controls ($F(4,68)=45.95$; $p<0.001$). The low concentration group were not impaired: their escape latencies were comparable to those of controls. The mid, high and very high concentration groups did, however, show impaired performance and took longer to escape the water. The mid and high concentration group showed an improvement across the 5 days of training (mid: ($F(4,48)=14.89$; $p<0.001$); high: ($F(4,60)=3.72$; $p<0.01$) whereas the very high concentration group did not show any significant improvement ($F(4,40)=1.35$; $p>0.20$).

Fig. 2.12:
DOSE-RESPONSE STUDY
Mean escape latencies (seconds) taken by each of the 5 groups during A: Pretraining, mean of 4 trials/day; B: First trial, day 1; C: Chronic infusion of drug, mean of 6 trials/day.



Analysis of Trials on Each Day

From the acquisition data, it was apparent that all animals except those in the high and very high concentration groups had reached their own assymptotic level of performance by day 3 and so a more detailed analysis of performance each day was carried out with particular emphasis on day 2.

On day 1 of the training, an analysis of variance showed that there was a significant difference between the trials for the controls ($F(5,60)=2.56$; $p<0.05$), low concentration group ($F(5,95)=5.36$; $p<0.001$) and the mid ($F(5,60)=2.82$; $p<0.05$) but not with the high group ($F(5,75)=1.04$; $p>0.30$) or the very high group ($F(5,50)=1.59$; $p>0.20$). The difference between trials with the control and the low concentration group was attributed to the first trial of the day because, by the second trial, the animals had usually reached a near as ymptotic level of performance (see Fig. 2.13A). Analysis of variance of trials 2-6 showed no significant difference for the controls ($F<1$) or the low group ($F(4,76)=1.29$; $p>0.2$). The mid concentration group continued to fluctuate in the time they took to escape the water ($F(4,48)=3.49$; $p<0.05$) and the high and very high groups showed no improvement in escape latencies after the first trial (high ($F(4,60)=1.38$; $p>0.25$); very high ($F(4,40)=1.86$; $p>0.10$; see Fig. 2.13A).

On day 2, controls ($F(5,60)=2.39$; $p<0.05$); low ($F(5,95)=3.47$; $p<0.01$), the mid ($F(5,60)=3.37$; $p<0.01$) and high ($F(5,75)=3.15$; $p<0.05$) concentration groups showed a significant difference between trials (see Fig. 2.13B). This difference was attributed to the first trial of the day for the controls, the low and mid concentration groups, because subsequent analysis of trials 2-6 showed no significant difference. The high concentration group continued to show a difference in the other 5 trials ($F(4,60)=4.06$; $p<0.001$). After a drop in the latency on the second trial, they began to show a steady increase in latency on each subsequent trial, (see Fig. 2.13B). The very high concentration group showed no difference between the escape latencies over the 6 trials ($F(5,50)=1.36$; $p>0.25$); they took between 72.9s on the first trial and 101.3s on the last trial.

Although the latencies for the high and very high groups were long, analysis of variance of the swim speeds showed that there was no difference between the groups on this measure ($F<1$) and the longer latencies were not merely due to the animals swimming more slowly. Furthermore, there was a group difference in pathlengths ($F(4,68)=21.17$; $p<0.001$) which correlated with the latencies ($r=.58$; $p<0.001$).

In general on days 3 and 4 all groups performed at an asymptotic level after the first trial. The exception was the low concentration group on day 4 but they reached an asymptotic level by the second trial (see Figs. 2.13C and 2.13D).

On day 5, however, the controls, the low and mid concentration groups, again, resumed a pattern of escape latencies where they reached an asymptotic level by the second trial of the day There was a significant difference between the trials (control: ($F(5,60)=4.07$; $p<0.01$); low ($F(5,95)=4.89$; $p<0.01$); mid: ($F(5,60)=2.44$; $p<0.05$) and this was attributed to the first trial (see Fig. 2.13E). The high and very high concentration groups showed no significant change in latencies after the first trial, (high: ($F<1$) and very high: ($F(5,50)=1.44$; $p>0.20$; see Fig. 2.13E).

Transfer Test

The amount of time spent in each quadrant of the pool during the 60 second transfer test where the platform had been removed was analysed. In this way, the amount of knowledge retained about the position of the escape platform could be quantified. The accepted analysis is to look for a groups by quadrant interaction (because there are always 4 quadrants and the summed time across quadrants is always 60s by necessity). This overall analysis was therefore backed up by a separate analysis of the training quadrant only.

The overall analysis of variance showed that there was a groups by quadrant interaction ($F(12,102)=7.94$; $p<0.001$). However, the groups difference (see Fig. 2.14) appeared to be due solely to the performance of the high and very high concentration groups. An analysis of variance carried out on the training quadrant only also showed an overall difference ($F(4,68)=10.36$; $p<0.001$) and Newman Keuls post hoc test showed that only the high and very high concentration groups differed from controls ($p<0.05$). The low and mid concentration groups which showed a spatial bias to the training

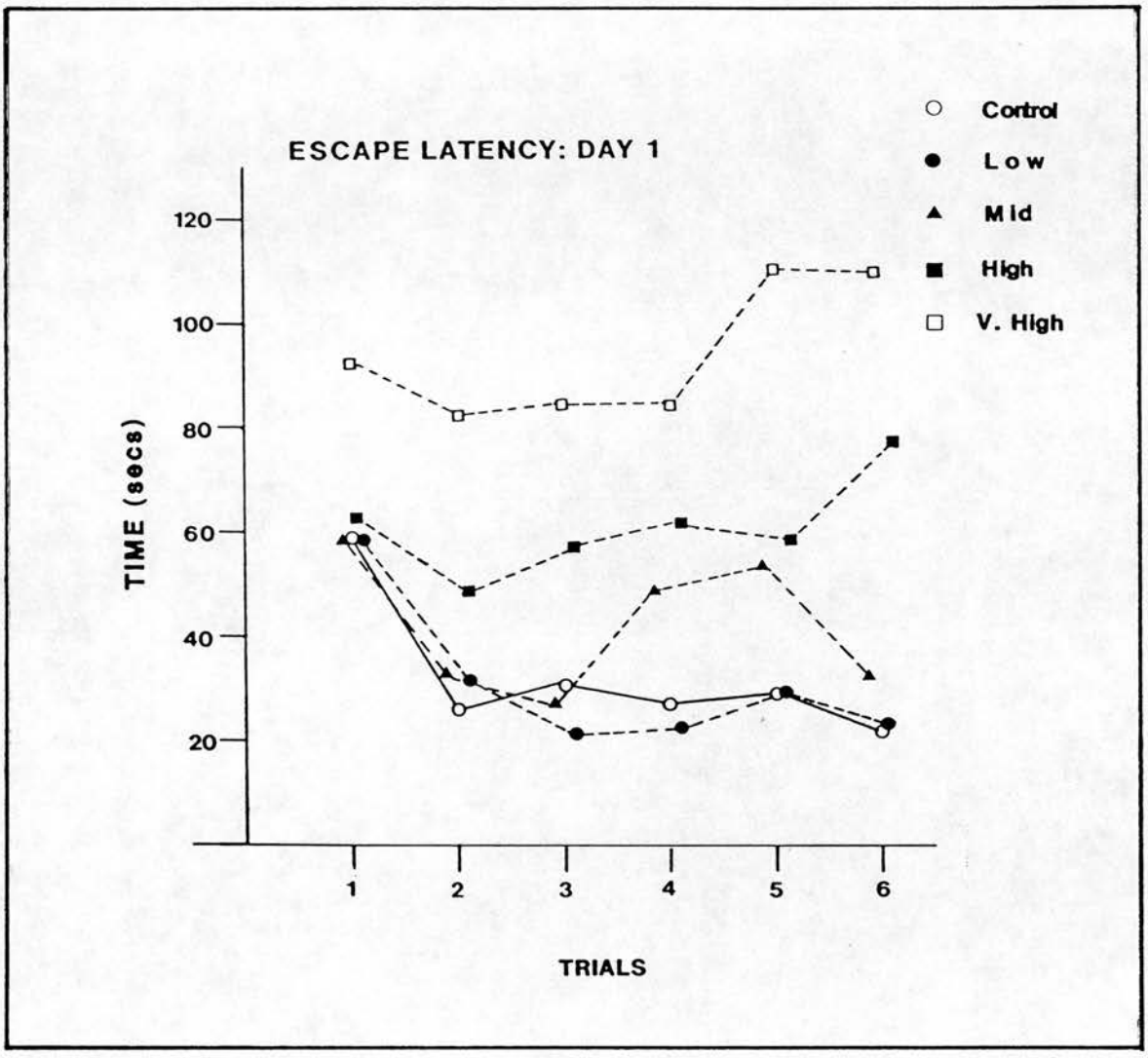


Fig. 2.13A:
DOSE-RESPONSE STUDY
Mean group escape latency for the 6 trials on day 1.

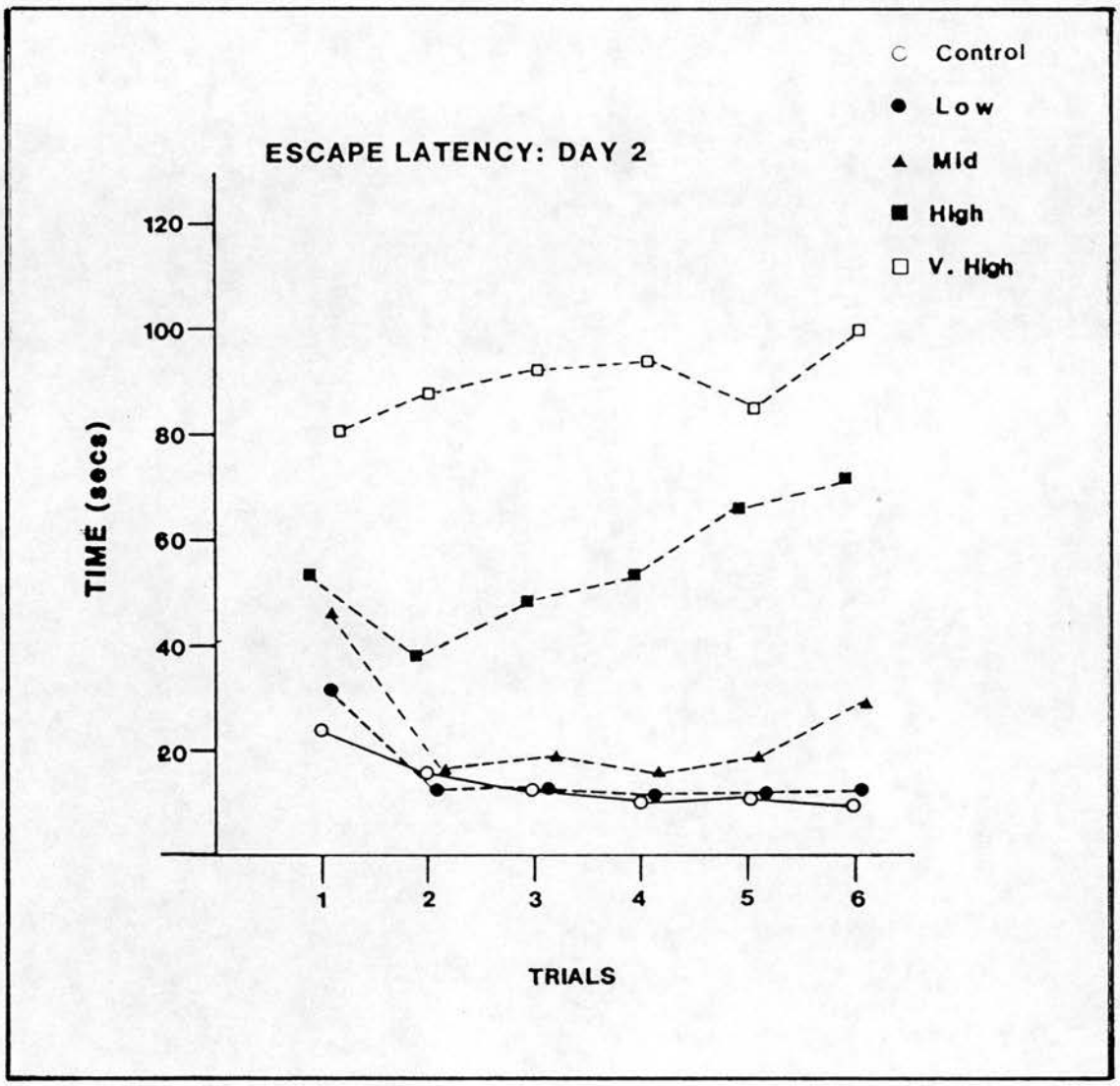


Fig. 2.13B:
DOSE-RESPONSE STUDY
Mean group escape latency for the 6 trials on day 2.

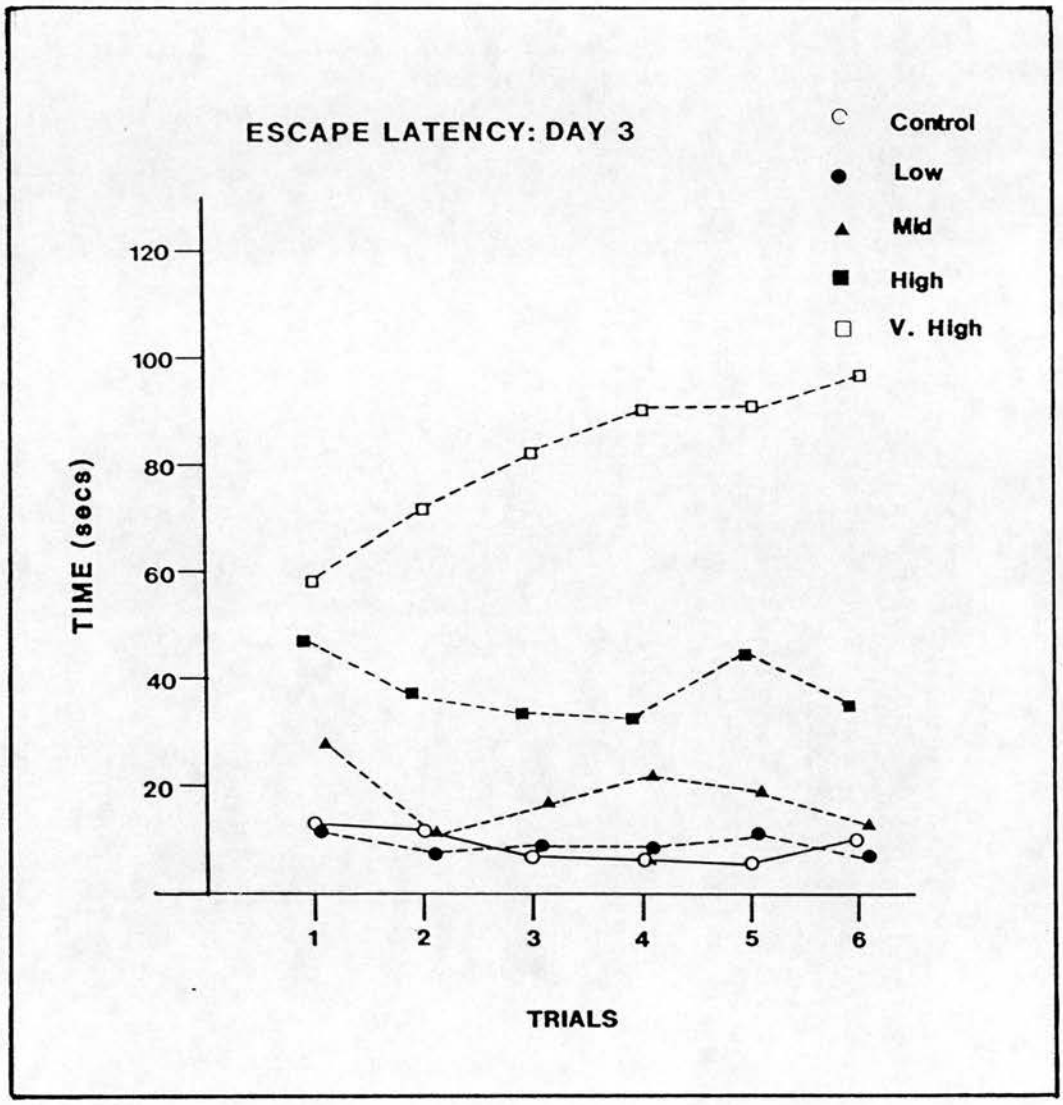


Fig. 2.13C:
DOSE-RESPONSE STUDY
Mean group escape latency for the 6 trials on day 3.

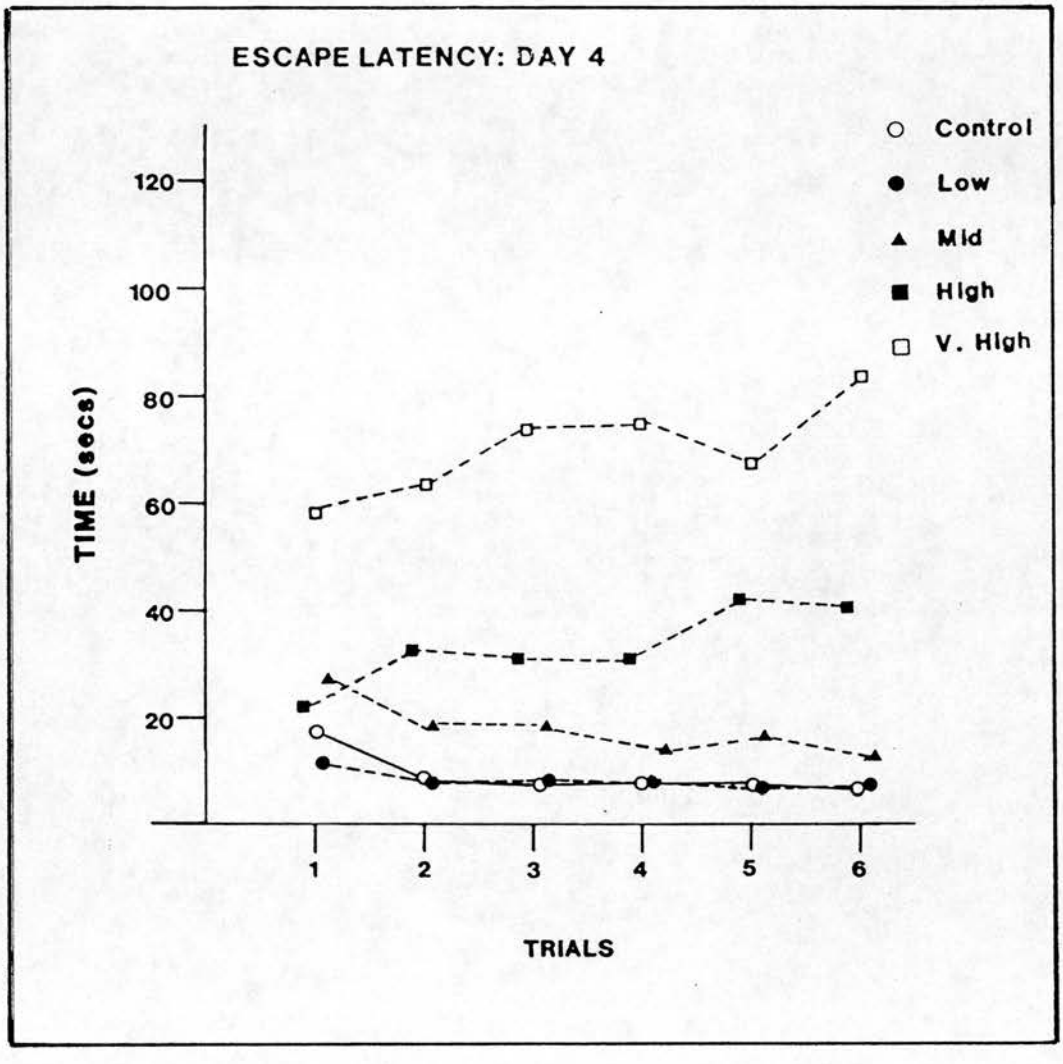


Fig. 2.13D:
DOSE-RESPONSE STUDY
Mean group escape latency for the 6 trials on day 4.

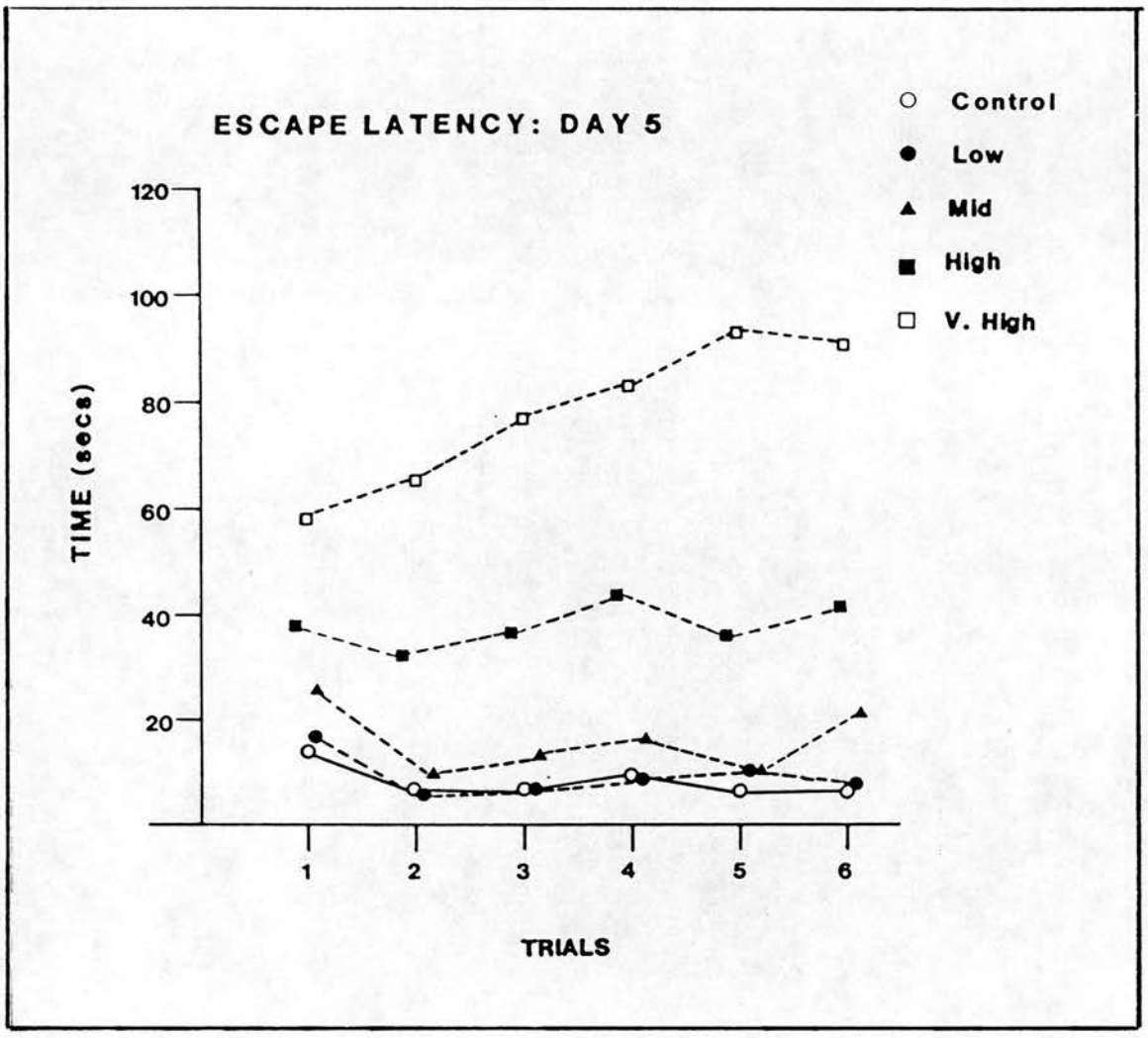


Fig. 2.13E:
DOSE-RESPONSE STUDY
Mean group escape latency for the 6 trials on day 5.

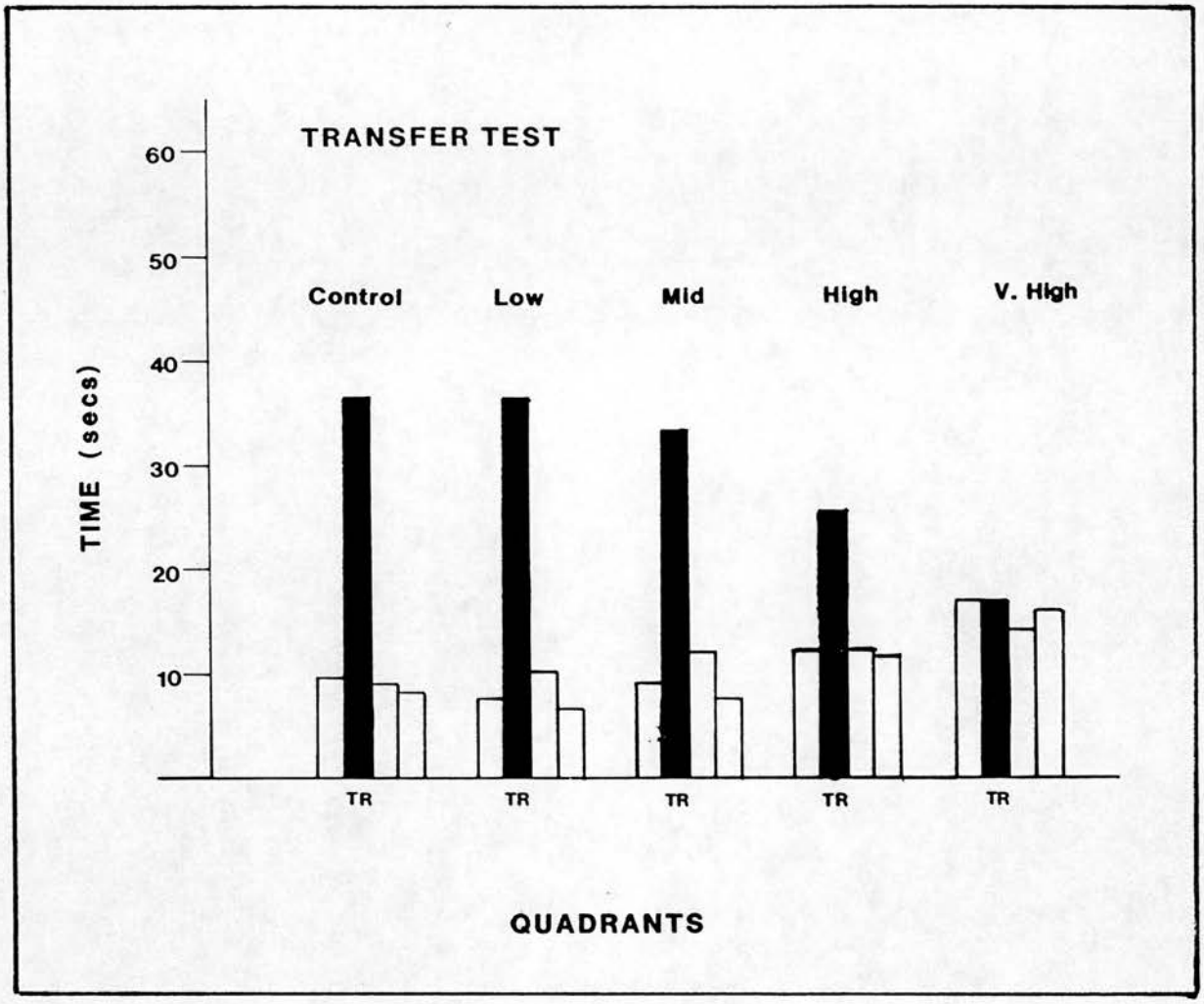


Fig. 2.14:
DOSE-RESPONSE STUDY
Histogram shows the amount of time spent in each quadrant during the 60s transfer test where the platform had been removed. Shaded bar is the training quadrant.

quadrant were not significantly different from control animals. They spent over 50% of their time in the training quadrant (control: 34.9(±1.5)s; low: 35.7(±2.0)s; mid: 32.2(±3.8)s; see Figs. 2.15A-C). The high concentration group distributed their time more evenly throughout the pool, spending only 24.9(±2.8)s in the training quadrant (see Fig.2.15D). The time that the very high concentration group spent in each of the quadrants was at the level of chance (see Fig.2.14) and from the swim path shown in Fig. 2.15E it can be seen that these animals were obviously thigmotaxic and showed no search pattern. This behaviour is discussed below with reference to sensorimotor impairment.

Discussion

Prior to surgery all animals had reached a comparable level in performance during pretraining. After surgery there was an AP5-dependent impairment over the next 5 days of training. Rather than a complete block, AP5 caused a slowing down of learning, and within the 5 days of testing, all groups reached an as ymptotic level of performance. The control low and mid groups showed a similar pattern of learning; they reached an assymptotic level of performance by day 3, with the mid concentration taking an average of 10 seconds longer to escape the water than the controls and the high concentration group taking on average, 30s longer. A similar pattern of spatial learning in the watermaze was reported by Morris et al (1986) in the original study using 40mM DL-AP5 and also by Robinson et al (1989) using 0.05 mg/kg (ip) MK-801, although the results from Robinson et al (1986) must be considered with some reservation because of the state-dependency for the activation of MK-801's receptor site. Until the assymptotic level of performance had been reached, most of the learning in the animals in the control, low, and mid concentration groups within each day was on the first trial, when animals had a delay of 24 hours since the last trial. The high concentration group showed no improvement in performance within each day, but did show some improvement between days; whereas the very high concentration group showed very little improvement in escape latencies over the 5 days testing.

The learning ability during the acquisition phase of each group was reflected in the transfer test. Unlike the 40mM DL-AP5 group (Morris, et al, 1986) and the mid MK-801 group (Robinson et al,

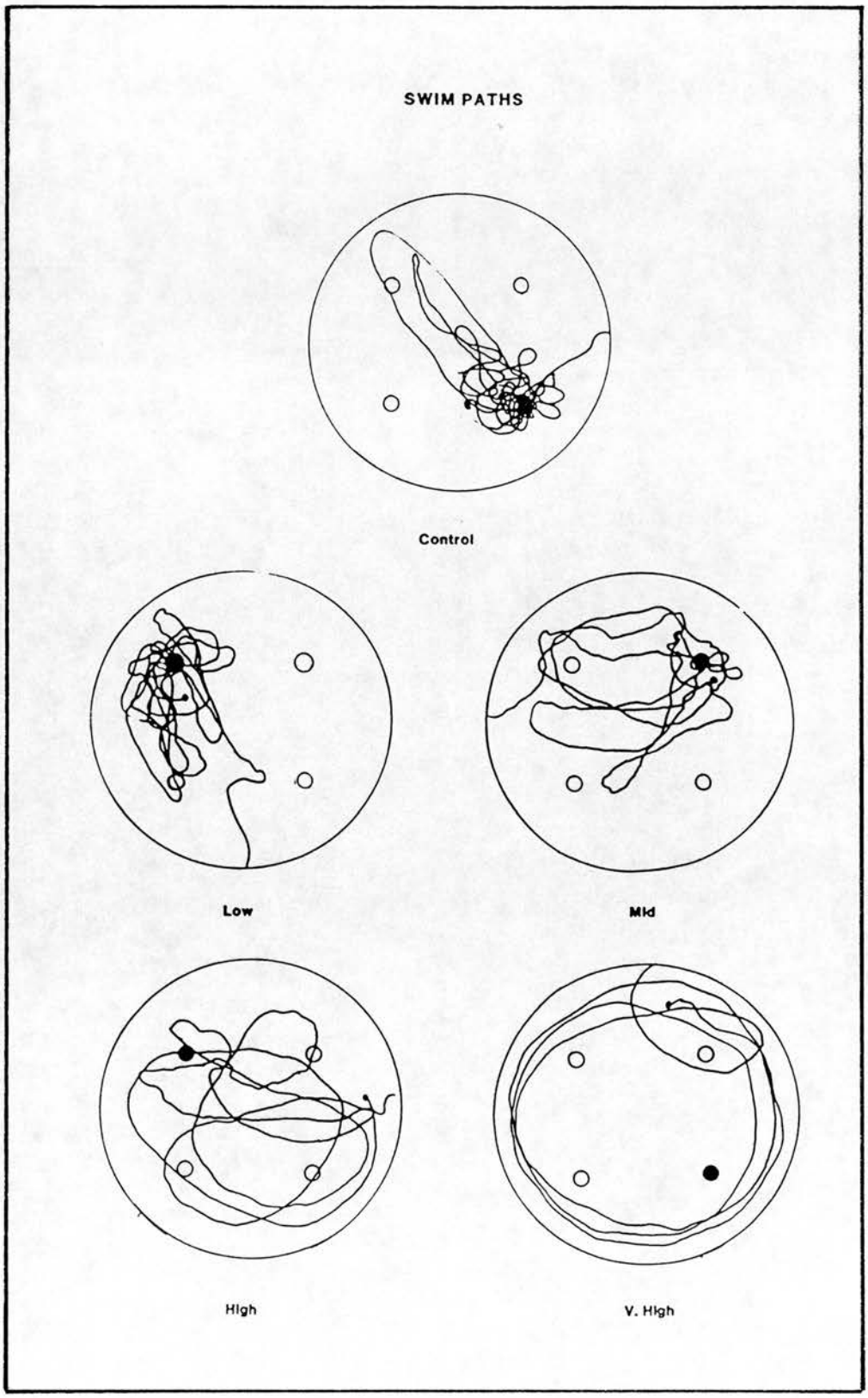


Fig. 2.15:
DOSE-RESPONSE STUDY
Swim paths from a single animal in each group represents the type of search pattern for the platform during the transfer test: (A) Control, (B) Low concentration, (C) Mid concentration, (D) High concentration and (E) Very high concentration.

1989) the mid concentration group in this experiment had gained enough information to show a pool bias for the training quadrant that was comparable to normal animals. These animals, however had 30 trials of acquisition by the time they were given the transfer test, whereas in the Morris study and the Robinson study animals had only 15 and 16 trials respectively before the transfer test. In a similar manner to the other two studies the high and very high concentration groups did not show a spatial bias. Comparisons of the type of learning impairment caused by blocking the NMDA receptor complex seems to be a slowing down in the acquisition of the task.

The level of impairment displayed by the very high concentration group was much more severe than that on all the behavioural measures: they showed no learning during the acquisition phase and, in fact, their escape latencies were longer than that in pretraining when they were prevented from using spatial cues. During the transfer test, the time spent in each quadrant showed that they had not gained any knowledge of the platform position and their swim path suggested they had adopted a thigmotaxic strategy. High concentrations of NMDA receptor antagonists have been known to cause sensorimotor impairment (Compton et al, 1987, Contreras et al, 1986, 1988; Koek et al, 1987), and these animals did display some of the signs of sensorimotor impairment. Typically, they showed signs of ataxia, inability to remain on the platform either by continuously falling or swimming off, continued swim movements when held out of the water and lateral sensory neglect (such that if the animal brushed its side against the platform it sometimes showed no sign that it had felt it).

These sensorimotor effects have been well documented; NMDA receptor-complex antagonists have been shown to have muscle relaxant (Turski et al, 1985a, 1987) and sedative effects (Bennet and Amrick, 1986). Local injections of NMDA antagonists to the spinal cord (Cahusac et al, 1984) or to the ventromedial thalamus (Klockgether et al, 1986) have been reported to cause analgesic and motor effects and response to sensory input is blocked by iontophoretic application to the thalamus (Salt, 1984; Eaton and Salt, 1989). These effects together with the more global effects of ataxia and stereotypy have led, inevitably, to the moot point of whether the impairment is one of performance rather than learning (Keith and Rudy, in press).

The question is not new and has been partly addressed by Morris (1989) who showed that the sensorimotor element could be reduced or eliminated by allowing animals to gain experience with the nonspatial requirements of the task prior to drug infusion. He found that if animals were first pretrained to find a randomly placed platform inside a pool surrounded by curtains, they showed as great an impairment on the subsequent drug-phase as AP5 treated animals but with a much reduced sensorimotor abnormality. This indicates that the sensorimotor side-effects of AP5 can be partially dissociated from the spatial learning impairment. Morris's (1989) finding led to the incorporation of 12 trials of pretraining into the general procedure used in this experiment.

In view of the fact that the sensorimotor effects may mask a learning impairment by causing an impairment in performance, analyses of variance were repeated on all the behavioural data excluding the very high concentration group. There was still a significant dose-dependent effect of AP5 on all measures. The animals in the very high group were defined as those animals having in excess of 0.31nmol/mg. In actual fact, the mean concentration of AP5 in the hippocampus of the animals in the "main series" of this group was 0.41nmol/mg and most of these animals had a concentration on at least 0.4nmol/mg; a concentration one and a half times greater than the high concentration group that showed impaired behaviour without sensorimotor effects or only showed mild effects that were transitory. The animals in the high concentration group occasionally showed mild motor symptoms that occurred transiently. In the early stages of the training they sometimes fell off the platform when they shook themselves on getting out of the water. After several trials, however they stopped falling in the water but simultaneously they stopped the wet dog shakes. It is therefore not certain whether the motor impairment stopped or whether the animals learned not to make certain movements that would cause them to fall in the water.

2.2.vi. Electrophysiological Analysis

Under conditions of low frequency stimulation, the mean field potential amplitude (mV) did not differ across groups ($F<1$). The slope function (mV/msec) did differ in a dose-dependent manner ($F(4,60)=3.25$; $p<0.05$; see Figs. 2.16A and B). However, a Newman

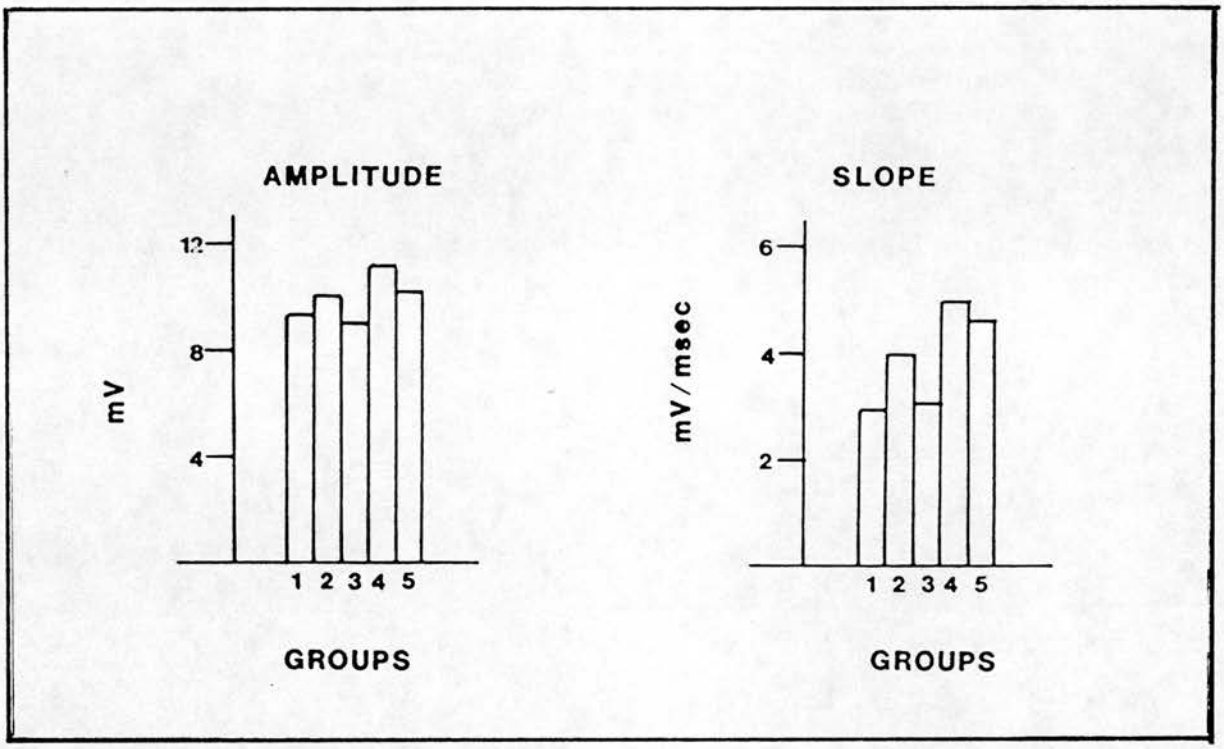


Fig. 2.16:
DOSE-RESPONSE STUDY
Change in (A) the amplitude (mV) and (B) the slope function (mV/msec) of the field potentials recorded at low frequency (7mV) across groups: (1) Control; (2) Low concentration; (3) Mid concentration; (4) High concentration and (5) Very high concentration.

Keuls post hoc test showed the difference to be only between the two groups that showed the biggest difference: the controls and the high concentration ($p<0.05$).

AP5 caused a dose-dependent decrease in the amount of LTP that could be evoked, such that the higher the concentration the less the amount of LTP. There was a significant difference in the percentage increase in the slope function of the field potentials measured 30 minutes after the tetanus when compared with the baseline, ($F(4,60)=17.01$; $p<0.001$). Control animals and the low concentration group showed essentially the same amount of potentiation 21.0% and 20.0% respectively. The Mid concentration group increased only 8.0% above baseline, the high concentration showed no potentiation and was below baseline by 1.0%, while the very high concentration group showed only 1.0% potentiation (see Figs. 2.17A-E).

The difference in the mean pre-LTP slope values under low frequency stimulation complicates the interpretation of the AP5 induced blockade of LTP. Specifically, it may have been the case that the high concentration group did not show LTP because its mean slope value was already at or near its assymptotic level, rather than because of the AP5. To investigate this possibility further, a correlation was carried out between the slope values under low stimulation conditions and the percentage LTP of each animal in the two groups in which LTP could be evoked, namely the control and low concentration groups. This correlation was found to be non significant ($r=.08$; $p>0.21$) and the finding implies that the blockade of LTP in the high concentration group is unlikely to be due to its high mean slope value.

Discussion

Measurement of the amount of LTP that could be evoked in the presence of different concentrations of AP5 in this experiment was a means to an end, and so the assessment of the electrophysiological measure was a simple one.

The effect of AP5 on the induction of LTP is quite clear cut: there was a dose-dependent impairment which showed that the higher the concentration, the greater the block of LTP. In the control group, 2 animals showed some post-tetanic-potentiation after the tetanus but no LTP. This did not seem to be because stimulation was

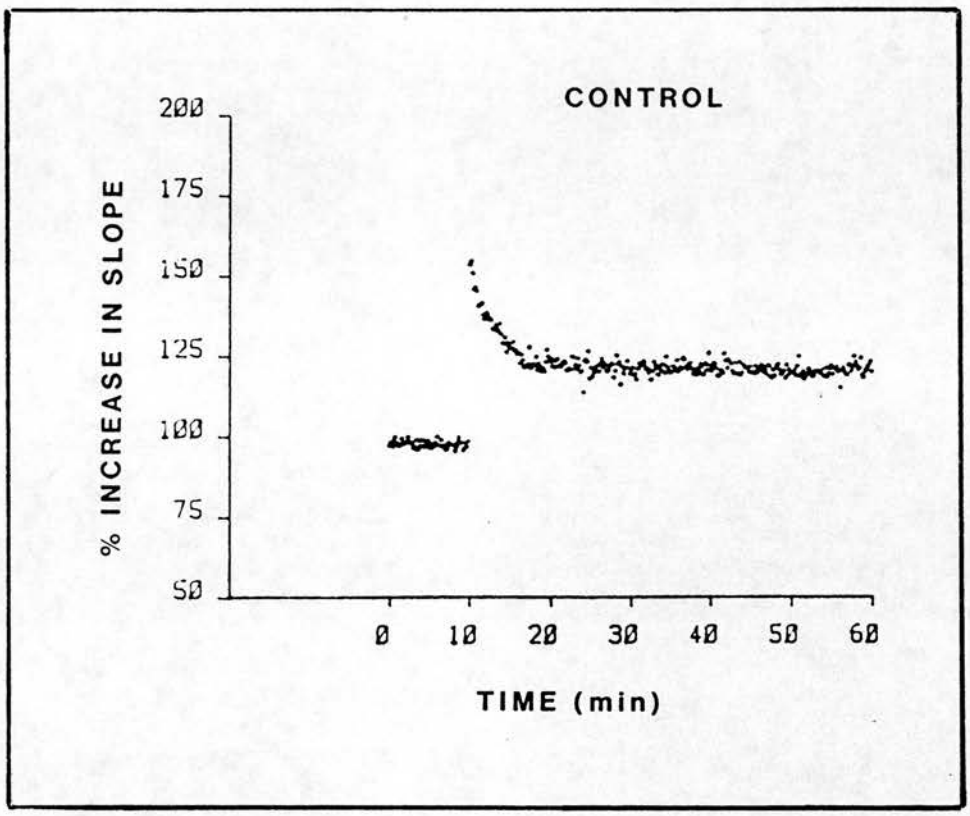


Fig. 2.17A:
DOSE-RESPONSE STUDY
Mean percentage change in slope function of field potentials (mV/msec) measured against the baseline for Control group after a high frequency tetanus.

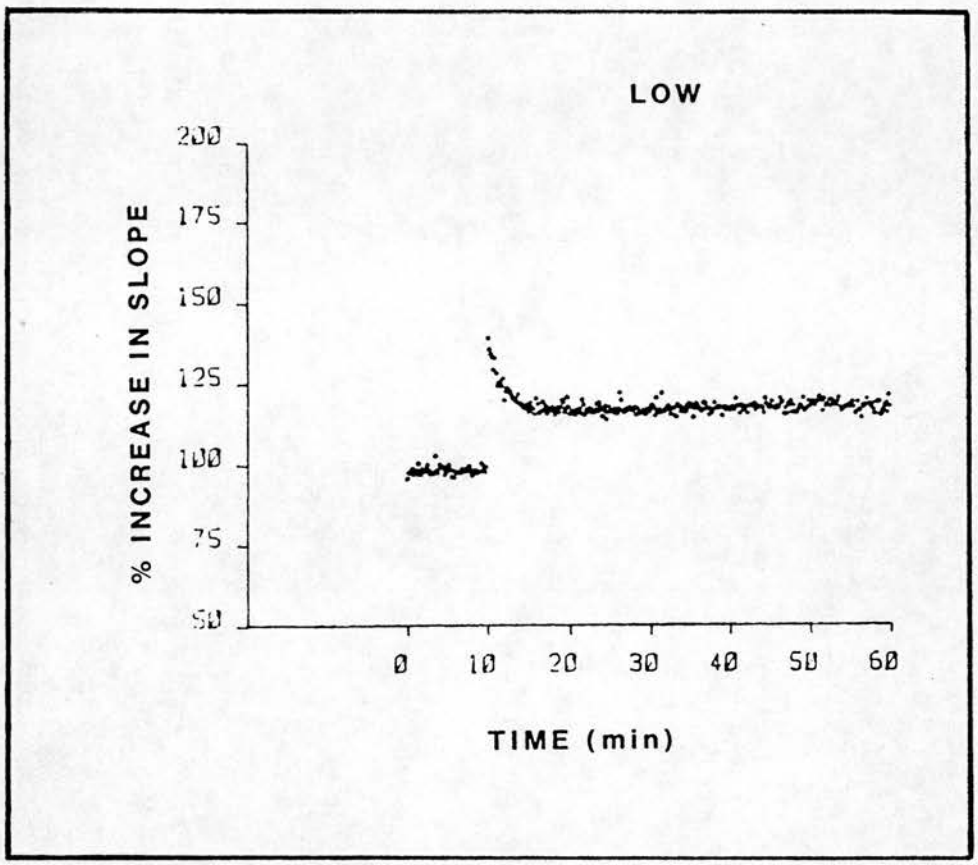


Fig. 2.17B:
DOSE-RESPONSE STUDY
Mean percentage change in slope function of field potentials (mV/msec) measured against the baseline for animals in the Low Concentration group after a high frequency tetanus.

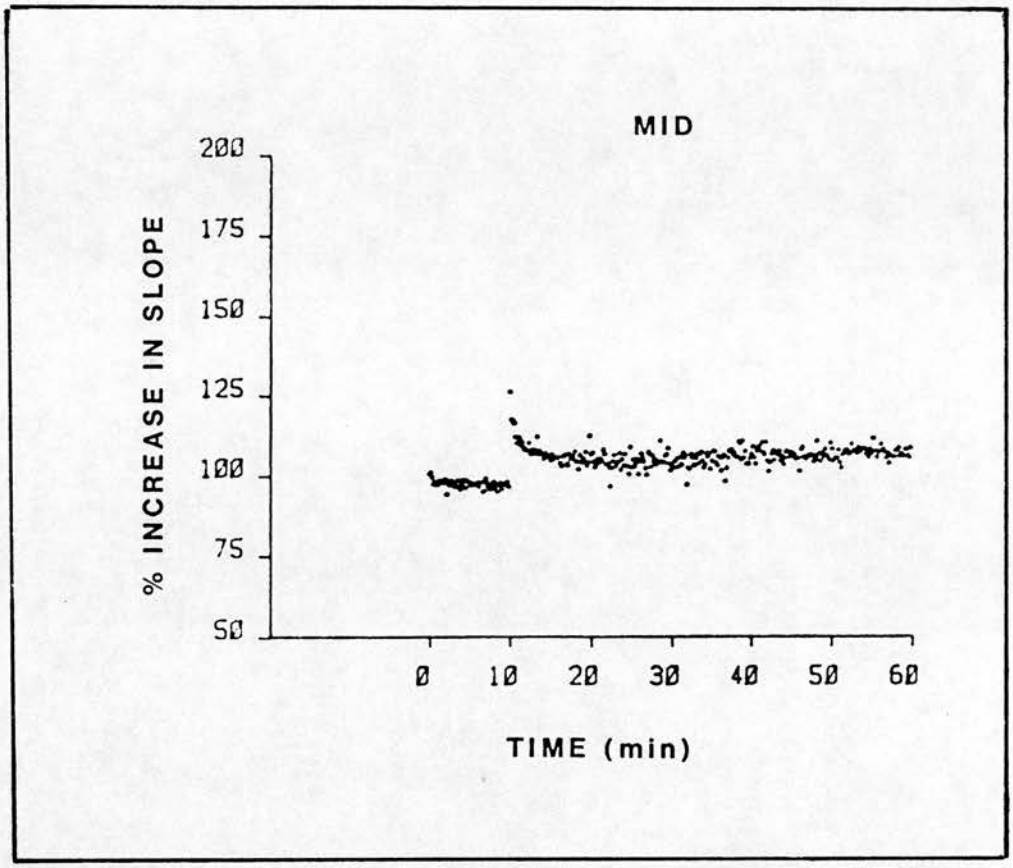


Fig. 2.17C:
DOSE-RESPONSE STUDY
Mean percentage change in slope function of field potentials (mV/msec) measured against the baseline for animals in the Mid Concentration group after a high frequency tetanus.

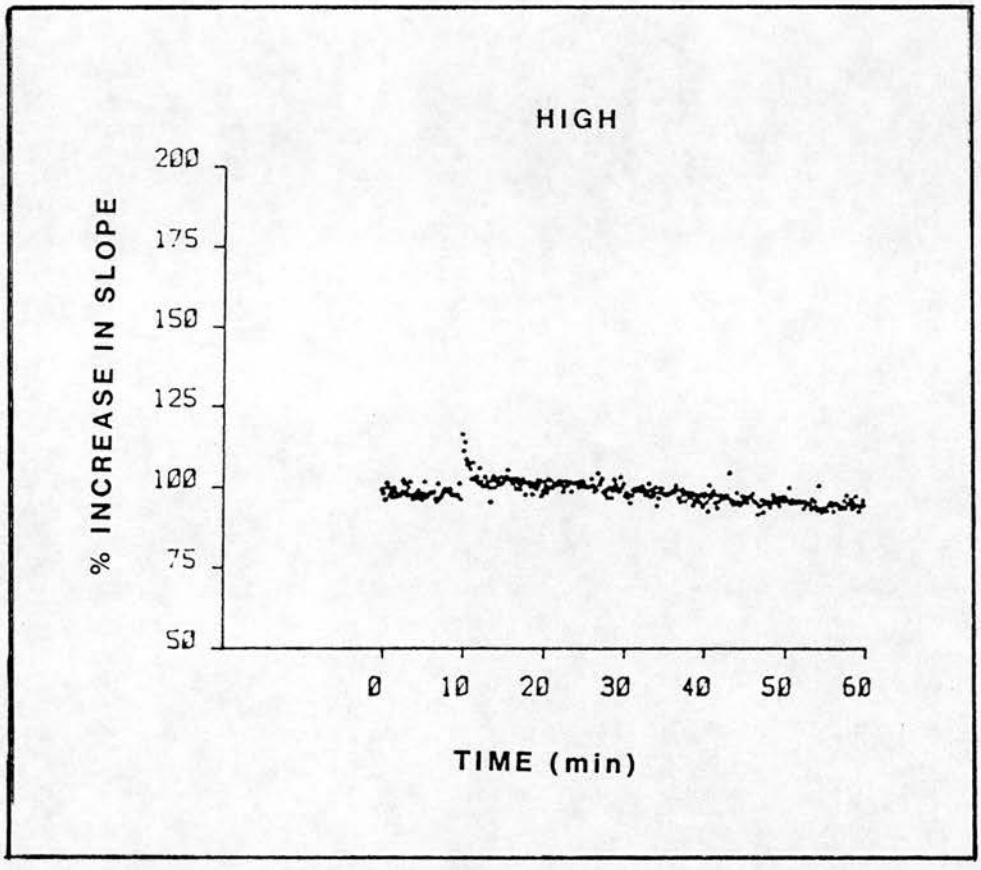


Fig. 2.17D:
DOSE-RESPONSE STUDY
Mean percentage change in slope function of field potentials (mV/msec) measured against the baseline for animals in the High Concentration group after a high frequency tetanus.

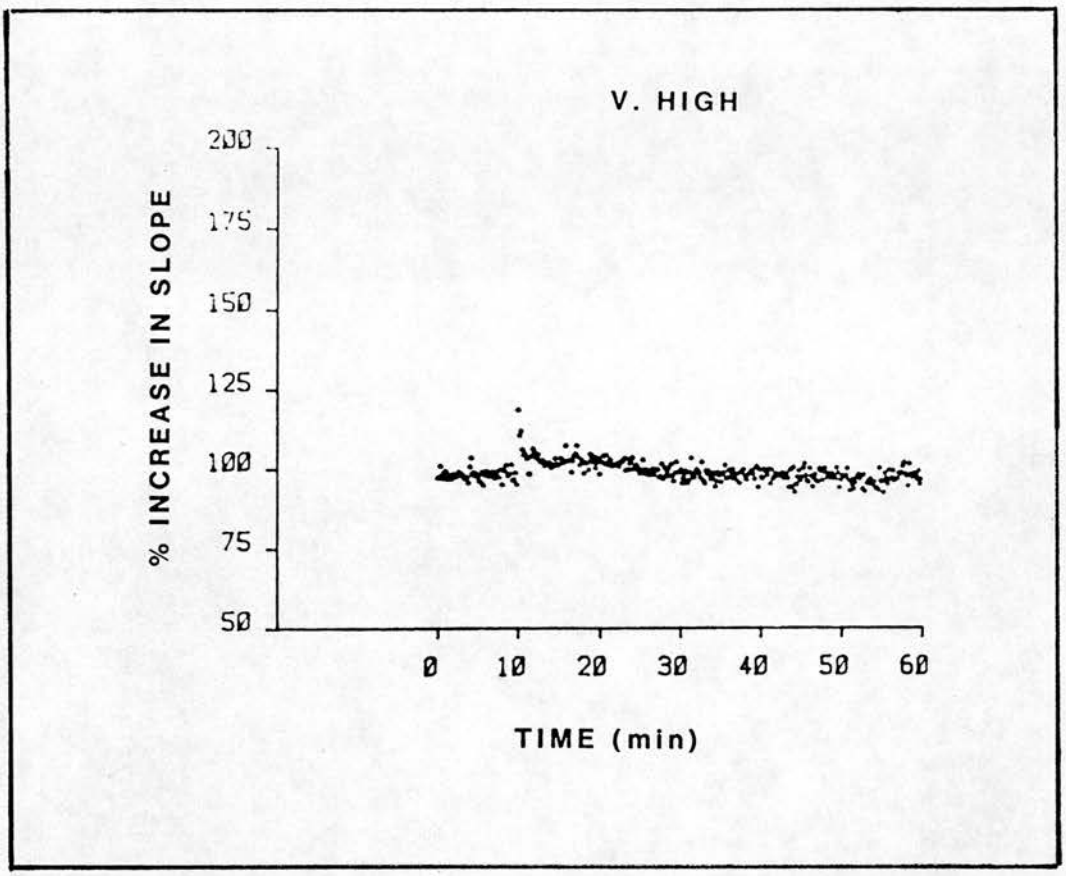


Fig. 2.17E:
DOSE-RESPONSE STUDY
Mean percentage change in slope function of field potentials (mV/msec) measured against the baseline for animals in the Very High Concentration group after a high frequency tetanus.

below the threshold required to evoke LTP (McNaughton et al, 1978) as the size of the potentials during low frequency, were well within the range of those animals in which LTP could be evoked. Teyler and Discenna (1987), in their review of LTP, find that between a 10 and 60% failure rate to induce LTP has been reported by various labs.

The results from the amplitude and slope function values at low frequency stimulation were not so clear cut, for two reasons: (i) The amplitude was not affected by AP5 in this study, whereas Errington et al (1987) and Abraham and Mason (1988) found that AP5 and CPP did caused a reduction in the size of the amplitude of the population spike. Errington et al (1987) point out that, in their preparation, although the amplitude decreased there was minimal effect on the slope of the population EPSP. (ii) The slope function during low frequency stimulation showed an increase with higher concentrations of AP5, unlike the small effect in the opposite direction reported by Errington et al (1987). In this study, the difference in slope values was also slight and only between the two groups showing the greatest difference. The question of whether there was a relationship between the size of the slope function and the percentage LTP was resolved by a lack of correlation between these two measures in the controls and the low concentration groups. One explanation for the slope value difference was that many of the control animals were run during the early replicates of the experiment. During this period, skills required for running the experiment improved, including optimal placement of electrodes. Although animals from most groups were run in each replicate, as the numbers increased in each group, the number of controls per replicate were reduced in number.

vii. Correlations

AP5 showed a dose-dependent impairment in spatial learning and in the amount of LTP that could be evoked. In order to see more explicitly whether these two dose-response effects were related, correlations were carried out on a single measure taken from each the three aspects of testing. The correlations were based on individual scores of animals in the "main series" (N=58) rather than group means.

The measures taken from each test aspect were:

(i) The amount of AP5 (nmol/mg wet weight) measured in the hippocampal tissue.

(ii) The amount of LTP that could be evoked. This measure was the mean of 10 slope values taken 30 minutes after the tetanus.

(iii) The mean overall escape latency for the 30 trials during the behavioural testing.

The results are shown in Figs. 2.18, 2.19, and 2.20 and have a high correlation factor between all measures:

(i) AP5 (tissue) and LTP: ($r = -.61$, $p<0.001$).

(ii) AP5 (tissue) and Escape Latency: ($r=.81$, $p<0.001$).

(iii) LTP and Escape Latency: ($r=.61$, $p<0.001$).

Discussion

The high correlations between the three measures suggests the two dose-response effects that AP5 has on spatial learning and on induction of LTP may be elicited via the same mechanism: blockade of the NMDA receptor. In this experiment, LTP could be evoked in the mid (8.0%) and low (20.0%) concentration groups, while the high and very high concentration groups showed a complete block of LTP. Similarly the mid and low concentration groups showed spatial learning, while the high and very high concentration groups showed impairments.

However, the mean concentration of AP5 in hippocampal tissue required to cause these behavioural and electrophysiological effects was, in absolute terms, very high. For example, the high concentration group had a mean tissue concentration of 0.27nmols/mg or, converting it to molar concentration, 270μM. In slice preparations (Collingridge et al, 1983; Harris et al, 1984), LTP can be completely blocked by 50μM D-AP5, a concentration that is approximately 5 times less than the concentration required to block LTP in this experiment. Given the fact that most of the AP5 had been removed from the tissue through excretion or metabolism, it would appear that there may be a fundamental difference between the

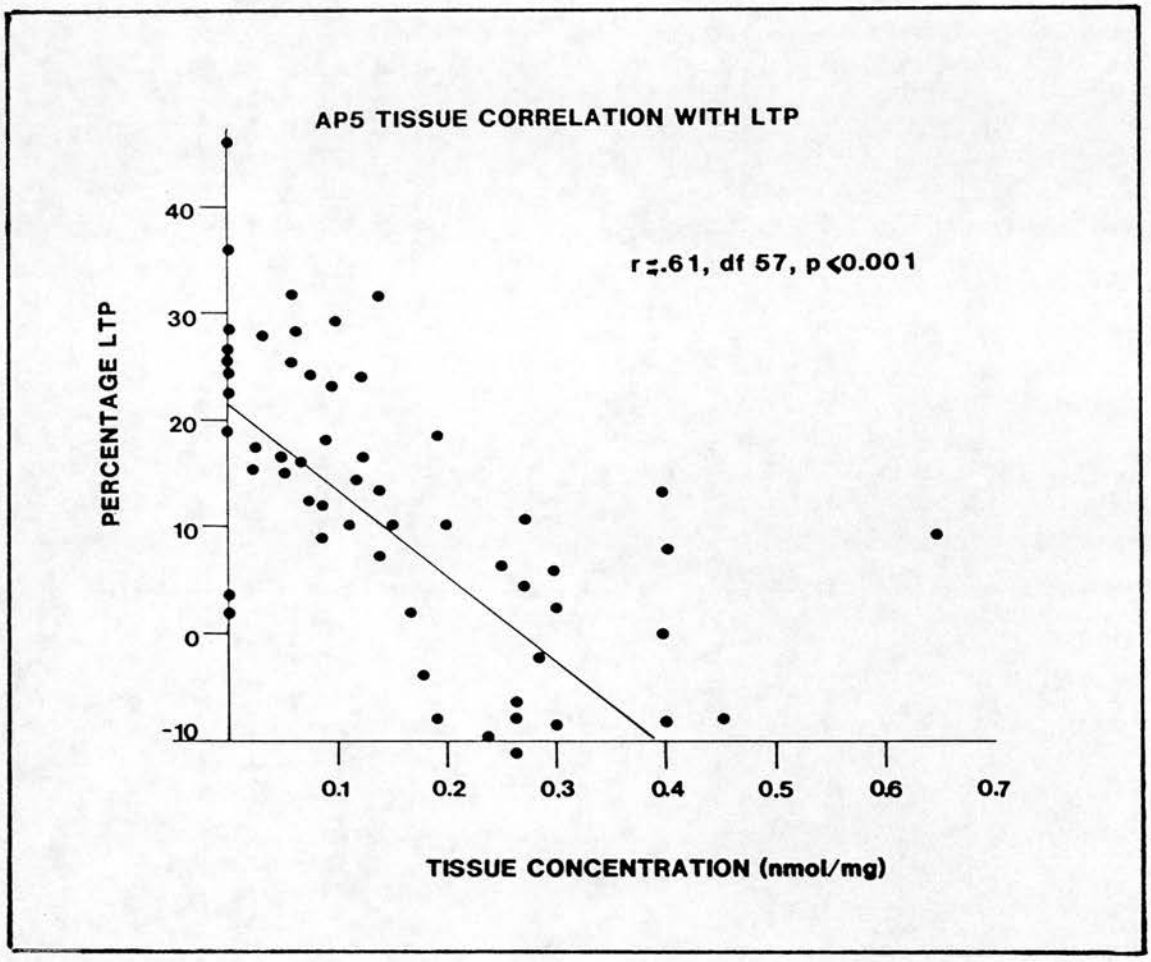


Fig. 2.18:
DOSE-RESPONSE STUDY
Correlation between AP5 concentration measured in hippocampal tissue (nmol/mg wet weight) and percentage increase in the slope function (LTP), based on individual scores.

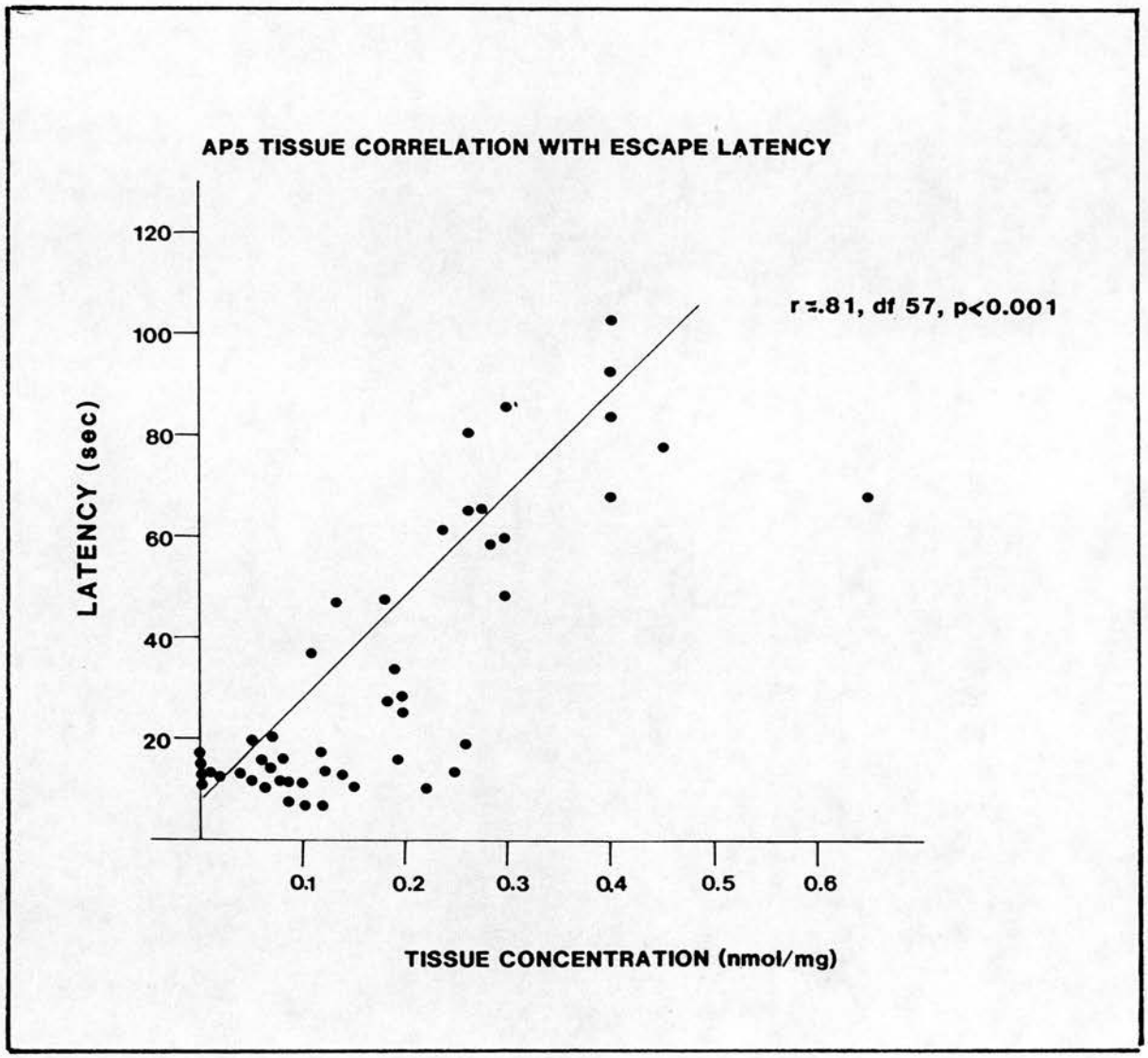


Fig. 2.19:
DOSE-RESPONSE STUDY
Correlation between AP5 concentration measured in hippocampal tissue (nmol/mg wet weight) and overall mean escape latency (seconds), based on individual scores.

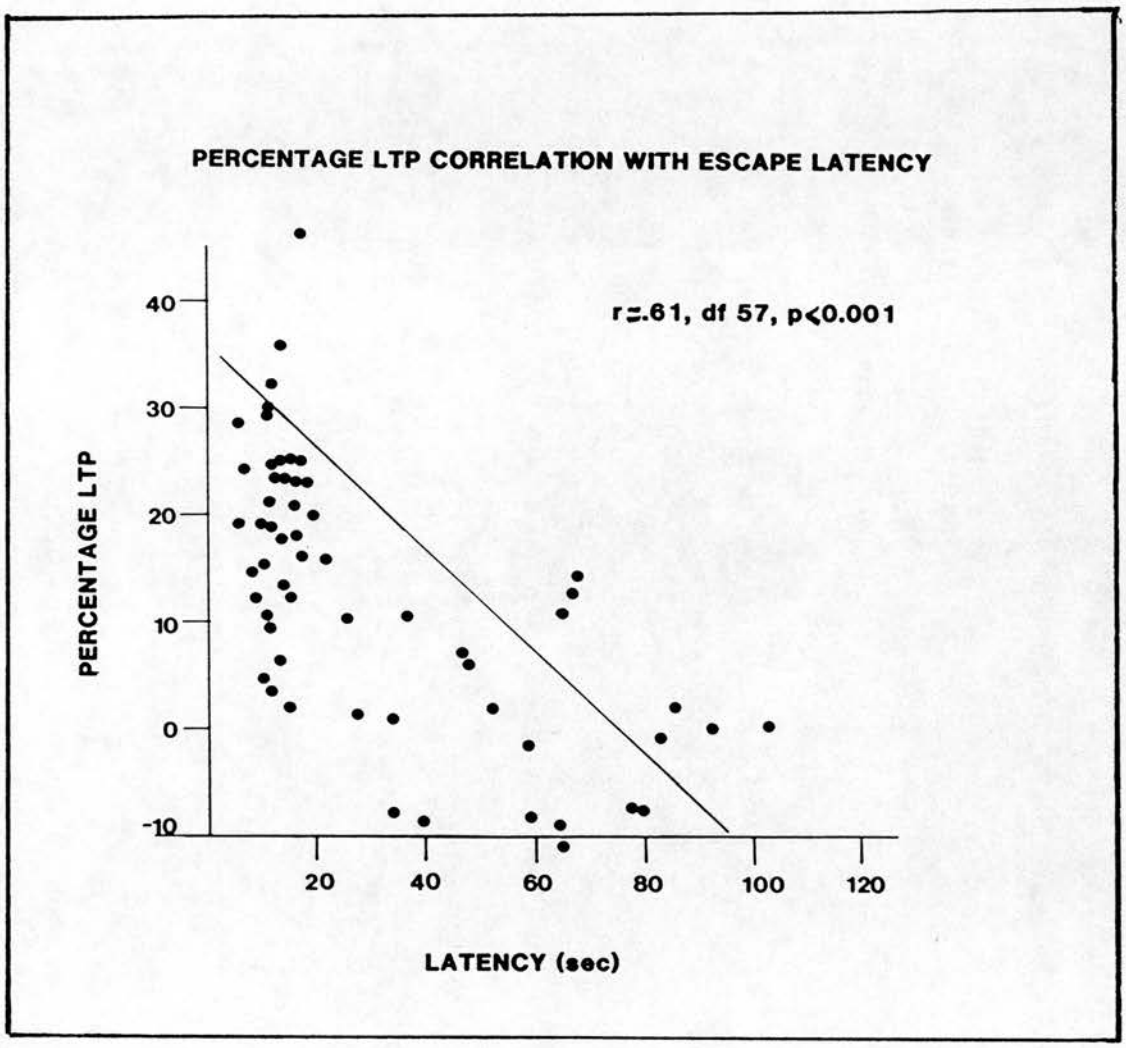


Fig. 2.20:
DOSE-RESPONSE STUDY
Correlation between the percentage increase in the slope function (LTP) and the overall mean escape latency (seconds) based on individual scores.

in vivo and *in vitro* preparations. It must be borne in mind, however, that the whole tissue content of AP5 does not indicate the amount of AP5 that is present in the extracellular fluid.

2.2.viii. Dialysate analysis

AP5 levels, and the levels of the primary amines (aspartate, glutamate, glutamine, taurine, alanine and valine) were sampled from the interstitial space in the hippocampus in order to estimate their concentrations in the extracellular fluid.

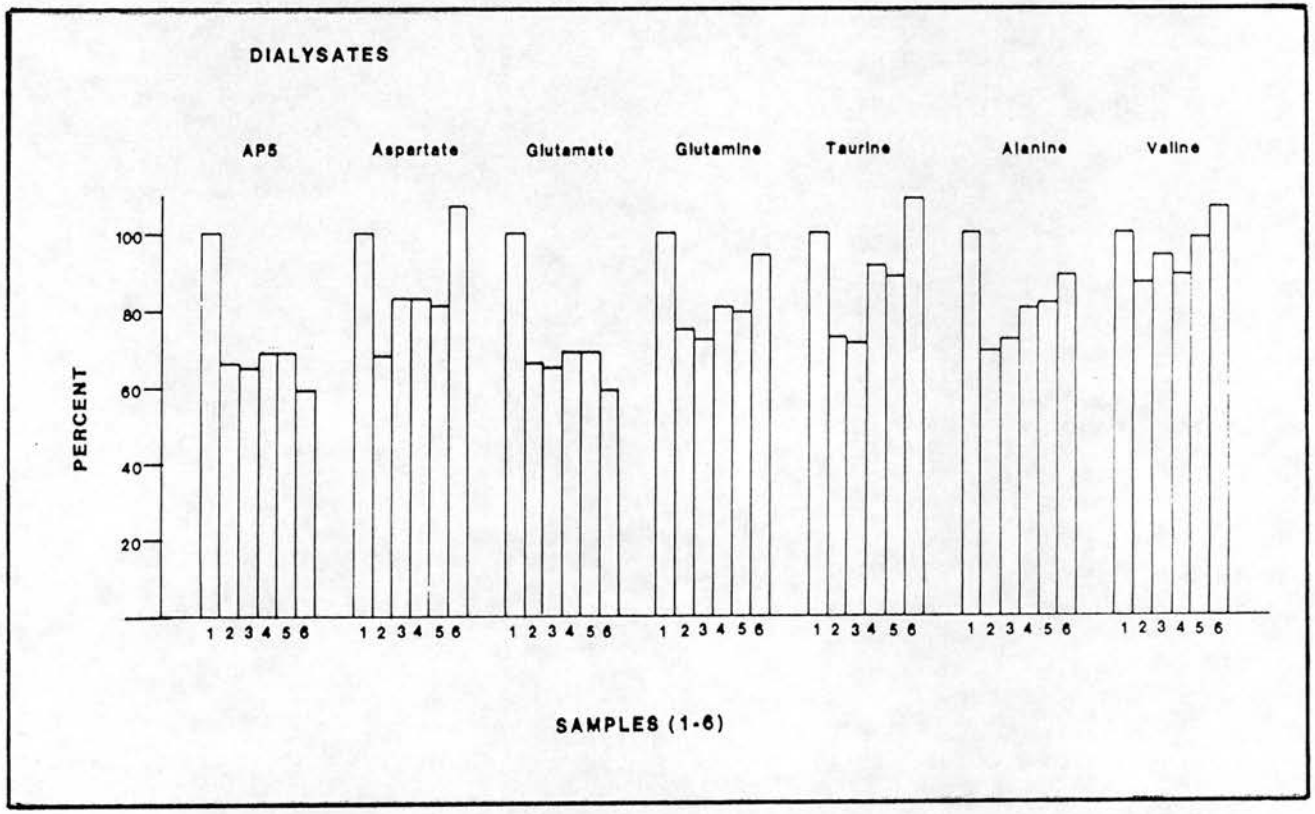
First of all, the change in recovery over time was monitored by collecting six 20 minute dialysate samples of AP5 and the amino acids from 8 animals in the high concentration group (see Fig. 2.21). From this group of animals, it was found that there was a considerable drop in the levels of AP5 and most of the amino acids over the first 20 minutes. After the second dialysate sample, there were some fluctuations, but the 4th and 5th dialysates remained the most stable and all analyses of variance carried out hereafter were done using these two dialysate samples.

Secondly, the mean dialysate concentration of AP5 measured in μM for each group (based on the 4th and 5th sample) was:

Control	0.00
Low concentration	0.11
Mid concentration	0.18
High concentration	0.55
Very high concentration	0.96

Analysis of variance (N=49: only a sample of 4 controls were used) showed that there was a significant dose-dependent increase in the amount of AP5 in each group ($F(4,44)=20.27$; $p<0.001$; see Fig. 2.22). Furthermore, a Pearsons product moment correlation carried out on the amount of AP5 measured in the dialysate and in the hippocampal tissue (using the individual animal values) was highly significant ($r=.80$; $p<0.001$; see Fig. 2.23). These values were between 350 and 850 times lower than the total hippocampal tissue concentration - a point which will be addressed later.

Fig. 2.21:
DOSE-RESPONSE STUDY
Percentage change in the levels of AP5 and amino acids measured in the six, 20 minute dialysate samples taken from 8 animals from the High concentration group.



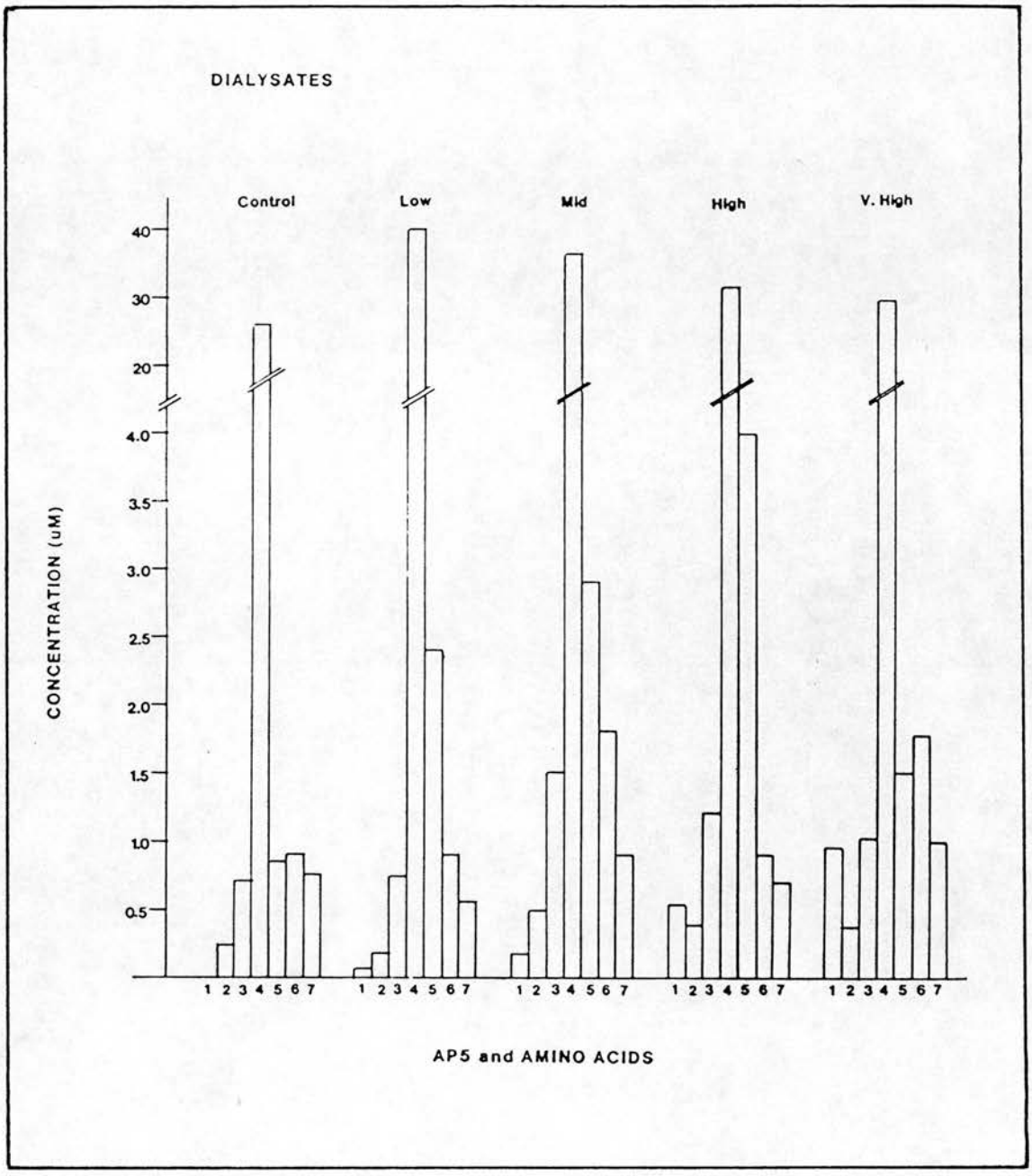


Fig. 2.22:
DOSE-RESPONSE STUDY
Histogram of the mean concentration of AP5 and the amino acids (μmol) in the 4th and 5th dialysate sample for each group: (1) AP5; (2) aspartate; (3) glutamate; (4) glutamine; (5) taurine; (6) alanine and (7) valine.

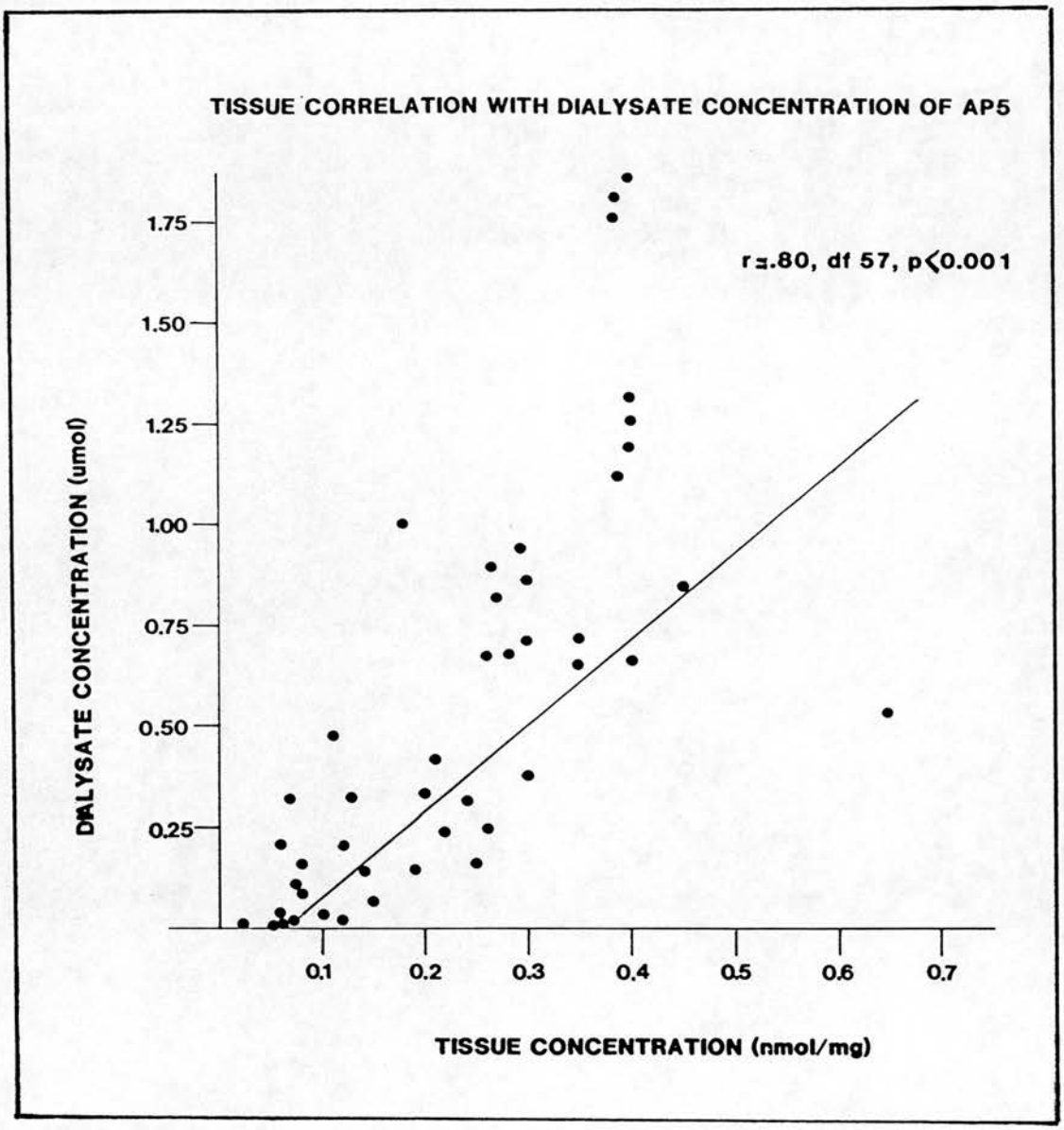


Fig. 2.23:
DOSE-RESPONSE STUDY
Correlation between AP5 concentration in dialysates (μmol) and combined hippocampal tissue (nmol/mg wet weight) based on individual scores.

Analysis of variance carried out on the amino acids showed the expected difference in levels ($F(5,220)=59.09$; $p<0.001$; glutamine being highest in concentration), but there was no significant difference between groups ($F<1$) nor, importantly, any interaction between groups and amino acid level ($F<1$; see Fig. 2.22).

Probe Recovery

The probes were calibrated *in vitro* by placing them in a beaker containing a known concentration of AP5 (50μM). Two flow rates were used (1.25μl/min, the rate used to sample from the hippocampus, and also 2.50μl/min) to calculate the amount of AP5 that could pass through the dialysis membrane and be "harvested" *in vitro*. The percentage recovery rate at 2.5μl/min was 2.90(±0.62)% and at 1.25μl/min, it was 6.2(±0.74)%.

The percent recovered at 1.25μl/min was used to calculate an estimated amount of AP5 in the ecf from the animals in the "main series" (see Nr. 4 in Table 2.2B for details of the calculation). Taking this recovery rate into account, the best estimates for mean extracellular concentration across groups, calculated in μM, were:

Control	0.00
Low concentration	1.51
Mid concentration	3.72
High concentration	8.76
Very high concentration	14.77

These concentrations were then compared with the mean concentration of AP5 in the hippocampal tissue (converted to the same units: see Nrs 2 and 5, Table 2.2B for calculations) and it was found that the tissue content of AP5 was between 22 and 51 times greater than that estimated to be in the extracellular fluid (see Table 2.2A).

Discussion

The AP5 concentration in the dialysates showed a dose-dependent increase in the same manner as the concentration of AP5 in the hippocampal tissue. The fact that the amino acid markers did not

differ between groups was an indication that AP5 was not exerting its effect through some secondary or modulatory effect on the metabolites or precursors of the amino acids that were measured.

There was a highly significant correlation between the concentration of AP5 in the hippocampal tissue and in the dialysates, but the amount of AP5 calculated to be in the ecf was between 22 and 51 times lower than the concentration in the tissue. The estimated value based on the probe recovery (rather than the exact amount measured) gives an indication of the amount of AP5 that is available to interact at the receptor (Ungerstedt, 1984; Hamberger, 1985; Benveniste et al, 1989). It must be pointed out, though, that the amount estimated to be in the ecf may be lower than the actual amount (see Benveniste, 1989, in section 2.1.ix on microdialysis theory). Compared to the amount measured in the tissue, the estimated value of AP5 in the extracellular fluid is more comparable to those concentrations shown to block the induction of LTP in the hippocampal slice (Collingridge et al, 1983; Harris et al, 1984) and shown in ligand binding studies that represent the Kd values for receptor occupancy (Monaghan et al, 1988).

The fact that there is such a difference between the tissue concentration and the extracellular concentration of AP5 raises the question of where the AP5 in hippocampal tissue is actually located. One possibility is that it is taken up into neurons or glia. A second possibility is that the AP5 may somehow be trapped to the tissue, making much of it inaccessible to the receptor.

2.2.ix. Summary

This experiment has shown that AP5 impairs spatial learning and blocks the induction of LTP in a dose-dependent manner. It substantiates the preliminary findings of Morris et al (1986) and lends support to the theory that a form of neural plasticity such as LTP may underlie learning and be involved in information storage (Hebb, 1949; Bliss and Lomo, 1973; Bliss and Gardner-Medwin, 1973 McNaughton, et al, 1986). These two parallel but independent dose response functions however do not alone lend definitive proof to the theory that the NMDA receptor is involved in learning through the induction of LTP. The high correlation between AP5 concentrations, the learning impairment and the amount of LTP that could be evoked in a single animal, however, does weight the data in favour of the

theory. Within the theoretical parameters of the experimental design used here, the study indicates that the link between the NMDA receptor and spatial learning is through the induction of LTP because there is no concentration of AP5 that blocks the induction of LTP without impairing spatial learning.

During the course of running these experiments several important issues which concern the interpretation of the data emerged. These relate to levels of AP5 in the brain during the experimental period and the nature of the learning impairment that was generated. These questions cast a complicating effect on the interpretation of the data in the light of the theoretical framework. Accordingly, a series of 4 control studies were carried out in an attempt to clarify the issues; these are described in the next section.

2.3 CONTROL STUDIES FOR THE DOSE-RESPONSE STUDY

Four experiments are described in this section which address the two points raised in the last section. The first experiment addresses the question of the discrepancy between the amount of AP5 in the tissue and the amount sampled from the interstitial space of the hippocampus in the dialysates. The second experiment was designed to investigate whether there was any change in the level of AP5 during the experimental period that may relate to the change in the animals performance during the behavioural testing. The third and fourth experiments examined whether the AP5 induced impairment in spatial learning could be attributed to disruption of hippocampal function or alternatively to a drug induced disruption of sensorimotor performance.

2.3.i. Effects of EGTA on tissue and dialysate levels of AP5

Throughout the 12 replicates for the dose-response study there was a consistent discrepancy between the amount of AP5 measured in the tissue and that in the ecf. This was a reliable 30 fold increase in the tissue content of AP5 over the estimated ecf content (see Table 2.2A). Attempts to resolve this discrepancy have shown that AP5 is not taken up into cells or glia via a high affinity glutamate uptake system (Griffiths, unpublished observations; Kessler, pers. comm. to RGM. Morris). The following experiment was based on one suggestion (Watkins, pers. comm.) that AP5 may be trapped to the tissue by a calcium sensitive mechanism. If this was the case, then infusion of the calcium chelator EGTA into the hippocampus would chelate calcium and release trapped AP5 into the ecf.

Procedure

This experiment was carried out on 12 animals infused with 30mM D-AP5 in the final replicate of the dose-response study. Following the normal pharmacological procedure, where six 20 minute dialysate samples of ecf were taken from the hippocampus, a further three 20 minute samples were taken, using a modified infusate. In the normal procedure aCSF (Alza methodology) is pumped in to the hippocampus via the probe, but for the final three samples the infusate was

changed to a Ca^{++} free aCSF containing 20mM EGTA. After the last EGTA sample, the animals were sacrificed and their brains removed for tissue and histological assessment.

Results

Two dialysate samples of the ecf with EGTA present were compared with the earlier 4th and 5th samples taken using the normal aCSF infusions. There was a large increase in the amount of AP5 measured in the dialysates after the infusion of EGTA. The mean concentration in μM (N=12) for each time-sample was:

Pre EGTA	Sample 4	0.71
	Sample 5	0.52
Post EGTA	Sample 1	4.02
	Sample 2	4.25

An analysis of variance carried out on these 4 samples of AP5 showed a highly significant difference ($F(3,33)=7.51$; $p<0.001$; see Fig. 2.24) and a post hoc Newman Keuls analysis confirmed the difference was between the samples before and after the infusion of EGTA ($p<0.01$). Analysis of variance of the amino acid markers in each sample showed that EGTA did not affect any of them ($F(3,33)=1.16$; $p>0.30$; see Fig. 2.25).

Based on the recovery rate of the probe (6.2%) the mean concentration of AP5 present in the extracellular fluid after the infusion of EGTA was 69.7μM as opposed to 8.29μM estimated to be present before the infusion of EGTA, an 8 fold increase (see Table 2.3).

Discussion

EGTA chelates calcium in a chemical reaction, thereby removing the divalent cation from the ecf. The increase in the concentration of AP5 in the ecf following the infusion of EGTA strongly suggests that calcium is involved, though not necessarily directly, in the trapping of AP5 to tissue. From table 2.3, it can be seen that although the concentration of AP5 in the EGTA samples is greater than the normal dialysates it is not as great as the amount in the tissue. It might be expected that the amount of AP5 in the dialysate when EGTA is present should be the same as the tissue

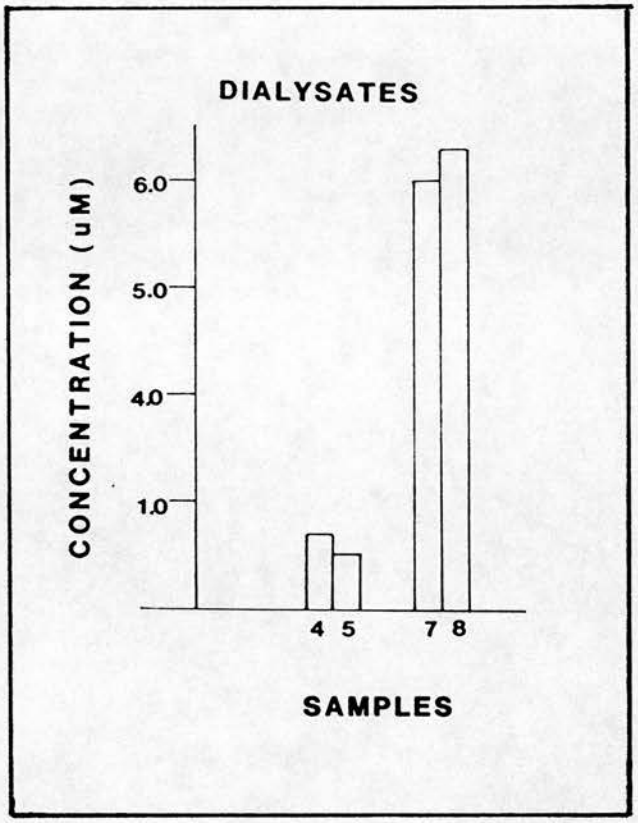


Fig. 2.24:
EGTA STUDY
Increase in level of AP5 measured in the dialysates before (samples 4 and 5) and after (samples 7 and 8) EGTA has been infused into the hippocampus through the dialysis probe.

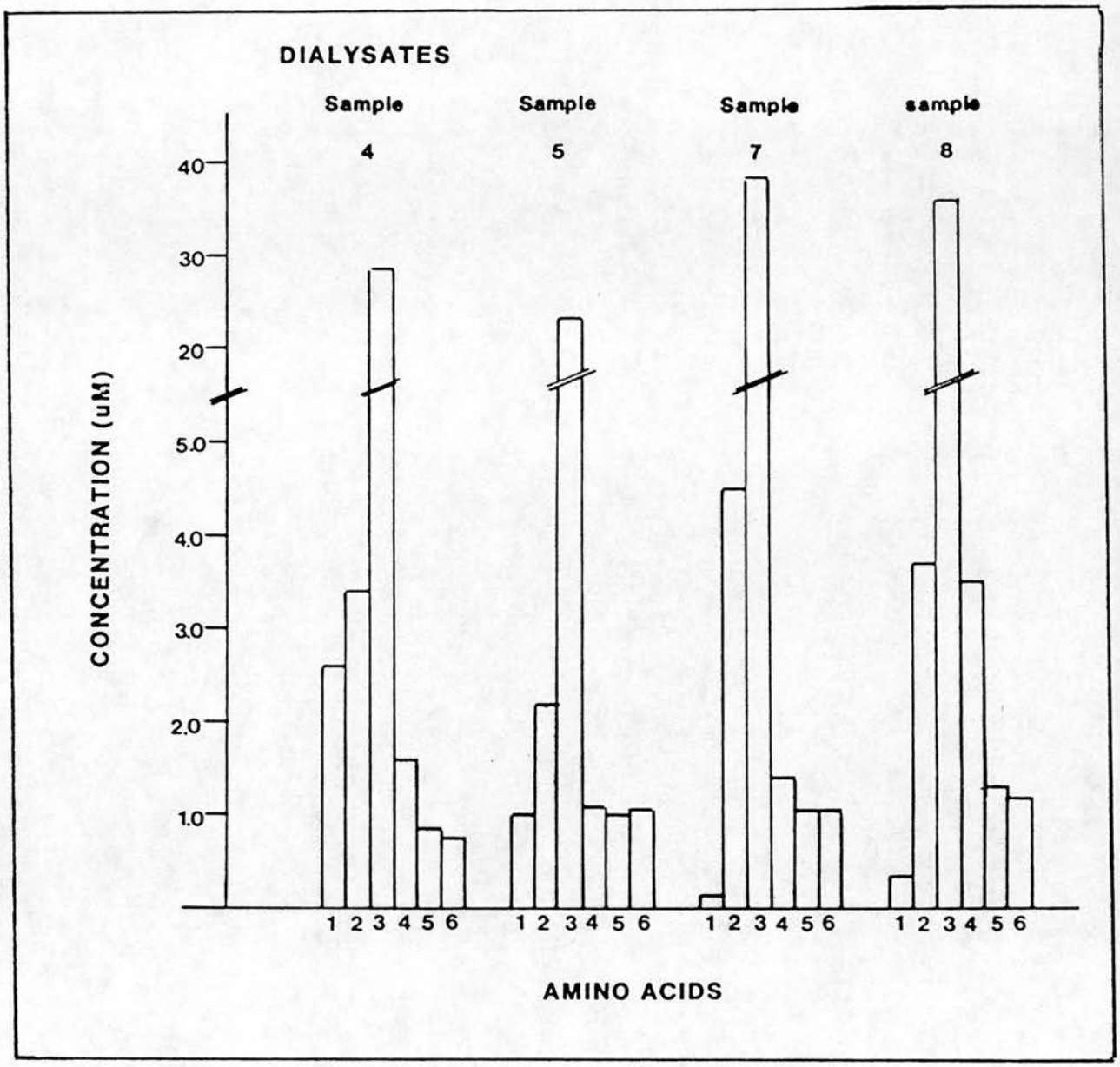


Fig. 2.25:
EGTA STUDY
Levels of amino acids measured in dialysate samples, 4 and 5 taken before infusion of EGTA and samples 7 and 8, take after infusion of EGTA: (1) aspartate, (2) glutamate, (3) glutamine, (4) taurine, (5) alanine, (6) valine.

AP5 CONCENTRATION			AFTER EGTA INFUSION		RATIOS		
Whole brain tissue	Dialysates µM	ECF Estimate µM	Dialysates µM	ECF Estimate µM	Pre EGTA Tissue : ECF	Post EGTA Tissue : ECF	ECF Estimate Post : pre EGTA
0.31(±.09) µmol/1.5gm							
203.3 µM	0.51(±0.27)	8.29(±4.37)	4.3(±4.3)	69.9(±70)	25	3	8

Table 2.3:
EGTA STUDY
Comparison between the levels of AP5 in the tissue, the dialysates and the ecf before and after the infusion of EGTA. See Table 2.2B for calculations used to derive comparisons.

because AP5 is now being displaced. However, it must be recognised that the concentration of EGTA (20mM) used may not be adequate to chelate all the calcium from the ecf. Furthermore, it is not known at what rate calcium is replenished in the ecf from the blood and brain tissue, or how far the EGTA can spread from the probe after infusion. Although these questions remain unanswered at the present, the intention of this experiment was to address the discrepancy in the AP5 levels in the tissue and in dialysates, and to find a plausible reason for why a much higher concentration of AP5 was required to block the induction of LTP in the whole animal as opposed to the *in vitro* preparation. The results from this control experiment give added confidence that the estimated extracellular concentration of AP5 is correct.

2.3.ii. Time Course Study of the levels of AP5

The nature of the behavioural impairment in the animals infused with AP5 was a slowing down of learning rather than a complete block. One important consideration to be made is whether the change in performance is caused by a change in the levels of the AP5 in the brain during the experimental period. As discussed in the dose response study, although the mini pumps reach a steady state within 6 hours of implant (Alza), much of the AP5 is removed from the csf and excreted. If AP5 levels changed over the experimental period then the amount of LTP and the amount of AP5 measured in the brain at the end of the experiment would result in ambiguous correlation with the behavioural impairment. In this control experiment a sample of ecf was taken from the right and the left hippocampus of each animal on two separate days - one day being when the animals would have been on day 2 of behavioural testing, the other being the first day of the electrophysiological testing.

Procedure

Animals (N=6) were surgically implanted with mini pumps containing 30mM D-AP5 under tribromoethanol anaesthetic (0.29gm/kg) as described in the dose-response study. At the same time, the co-ordinates to locate a microdialysis probe in both the right and left hippocampus were measured relative to Bregma (Paxinos and Watson, 1982; AP: -5.8mm; ML: ±5.2mm).

The first sample was taken from the right hippocampus on day 5 after mini pump implantation (day 2 of behavioural testing). The animals were anaesthetised with tribromoethanol and supplemented throughout the period as necessary (see general surgical procedure for details). The skull was exposed to place the microdialysis probe *in situ* and six, 20 minute samples of ecf were "harvested". At the end of the sampling period, the incision was re-sutured and the animals placed back in their home cages for a further 3 days.

The second sample was taken on day 8 after mini pump implantation (first day of LTP induction). To maintain as much consistency with the original procedure, the second sample was taken from the left hippocampus and animals were anaesthetised with urethane carbamate (1.5gm/Kg) this time. The midline incision was opened again, the probe placed, and six 20 minute samples were taken. The same probe was used to recover both samples in each animal in order to maintain consistency in the recovery rate.

Results

The mean concentration of AP5 recovered from the 6 animals on the two different days is shown below, measured in μM (Fig. 2.26). An analysis of variance showed that there was no significant difference in the concentrations of AP5 ($F(1,5)=2.01$; $p>0.22$) or of the 6 amino acids ($F(1,5)=0.59$; $p>0.40$) measured on the two different days.

Discussion

Animals were used as their own internal controls for the two samples and the same probe was also used each time. Therefore the correlations were free of any ambiguity relating to the use of different animals and any difference in probe recovery. The fact there was no difference in the levels of the AP5 or the amino acids between the 2 days strongly suggests that the levels of AP5 are stable, at least after the 5th day of the drug infusion.

This is important because the amount of AP5 that remained in the brain tissue at the end of the dose-response experiment was approximately 10% of that infused and it could be assumed that the amount of AP5 was not decreasing slowly across the testing days due to excretion of the drug. In particular, the stability of the AP5 level over the period when animals showed the biggest impairment in

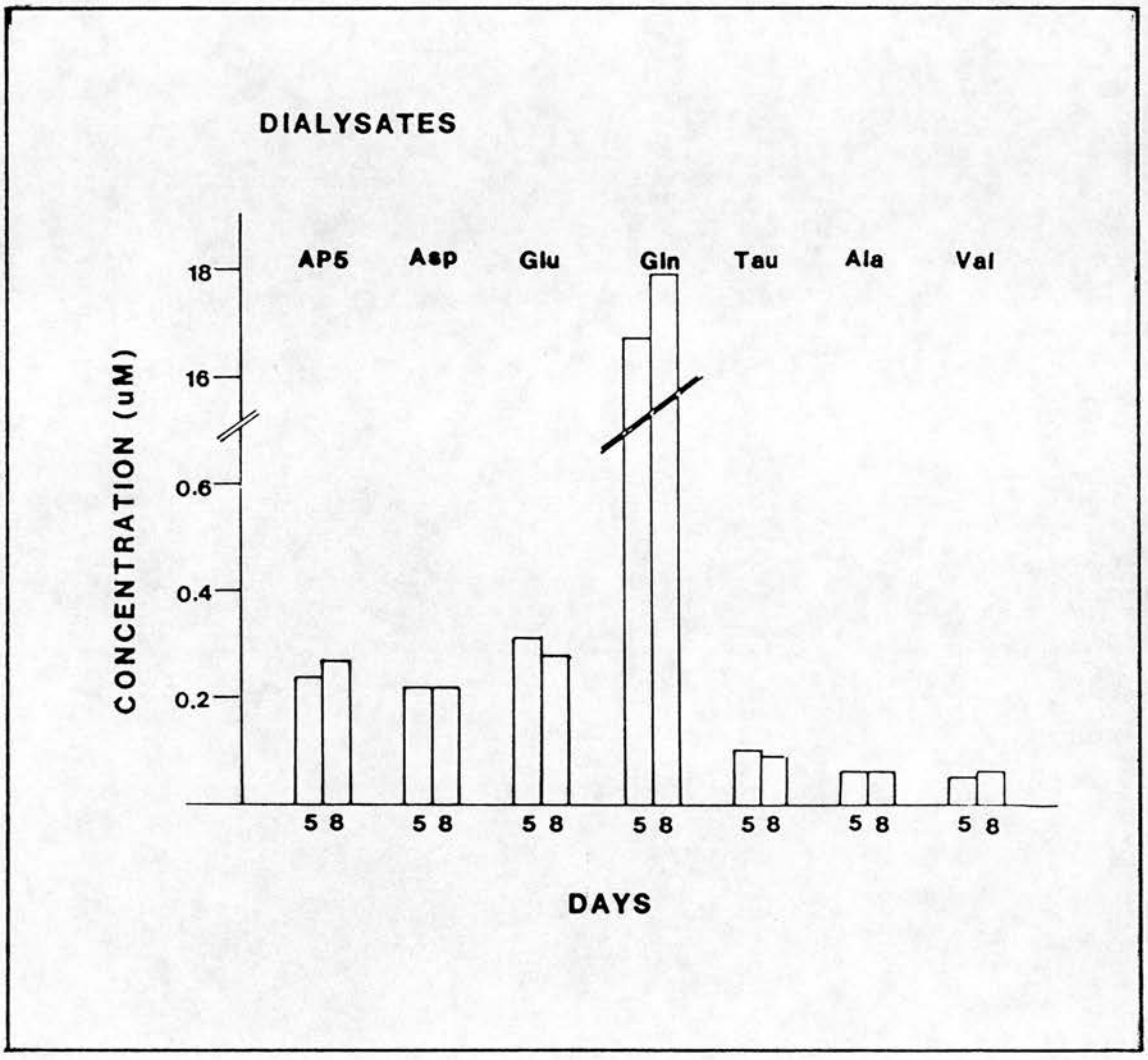


Fig. 2.26:
TIME COURSE STUDY
Levels of (1) AP5 and 6 amino acids: (2) aspartate, (3) glutamate, (4) glutamine, (5) taurine, (6) alanine, and (7) valine taken on days 5 and 8 after mini pump implant.

spatial learning and the first day of the induction of LTP gives confidence to the correlations made between the block of LTP and the impairment in spatial learning.

2.3.iii Hippocampal Lesion Study

The behavioural testing in the dose response study was based on studies that showed animals with damage to the hippocampus were impaired in spatial learning tasks (see Chapter 1.1.iii). To test the similarity of the effect of AP5 on spatial learning with that of animals with damage to the hippocampus, a group of animals with complete hippocampal lesions were tested in the watermaze, using an identical procedure as that in the dose-response study.

Procedure

Surgery

Ibotenic acid (Sigma chemicals) was used to make lesions to the hippocampus in 12 animals taken from the Department of Pharmacology breeding stock. It was dissolved in phosphate buffered saline (pH 7.4) at a concentration of (10mg/ml). Animals were anaesthetised with tribromoethanol (0.29gm/kg) and placed in a Kopf stereotaxic frame as previously described. Anaesthetic was supplemented as required. An incision was made along the midline of the scalp to expose the skull, and bilateral sections of the skull over the area where the hippocampus was located were removed. Within this area 12 injections of ibotenic acid were made in each side of the hippocampus (see Table 2.4 below for co-ordinates). Injections of 0.10μl of ibotenic acid were made at all sites except where indicated on the table. The injections were made using a (1.0μl) Hamilton Syringe held by a vertical manipulator on the stereotaxic frame. In order to reduce damage to the overlaying cortex a glass micropipette was attached to the syringe such that only the pipette entered the brain and used to inject the drug. The pipette was left in place for 1 to 2 minutes at each site to prevent spread of ibotenic acid up the pipette tract. At the end of surgery animals were given a period of 15 days before commencing training to allow for substantial cell death. Lesions were carried out with Prof. L.E.Jarrard; (Dept. Psychology, Washington and Lee University, Lexington, Virginia, U.S.A.) in Edinburgh.

COORDINATES FOR LESION (mm from Bregma)

A-P	M-L	D-V
-2.4	1.0	-3.4
-3.0	1.0	-2.6*, -3.4*
-3.0	3.0	-3.0
-4.0	2.6	-2.3*, -3.3*
-4.0	3.7	-3.0
-4.9	3.9	-3.5*, -7.0*
-5.7	5.1	-4.0, -4.9, -5.8

0.10μl IBO is injected at all sites except those marked with an asterisk. 0.5μl are injected at the sites. (Taken from Jarrard, 1989).

Table 2.4:
HIPPOCAMPAL LESION STUDY
Co-ordinates for the sites for injection of ibotenic acid into the hippocamus.

Behavioural Testing

Animals were given 12 trials of pretraining as described previously and, following 15 days of post surgery recovery, they were then tested using the identical procedures as those in the dose-response study (ie. 6 trials a day for 5 days, trained to a fixed platform location). Following the final trial a 60 second transfer test was given.

Histological Procedure

Following the training, animals were perfused transcardially with physiological saline and 10% formalin and 30μ horizontal sections were stained with fast cresyl violet to assess cell loss.

Results

Histology

The extent of the damage was at least 85-90% removal of the cells in the hippocampus (see Fig. 2.27 for photomicrographs of the range of damage). In most animals, the subiculum and the entorhinal cortex were left entirely intact. In some animals part of the dentate gyrus was spared and there was sometimes a difference in the amount of damage between the right and left hippocampus. The amount of damage in these animals was comparable to that reported by other authors, (Jarrard et al, 1986; Bouffard and Jarrard, 1988; Davidson and Jarrard, 1989).

Pretraining

Over the 12 trials of pretraining, animals were given the opportunity to learn the non-spatial aspects of the task. There was a significant difference in escape latencies over this period ($F(11,66)=2.08$; $p<0.05$; see Fig. 2.28A), and at the end of the training all animals were escaping the water in an average of 67.5(±11.3)s, a latency comparable to the last trial of pretraining in the dose-response study (see Fig. 2.12A).

Acquisition

The escape latencies were compared and analysed (using analysis of variance) with the 5 groups in the dose-response study (see Fig. 2.28C). On the first trial of day 1, the hippocampal group escaped in approximately 56.3(±13.9)s (see Fig. 2.28B) and, over the next 5

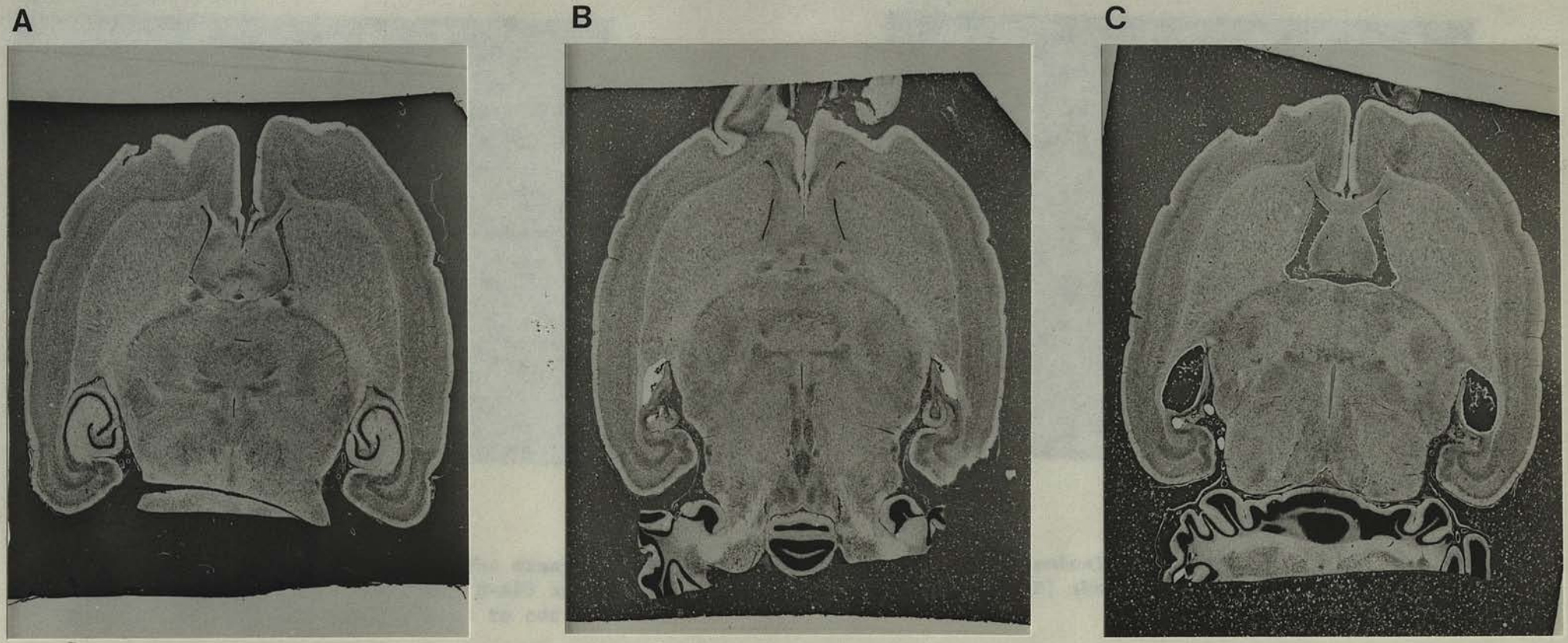
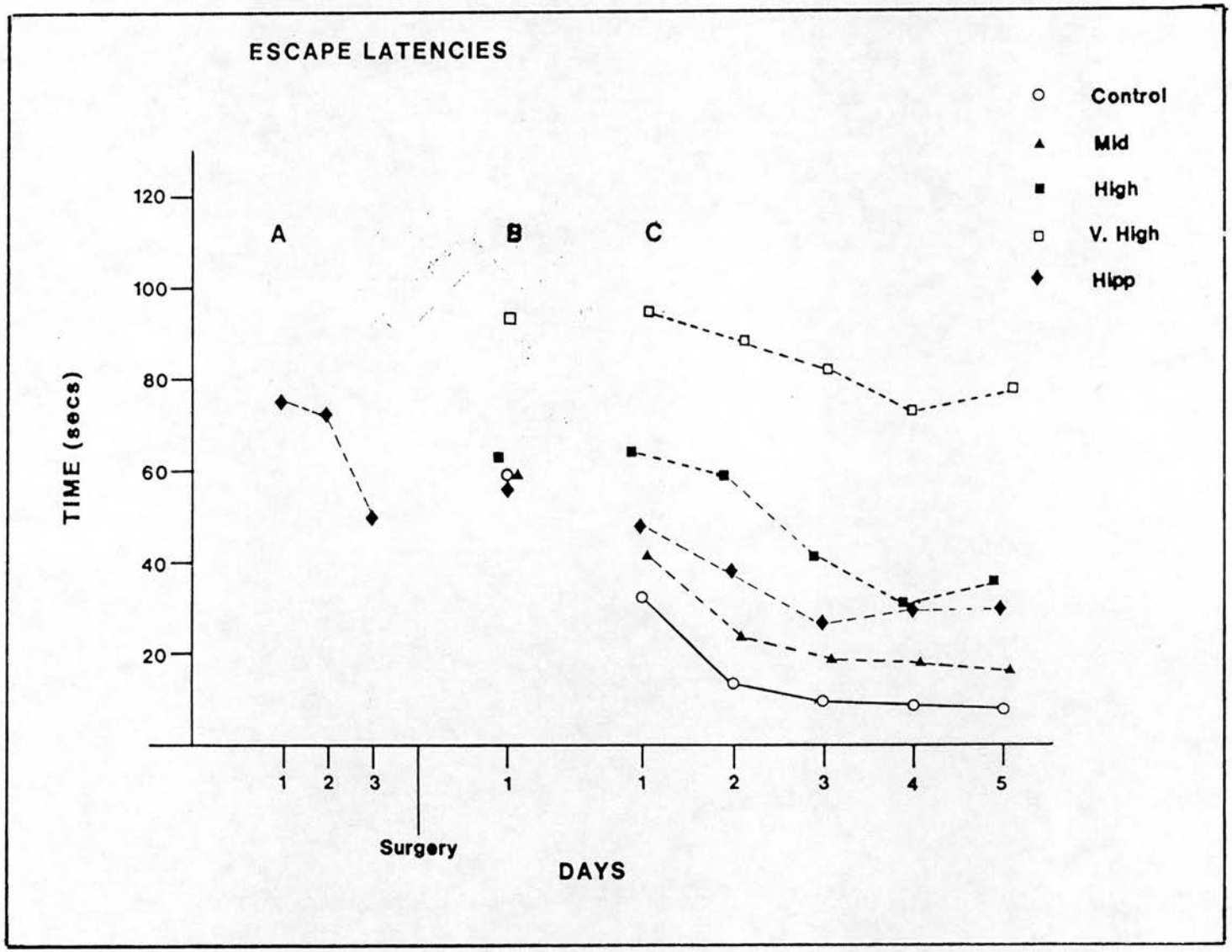


Fig. 2.27:
HIPPOCAMPAL LESION STUDY
Microphotographs of horizontal sections taken from the hippocampus are stained with fast cresyl violet to show the amount of cell loss (B and C) compared with Controls (A) 14 days after injections of ibotenic acid.

Fig. 2.28:
HIPPOCAMPAL LESION STUDY
Mean escape latencies (seconds) taken by the Hippocampal lesion group during A: Pretraining, mean of 4 trials/day; B: First trial, day 1; C: Chronic drug infusion, mean of 6 trials/day. The graph shows performance compared with the controls and the Mid, High and Very high concentration groups from the dose-response study. Low concentration group is not represented because it showed comparable escape latencies to controls.



days of training, they showed a non-significant trend towards a decrease in escape latencies ($F(4,24)=1.33$; $p>0.25$) that reached an assymptote by day 3. By day 3, this group escaped the water in approximately 30 seconds. When compared with the groups in the dose-response study, there was an overall groups effect ($F(5,74)=32.70$; $p<0.001$). A Newman Keuls post hoc analysis was carried out on the overall mean escape latency and showed that the hippocampal group escaped the water more rapidly than the high and very high concentration groups but was no different from either the controls, the low and the mid concentration group ($p<0.05$). The very high concentration group escaped the water more slowly than all other groups.

Transfer Test

Animals' performance on the transfer test was also compared and analysed with the 5 groups in the dose response study. As would be expected there was a significant difference between the amount of time the groups spent in each of the 4 quadrants ($F(15,222)=8.04$; $p<0.001$; see Fig. 2.29). Further analysis of only the training quadrant also showed a significant group difference ($F(5,74)=10.67$; $p<0.001$) and Newman Keuls post hoc test showed that the hippocampal group, the high and the very high concentration groups showed less of a spatial bias to the training quadrant than the control, low and mid concentration groups ($p<0.05$). Interestingly, the hippocampal group showed random performance but still searched throughout the area of the pool without displaying the thigmotaxic behaviour shown by the very high concentration group (see Fig.2.30).

Discussion

After the initial pretraining, where the hippocampal group learned the nonspatial aspects of the task, they were then unable to use spatial navigation adequately after the lesion and showed a non significant decrease in escape latencies over the 5 days of testing. Furthermore, they showed no spatial bias for the training quadrant in the transfer test. By the third day of the acquisition period (18 trials), they had reached an assymptotic level of approximately 30 seconds to escape the water. These latencies are comparable to those shown by rats with aspiration (Morris et al, 1982) or electrolytic lesions (Sutherland et al, 1983).

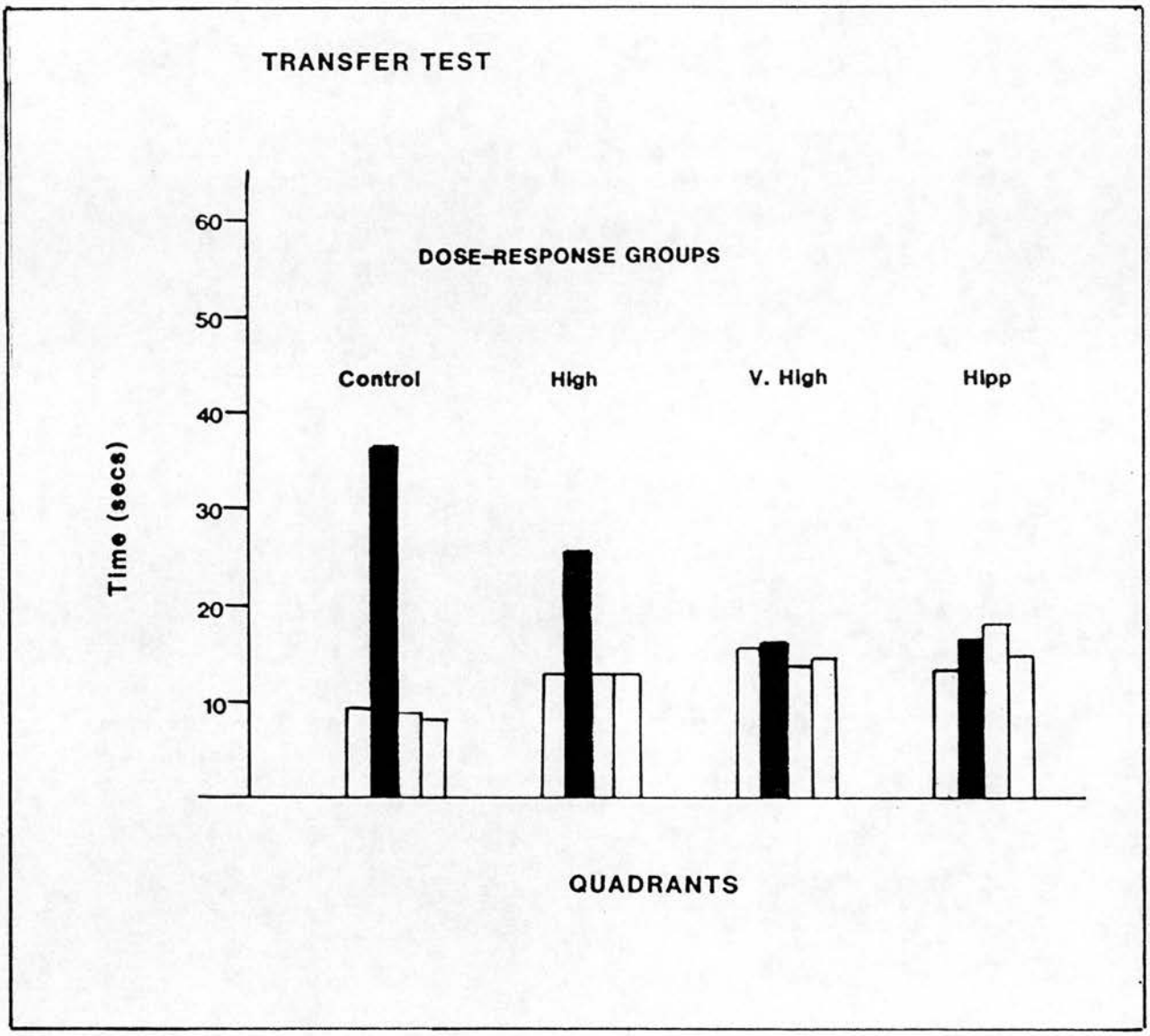


Fig. 2.29:
HIPPOCAMPAL LESION STUDY
Histogram shows the amount of time spent in each quadrant during the 60s transfer test for the Hippocampal lesion group compared with Control, High and Very high concentration groups in the dose-response study. Low and Mid concentration groups are not represented because they showed comparable spatial bias for the training quadrant as controls.

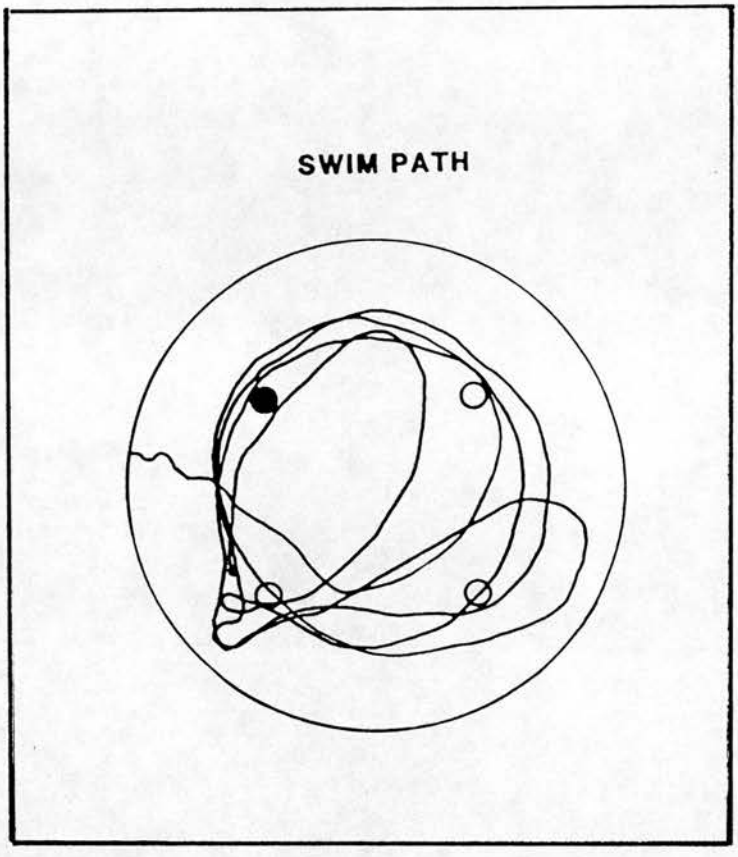


Fig. 2.30:
HIPPOCAMPAL LESION STUDY
Swim path of a Hippocampal lesioned animal during a 60s transfer test where the platform had been removed.

Compared with animals infused with AP5, the escape latencies of the hippocampal group fell half way between the mid and the high concentration groups. Although the hippocampal group did no worse than the mid concentration group and no better than the high concentration group there was a trend for the high concentration group to take consistently longer to escape the water than the hippocampal group, particularly in the first 2 to 3 days. In the transfer test, however, the high concentration group showed some spatial bias for the training quadrant, whereas the hippocampal group fell barely above the chance level of 15s in the training quadrant. This difference is interesting because the crossover in level of performance on the acquisition and the transfer test suggests a qualitative difference between the groups that may relate to the blockade of LTP. In the AP5-treated animals only the NMDA receptors are inactive, preventing the induction of LTP, whereas in the lesioned group all the cells are destroyed, preventing the activation of other transmitter systems. It also emphasises the sensorimotor impairment in the very high concentration group and one could interpret this NMDA-related affect to be mediated outside the hippocampus.

The main aim of this experiment was to assess, under the testing regimen used, whether the slowing down of learning in the AP5-treated animals could be attributed to an inability of the hippocampus to function optimally. The broadly comparable performance of the two groups is consistent with the hypothesis that one effect of chronic AP5 treatment is to cause a "hippocampal learning syndrome".

2.3.iv. Random Platform Study

The aim of this experiment was to make a further investigation into the nature of the impairment in spatial navigation caused by AP5. Although impaired, the AP5-treated animals showed some learning - their escape latencies decreased over the training period and they showed a spatial bias in the transfer test. However, at very high concentrations, AP5 causes a sensorimotor impairment such that it is difficult to dissociate effects on performance from those on learning, and the question arises of whether similar but more subtle effects may be occurring at lower AP5 concentrations also.

Previous experiments have attempted to address this issue and shown that the sensorimotor impairment can be reduced or eliminated by predrug exposure to aspects of the task, without affecting the AP5-induced learning impairment (Morris, 1989, exp. 3). In this control experiment, the effect of AP5 on motor performance was only tested by preventing spatial learning during the drug phase. The working hypothesis was that, with the escape platform randomly repositioned on each trial, allocentric cues would be of no use to control animals in finding the platform. Thus, if AP5 affects only learning but not some other motor aspect of the task, AP5-treated animals should perform at the same level as controls under these conditions.

Procedure

Lister-hooded rats from departmental breeding stock, weighing between 250 and 350gm were used. They were maintained on the same feeding and housing regimen described in the dose response study. All animals underwent 12 trials of pretraining in the watermaze before surgery to implant mini pumps (described in the dose response study) containing either aCSF or 30mM D-AP5. The single concentration of 30mM D-AP5 was used because it was the most appropriate concentration for reliably impairing spatial learning and blocking the induction of LTP. Animals were given two days post-operative recovery before starting the drug phase of behavioural testing.

The testing procedure during the acquisition period was altered to prevent animals from using spatial navigation, by randomly relocating the platform to a new position for every trial. Other than this change, the testing procedure remained identical to that used in the dose-response study, (ie. 6 trials/day for 5 days; a maximum swim time of 120s with a 30s ITI on the platform). Immediately following the last trial animals were given a 60 second transfer test where the platform was removed and the distribution of time spent in each quadrant assessed.

Results

Pretraining

Animals learned the non-spatial aspects of the task by showing a significant decrease in escape latencies over the 12 trials of pretraining ($F(11,110)=2.4$; $p<0.01$). By the 12th trial, the average escape latency was 69.9(±12.4)s (see Fig. 2.31A).

Acquisition

On trial 1 of acquisition all animals escaped the water with comparable latencies: control animals took an average of 70.4 ($\pm$45.5)s and the AP5 group took 64.2($\pm$45.5)s, which was comparable to the last day of pretraining (see Fig. 2.31A and B). Throughout the rest of the training period, however, all animals maintained long latencies between 70 and 90 seconds to escape the water (see Fig. 2.31C). Analysis of variance shows no overall difference between the groups ($F<1$) but a significant difference between trials ($F(5,50)=2.4$; $p<0.05$) and between the groups on different trials ($F(5,50)=2.8$; $p<0.3$). These differences were merely due to a random difference in escape latencies between each group on different trials. No group was consistently better or worse.

Transfer Test

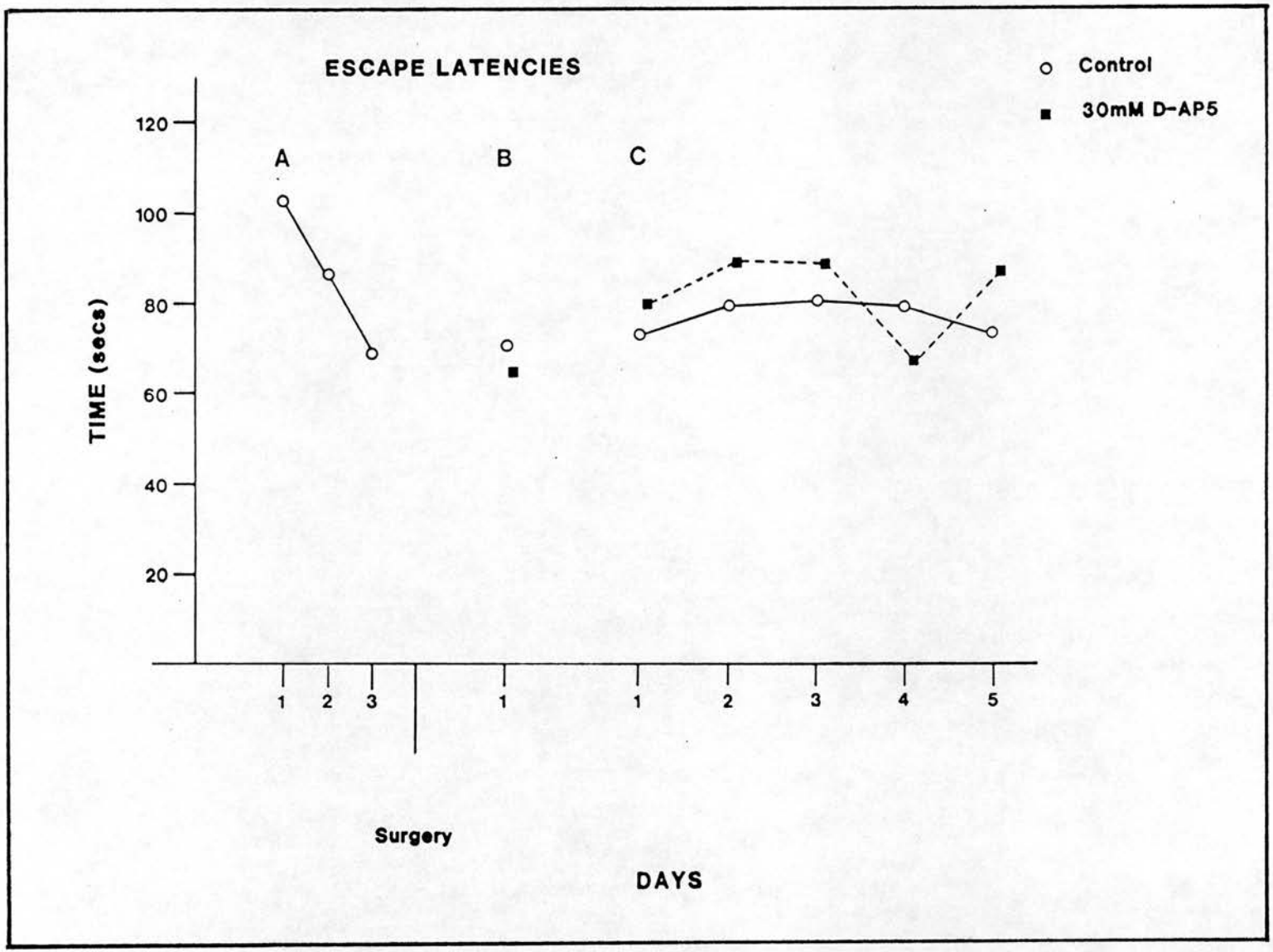
All animals spent an evenly distributed amount of time in each quadrant, seen by a failure of the F value to reach a level of significance in the analysis of variance ($F<1$). Both groups failed to show a bias for the training quadrant unlike animals that had learned a specific location of a fixed platform (see Fig. 2.32).

Discussion

When the crucial requirement for spatial learning (a fixed platform position) was removed, the performance of animals infused with AP5 was no worse than that of control animals. The transfer test also reflects the lack of spatial learning because none of the animals showed any particular preference for any one quadrant.

These results support the interpretation of the dose-response study which was that AP5 impairs the ability to learn the fixed location of an escape platform rather than disrupting motor performance in some non-specific way. In conjunction with the study carried out by Morris (1989, exp. 3) who controlled for sensorimotor

Fig. 2.31:
RANDOM PLATFORM STUDY
Mean escape latencies (seconds) taken by the Control and 30mM D-AP5 in random platform experiment. A: Pretraining, mean of 4 trials/day; B: First trial, day 1; C: Escape latencies each day during chronic infusion of drug, mean of 6 trials/day.



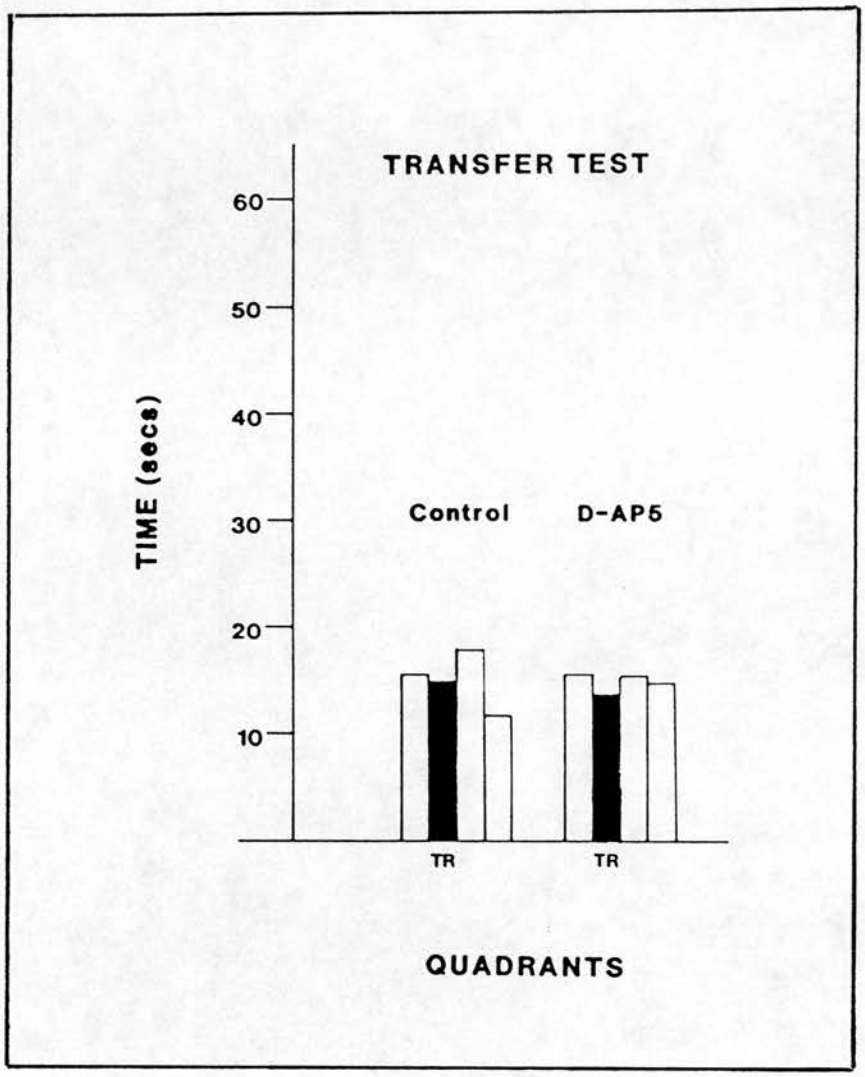


Fig. 2.32:
RANDOM PLATFORM STUDY
Histogram shows the amount of time spent in each quadrant during the 60s transfer test when the platform has been removed for Control and 30mM D-AP5 groups.

impairment, the random platform experiment emphasises the dissociation of learning impairment from the impairment in performance seen in animals infused with high concentrations of AP5.

2.3.v. Summary

The results from these four experiments present empirical evidence to some of the questions generated by the data from the dose-response study that would otherwise have been based on speculation or related to other similar experimental research. The discovery that AP5 was somehow being trapped to the tissue goes some way to explain the discrepancy between the concentrations of AP5 in the whole tissue and the dialysates and the need for such a high concentration of AP5 to block LTP *in vivo*. It leaves many questions open as to what is happening to AP5 at the molecular level but further explanation is beyond the scope of this thesis. The steady state of the level of AP5 during the crucial period of behavioural testing implies the rate of learning is determined by the parameters selected for testing such as the number of trials and the time intervals between them. At a concentration of AP5 which blocks the induction of LTP in the hippocampus, the learning impairment is broadly comparable to that of hippocampally lesioned animals. At higher concentrations, however, AP5 causes sensorimotor deficits which may be due to AP5 interaction at NMDA receptors outside the hippocampus. This interpretation is made because when preventing animals from learning the task at a concentration that does not induce lasting sensorimotor effects, AP5 animals perform at the same level as controls.

CHAPTER 3: WORKING MEMORY STUDY

3.1 INTRODUCTION

The results of the dose-response study showed that the impairment in spatial reference memory could be attributed mainly to the first trial of the day where there was (approx) 24 hours delay since the last trial. On the following 5 trials, where the delay was 30s, the AP5-treated animals showed less or no impairment (see Figs 2.13A-E) except at very high concentrations. This suggests that there may be a time dependent factor contributing to the AP5 effect, such that over long periods of time when information requires some further processing and/or storage, AP5 may interfere with this process. Over short periods of time, however, where information can be held and manipulated without further processing, AP5 would have no effect. If this was the case, then it could be argued that the mechanisms involved in LTP (ie, activation of the NMDA receptor) were required for the transfer of information from short term to a more permanent form of memory, but not for processing information over a brief period of time.

The distinction between long-term and working memory, with particular reference to the function of the hippocampus, has long been debated between Olton et al (1979) and O'Keefe and Nadel (1978). Olton maintains the position that the hippocampus is involved in working memory, while O'Keefe and Nadel's spatial mapping theory is essentially a theory of long term memory (see Chapter 1.1.i and ii). In general, hippocampal lesion studies have not supported either theory with any level of consistency. This has been due to confounding procedural effects, such as type of lesion or whether training is given before the lesion or only after it (see Chapter 1.1.iii). In one sense, there is no reason to believe that the dichotomy between reference and working memory need be dissociated in terms of one form of information being processed within the hippocampus and the other being processed somewhere else in the brain. Alternatively, the dichotomy could be described as reflecting different types of cellular activity, but both happening within the hippocampus: When information is stored more permanently a more enduring form of synaptic activity (LTP) may occur, whereas information used for only a brief period of time may occur in conjunction with normal fast synaptic transmission.

For this reason, it is of interest to investigate the effect of NMDA antagonists on working memory. Over the past year, a number of studies investigating this possibility have been reported. These show mixed results (see Chapter 1.3.vi.) and the lack of clarity is compounded by their use of the non-competitive antagonist, MK-801 (which causes sensorimotor effects and is use-dependent).

An example of the conflict is seen in the experiments carried out by Butelman (1989) who showed that MK-801 impaired working memory in the radial arm maze and Shapiro and Caramanos (1989) who showed the impairment was only on acquisition of the task and did not occur when training was given before injections of MK-801. In addition, Shapiro and Caramanos (1989) also found MK-801 impaired acquisition of spatial reference memory. A study by Ward et al (1990) showed that both CPP (a competitive antagonist) and MK-801 disrupted general maze performance, with maximal impairment after 2 hours. The impairment caused by CPP was at a concentration they had previously shown to block LTP (Abraham and Mason, 1988). However, the concentration at which MK-801 could cause an impairment in learning without sensorimotor side-effects was sufficient to block only 50% LTP, and only after a delay of 2 hours (Abraham and Mason, 1988). When they tested reference vs working memory, after 1 hour CPP disrupted performance on both tasks but the working memory deficit was statistically more robust. MK-801, on the other hand, had no effect on either working or reference memory at this time delay. The only working memory experiment to date that has used a competitive antagonist (AP5 (icv) injections), showed an impairment in working memory (Danysz et al, 1988).

The aim of this experiment was, therefore, to test the effect of AP5 on a spatial working memory task, taking advantage of the results obtained in the dose-response study. The watermaze procedure was adapted to test working memory.

3.2. RATIONALE FOR THE WORKING MEMORY PROCEDURE

The underlying rationale for a working memory experiment is to test animals' ability to utilize items of information that are varied over a brief period of time (Olton, 1983). In this way, information about the particular variation of a list of items is of no use from one trial to the next. For example, in the radial arm

maze, an animal may have to visit arms 2, 3, 5 and 8 to pick up a single reward in each arm and must do so on every trial. Within a single trial, the order may not matter, but the animal must remember which arms it has already visited and not re-enter them.

This form of learning can be tested in the watermaze by changing the location of the platform on every test session but giving the animal more than one trial to the platform within each session. In this way, the procedure follows a matching-to-sample paradigm (Honig, 1978). On the first trial, animals have no way of "knowing" the location of the platform and take an arbitrary length of time to find it. On the second trial, they should be able to use the potential information gained on trial 1 to locate the platform more rapidly, thus showing a savings in escape latency on the second trial. Notional data (Fig. 3.1) shows the difference between a spatial reference memory task and a spatial working memory task over 4 trials. In the reference task, the platform position remains in the same location throughout the entire testing period and after several days of training animals go rapidly to the platform on the first trial of each session and eventually reach an assymptotic level in escape latency. In the working memory procedure, animals would always show a longer escape latency on the first trial of each day (because the platform is in a different position to that of the previous day) but, on the second trial, they should reach their assymptotic level of performance with subsequent trials showing little further savings in escape latency. In the working memory procedure, retention of information about the location of the platform on the previous day is in fact detrimental to performance.

In order to be able to manipulate information in a working memory task, animals must first have the necessary reference memory information "which involves repetition of a general set of rules and procedures" (Olton, 1983). In the procedure to be described, it entails learning the arrangement of spatial cues, becoming accustomed to the water and learning to use the platform as a means of escape. In this way, any impairment in performance would be attributed only to working memory and not be confounded by possible effects of spatial reference memory. Three experiments were carried out in which the effect of AP5 on working memory was tested at different time intervals. The rationale behind varying the time intervals was to investigate whether AP5 would only disrupt working

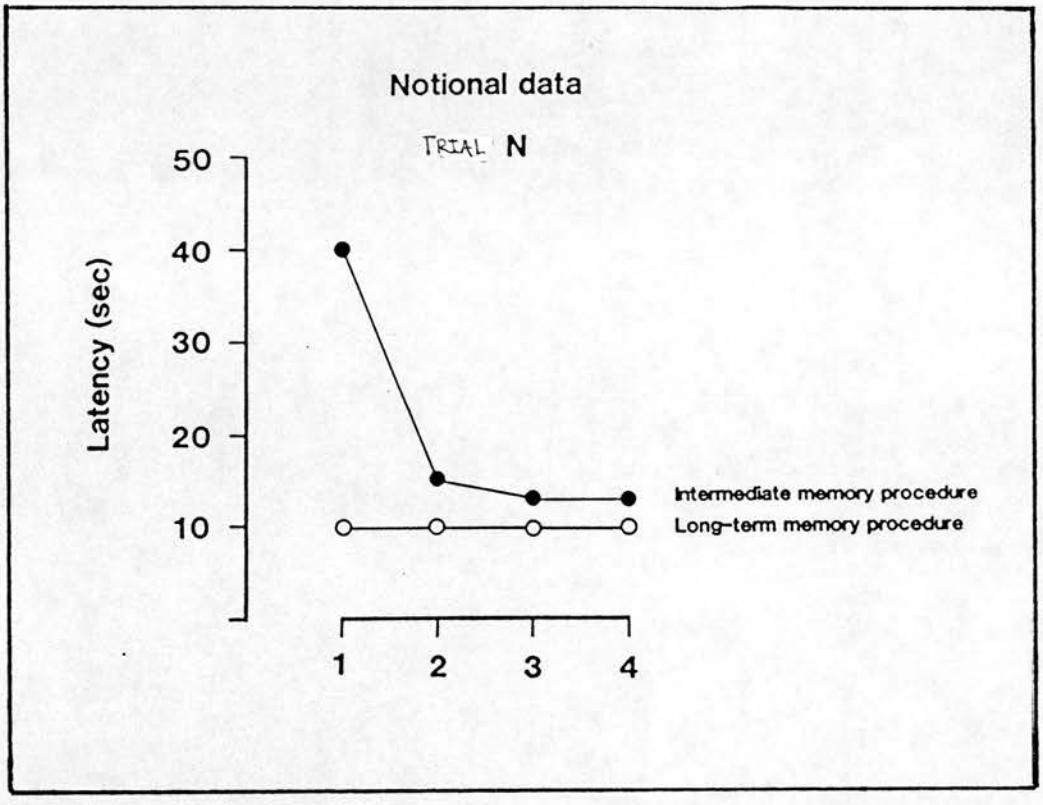


Fig. 3.1:
WORKING MEMORY
Notional data shows the difference in performance expected with animals learning a spatial reference memory task, requiring long term memory processing and a spatial working memory task, requiring short term or intermediate memory processing.

memory at longer time intervals. Panakhova, Buresova and Bures (1984) have shown that normal animals performing a working memory task show rapid forgetting within the first hour and then the decay rate slows down exponentially after 1 hour. It might be the case that when the type of synaptic plasticity modelled by LTP was blocked, information required for a longer period of time may be hindered.

3.3. METHODS

3.3.i. Subjects

Male Lister-hooded rats weighing between 250 and 350 grams, taken from breeding stock supplied by the Department of Pharmacology were used. They were housed and fed under the same conditions as the animals used in the dose-response study. Testing was carried out between 0800h and 2000h.

3.3.ii. Drugs

A single concentration of D-AP5 (30mM) shown to reliably impair spatial reference memory and block induction of LTP was used in the experimental group. Control animals were infused with aCSF made up using the Alza methodology, or were sham and unoperated animals. All drugs and anaesthetics used in these experiments were made up in millipore water using the same methodology as that described in the dose-response study.

3.3.iii. Surgical procedure

Surgery to implant osmotic minipumps containing aCSF or 30mM D-AP5 was identical to that previously described in the dose response study. The animals were allowed 2 days post operative recovery before starting the drug phase. Surgery was counted as day 1 of drug infusion.

3.3.iv. General Procedure

Animals in experiment 1 were given 3 phases of training: a predrug phase in which the animals were trained on the working memory procedure, a drug phase in which the working memory procedure was tested in the presence of AP5, and a post drug phase where the testing continued after exhaustion of the minipumps. In experiments 2 and 3, animals were only tested on the predrug and drug phases so

that the levels of AP5 could be measured in the hippocampal tissue using HPLC. Each phase consisted of 10 sessions with 4 trials per session. At the start of each session, the platform was placed in a different location to that of the previous session. There were 5 possible platform positions and these were used twice in a semi random order through each phase. The maximum swim time was 120s and a 30s ITI was spent on the platform after the animals had escaped the water. After the pre drug phase, animals were surgically implanted with osmotic minipumps containing either aCSF or D-AP5. The animals in each group were matched for their performance in the predrug phase.

During the drug phase, the time interval between trials 1 and 2 was varied in the 3 experiments (EXP 1: 30s; EXP 2: 2h; EXP 3: 30s, 2h and 5h assigned to each animal over 12 sessions in accordance with a latin square design). The ITI between trials 2 and 3, and 3 and 4 were always 30s. At the end of each experiment, a coronal wedge of brain was taken from the ventricular area for histological assessment of brain damage and verification of the cannula position. At the end of experiments 2 and 3, the hippocampus was dissected out for HPLC analysis of AP5 levels.

3.3.v. Data Handling

The criteria used for accepting or rejecting animals from the analysis at the end of each experiment was a subset of that used in the dose-response study: the general health of the animals and histological assessment of damage to the brain were considered. As no LTP or microdialysis phases were tested, these criteria were not relevant.

Weight

Animals weights were recorded every day because it was shown in the dose-response study that the surgery and/or the pumping of a solution into the ventricle may have been contributing to a weight loss. The weights of the animals taken on the day of surgery were used as a baseline.

Pharmacological Analysis

The hippocampal tissue was analysed using HPLC to measure the exact concentration of AP5 present at the end of experiments 2 and 3. The methodology for tissue preparation and HPLC analysis was as described previously in the dose-response study.

Behavioural Analysis

In addition to qualitative observations, the mean escape latency for each of the 4 trials during the successive phases were analysed with an analysis of variance using the statistical package, Alice (Alice Associates, Mass) on a mainframe computer at the University of Edinburgh.

Predrug phase (5 days): The animals were trained for 10 days but only the last 5 days in this phase were analysed. (During the first 5 days the animals learned more about the global aspects of the task, such as swimming away from the side walls, learning there was an escape platform, etc).

Drug phase (10 days): The mean escape latencies on each of the 4 trials across the 10 days of training were analysed.

Postdrug phase (10 days): The latencies of the 4 daily trials were analysed (as in the drug phase).

Trial 2 in the drug phase: Emphasis was placed on this trial because it was the trial in which animals had the first opportunity to use the information potentially gained about the location of the platform from trial 1 (see notional data, Fig. 3.1). The amount of savings in escape latency on trial 2, relative to that on trial 1 is a reflection of optimal performance in the working memory task and would best show any effect that AP5 was causing.

3.4. WORKING MEMORY 1: 30s ITI BETWEEN TRIALS 1 AND 2

Animals were assigned to a control group (n=8) implanted with minipumps containing aCSF or an experimental group (n=7) implanted with minipumps containing 30mM D-AP5 after the end of the predrug phase.

3.4.i. Results

Histology

In most animals, the cannula was positioned directly into the right lateral ventricle. The damage consisted mainly of infection to cortical areas along the cannula tract (see Fig. 3.2A and B). In some cases, the infection had spread to the septum (Fig. 3.2C) or the fimbria-hippocampal area (Fig. 3.2D). However, as these animals showed no discernible sensorimotor impairment or weight loss which could affect their performance, their data was included in the analysis. Moreover, the damage to the brains was comparable in both groups. This type of damage was similar in working memory experiments 2 and 3 and was also within the range of damage seen in the dose-response study (Compare Fig.3.2 for damage in animals in this experiment with Fig.2.8 for damage in animals in dose-response study).

Weight

There was an initial increase in weight on the day following surgery in all animals because they had been water deprived the night before and made a consequent increase in water and food intake. A similar effect was seen in the animals in the dose-response study. Controls (all animals were aCSF-operated) showed a drop in weight for 3 days but then started to show a weight gain that continued throughout the rest of the experimental period. AP5 animals, however, lost weight over the first 4 days and remained at that level for a further 6 days. Thereafter, they began to gain weight (see Fig.3.3). An analysis of variance of these data showed no overall difference between the groups ($F(1,13)=2.44$; $p>0.1$) but there was a group by day interaction ($F(13,169)=2.31$; $p<0.01$).

Pharmacological Analysis

HPLC analysis of the AP5 content in hippocampal tissue was not measured because animals were tested for a further 10 days after the pumps were exhausted.

A

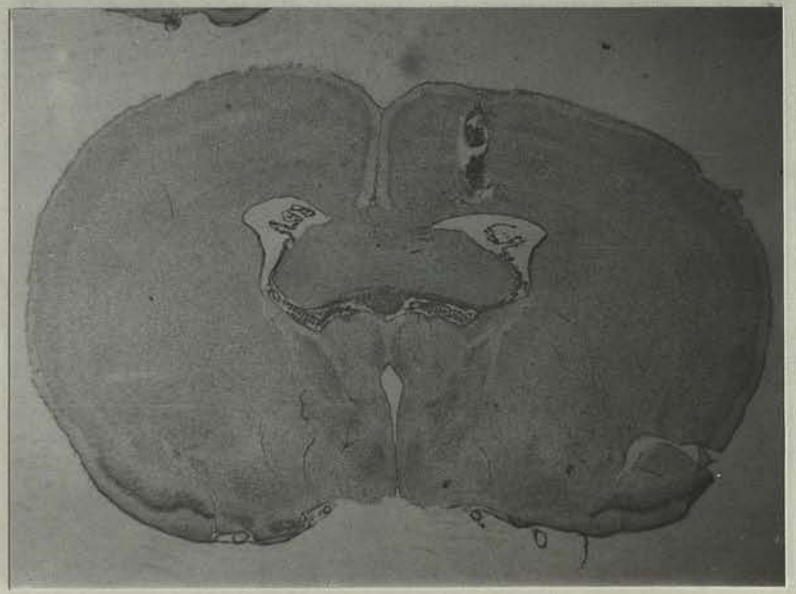
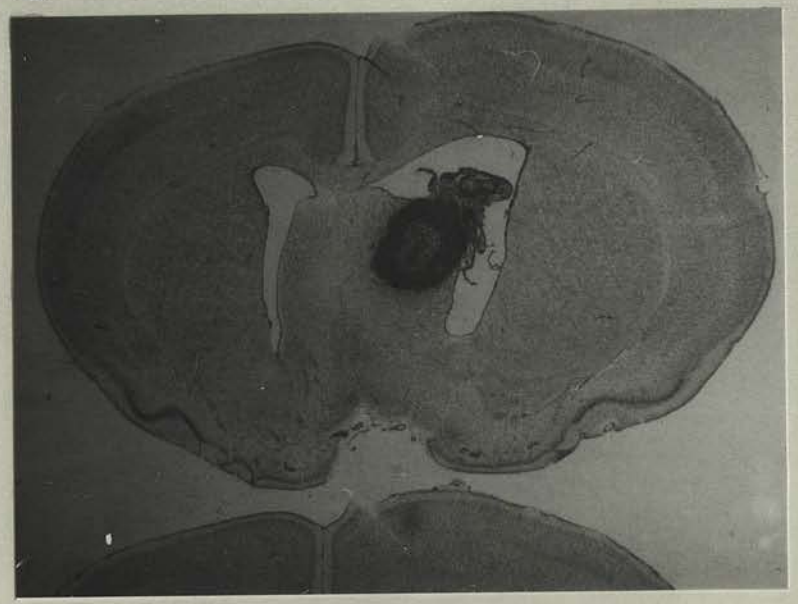


Fig.3.2:
WORKING MEMORY 1
Microphotographs show the extent of the damage caused by infection and by the mechanical damage caused by the presence of the cannula in both aCSF and 30mM D-AP5 animals. (A) shows minimal damage and (B) shows infection rising up along the cannula tract from the ventricle into to cortical area.

C



D

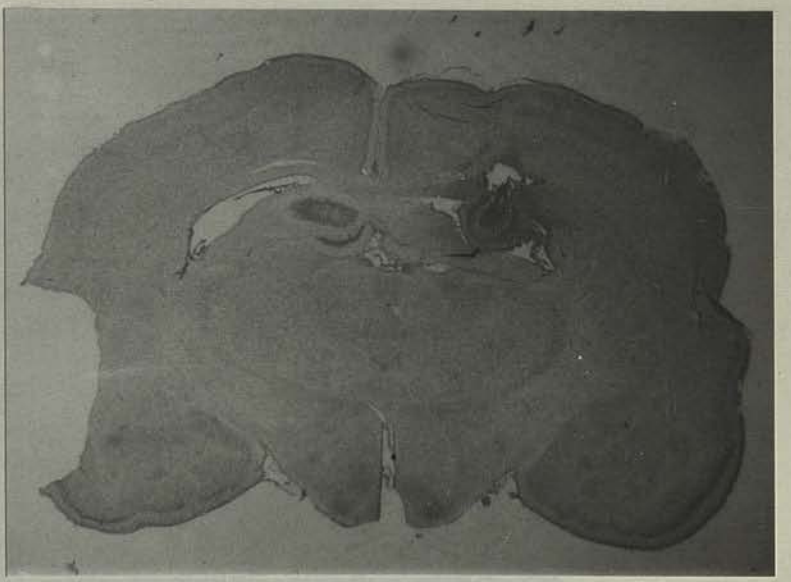


Fig.3.2:
WORKING MEMORY 1
Microphotographs show the extent of the damage that is caused by spreading infection from the ventricle in both aCSF and 30mM D-AP5 animals. (C) shows both damage to the septum and enlargement of the ventricle. (D) shows damage to the fimbria-hippocampal area.

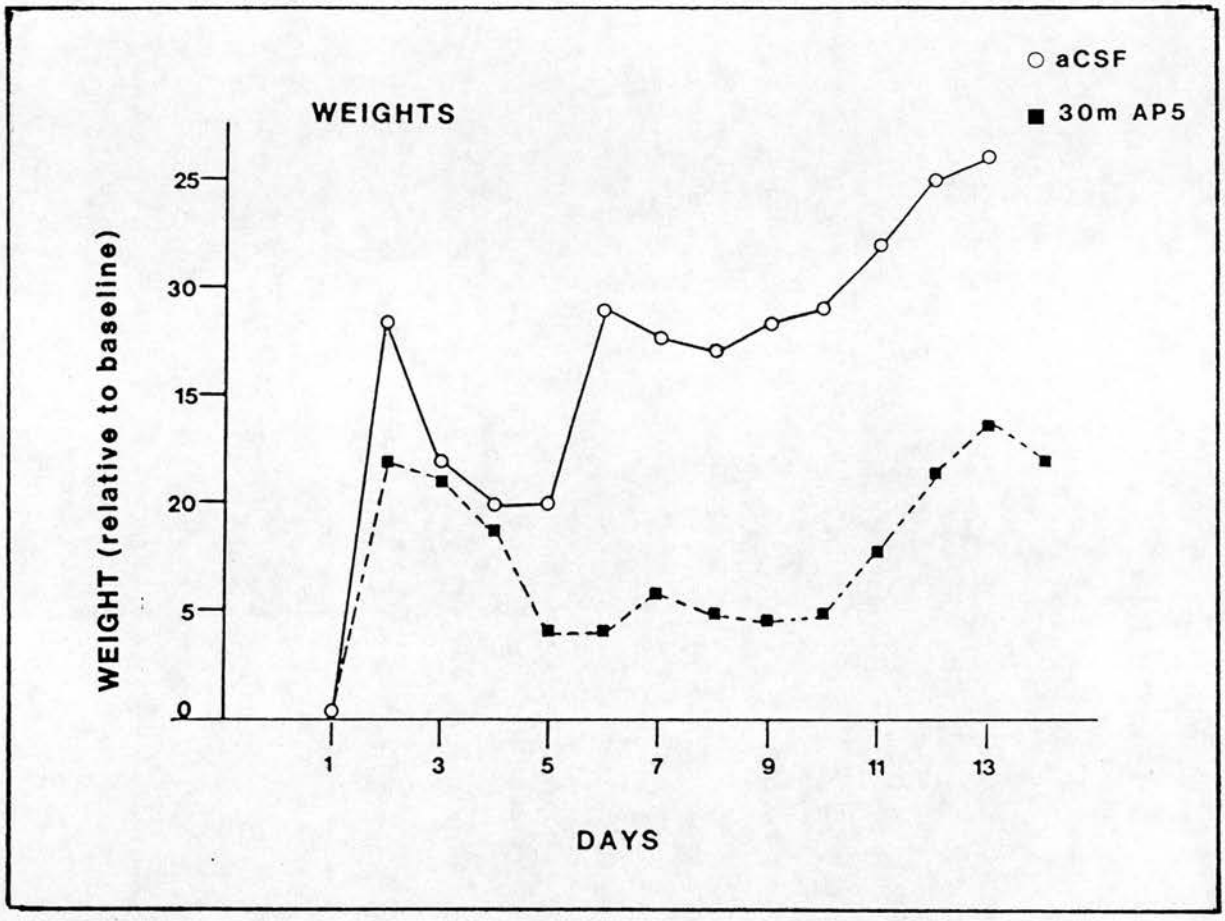


Fig.3.3:
WORKING MEMORY 1
Graph shows the amount of weight (gm) increase or decrease each day of the experimental period for the Control (all aCSF-infused) and the 30mM D-AP5 groups. Weights are measured against a baseline weight taken on the day of surgery.

Behavioural Data

Predrug phase: Trials 1 to 4

Over the last 5 days of training, all animals were performing well on the working memory task. They escaped the water on trial 1 in an average time of 58.4s, and on trial 2 in 14.9s, showing a mean savings in latency of 43.5s. They had reached an asymptotic level of performance by trial 2 because they showed no further savings on trials 3 and 4 (see Fig. 3.4). An analysis of variance of latencies over the 4 daily trials showed that was a significant difference between each trial of the day ($F(3,39)=79.5$; $p<0.001$) and a Newman Keuls post hoc test showed the difference could be attributed to the savings between trials 1 and 2 ($p<0.01$). Grouping the animals into their subsequent control or AP5 groups also showed no difference in performance between each group ($F<1$).

Drug phase: Trials 1 to 4

Analysis of variance carried out over 10 days of training in this phase showed that there was no difference in escape latencies between the 2 groups ($F<1$). Both groups escaped the water in approximately 54.2s in the first trial of each day (controls: 52.9(±6.9), AP5: 55.5 (±5.3)). This is comparable to the average latency taken by all animals to escape the water on the first trial of each day during the pre-training (58.4s). On trial 2, both groups escaped the water in an average of 14.5s (controls: 15.4(±1.9), AP5: 13.7(±1.4)), showing a savings of 37.4s and 41.9s respectively. As in the predrug phase, both groups had reached an asymptotic level of performance by trial 2, showing no further savings between trials 3 and 4 (see Fig. 3.5). There was a significant difference between trials each day ($F(3,39)=84.1$; $p<0.001$), obviously attributed to the savings between trials 1 and 2.

Post-Drug Phase: Trials 1 to 4

The post drug training showed that animals previously infused with AP5 were not impaired as they made no further improvement in their performance now that the pumps were exhausted. Control animals took 35.4(±4.6)s on trial 1 and 12.5(±1.7)s on trial 2 (a savings of 22.9s). The AP5 group took 37.3(±3.7)s on trial 1 and 11.1(±1.4)s on trial 2 (a savings of 26.2s). On trials 3 and 4 no

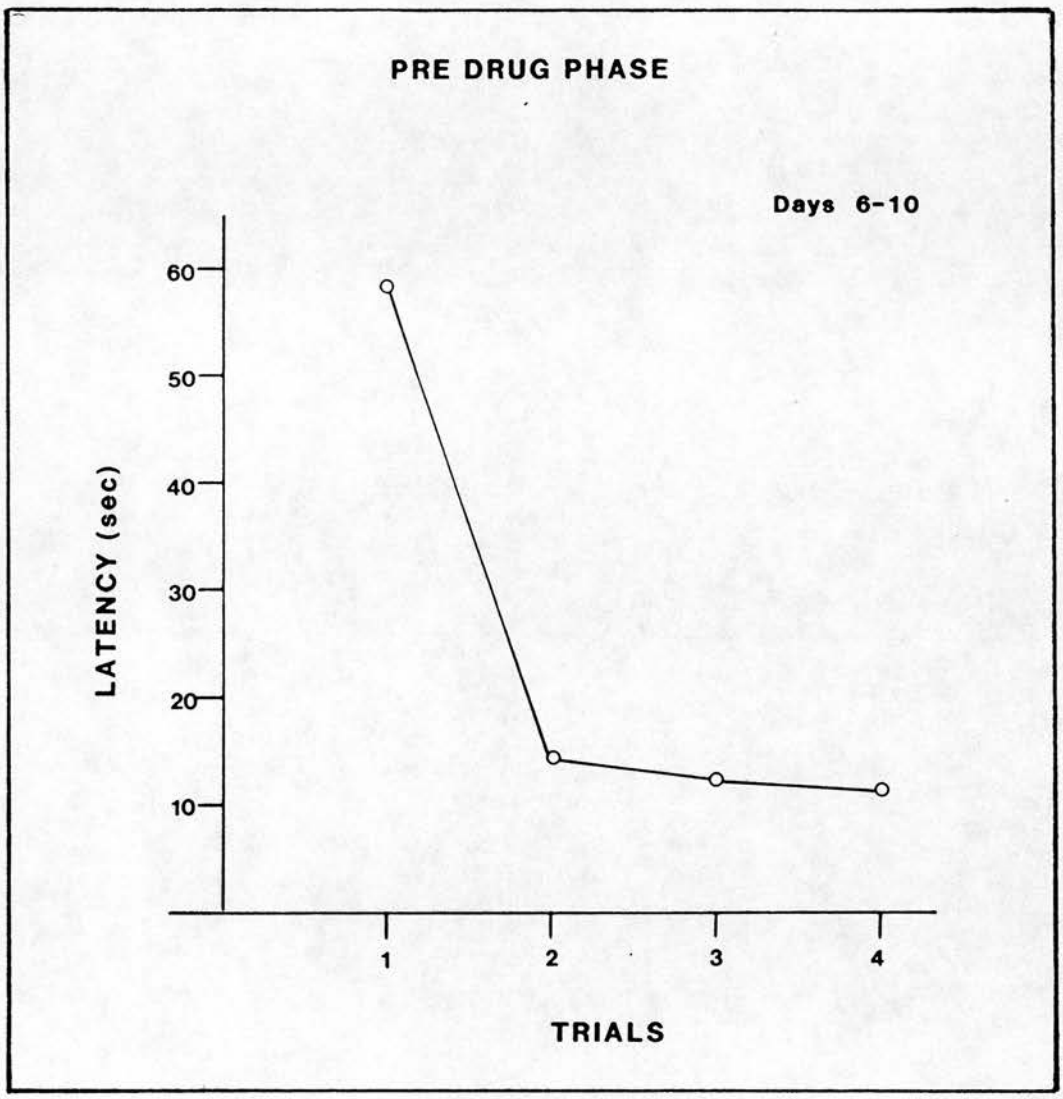


Fig.3.4:
WORKING MEMORY 1
The average escape latency of all animals for each of the 4 trials are plotted. The escape latencies from days 5-10 are plotted only.

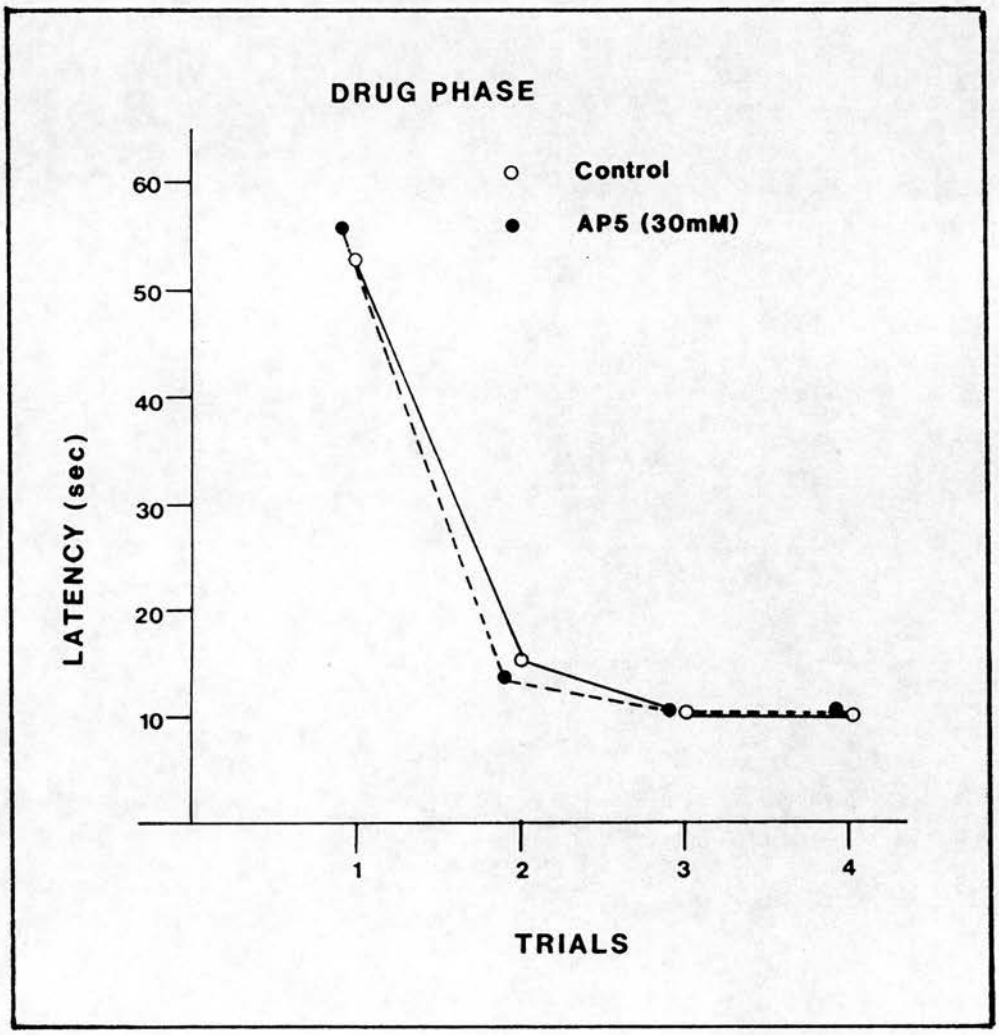


Fig.3.5:
WORKING MEMORY 1
The mean escape latencies for each of the 4 trials over the 10 day drug phase are plotted for the control and 30mM D-AP5 groups.

further decrease in latencies occurred indicating that an asymptotic level of performance had been reached by trial 2 (see Fig.3.6). There was a significant difference between trials ($F(3,39)=104.2$; $p<0.001$) but not between groups ($F<1$).

One obvious improvement in performance seen in both groups was on the first trial of the day between the drug and the post drug phase (see Figs. 3.4 and 5). There was a reduction in latency of 18.9s for controls and 18.2s for the AP5 group. Analysis of variance of the latencies on the first trial of the day indicated that both groups of animals showed an overall improvement in performance over the course of the drug and post drug phases ($F(9,117)=6.17$; $p<0.001$), although there was no difference between the 2 groups ($F<1$).

Drug phase: Trial 2

Separate analyses of trial 2 latencies were not conducted because the data in the overall analysis of the drug phase clearly indicates that there were no important group differences.

3.4.ii. Discussion

The results from the experiment show quite clearly that AP5 did not have any effect on working memory at short delays. This is shown by the fact AP5 animals performed no differently from controls and no better when the drug had been washed out. These results do not reflect those of Butelman (1989) or Danzyl et al (1988) who showed an NMDA-antagonist induced impairment. They do, however, support the Shapiro and Caramanos (1989) data which indicated that if training was given before the administration of MK-801, animals were impaired on neither working memory nor spatial reference memory in the radial arm maze. Although this experiment differs considerably from those of Butelman (1989), Danzylet al (1988), and Shapiro and Caramanos (1989) in terms of apparatus and testing procedures, all the experiments were designed to test the effects of NMDA antagonists on working memory.

Interpretation of the results from this experiment requires several considerations. First of all, the post drug training phase made it impossible to measure the concentration of AP5 in the hippocampal tissue. Without assessment of the exact level of AP5 in the tissue it cannot be ruled out that the lack of effect may

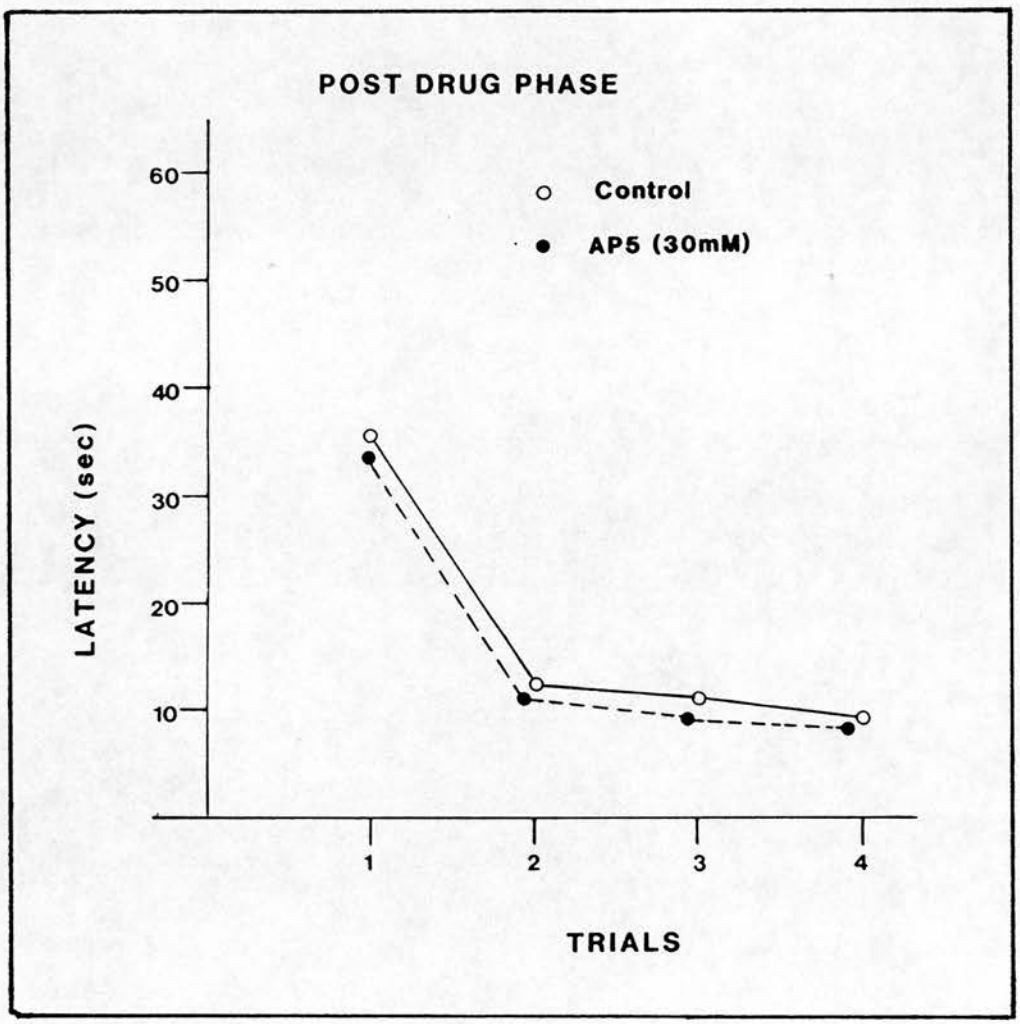


Fig. 3.6:
WORKING MEMORY 1
The mean escape latencies for each of the 4 trials over the 10 day post drug phase are plotted for the control and 30mM D-AP5 group.

have been due to an insufficient amount of AP5 in the brain. The second possibility for the lack of an AP5 effect was the predrug training. Shapiro and Caramanos (1989) showed no impairment in performance when pretraining was given. Although Danysz et al (1988) did show an impairment, it is difficult to ascertain from their report whether they gave the animals formal pretraining, it saying only that the animals were "habituated to the maze for 5 days". Moreover, with the exclusion of the Olton and Pappas (1979) experiment, hippocampal lesions have little or no effect on working memory alone if pretraining is given (Jarrard, 1983, 1986, Jarrard et al, 1984; Gage, 1985; Nadel and McDonald, 1980). The third possible explanation is the effect of the time delay. The results in this experiment are consistent with those at the 1 minute interval shown by Panakhova et al (1984). The lack of effect of AP5 may be merely a reflection of task insensitivity to a short delay or, more importantly, it may be the case that when information is held for only a brief period of time the type of neural plasticity that is modelled by LTP does not occur. It was difficult from this experiment to make a conclusive statement as to the effect of AP5 on working memory so the next experiment was devised to attempt to answer some of the questions raised in this experiment.

3.5. WORKING MEMORY 2: 2 HOUR ITI BETWEEN TRIALS 1 AND 2

Animals were assigned to a control group (sham-operated (N=4); aCSF-infused (N=4)) or a 30mM D-AP5 group (N=8). They were tested on only the predrug and drug phase so that AP5 levels could be measured in the hippocampus. The delay between trials 1 and 2 was extended to 2 hours during the drug phase only, to investigate whether AP5 had an effect on working memory at this longer time interval. The intervals between the other trials in the drug phase remained at 30s to allow all animals to reach the same level of performance by the end of each session. As the drug phase was limited to 10 days (because of the minipumps), testing was increased to 2 sessions per day (AM and PM) thereby increasing the amount of data. The predrug phase remained at 1 session per day.

3.5.i. Results

Histology

Two AP5 animals were dropped from the analysis based on poor health and the amount of damage to the brain (N=6). In the remaining animals, there was some infection in all brains confined mainly to the right lateral ventricle but in some cases involving the septum or the fimbria-hippocampal area. This damage was similar to that seen in animals in working memory 1 (see Fig. 2.8). There was also some enlargement of the ventricles (see Fig. 3.7), but there was no difference in performance in the drug phase between the aCSF-infused controls and the sham operated animals ($F(1,6)=1.17$; $p>0.3$). The worst level of infection found in the aCSF group was therefore used as a threshold with which to reject AP5 animals.

Weight

All animals showed an initial increase in weight on the day after surgery that was caused by an increase in food and water intake. Controls thereafter showed a steady weight gain over the rest of the experimental period. An analysis of variance showed there was no overall difference between Shams, aCSF-infused controls and AP5 animals ($F(2,11)=3.35$; $p>0.05$), but there was a group by day interaction ($F(22,121)=3.88$; $p<0.001$). AP5 animals steadily lost weight after surgery until day 11 when they started slowly to gain weight (see Fig.3.8).

Pharmacological analysis

Analysis of AP5 content in the hippocampal tissue showed that the average concentration was 0.21(±0.10)nmol/mg wet weight. There was no difference in the levels of the amino acids measured in the hippocampus between the control and AP5 group ($F(1,9)=1.98$; $p>0.20$; see Fig. 3.9). Based on the results of the dose-response study, the mean concentration of AP5 falls within the range of the high concentration group in which animals were impaired on the spatial reference memory task and no LTP could be evoked. The individual animals concentrations, however, range between 0.11nmol/mg and 0.40nmol/mg covering the mid to very high concentration groups.

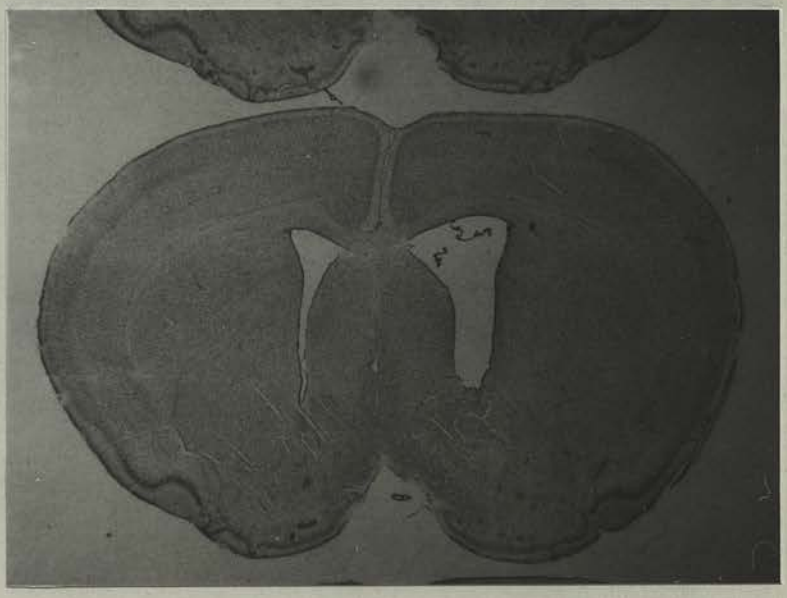
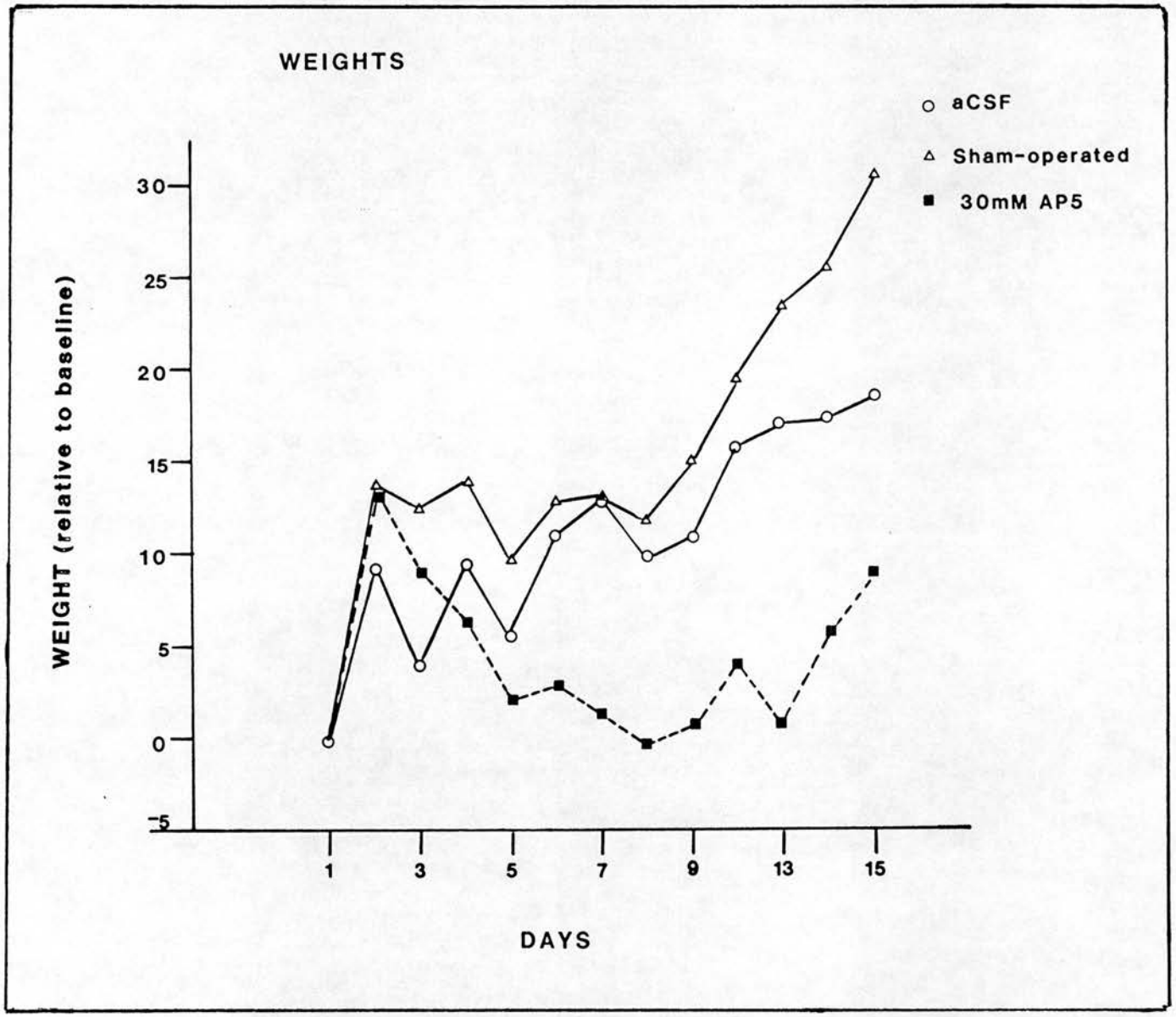


Fig.3.7:
WORKING MEMORY 2
Microphotographs show enlargement of the ventricle in both aCSF and 30mM D-AP5-treated animals.

Fig.3.8:
WORKING MEMORY 2
Graph shows the amount of weight (gm) increase or decrease each day of the experimental period for the sham-operated controls, the aCSf-infused controls and the 30mM D-AP5 animals. Weights are measured against a baseline weight taken on the day of surgery.



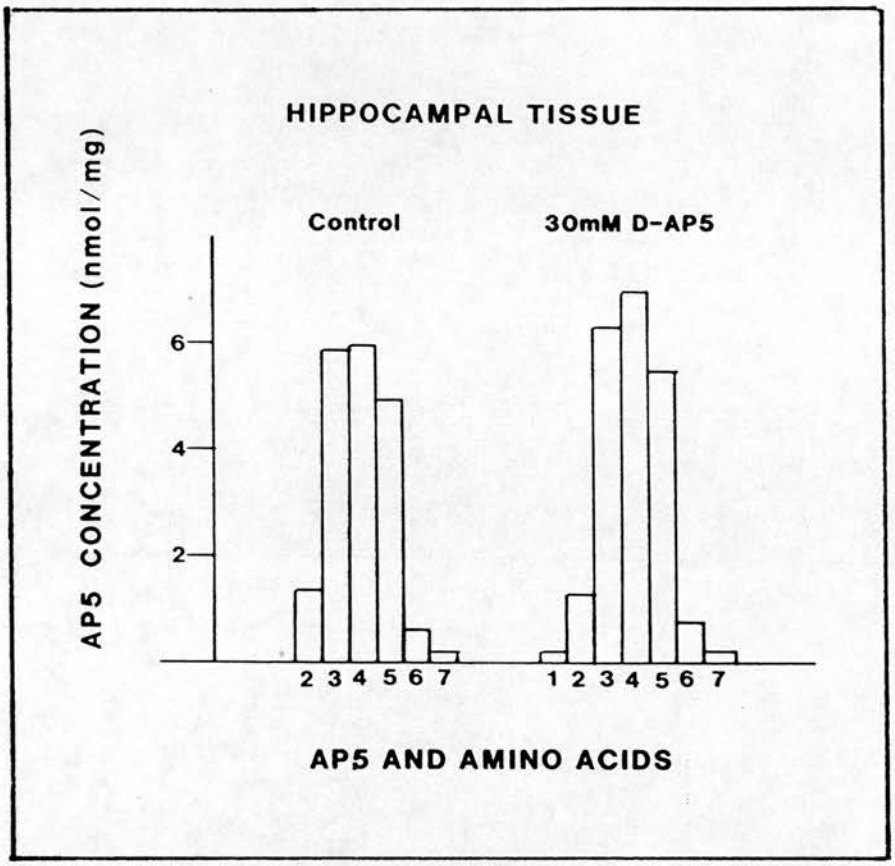


Fig.3.9:
WORKING MEMORY 2
Histogram shows the amount of AP5 and amino acids measured in the combined right and left hippocampus using HPLC: (1) AP5; (2) aspartate; (3) glutamate; (4) glutamine; (5) taurine; (6) alanine; (7) valine.

Behavioural data

Predrug phase: Trials 1 to 4

In the last 5 days of testing, all animals escaped the water in an average time of 60.6s on trial 1 and in 14.3s on trial 2, showing a saving of 46.3s. The average latencies for trials 3 and 4 were 8.7s and 9.2s (respectively) showing that they had very little reduction in escape latencies after trial 2 (see Fig. 3.10). An analysis of variance showed that there was a significant difference between trials ($F(3,36)=92.59$; $p<0.001$) but not between the animals on subsequent grouping into controls or AP5 ($F(1,12)=1.56$; $p>0.20$). These results are comparable with those of the predrug training in working memory 1.

Drug phase: Trials 1 to 4

In this phase, the animals received 2 sessions of 4 trials per day with a 2 hour delay between trials 1 and 2 (making a total of 20 sessions of data). An overall analysis of variance of these data showed that controls escaped the water more rapidly than the AP5 group ($F(1,12)=16.95$; $p<0.001$) and that there was a group by trial interaction ($F(3,36)=3.51$; $p<0.05$). Controls took an average of 36.4 (±3.4)s to escape the water on trial 1 and 12.3 (±1.7)s on trial 2 - making a savings of 24.1s. Their latencies decreased marginally on trials 3 and 4 to 8.9 (±0.9)s and 8.3 (±0.2)s respectively. This implied that, despite the 2 hour delay, the controls had reached a near asymptotic level of performance by trial 2. AP5 animals escaped the water in 42.9(±3.3)s on trial 1. A Newman Keuls post hoc test showed that this mean latency was not significantly different from that taken by controls on the first trial ($p<0.05$). On trial 2, however, the AP5 group took 25.4 (±3.1) - a savings of only 17.5s. They made a further savings of 11.6s between trials 2 and 3, and by the 4th trial they were escaping the water in 8.9(±0.4s) - a similar latency to controls (see Fig.3.11).

Trial 2: Drug phase

On trial 2, AP5 animals took longer to find the platform than controls (25.4s vs 12.3s; $F(1,12)=19.27$; $p<0.001$). Newman Keuls post hoc analysis ($p<0.01$) of the latencies on the 4 trials showed

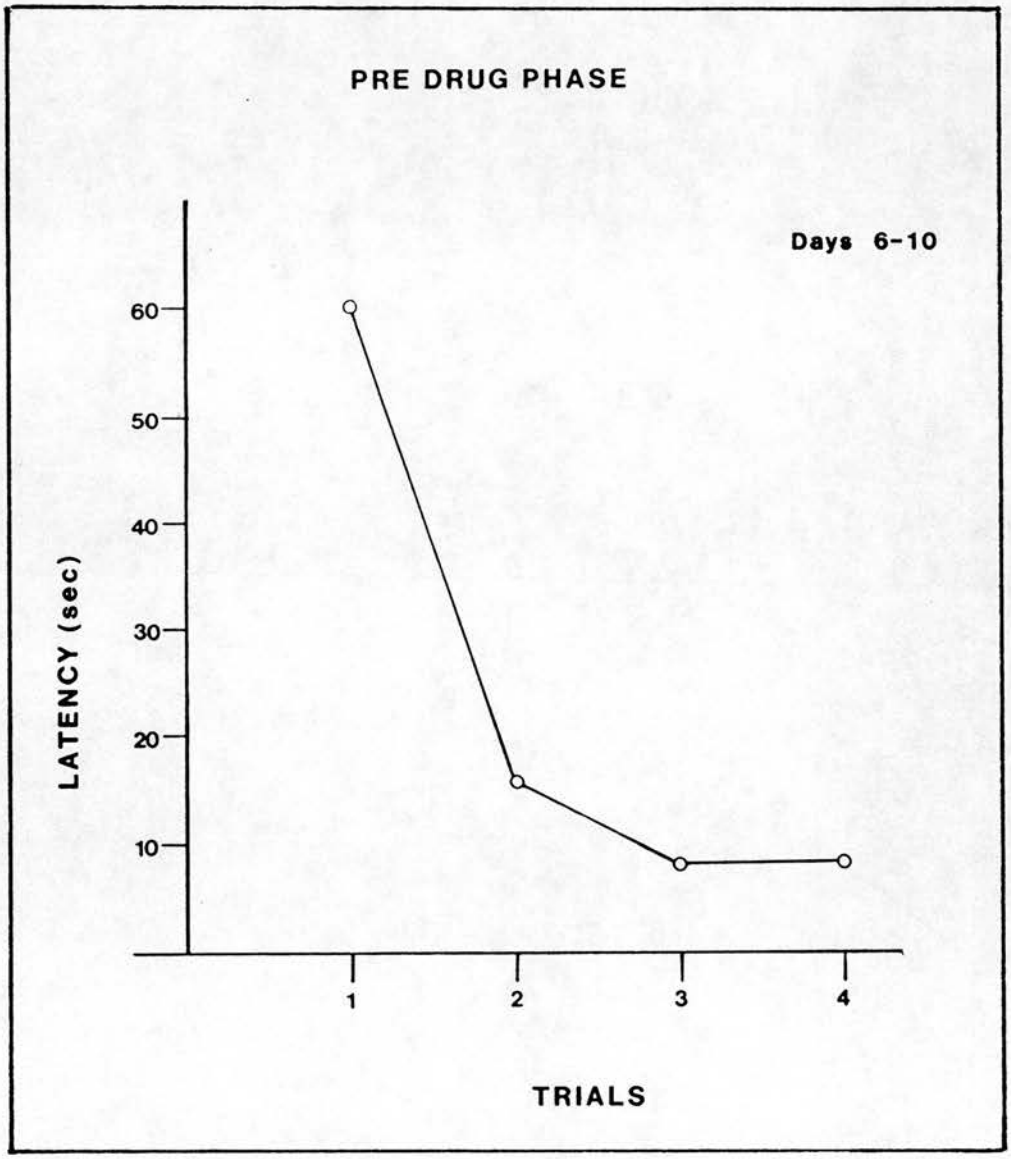


Fig.3.10:
WORKING MEMORY 2
Average escape latency of all animals on each of the 4 trials are plotted. The escape latencies from days 5-10 are plotted only.

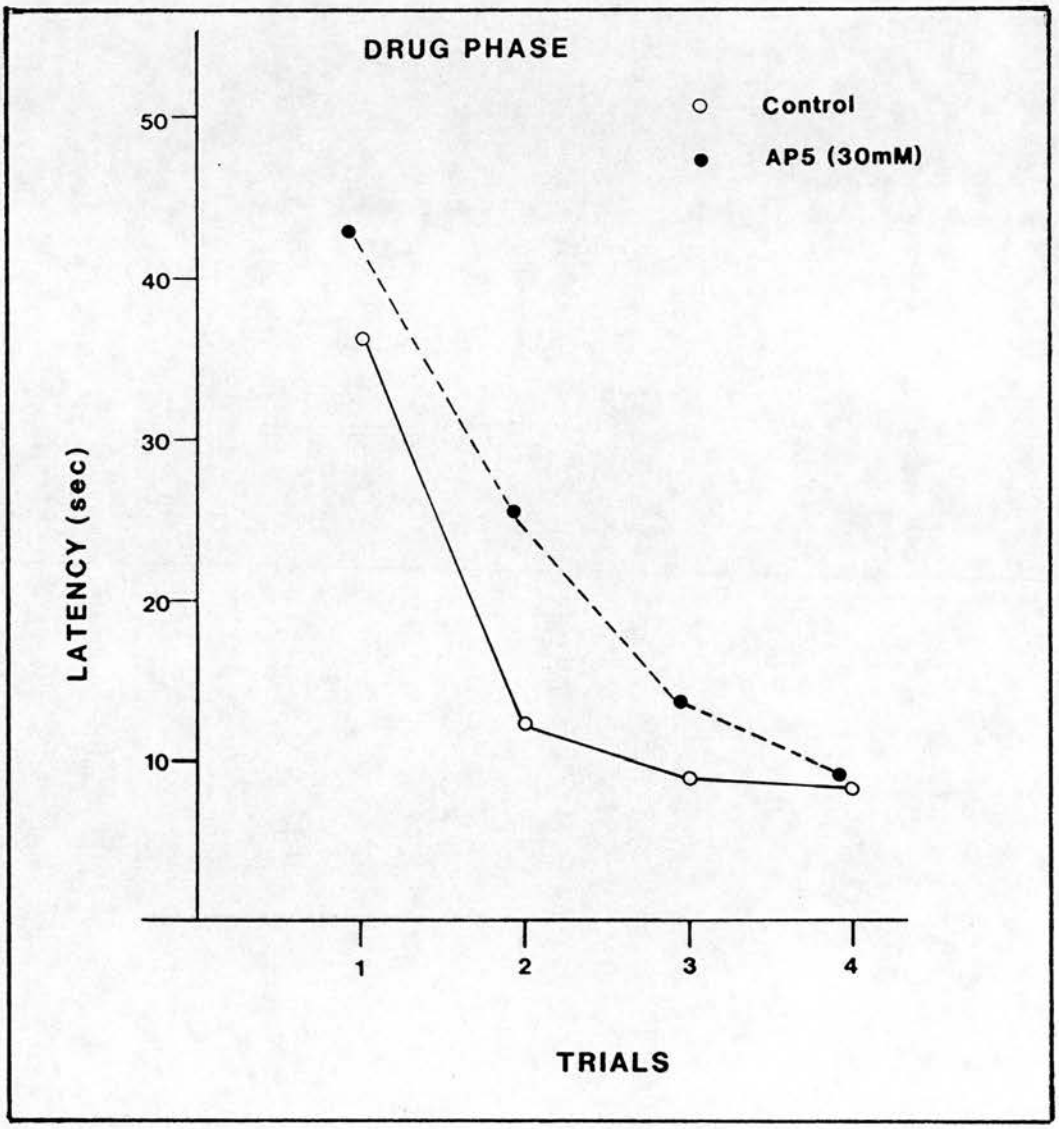


Fig.3.11:
WORKING MEMORY 2.
The mean escape latencies for each of the 4 trials over the 10 day drug phase are plotted for the control and 30mM D-AP5 groups.

they improved only on the fourth trial ($p<0.01$). Comparing their latency on this trial (8.9s) with that of controls (8.8s) indicates that they had caught up to the control level by this time.

3.5.ii. Discussion

By the end of the pre drug phase, all animals reached an optimal level of performance in working memory. When the delay between trials was extended to 2 hours during the drug phase, the AP5 animals took significantly longer to find the platform than controls. However, when the interval between trials was returned to the shorter delay of 30 seconds, the AP5 animals caught up to the same level of performance as controls after 1 extra trial. The amount of AP5 measured in the brain was within the range of concentrations which cause an impairment in spatial reference memory and LTP. The present results are consistent with those of the first working memory experiment inasmuch as the AP5 animals were as good as controls on the fourth trial. However, the AP5-induced impairment at the 2 hour delay suggests that blockade of NMDA receptors alters the time-dependent pattern of forgetting shown by normal animals in this task. This may be attributed to the inability to process information over a long period of time using an LTP-type mechanism.

3.6 WORKING MEMORY 3: VARIED ITI (30s, 2h, 5h) BETWEEN TRIALS 1 AND 2

The results from experiments 1 and 2 suggest there may be an interaction between the effects of AP5 and time. In this experiment, a within subjects design was used to investigate this interaction, specifically to see whether the impairment caused by AP5 was due only to the increased time interval, or whether there might have been some unaccountable difference between experiments 1 and 2. For example, the levels of AP5 were not measured in experiment 1 and the lack of difference in performance between the 2 groups may have been due to insufficient levels of the drug. The use of a within subjects design and the monitoring of drug levels in the brain provides a more rigorous way of investigating the possibility of an interaction between AP5 and time.

Animals were assigned to either a control group (shams=6, aCSF=5) or a 30mM D-AP5 group (N=12). Two drug phases were used: predrug and drug, with one session per day. In the drug phase animals were tested on 12 sessions (rather than 10 in experiments 1 and 2). Due to the 5 hour time interval, it was not possible to test the animals more than once a day, and so they were tested on 4 sessions at each time interval (30s, 2h and 5h) between trials 1 and 2. Only 4 platform positions were used to ensure a completely balanced and randomised design with respect to start positions, ordering of time intervals and platform position across both groups. Latin squares were used to balance each animal's training schedule.

3.6.i. Results

Histology

Histology showed that at least the right lateral ventricle was enlarged in most animals, as in working memory 2 (see Fig. 3.7). There was also some infection in the ventricle which was surrounded by gliosis and in some cases it had spread to the septum or the fimbria similar to that seen in experiments 1 and 2 (see Figs 3.2.C and D, and Fig. 3.12). Based on the histology and on their health, 4 animals were dropped from the experiment (1 aCSF and 3 AP5). There was extensive infection spreading from the ventricle to the septum and the fimbria, and in the case of the aCSF animal that was dropped, it was bilateral. After elimination of animals based on the histological assessment, the relative performance of the aCSF and sham-operated animals in the drug phase was analysed as a further criterion for accepting or rejecting animals. Analysis of variance of their performance showed no significant difference ($F<1$), and the worst level of damage seen in aCSF animals was used as a threshold for acceptance of animals in the AP5 group.

Weight

After the day of surgery, there was an initial increase in the weights of the aCSF and sham operated animals due to increase in food and water intake, as described in experiments 1 and 2, while AP5 animals showed no increase on this day. Throughout the experimental period, there was a significant overall difference between the groups in weight ($F(2,16)=4.72$; $p<0.05$). The sham-operated animals made a steady increase in weight over the rest of

Fig.3.12:
WORKING MEMORY 3
Microphotographs show damage to the fimbria hippocampal area in both aCSF and 30mM D-AP5-treated animals.

the experimental period but the aCSF and the AP5 animals did not. The aCSF animals lost weight for the next 6 days, but then showed a steady increase. AP5 animals, however, showed a steady decrease in weight, and only at the end of the experiment did they show any sign of gaining weight; however, at this time they were still below the original baseline weight (see Fig. 3.13).

Pharmacological Analysis

Analysis of the hippocampal tissue showed that all animals had an AP5 concentration of either 0.3 or in excess of 0.3nmol/mg wet weight. The average concentration was 0.55(±0.18) nmols which was above the level of AP5 accepted in animals in the dose-response study. Other primary amino acids showed no discernible changes between the 2 groups ($F(1,12)=2.07$; $p>0.1$; see Fig. 3.14).

Behavioural data

Trials 1-4: Predrug phase

In the last 5 days of training, all animals were escaping the water rapidly on trial 2, indicating they had learned the working memory task. On trial 1 of each day, the mean escape latency was 48.4s, and on trial 2 it was 11.5s, showing a saving 36.9s. On the 3rd and 4th trials animals found the platform in 9.5s and 8.9s respectively, indicating that animals had reached a near asymptotic level of learning (see Fig.3.15). Analysis of variance showed a significant difference between trials ($F(3,51)=112.17$; $p<0.001$), but from the graph it can be seen that this effect could mainly be attributed to the savings between trials 1 and 2. Separating animals into their subsequent groups showed that, in pretraining, there was no difference in their performance ($F<1$).

Trials 1-4: Drug phase

Analysis of variance was carried out on the 12 days of testing. During this time, all animals were tested 4 times on 3 different delays of 30s, 2h and 5h between trials 1 and 2; the ITI between trials 2 and 3 and 3 and 4 was always 30s. Overall, the AP5 animals took longer to escape the water than controls on all trials ($F(1,17)=7.76$; $p<0.02$; see Fig.3.16), but there was no group by delay interaction ($F(2,34= 1.70$; $p>0.19$). Controls showed a trend towards taking longer to escape the water as the delay increased;

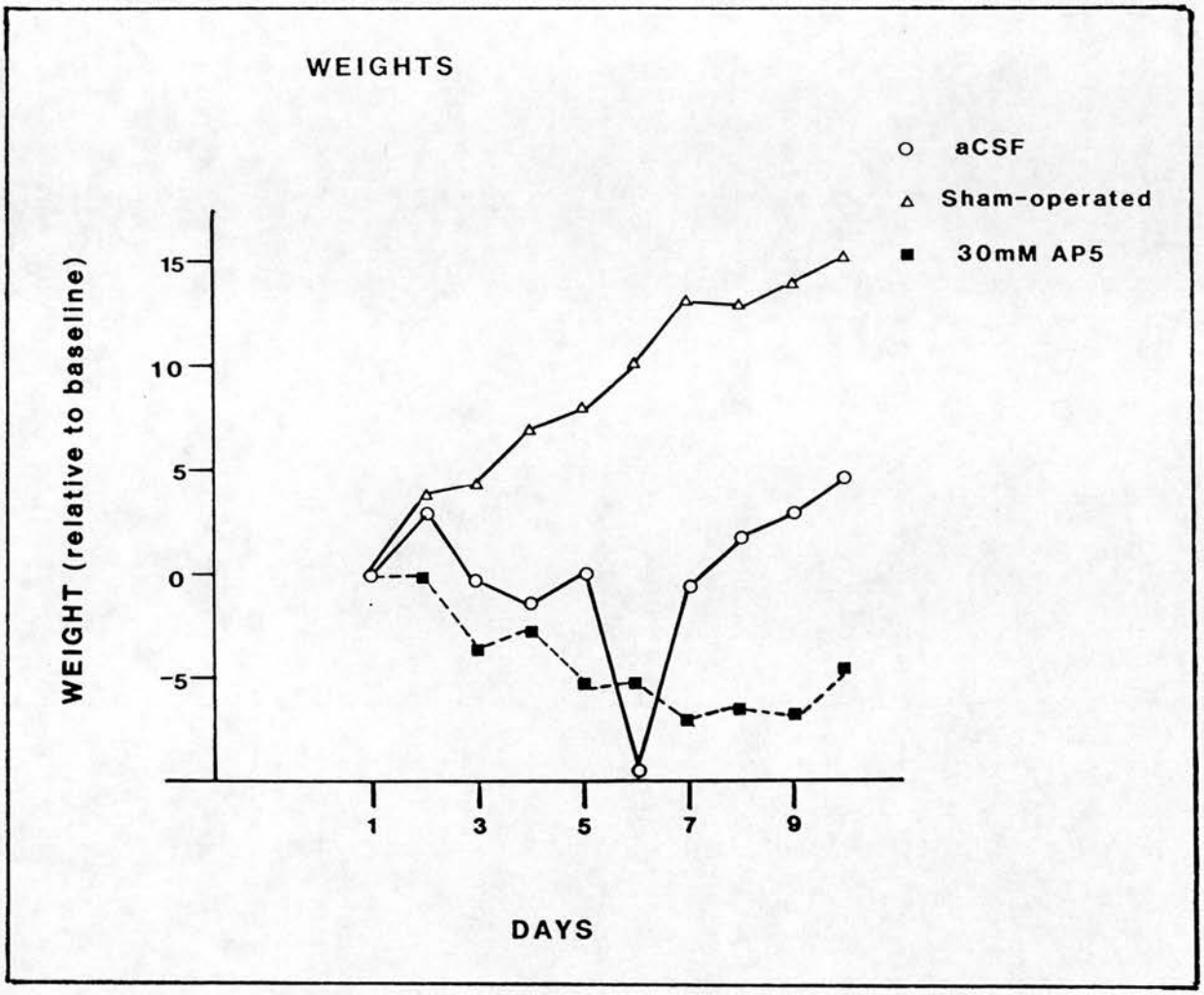


Fig.3.13:
WORKING MEMORY 3
Graph shows the amount of weight (gm) increase or decrease each day of the experimental period for the sham-operated, aCSF-infused controls and 30mM D-AP5 infused groups. Weights are measured against a baseline weight taken on the day of surgery.

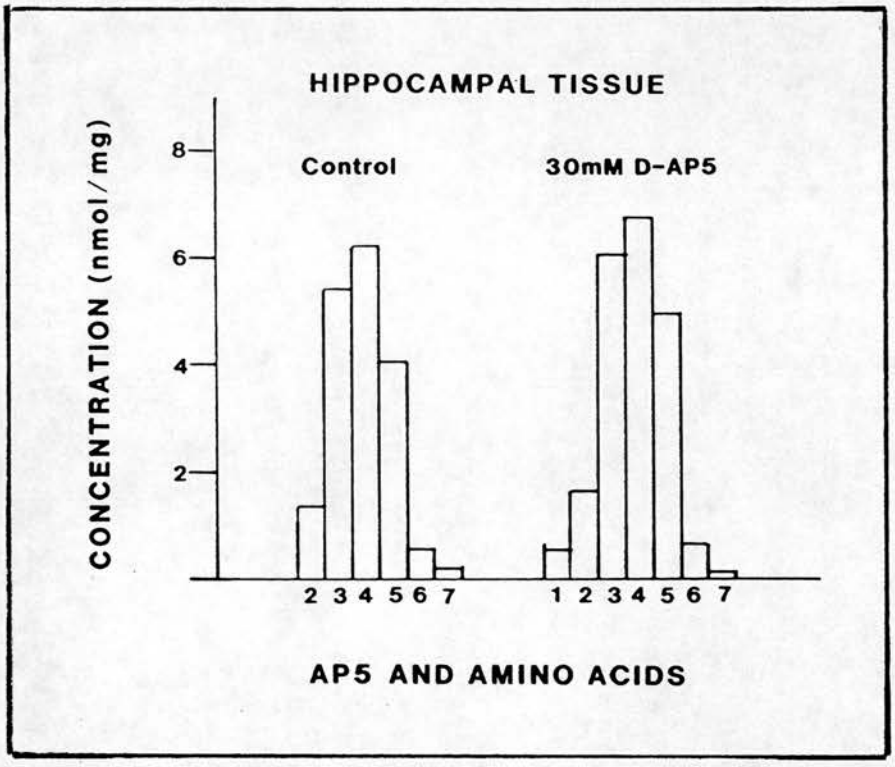


Fig.3.14:
WORKING MEMORY 3
Histogram shows the amount of AP5 and amino acids measured in the combined right and left hippocampus using HPLC: (1) AP5; (2) aspartate; (3) glutamate; (4) glutamine; (5) taurine; (6) alanine; (7) valine.

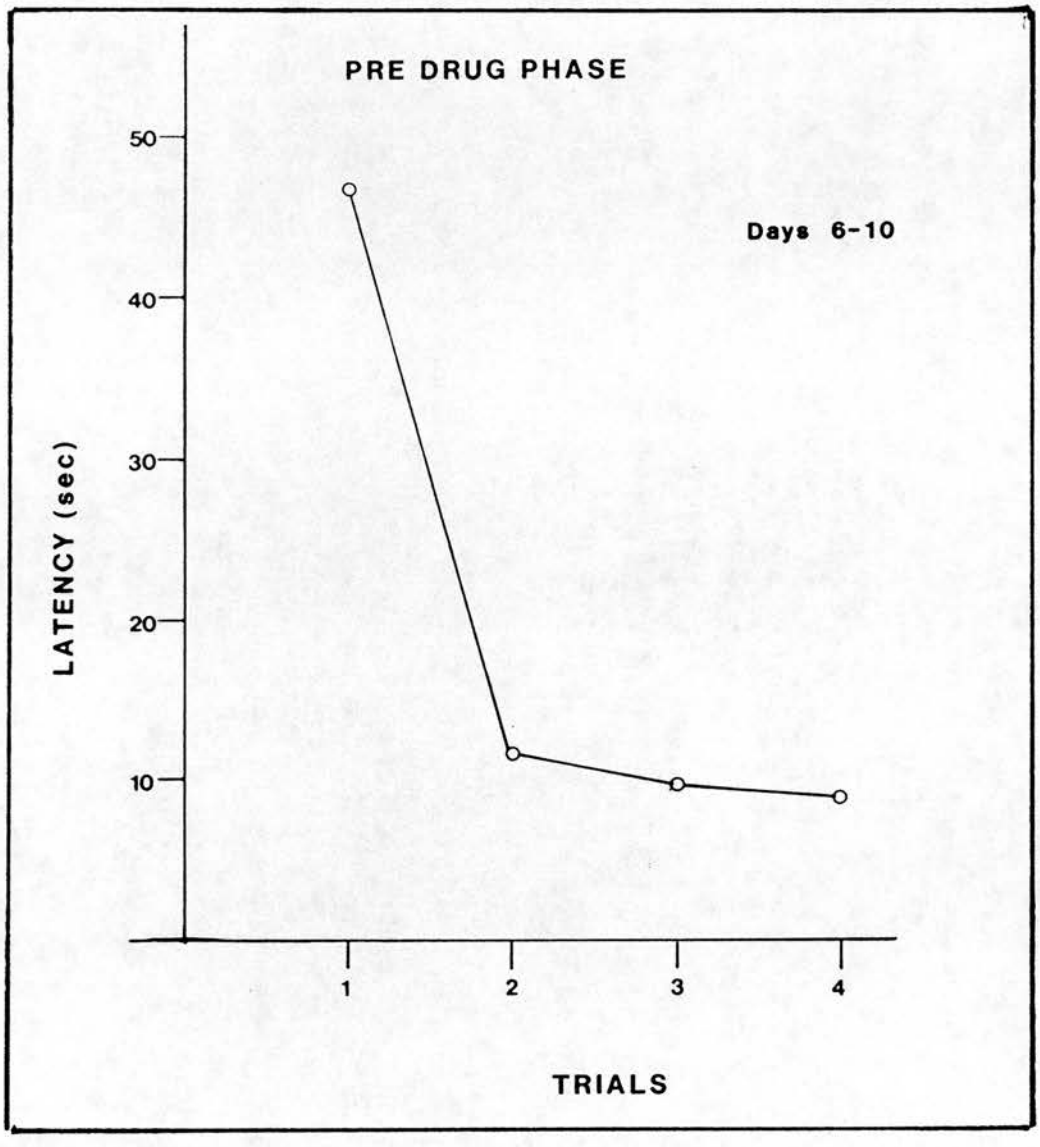


Fig.3.15:
WORKING MEMORY 3
The average escape latency of all animals for each of the 4 trials are plotted. The escape latencies from days 5-10 are plotted only.

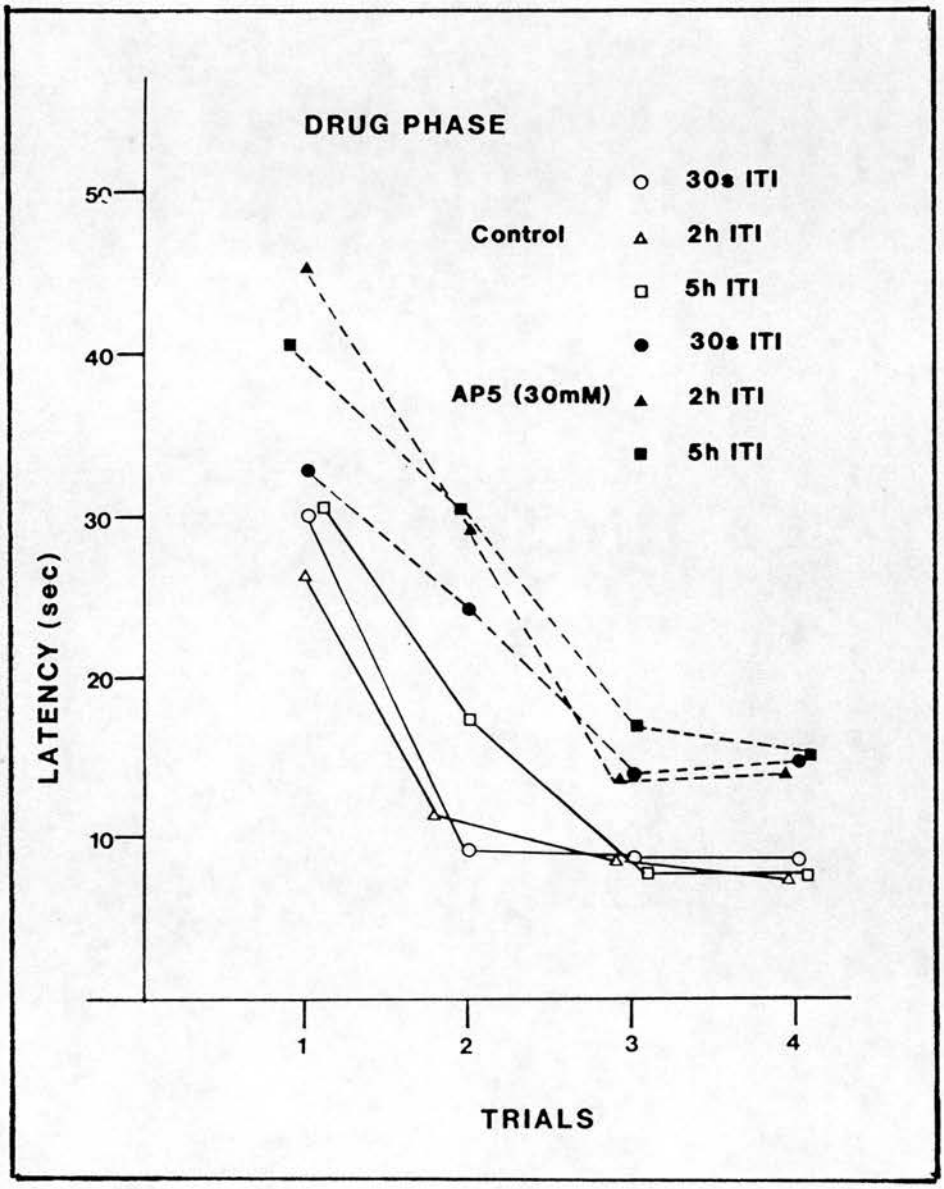


Fig.3.16:
WORKING MEMORY 3
The mean escape latencies for each of the 4 trials, at each delay period (30s, 2h and 5h) over the 10 day drug phase are plotted for the controls and 30mM D-AP5 group.

seen as a reduction in the savings between trials 1 and 2 at each interval were: 30s, 20.9s; 2h, 14.7s; 5h, 13.6s). By trials 3 and 4 (30s ITI) they escaped the water in approximately 8.0s. The savings for AP5 animals between trials 1 and 2 were: 30s, 8.6s; 2h, 15.6s; 5h, 10.0s. By trials 3 and 4 they were escaping the water in approximately 12.0s. Post hoc analysis of these latencies using Newman Keuls test showed the difference was not significant ($p>0.05$).

Trial 2

Separate analysis of trial 2 only showed that there was a highly significant difference between groups for the time taken to locate the platform and escape the water ($F(1,17)=10.6$; $p<0.01$). However, Newman Keuls post hoc analysis carried out on each group separately showed the 3 different time delays had no effect.

3.6.ii. Discussion

By the time animals started the drug phase, they had clearly demonstrated an ability to use working memory to an optimal level. During the drug phase, control animals continued to use working memory to an optimal level except for the 5 hour delay where there was a trend towards forgetting over that period of time. The AP5 group, however, showed impairment at all delays, including the 30 second delay between trials 1 and 2, unlike the AP5-treated animals in experiment 1. Comparing the savings between trials in the AP5-treated groups in Working Memory Experiments 2 and 3 the animals in experiment 3 showed less savings on trial 2 (averaged across all intervals) than the animals in experiment 2 (11.3s vs 17.5s). Furthermore, compared with their respective control groups, on trial 4 (when the ITI had been at 30s for two trials) the AP5 animals in experiment 2 had caught up to the same escape latency (a difference of 0.3s) whereas in experiment 3 the AP5 animals took 6.4s longer (although this was not significant). Thus, the results have failed to bear out the prediction of an interaction between AP5 and time.

One obvious possibility to account for these results is the amount of AP5 in the brain: the levels measured in the hippocampal tissue in experiment 3 were very high, in some of the animals equivalent to or greater than the amount in the very high concentration group of the dose-response study. This concentration

of AP5 is much higher than that shown to be necessary to block LTP or impair spatial reference memory (30s ITI) and higher than that found in Working Memory 2. It is interesting to note, however, in experiment 3 (and also experiment 2) that the AP5-treated animals did not show a sensorimotor impairment like that of the very high concentration group. A difference in procedure is that the present group had 40 trials of training before the AP5 was infused. Their lack of sensorimotor disturbance supports Morris' (1989) finding that prior exposure to the task reduces or eliminates sensorimotor effects.

Another possibility to consider is the complexity of the task. To test working memory in exclusion of reference memory, animals should be given training on the task first (Olton and Pappas, 1979). In this experiment, animals were pretrained on the task, using only a 30s delay. During the drug phase, 2 new aspects were introduced: two additional time delays, and the random dispersion of these delays throughout the testing period. The same procedure of pretraining was used in the 2nd experiment and animals did show impairment on the extended delay, but not on the brief delay. However these animals had nearly twice as many test sessions. Also in that experiment, the procedure used in each session was identical. In this experiment not only did an animal never know what delay to expect at the start of each session, it was also only tested on each time delay four times. In one sense each session was an unexpected new task. The combined effect of these 3 aspects may have made the task too complex to test working memory.

The aim of this experiment was to test the possibility of an interaction between time and the effect of AP5 suggested by experiments 1 and 2. The results were primarily confounded by the high concentration of AP5 measured in the brain but they also hinted at the possibility that the task had become one that required more than working memory to learn it. For these reasons it is recommended that the experiment be repeated, using a larger number of animals and selecting a subset whose AP5 levels are comparable to the level to block LTP. The effect may be tested more successfully by using a more "true" matching-to-sample task, by having a visible platform on trial 1 and then a hidden platform in the same position

on trial 2. The second possibility is to explore the effect of exposing animals to the three time intervals during the pretraining.

3.7. SUMMARY

The data from the working memory experiments indicates that animals are able to perform a working memory task at brief delays when NMDA receptors are blocked to a level that would impair spatial reference memory and the induction of LTP. If, however, the time interval is increased, the ability of AP5-treated animals to retain information about the location of the platform is impaired relative to controls and their own performance at brief delays. The effect of the AP5 appears to have an earlier onset of a time-dependent effect on normal animals first described by Panakhova et al (1984). The results suggest that information required for only a brief period of time does not require the type of ongoing neuronal processing in which the NMDA receptor is involved. Activation of cells through normal fast synaptic transmission at non-NMDA excitatory amino acid receptors or even receptors activated by other transmitters may underlie this form of information processing. However, as stated earlier, further research is required before a more definitive statement can be made.

CHAPTER 4: GENERAL DISCUSSION

The aim of this thesis has been to investigate a possible cellular mechanism underlying learning, specifically, an enduring form of synaptic plasticity modelled by LTP. This aim was tested by blocking NMDA receptors in the hippocampus, thereby preventing the induction of LTP, and testing animals on two behavioural tasks sensitive to hippocampal function.

4.1 Summary of results

Following chronic blockade of the NMDA receptor by icv infusion of the competitive antagonist, AP5, animals were impaired on a reference memory spatial navigation task but not in a working memory task when the delay period was short. Control studies showed that the AP5 impairment was broadly similar to that seen in animals with hippocampal lesions excepting that AP5 animals were more impaired on acquisition of the spatial navigation task than hippocampally-lesioned animals but less impaired on the transfer test. Furthermore, the AP5 effect was not due to impairment of sensorimotor function - drug-treated animals performed no differently from controls when prevented from using spatial learning. The high correlations between the levels of AP5 in the hippocampus and the blockade of both LTP and learning indicate that this type of learning requires an ongoing form of synaptic enhancement shown by LTP.

One qualitative difference noted in AP5 animals' performance (with exclusion of the very high concentration group) was that they were more impaired on the first trial of the day. This implies that blocking LTP interfered with the processing and/or storage of memories over a longer period of time. The results from the working memory experiments bear out this time-dependent factor to a certain extent because animals were unimpaired at short delays but affected at longer delays (despite the fact that the results from the final experiment were confounded by the very high levels of AP5 measured in the hippocampal tissue).

These results support the original finding of Morris et al (1986) and go a long way to support the NMDA receptor theory of learning. The correlated dose-dependent effect of spatial impairment and blockade of LTP overcome, to a certain extent, the problems associated with making a causal link between two of the independent events mediated via the NMDA receptor. The hypothesis

which the experiments were designed to test, however, was somewhat restricted because it is based on data showing the effects of the NMDA receptor in the hippocampus and a single form of plasticity measured in the hippocampus. It is important to point out that (i) The NMDA receptor is widely distributed throughout the cns, (ii) LTP can be induced in brain areas other than the hippocampus, and, conversely other brain areas have been implicated in spatial learning, and (iii) the NMDA receptor is also involved in generating cell excitability, and involved a range of functional consequences such as movement, neuronal development, kindling, epilepsy and neuronal degeneration. One must consider the wider implications of these other NMDA mediated effects in an attempt to understand how its involvement in learning may relate to a more unified notion of the mechanisms involved in cell excitability.

4.2. Implications of other brain areas that support LTP and other brain areas involved in spatial learning

In relating the effects of AP5 to a type of learning attributed to the hippocampus, it must be stressed that in the experiments described in this thesis, AP5 was infused into the brain through the right lateral ventricle. This method was used because the hippocampus lies alongside the ventricles and AP5 could diffuse to receptors quickly at high concentrations without causing damage to the hippocampus of a kind that might occur if injections of AP5 were made directly into the tissue. This method, however, ensured that AP5 was infused throughout the entire cns, although the concentration decreased with distance from the site of infusion. Two points of interest are (i) LTP, as mentioned earlier (Chapter 1.2.1) can be evoked in different areas of the brain, such as cortical areas (Komatsu et al, 1981; Lee, 1982; Sakimoto et al, 1986; Bindman, 1987; Artola and Singer, 1987); pyriform cortex (Stripling and Patneau, 1985); medial geniculate nucleus (Gerren and Weinberger, 1983) and amygdala (Racine et al, 1983). (ii) Lesion studies have shown that damage to brain structures other than the hippocampus such as the posterior cingulate areas (Sutherland, Whishaw and Kolb, 1988), medial frontal cortex (Sutherland, Kolb and Whishaw, 1982) and medial septum (Winson, 1978) also impair spatial learning. It seems highly likely, however, that the effect of AP5 on spatial learning was due to the interaction of AP5 at NMDA

receptors in the hippocampus because of the similarity in behavioural impairment to hippocampally lesioned animals. Furthermore, both the correlation between LTP and spatial learning and the fact that AP5 decreased in concentration the further away it was from the site of infusion also support this hypothesis. LTP, however, was not measured in any other brain area that has been shown to support LTP or where lesions impaired spatial learning. It seems likely that other brain areas contribute to spatial learning by feeding in information from other sensory modalities (see Swanson, 1983) and one must consider the possibility that blocking NMDA receptors in these areas may contributed to the learning deficit.

4.3. Activation of NMDA receptors is required in some forms of endogenous cell excitability involved in movement

NMDA receptor involvement in sensorimotor control has already been discussed (see Chapter 1.3 and discussions in the Dose-response study). Further evidence to suggest its involvement in movement has been demonstrated in detail using the lamprey and Xenopus embryo (Poon, 1980; Grillner, Wallen, McClellan, Sigvardt, Williams and Feldman, 1981; Wallen and Williams, 1984; Dale and Roberts, 1984; Dale, 1986) and it has also been shown in neonatal rat (Kudo and Yamada, 1987), amphibia (McClellan and Farel, 1985) and in turtles (Stein and Schild, 1988).

Control of rhythmic movement, such as walking, jumping swimming, breathing and scratching is thought to be mediated by a network of neurons called the "central pattern generators" (see Dale, 1986). These networks generate alternating periods of excitation and inhibition in a single movement cycle and occur in both vertebrates and invertebrates (Delcomyn, 1980).

In the isolated lamprey spinal cord, fictive swimming can be generated by the application of both Glu (Poon, 1980) and at lower concentrations of NMDA (Grillner et al, 1981). The firing patterns of the intact lamprey (whilst swimming) have been compared with those patterns in lamprey with spinal transection and the isolated lamprey spinal cord (Wallen and Williams, 1984). Similar patterns of firing seen in natural swimming were found when the spinal transection was stimulated to swim by light mechanical stimulation and D-Glu was applied to the *in vitro* preparation. In the *in vitro*

Xenopus embryo spinal cord, Dale and Roberts (1984) showed that application of EAA agonists to the bath medium also evoked fictive swimming patterns similar to the natural swimming patterns and these effects could be depressed by antagonists, although AP5 was not as effective as the broader band antagonists, PDA and DGG. A similar parallel between the isolated spinal cord of tadpoles at different stages of development and frogs was seen with the application of NMA to the bath medium in the *in vitro* preparation that coincided with the type of movement at the equivalent stages of development in the intact animal, such as swimming in the early stages and jumping and kicking in the later stages (McClellan and Farel, 1985).

Further evidence to implicate the NMDA receptor in this type of movement is that the presence of Mg^{++} (which also blocks the NMDA receptor) alters the patterns of firing which result in more stable and slower swimming in the lamprey (Brodin and Grillner, 1985). Dale speculates that the NMDA receptor may be involved in the generation and maintenance of the rhythmic patterns for swimming via a self-exciting network of rhythmically active neurons (including both NMDA and non-NMDA receptors), allowing the network to sustain itself by positive feedback and limiting itself, by using Ca^{++} sensitive conductances (see Dale, 1989).

Another form of rhythmic activity involved in locomotion, in which the NMDA receptor has been implicated is theta rhythm (Leung and Desborough, 1988; Vanderwolf and Leung, 1983). Theta rhythm is a form of EEG activity that is based on the synchronous firing (5-7 Hz) of theta cells in the hippocampus (O'Keefe and Dostrovski, 1971; Fox and Ranck, 1983) and is strongly influenced by input from the septum (see Bland, 1986). This form of activity has been recorded in animals engaged in movement or exploratory behaviour (Vanderwolf, 1961, 1975; Whishaw and Vanderwolf, 1973) and has been implicated in spatial learning (Winson; 1978; Berry and Thompson, 1978). Theta rhythm can be blocked by AP5 (Leung and Desborough, 1988) and the non-competitive antagonist, PCP (Vanderwolf and Leung, 1983). It must be pointed out, however, the infusion rate of AP5 used by Leung and Desborough (1988) was very high (approx 100 times higher than the 30mM infusion used in this thesis) and Vanderwolf and Leung (1983) used the non selective sigma opioid antagonist, PCP, which, although blocks the NMDA response, also blocks opioid receptor sites outwith the NMDA receptor-channel complex. Other research groups

using the hippocampal slice, have mimicked the frequency at which theta rhythm is generated and shown that low frequency stimulation on the peak of theta rhythm induces LTP (Larson et al, 1986; Lynch, 1978) and the effect is blocked by AP5 (Larson and Lynch, 1988).

This is particularly interesting because, from these results, one cannot rule out the possibility that hippocampal NMDA receptors may be involved in spatial learning through a physiological mechanism other than LTP, such as theta rhythm. It is also possible to consider that theta rhythm creates depolarisation of the post synaptic membrane of cells in the hippocampus, such that a form of synaptic plasticity similar to LTP can be induced with lower frequency stimulation that may occur via other inputs to the hippocampus, as suggested by Witter, Groenewegen, Lopez da Silva and Lohman, (1989). This would seem possible in the light of the fact that LTP can be evoked by converging inputs seen with co-activation of fibres in the same input or association of different inputs to the same synaptic regions evoked LTP. The hippocampus receives input from 2 major pathways, the perforant path input from the entorhinal cortex (which itself receives input from many cortical and subcortical areas) and the fimbria-fornix system. Normal stimulation of these 2 pathways simultaneously (Robinson, 1986) or stimulation of the contra and ipsilateral fibres of the same pathway (Levy and Steward, 1979) have been shown to evoke LTP.

4.4. NMDA receptors are involved in other forms of plasticity and cell excitability

In addition to its involvement in a form of synaptic plasticity thought to underlie learning, NMDA receptors are also involved in other forms of plasticity and cell excitability as well. In more general terms, plasticity is a phenomenon, that could be described as experience-dependent alterations of cellular properties which could include a number of functional changes such as reactive synaptogenesis (Liu and Chambers, 1958), sensitisation (Kandel, 1981) habituation (Thompson and Spencer, 1966). These effects could also be described as learning, although these span several levels of biological explanation. In higher order learning, such as spatial learning, a multicellular network of representations is thought to occur, based on information that is received, processed and emitted as as output, depending on their contextual involvement (O'Keefe

1983). Below, the NMDA receptor's involvement in some forms of synaptic plasticity, such as that involved in early development, cell excitability involved in epilepsy and the other cellular processed associated with neuronal degeneration are discussed. By relating the NMDA receptors involvement in the induction of LTP to other forms of plasticity, specifically to cell excitability, it incorporates LTP into a more global mechanism subserving different functions and places it in a more meaningful context.

4.4.i. NMDA receptor and development

The appearance of NMDA receptors occurs early in postnatal development; although very low in density they are present at postnatal day (PND) 0 (see McDonald and Johnston, 1990). In the hippocampus and cortical areas of the brain, there is a critical period where cells show an increase in binding to Glu and NMDA, which peaks at a higher level than that seen in the adult brain. This critical period varies with different species and in different brain areas. For example, in rat visual cortical areas, the response to [^{3}H]Glu binding transiently peaks at PND 15 and rapidly declines to adult level by PND 25 (Schliebs, Kullman and Bigl, 1986). In the cat, high-affinity uptake of D-aspartate (HA-D-asp) peaks to a level between postnatal week (PNW) 8 and 9 that is 7-fold higher than that in the adult cat. It begins to decline at PNW 10, but is still 80% higher than the adult level by PNW 17 (Fosse, Heggelund and Fonnum, 1989). A similar time course is seen with AP5-sensitive inhibition of [^{3}H]Glu binding (Bode-Greuel and Singer, 1989). An overshoot in binding to NMDA sites also occurs in the temporal cortex of rats, where the binding rises from PND 1 and then plateaus out at the adult level by PND 28 (McDonald, Johnston and Young, 1989). In the hippocampus of rats, [^{3}H]Glu binding overshoots the adult level at PND 9 and then declines to adult level at PND 23 (Baudry, Arst, Oliver and Lynch, 1981), and AP5-sensitive binding of [^{3}H]Glu exceeds adult density level between PNDs 4 and 13 in stratum radiatum of CA_1 (Tremblay, Roisin, Represa, Charriut-Marlangue and Ben-Ari, 1988). In other subfields of the hippocampus AP5-sensitive binding of [^{3}H]Glu differ in the peak level of densities, but in general they overshoot the adult level between PNDs 10 and 28.

During these periods, physiological and neurotrophic changes also occur which seem to parallel the ontogeny of electrophysiological responses to glutamate. In CA_3 of the hippocampus epileptogenic activity in response to NMDA increases from PND 1, reaching a peak at PND 10 (King, Cherubini, and Ben-Ari, 1989). This susceptibility is greater in the immature brain, (Swann and Brady, 1984) and the seizure activity can be blocked by NMDA receptor antagonists (Brady and Swann, 1986). Moreover, up to PND 10, cells in the hippocampus are not susceptible to the voltage-dependency of the NMDA receptor, as they are in the adult brain. Ben-Ari, Cherubini and Krnjevic (1988) have shown immature cells do not consistently exhibit the region of negative slope conductance characteristic of Mg^{++}-gating. Furthermore, the amount of LTP that can be evoked in CA_1 increases from PND 5 to PND 15, where it is greater than the amount seen in the adult (PND 60) (Harris and Teyler, 1984). This peak level corresponds to the ontogeny of the development of the NMDA recognition site in CA_1 (McDonald et al, 1989).

In young kittens, a form of experience-dependent plasticity occurs during the early months of development in the visual cortex. At first, cells in the visual cortex are functionally connected to both eyes and visual input between PNW 5 and PNM 4 leads to occular dominance (Hubel and Weisel, 1970; Blakemore and Van Slutyers, 1974). If a single eye is sutured between PND 1 to PNW 5, a shift in occular dominance occurs, where every neurone in the visual cortex becomes dominated by the still active eye. If the eye was sutured at PNW 14, occular dominance did not occur (Blakemore and Van Slutyers, 1974), suggesting there was a critical period of plasticity up to PNW 5. The NMDA receptor has been implicated in this form of synaptic plasticity because chronic infusion of AP5 into the visual cortex prevented the shift in dominance in kittens aged 4 to 5 weeks (Klineschmidt, Bear and Singer, 1987). Furthermore, Tsumoto and colleagues (Tsumoto, Hagihara, Sato and Hata, 1987) showed that AP5 had a greater effect at suppressing the visual response of cortical neurons in the kitten, aged between 4 and 8 weeks than it did in the adult cat. During this period of development NMDA receptors have also been demonstrated as having a neurotrophic function and to be involved in neuritic outgrowth (see McDonald, 1990). Applications of endogenous NMDA to cerebellar

granule cell cultures promotes cell survival in a dose-dependent manner and addition of competitive NMDA antagonists block this effect (Balazs, Hack, and Jorgenson, 1988; Balazs, Hack, Jorgenson and Cotman, 1989). The ability of NMDA to promote cell survival coincides with the same time that differentiating granule cells migrate to the granule layer and are innervated by glutamate *in vivo* (Altman, 1982).

Kynurenate and D-AP5 applied to dissociated cerebellar granule cell cultures from 4 to 5 day old rats inhibited the number of cells extending neurites that would normally occur by approximately 50% and this inhibitory effect could be reversed in a dose-dependent manner by the addition of endogenous Glu (Pearce, Cambray-Deakon and Burgoyne, 1987). Brewer and Cotman (1989) showed that NMDA applied to cultured hippocampal granule cells from 4 day old rat increased the number of neurite branch points and the total length of neurites and this effect could be blocked by co-application of MK-801. However, it is not known whether these outgrowths are axonal or dendritic. This is important because the effects non-NMDA agonists were not tested, and other studies looking at the effect of EAAs on dendritic outgrowth have shown Glu regulated growth of dendritic growth can be blocked by the non-selective antagonist DGG but not AP5 (Mattson, Dou and Kater, 1988).

These data suggest that during development, NMDA receptors are malleable and subserve a variety of functions during specific windows of time. This is emphasised by the overshoot in the response of cells to the application of endogenous ligands which reduce with maturation. It further suggests that during the developmental period, NMDA receptors are involved in the establishment of more permanent functions depending on the chemical and electrophysiological events and stimulus input that occur at specific times.

4.4.ii. NMDA receptor and epilepsy

A body of research has been carried out investigating the NMDA receptors' involvement in cell hyperexcitability that leads to seizure activity and, in the extreme, neuronal degeneration. This has clinical implications for suggesting the NMDA receptor is involved in epilepsy (Croucher, Collins and Meldrum, 1982).

The role of the NMDA receptor in epilepsy was first implied when Hayashi (1952, 1953 cf Meldrum, 1988a) injected Glu into the cortex or subcortical nucleus and found that it caused convulsions. Since then, genetic and chemically induced models of epilepsy have been developed and it has been found that NMDA receptor antagonists suppress seizure activity (see Meldrum, 1989). For example, Croucher et al (1982) showed that AP7 had anticonvulsant effects in sound-induced seizures in DBA/2 mice and pentylenetrazol seizures in Swiss mice. A range of competitive NMDA antagonists including AP7 and AP5 when administered iv (Meldrum, Croucher, Badman and Collins, 1983; Meldrum, 1984) and CGP 37849 and CPP-ene when administered orally suppress photically-induced myoclonic responses in photosensitive baboons (Meldrum, 1989). NMDA receptor antagonists also prevent a variety of excitatory amino acid and cholinergic muscarinic agonist induced seizures evoked in the limbic system (Patel, 1988) and cobolt implantation-induced seizures in the sensorimotor cortex (Coutinho-Netto, Abdul-Ghani, Collins and Bradford, 1981).

Perhaps the most relevant model of epilepsy to this thesis is the kindling model because there are some similarities between the stimulation used to kindle an animal and those involved in the induction of LTP (Sutula and Steward, 1986; Cain, 1989).

Goddard et al (1969) showed, *in vivo*, that brief bursts of high frequency stimulation in limbic regions of the brain such as the amygdala, septum and hippocampus, repeated at 24 hour intervals for several days resulted in seizure activity. At first there was little or no effect but with repeated application a slow build up of epileptiform activity occurred, which resulted in a behavioural seizure response. Reducing or increasing the interval between stimulation did not evoke convulsions. A number of *in vivo* experiments have shown that both selective (AP7 and MK-801) and non selective NMDA antagonists, Ketamine and PCP can have two effects on kindling: they can prolong or prevent the development of seizures (Callaghan and Schwartz, 1980; Bowyer and Winters, 1981; Bowyer, 1982; McNamara, Russell, Rigsbee and Bonhous, 1988; Vezzani, Wu, Moneta and Samanin, 1988) and they can reduce the intensity of the seizures after kindling (Peterson, Collins and Bradford, 1983; Vezzani et al, 1988; Gilbert, 1988; Sato, Morimoto and Okomoto, 1988). However, McNamara et al (1988) failed to prevent already

established kindling with MK-801 and Wilson and colleagues (Wilson, Stasheff, Swartzwelder, Clark, Anderson and Lewis, 1989) point out that Gilbert (1988) and Sato et al (1988) used high concentrations of MK-801 that cause behavioural depression.

Because the time course for the development of kindling is prolonged (usually a single train of 1-2s at 60Hz: see Wilson et al, 1989), it is difficult to demonstrate where, during the time course, the NMDA receptor may be involved in kindling in the *in vivo* model. A form of epileptogenesis that is very similar to kindling was shown in the hippocampal slice by Wilson and colleagues (Stasheff, Bragdon and Wilson, 1985; Anderson, Schwartzwelder and Wilson, 1987). Not only was it characterised by interictal spiking and paroxysmal depolarising shifts (which are established indicators of epileptiform activity in the slice: see Meldrum, 1988b) but it also showed similar electrographic seizures to those seen in the *in vivo* model of kindling. Application of AP5 to the slice prevented the development of electrogenic seizures but application of AP5 after the seizure threshold had been reached had little effect (Wilson et al, 1989). They suggested that the role of the NMDA receptor in seizure activity is in seizure induction but not expression. This contradicts the effects of NMDA antagonist on seizures once they have been established as shown by other groups using interictal spiking in the slice (Slater, Stelzer and Galvin, 1985) and *in vivo* models of kindling described above.

One important similarity in the induction of kindling and LTP is that, during the stimulation time course for kindling, some groups have found that LTP occurs before the onset of convulsions (Slater et al, 1985; Sutula and Steward, 1986). This suggests that the NMDA receptor adds to the overall level of excitability, and although one view of LTP is to consider it as a trigger for kindling induced epileptogenesis (Slater et al, 1985), it is also possible to consider LTP and kindling on a continuum of cell excitability to which activation of the NMDA receptor contributes. At the lower end of the scale where LTP can be evoked, this level of excitability may enhance synaptic efficacy such that memories can be processed and stored, beyond that level of excitability or if the excitability spreads, it may become detrimental and cause seizures.

4.4.iii. NMDA receptors and neuronal degeneration

NMDA receptors involvement in neuronal degeneration, was first indicated when systemic injections of Glu destroyed cells in the retina of the immature mouse (Lucas and Newhouse, 1957). The neurotoxic effects of Glu in brain cells has also been shown in monkeys and rats (see Rothman and Olney, 1987) and the effect has been localised to a post synaptic membrane site. This led to the formulation of the excitotoxic hypothesis which suggested that neurotoxicity was mediated by prolonged depolarising effects of Glu (Olney, Ho and Rhee, 1971; Olney, 1978), resulting in a pathological condition of membrane permeability and impaired cellular homeostasis. It was further postulated that the underlying mechanism was a persisting influx of Ca^{++} through the membrane which led to the breakdown of ion homeostasis (Seisjo and Bengtsson, 1989). Choi (1987) showed in dissociated murine cortical cell cultures that the Glu neurotoxicity was dependent on two components. First there was a swelling in cells occurred which was dependent on extracellular Na^{+} and Cl^{-} and then Ca^{++} dependent neuronal disintegration occurred. Although each independent component could cause irreversible injury to the cells, the Ca^{++} component was more effective with lower exposures to Glu. This is particularly relevant in view of the Ca^{++} permeability of the NMDA receptor channel occurring during the induction of LTP.

In vitro models of brain damage, using *ex vivo* chick embryo retina, exposure of the retina to the EAA agonists, KA and NMDA resulted in brain damage similar to that caused by Glu (Rothman and Olney, 1987). It is therefore of interest that NMDA receptor antagonists have exhibited neuroprotective properties in various *in vivo* models of ischaemia.

In focal ischaemia, induced by middle cerebral artery occlusion, the non-competitive NMDA antagonist, MK-801 reduces the area of cortical infarct (Park, Nehls, Graham, Teasdale and McCulloch, 1988; Park, Nehls, Graham, Teasdale and McCulloch, 1988). Protection is also offered by AP7 in hypoglycemic brain damage caused by reducing the supply of glucose (Weiloch, 1985). However, studies on global ischaemic damage induced by two vessel (Smith, Auer and Siesjo, 1984) or four vessel occlusion (Pulsinelli, Brierley and Plum, 1982) have produced some contradictory results. Some groups have shown that both competitive and non-competitive

antagonists protect against damage when injections of antagonists were given prior to the occlusion (Swan, Evans and Meldrum, 1988; Gill, Foster and Woodruff, 1987) but this is has not been supported by others (Block and Pusinelli, 1987; Jensen and Auer, 1988; Weiloch, Gustafsson and Westberg, 1988). In the *in vitro* studies, the selective NMDA antagonist, AP5 was 100-fold more powerful at protecting NMA induced toxicity than the non selective antagonist, D-(α)aminoadipate (Olney, LaBruyere, Collins and Curry, 1981) but non-competitive NMDA antagonists were weak at blocking Glu toxicity in the chick retina (Rothman and Olney, 1987). Both the non-selective EAA antagonists (Rothman, 1984) and the selective antagonists (Rothman, Thurston and Haurhart, 1987) protect against hypoxia-induced damage in rodent hippocampus and cortex cultures (Rothman, 1984; Rothman et al, 1987).

The Glu hypothesis of neuronal death, as mentioned above, states that it occurs as a result of prolonged depolarisation of Glu sites, which leads to a breakdown in the homeostatic control of the cell. There some similarities between neuronal degeneration and the induction of LTP. For example, there is the common factor of the influx of Ca^{++} through the membrane. In LTP, this is the mechanism thought to trigger off the sequence of second messenger activity that leads to the expression of the synaptic enhancement. In cell, death the entry of Ca^{++} from the extracellular fluid is sustained and coupled with a progressive failure of membrane ion permeability and intracellular buffering of Ca^{++}, such that the cell loses control of its homeostatic mechanism. In LTP, however, the cells maintain control over their ion homeostasis.

4.5. Conclusion

In considering how hippocampal NMDA receptor may be involved in spatial learning in more global terms of plasticity and cell excitability, it can be speculated that a general continuum of cell excitability exists, subserving different functions in different areas of the brain. In more general terms of synaptic plasticity, one might imagine this continuum to resemble the sigmoidal curve in a dose-response function, where at the lower end a form plasticity involving the NMDA receptor is required during early development and at the other end it is involved in neuronal degeneration. Between these two extremes, NMDA receptors are involved in the induction of

LTP, creating an appropriate physiological environment in which information is processed during learning. Further along the continuum where cell excitability is increased seizure activity occurs. Two important riders must be added: (i) First, in the case of neuronal degeneration, it might be the case that the level of cell excitation crosses a threshold where cells loose control of their homeostasis and this results in death. (ii) Second, loss of inhibition by blocking GABA receptor function also contributes to the induction of LTP, epilepsy and neuronal death. This suggests that there is a fine balance between excitation and inhibition that controls the level of excitability and one might look to future research involved manipulating the fine tuning of this event with peptides and second messengers.

REFERENCES

Abraham, W.C. and Mason, S.E. (1988)
Effects of NMDA receptor/channel antagonist CPP and MK-801 on hippocampal field potentials and long term potentiation in anaesthetised rats. BRAIN RES., vol 462: pp 40-46.

Aggleton, J.P., Hunt, P.R. and Rawlins, J.N.P. (1986)
The effects of hippocampal lesions upon spatial and non-spatial tests of working memory. BEHAV. BRAIN RES., vol 19: pp 133-146.

Alger, B.E. and Teyler, T.J. (1976)
Long-term and short-term plasticity in the CA_1, CA_3 and dentate regions of the rat hippocampal slice. BRAIN RES., vol 110: pp 463- 480.

Altmann, J. (1982)
Morphological development of the rat cerebellum and sources of its mechanism. In: The cerebellum: New vistas, (eds) Chan Palay, V. and Palay, P. Springer, Berlin.

Amaral, D.G. and Witter, M.P. (1989)
The three dimensional organisation of the hippocampal formation: A review of anatomical data. NEUROSC., vol 31: pp 571-591.

Andersen, P. (1960)
Interhippocampal impulses. II. Apical dendritic activation of CA_1 neurons. ACTA PHYSIOL. SCAND., vol 48: pp 178-208.

Andersen, P., Holmqvist, B. and Voorhoeve, P.E. (1966)
Entorhinal activation of dentate granule cells. ACTA PHYSIOL. SCAND., vol 66: pp 448-460.

Andersen, P., Bliss, T.V.P. and Skrede, K.K. (1971a)
Lamellar organisation of hippocampal excitatory pathways. EXP. BRAIN RES., vol 13: pp 222-238.

Andersen, P., Bliss, T.V.P. and Skrede, K.K. (1971b)
Unit analysis of hippocampal population spikes. EXP. BRAIN RES., vol 13: pp 208-221.

Andersen, P., Sundberg, S.H., Sveen, O. and Wigstrom, H. (1977)
Specific long-lasting potentiation of synaptic transmission on hippocampal slice. NATURE, vol 266: pp 736-737.

Andersen, P., Sundberg, S.H., Sveen, O., Swann, J.W. and Wigstrom, H. (1980)
Possible mechanisms for long-lasting potentiation of synaptic transmission in hippocampal slices from guinea-pigs. J. PHYSIOL., vol 302: pp 463-482.

Anderson, W.W., Scartzwelder, H.S. and Wilson, W.A. (1987)
The NMDA receptor antagonist 2-Amino-5-phosphonovalerate blocks stimulus train-induced epileptogenesis but not epileptiform bursting in the rat hippocampal slice. J. NEUROPHYSIOL., vol 57(1): pp 1-21.

Artola, A. and Singer, W. (1987)
Long-term potentiation and NMDA receptors in rat visual cortex. NATURE, vol 330: pp 649-652.

Ascher, P. and Nowak, L. (1986)
Calcium permeability of the channels activated by N-Methyl-D-aspartate (NMDA) in isolated mouse central neurones. J. PHYSIOL., vol 337: 35P.

Ascher, P and Nowak, L. (1987)
Electrophysiological studies of NMDA receptors. TRENDS NEUROSCI., vol 10(7): pp 284-293.

Ascher, P., Bregestovski, P. and Nowak, L. (1988)
N-Methyl-D-aspartate-activated channels of mouse central neurones in magnesium-free solutions. J. PHYSIOL., vol 399: 207- 226.

Ault, B., Evans, R.H., Francis, A.A., Oakes, D.J. and Watkins, J.C. (1980)
Selective depression of excitatory amino acid induced depolarisations by magnesium ions in isolated spinal cord preparations. J. PHYSIOL., vol 307: pp 413-428.

Auyeung, A., Goh, J.W., Li, I.S., May, P., Palleta, M.J. and Sastry, B.R. (1986)
Is calcium required for the induction of long-term synaptic potentiation in the hippocampus? BR. J. PHARMACOL., vol 89: 779P.

Balazs, R., Hack, N. and Jorgenson, O.S. (1988)
Stimulation of the N-Methyl-D-aspartate receptor has a trophic effect on differentiating cerebellar granule cells. NEUROSCI. LETT., vol 87: pp 80-86.

Balazs, R., Hack, N., Jorgenson, O.S. and Cotman, C.W. (1989)
N-Methyl-D-aspartate promotes the survival of cerebellar granule cells: Pharmacological characterisation. NEUROSCI. LETT., vol 101: pp 241-246.

Barnes, C.A. (1979)
Memory deficits associated with senescence: A neurophysiological and behavioural study in the rat. J. COMP. PHYSIOL. PSYCHOL., vol 93(1): pp 74-104.

Barnes, C.A. and McNaughton, B.L. (1980)
Spatial memory and hippocampal synaptic plasticity in senescent and middle aged rats. In: The Psychology of Aging: Problems and perspectives, (ed) Stein, D. Elsevier, Amsterdam.

Barnes, C.A. and McNaughton, B.L. (1985)
An age comparison of rates of acquisition and forgetting of spatial information in relation to long-term enhancement of hippocampal synapses. BEHAV. NEUROSCI., vol 99(6): pp 1040-1048.

Barnes, C.A. (1988)
Spatial learning and memory processes: the search for their neurobiological mechanisms in the rat. TRENDS NEUROSC., vol 11(4): pp 163-169.

Barrioneuvo, G. and Brown, T.H. (1983)
Associative long-term potentiation in hippocampal slices. PROC. NATL. ACAD. SCI. USA., vol 80: pp 7347-7351.

Bashir, Z.I., Tam, B. and Collingridge, G.L. (1990)
Activation of the glycine site in the NMDA receptor is necessary for the induction of LTP. NEUROSCI. LETT., vol 108: pp 261-266.

Baudry, M., Arst, D., Oliver, M. and Lynch, G. (1981)
Development of glutamate binding sites and their regulation by calcium in rat hippocampus. DEV. BRAIN RES., vol 1: pp 37-48.

Ben-Ari, Y., Cherubini, E. and Krnjevic, K. (1988)
Changes in voltage dependence of NMDA currents during development. NEUROSCI. LETT., vol 94: pp 88-92.

Bennett, D.A. and Amrick, C.L. (1986)
2-Amino-7-phosphonoheptanoic acid (AP7) produces discriminitive stimuli and anti-conflict effects similar to diazepam. LIFE SCI. vol 39: pp 2455-2461.

Benveniste, H. (1989)
Brain Microdialysis. J. NEUROCHEM. vol 52(6): pp 1667-1679.

Berger, T. (1984)
Long-term potentiation of hippocampal synaptic transmission affects rate of behavioural learning. SCIENCE, vol 224: pp 627-629.

Berry, S.D. and Thompson, R.F. (1978)
Prediction of learning rate from the hippocampal electroencephalogram. SCIENCE, vol 200: pp 1298-1300.

Bindman, L.J., Meyer, T. and Pockett, S. (1987)
Long-term potentiation in rat neo-cortical neurones in slices, produced by repetitive pairing of an afferent volley with intracellular depolarising current. J. PHYSIOL., vol 386: 90P.

Bisco, T.J., Evans, R.H., Francis, A.A., Martin, M.R., Watkins, J.C., Davies, J. and Dray, A. (1977)
D-(alpha)-Aminoadipate as a selective antagonist of amino acid-induced and synaptic excitation of mammalian spinal neurones. NATURE, vol 270: pp 743-745.

Blackstadt, T.W., Brink, K., Hem, J. and Jeune, B. (1970)
Distribution of mossy fibres in the rat. An experimental study with silver impregnation methods. J. COMP. NEUROL., vol 138: pp 433-450.

Blakemore, C. and Van Sluyters, R.C. (1974)
Reversal of physiological effect of monocular deprivation in kittens: Further evidence for a sensitive period. J. PHYSIOL. vol 237: pp 195-216.

Bland, B.H. (1986)
The physiology and pharmacology of hippocampal formation theta rhythms. PROGS. NEUROBIOL., vol 26: pp 1-54.

Bliss, T.V.P. and Lomo, T. (1973)
Long lasting potentiation of synaptic transmission in the dentate area of the anaesthetised rabbit following stimulation of the perforant path. J. PHYSIOL. (Lond), vol 232: pp 331-356.

Bliss, T.V.P. and Gardner-Medwin, A. (1973)
Long lasting potentiation of synaptic transmission in the dentate area of the unanaesthetised rabbit following stimulation of the perforant path. J. PHYSIOL. (Lond), vol 232: pp 357-374.

Bliss, T.V.P., Lancaster, B. and Wheal, H.V. (1983)
Long-term potentiation in commissural and Schaeffer projections to hippocampal CA_1 cells. An *in vivo* study in the rat. J. PHYSIOL., vol 341: pp 617-626.

Bliss, T.V.P. and Lynch, M.A. (1988)
Long-term potentiation of synaptic transmission of the hippocampus: properties and mechanisms. In: Synaptic potentiation of the brain: a critical analysis, (eds) Landfield, P.W. and Deadwyler, S.A. Alan R. Liss Inc. New York.

Block, G.A. and Pulsinelli, W.A. (1987)
Excitatory amino acid receptor antagonists: Failure to prevent ischaemic neuronal damage. J. CEREB. BLOOD FLOW METAB., vol 7 (Suppl 1): S149.

Bode-Grueul, K.M. and Singer, W. (1989)
The development of N-Methyl-D-aspartate receptors in cat visual cortex. DEV. BRAIN RES., vol 46: pp 197-204.

Bouffard, J.P. and Jarrard, L.E. (1988)
Acquisition of a complex place task in rats with selective ibotenate lesions of hippocampal formation: Combined lesions of subiculum and entorhinal cortex vs hippocampus. BEHAV. NEUROSCI., vol 102(6): pp 828-834.

Bower, J.F. and Winter, W.D. (1981)
The effects of various anaesthetics on amygdaloid kindling seizures. NEUROPHARM., vol 20: pp 199-209.

Bower, J.F. (1982)
Phencyclindine inhibition of the rate of development of amygdaloid kindled seizures. EXP. NEUROL., vol 75: pp 173-183.

Bradler, J.E. and Barrionuevo, G. (1989)
Long-term potentiation in hippocampal CA_3 neurons: Tetanised input regulates hetrosynaptic efficacy. SYNAPSE, vol 4: pp 132- 142.

Brady, R.J. and Swan, J.W. (1986)
Ketamine selectively suppresses synchronised afterdischarges in immature hippocampus. NEUROSCI. LETT., vol 69: pp 143-149.

Brewer, G.J. and Cotman, C.W. (1989)
NMDA receptor regulation of neuronal morphology in cultured hippocampal neurons. NEUROSCI. LETT., vol 99: pp 268-273.

Brodin, L. and Grillner, S. (1986)
Effects of magnesium on fictive locomotion induced by activation of N-Methyl-D-Aspartate (NMDA) receptors in the lamprey spinal cord *in vitro*. BRAIN RES., vol 380: pp 244-252.

Butelman, E.R. (1989)
The effect of MK-801 an NMDA receptor antagonist, on the radial arm maze task in the rat. PHARMACOL. BIOCHEM. BEHAV., vol 34(1): pp 13-16.

Buzsaki, G. (1980)
Long-term potentiation of commissural path CA_1 pyramidal cell synapse in hippocampus of freely moving rat. NEUROSCI. LETT., vol 19: 293-296.

Cahusac, P.M.B., Evans, R.H., Hill, R.G., Rodriquez, R.E. and Smith, D.A.S. (1984)
The behavioural effects of an NMDA receptor antagonist following application to the lumbar spinal cord of conscious rats. NEUROPHARM., vol 23(7A): pp 719-724.

Cain, D.P. (1989)
Long-term potentiation and kindling: How similar are the mechanisms? TRENDS NEUROSCI., vol 12(1): pp 6-10.

Cajal, Ramon y. (1911)
Histologie du systeme nerveux de l'homme et des vertebres, vol II. Maluine, Paris.

Callaghan, D.A. and Schwartz, W.S. (1980)
Pharmacological modifications of amygdaloid-kindling seizures. NEUROPHARM., vol 19: pp 1131-1136.

Castro, C.A., Silbert, L.H., McNaughton, B.L. and Barnes, C.A. (1989)
Recovery of spatial learning deficits after decay of electrically induced synaptic enhancement in the hippocampus. NATURE, vol 342: pp 545-548.

Chang, F.L.F. and Greenough, W.T. (1984)
Transient and enduring morphological correlates of synaptic activity and efficacy changes in the rat hippocampal slice. BRAIN RES., vol 309: pp 35-46.

Choi, D.W. (1987)
Ionic dependence of glutamate neurotoxicity. J. NEUROSCI., vol 7(2): pp 369-379.

Church, J., Davies, S.N., Lagnado, L., Lodge, D., Martin, D. and Marshall, J. (1987)
Studies on the use-dependency of phencyclidine-like compounds as N- Methyl Aspartate antagonists *in vivo* and *in vitro*. BR. J. PHARM. Proc. Suppl., 90, 10P.

Collingridge, G.L., Kehl, S.J. and McLennan, H. (1983)
Excitatory amino acids in synaptic transmission in the schaeffer collateral-commissural pathway of the rat hippocampus. J. PHYSIOL., vol 334: pp 33-46.

Collingridge, G.L., Coan, E.J., Herron, C.E. and Lester, R.A.J. (1987)
Role of excitatory amino acid receptors in synaptic transmission and plasticity in hippocampal pathways. In: Excitatory amino acid transmission, (eds) Hicks, T.P., Lodge, D. and McLennan. H., Alan. R. Liss Inc., New York.

Collingridge, G.L. and Davies, S.N. (1989)
NMDA receptors and long-term potentiation in the hippocampus. In: The NMDA Receptor, (eds) Watkins, J.C. and Collingridge, G.L. Oxford University Press.

Compton, R.P., Contreras, P.C., O'Donoghue, T.L. and Monaghan, J.B. (1987)
The N-Methyl-D-Aspartate antagonist, 2-Amino-7-phosphonohaptanoate produces phencyclidine-like behavioural effects in rats. EUROP. J. PHARMACOL., vol 136: pp 133-134.

Contreras, P.C., Rice, K.C., Jacobson, A.E. and O'Donohue, T.L. (1986).
Stereotyped behaviour correlates better than ataxia with phencyclidine-receptor interactions. EUR. J. PHARMACOL., vol 121: pp 9-18.

Contreras, P.C., Contreras, M.L., O'Donohue, T.L. and Lair, C.C. (1988)
Biochemical and behavioural effects of sigma and PCP ligands. SYNAPSE, vol 2: pp 240-243.

Cotman, C.W., Monaghan, D.T. and Ganong, A.H. (1988)
Excitatory amino acid neural transmission: NMDA receptors and Hebb-type synaptic plasticity. ANN. REV. NEUROSCI., vol 11: pp 61-80.

Coutinho-Netto, J., Abdul-Ghani, A.S., Collins, J.F. and Bradford, H.F. (1981)
Is glutamate a trigger factor in epileptic hyperactivity? EPILEPSIA, vol 22: pp 289-296.

Croucher, M.J, Collins, J.F. and Meldrum, B.S. (1982)
Anticonvulsant action of excitatory amino acid antagonists. SCIENCE, vol 216: pp 899-901.

Dale, N. and Roberts, A. (1984)
Excitatory amino acids in xenopus embryo spinal cord and their role in activation of swimming. J. PHYSIOL., vol 384: 527- 543.

Dale, N. (1986)
Excitatory synaptic drive for swimming mediated by amino acid receptors in the lamprey. J. NEUROSCI., vol 6(9): pp 2668-2675.

Dale, N. (1989)
The role of NMDA receptors in synaptic intergration and the organisation of complex neural patterns. In: The NMDA receptor, (eds) Watkins, J.C. and Collingridge, G.L., Oxford Univ. Press.

Danysz, W., Wroblewski, J.T. and Costa, E. (1988)
Learning impairment in rats by N-methyl-D-aspartate receptor antagonists. NEUROPHARMACOL., vol 27(6): pp 653-656.

Davidson, T.L. and Jarrard, L.E. (1989)
Retention of concurrent conditional discrimminations in rats with ibotenate lesions of hippocampus. PSYCHOBIOL., vol 17(1): pp 49-60.

Davies, J., Francis, A.A., Jones A.W. and Watkins, J.C. (1981)
2-Amino-5-phosphonovalerate (2APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. NEUROSCI. LETT., vol 21: pp 77-81.

Davies, J, Evans, R.H., Herrling, P.L., Jones A.W., Olverman, H.J., Pook, P. and Watkins, J.C. (1986)
CPP, a new potent and selective NMDA antagonist. Depression of central neuron responses, affinity for [^{3}H]D-AP5 binding sites on brain membranes and anticonvulsant activity. BRAIN RES., vol 382: pp 169-173.

Davies, S.N., Lester, R.A.J., Reymann, K.G. and Collingridge, G.L. (1989)
Temporally distinct pre- and post- synaptic mechanisms maintain long-term potentiation. NATURE, vol 338: pp 500-503.

Dawborn, D. and Pycock, C.J. (1981)
Motor effects following application of putative excitatory amino acid antagonists to the region of the mesencephalic dopamine cell bodies in the rat. NAUNYN-SCHMEID. ARCH PHARMACOL., vol 318: pp 100-104.

Delcomyn, F. (1980)
Neural basis of rhythmic behaviour in animals. SCIENCE, vol 210: pp 492-498.

Desmond, N.L. and Levy, W.B. (1986)
Changes in numerical density of synaptic contacts with long-term potentiation in the hippocampal dentate gyrus. J. COMP. NEUROL., vol 253: pp 466-475.

Doller, H.J. and Weight, F.F. (1985)
Perforant pathway evoked long-term potentiation of CA_1 neurons in the hippocampal slice preparation. BRAIN RES., vol 333: pp 305-310.

Douglas, R.M. and Goddard, G.V. (1975)
Long-term potentiation in the perforant path granule cell synapses in the rat hippocampus. BRAIN RES., vol 86: pp 205-215.

Douglas, R.M. (1977)
Long-lasting synaptic potentiation in the rat dentate gyrus following brief high frequency stimulation. BRAIN RES., vol: pp 361-365.

Douglas, R.M. (1983)
Temporal characteristics of stimuli that produce long-term potentiation. ADV. PHYSIOL. SCI., vol 36: Cellular analogues of conditioning and neural plasticity. (eds) Feher, O and Joo, F.

Dunn, R.W., Corbett, R. and Fielding, S. (1989)
Effects of 5-HT_{1A} receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. EUR. J. PHARMACOL., vol 169: pp 1-10.

Dunwiddie, T. and Lynch, G. (1978)
Long-term potentiation and depression of synaptic responses in rat hippocampus: Localisation and frequency dependency. J. PHYSIOL., vol 276: pp 353-367.

Dunwiddie, T.V. and Lynch, G. (1979)
The relationship between extracellular calcium concentrations and the induction of hippocampal long-term potentiation. BRAIN RES., vol 169: pp 103-110.

Eaton, S.A. and Salt, T. E. (1989)
Effects of CNQX and CPP on responses of ventrobasal thalamus neurones to excitatory amino acids and sensory stimulation in the anaesthetised rat. J.PHYSIOL., vol 413: 35P.

Engberg, I., Flatman, J.A. and Lambert, J.D.C. (1979)
The actions of excitatory amino acids on motorneurones in the feline spinal cord. J. PHYSIOL., vol 288: pp 227-261.

Errington, M.L., Lynch, M.A. and Bliss, T.V.P. (1987)
Long-term potentiation in the dentate gyrus: Induction and increased glutamate release are blocked by D(-)amino-phosphonovalerate. NEUROSCI. vol 20(1): pp 279-284.

Evans, R.H., Francis, A.A. and Watkins, J.C. (1978)
Mg^{++}-like selective antagonism of excitatory amino acid-induced responses by (α)ϵ-diaminopimetic acid, D-aminoadipate and HA- 966 in isolated spinal cord of frog and immature rat. BRAIN RES., vol 148: pp 536-542.

Evans, R.H., Francis, A.A., Jones, A.W., Smith, D.A.S. and Watkins, J.C. (1982)
The effects of a series of ω-phosphonic (α)-carboxylic amino acids on electrically evoked and excitant amino acid-induced responses in isolated spinal cord preparations. BR. J. PHARMACOL., vol 75: pp 65-75.

Fadda, E., Wroblewski, J.T. and Costa, E. (1988)
Glycine and D-serine increase the affinity of the N-Methyl-D-aspartate sensitive glutamate binding sites in rat brain synaptic membranes. NEUROPHARMACOL., vol 27(11): pp 1183-1188.

Fagg, G.E., Pozza, M.F., Olpe, H-R., Brugger, F., Baumann, P., Bittiger, H., Schmutz, M., Angst, C., Brundish, D., Allgeier, H., Heckendorn, R. and Dingwall, J.G. (1989)
CGP 37849: Characterisation of a novel and potent competitive N-Methyl-D-Aspartate receptor antagonist. PROC. BRIT. PHARM. SOC., SP8.

Fosse, V.M., Heggelund, P. and Fonnum, F. (1989)
Post natal development of glutamatergic, GABAergic and Cholinergic neurotransmitter phenotypes in visual cortex, lateral geniculate nucleus pulvinar and superior colliculus in cats. J. NEUROSCI., vol 9(2): pp 426-435.

Foster, A.C. and Fagg, G.E. (1984)
Acidic amino acid binding sites in mammalian neuronal membranes: Their characteristics and relationship to synaptic receptors. BRAIN RES. REV., vol 7: pp 103-164.

Foster, A.C. and Fagg, G.E. (1987)
Taking apart NMDA receptors. NATURE, vol 329: pp 395-396.

Fox, S.E. and Ranck, Jr., J.B. (1981)
Electrophysiological characteristics of hippocampal complex-spike cells and theta cells. EXP. BRAIN RES., vol 41: pp 399-410.

Gabriel, M. Sparenborg, S.P. and Stolar, N. (1987)
An executive function of the hippocampus: Pathway selection for thalamic neuronal significance code. In: The Hippocampus, vol 4. (ed) Isaacson, R.L. and Pribham, K.H. Plenum Press, New York.

Gaddum, J.H. (1961)
Push-pull Cannulae. J. PHYSIOL., vol 155, 1P.

Gage, P.D. (1985)
Performance of hippocampectomised rats in a reference/working-memory task: Effects of preoperative vs post-operative training. PHYSIOL. PSYCHOL., vol 13(14): pp 235-242.

Gerren, R.A. and Weinberger, N.M. (1983)
Long-term potentiation in the magno-cellular medial geniculate nucleus of the anaesthetised cat. BRAIN RES., vol 265: pp 138-142.

Gilbert, M.E. (1988)
The NMDA-receptor antagonist, MK-801, suppresses limbic kindling and kindling seizures. BRAIN RES., vol 463: 90-99.

Gill, R., Foster, A.C. and Woodruff, G.N. (1987)
Systemic administration of MK-801 protects against ischaemia-induced hippocampal hippocampal neurodegeneration in the gerbil. J. NEUROSCI., vol 7: pp 3343-3349.

Goddard, G.V., McIntyre, D.C. and Leech, C.K. (1969)
A permanent changes in brain function resulting fron daily electrical stimulation. EXP. NEUROL., vol 25: pp 295-330.

Gotlieb, D. and Cowan. W. (1973)
Autoradiographic studies of the commissural and ipsilateral association connections of the hippocampus and dentate gyrus. J.COMP. NEUROL., vol 149: pp 393-422.

Green, E.J. and Greenough, W.T. (1986)
Altered synaptic transmission in dentate gyrus of rats reared in complex environments: Evidence from hippocampal slices maintained *in vitro*. J. NEUROPHYSIOL., vol 55(4): pp 739-749.

Green, E.J., McNaughton, B.L., Keith, J.R. and Barnes, C.A. (submitted)
Exploration-dependent modulation of evoked responses in fascia dentata II. Dissociation of behavioural and environmental factors and evidence for a synaptic conductance change.

Grillner, S., McClellan, A., Sigvardt, K., Wallen, P. and Wilen, M. (1981)
Activation of NMDA receptors elicit "fictive locomotion" in the lamprey spinal cord *in vitro*. ACTA PHYSIOL. SCAND., vol 113: 459-551.

Gustaffson, B. and Wigstrom, H. (1986)
Hippocampal long-term potentiation produced by pairing single volleys and brief conditioning trains evoked in separate afferents. J.NEUROSCI., vol 6(6): pp 1575-1582.

Halliwell, R.F. and Morris, R.G.M. (1987)
Anticonvulsant doses of MK-801 fail to block Hippocampal LTP *in vivo* or impair spatial learning in the rat. NEUROSCI. LETT. SUPPL., vol 29: pp 599.

Hamberger, A., Berthold, C-H., Jacobson, I., Karlsson, B., Lehmann, A., Nystrom, B. and Sandberg, M. (1985)
In vivo brain dialysis of extracellular nontransmitter and putative transmitter amino acids. In: *In vivo* perfusion and release of neuroactive substances, (eds) Bayou, A. and Drucker-Coln, N. Academic Press, Lond.

Handelmann, G.E., Contreras, P.C. and O'Donohue, T.L. (1987)
Selective memory impairment by phencyclidine in rats. EUR. J. PHARMACOL., vol 140: pp 69-73.

Harris, E.W., Ganong, A.H. and Cotman,C.W. (1984)
Long-term potentiation in the hippocampus involves activation of N-Methyl-D-Aspartate receptors. BRAIN RES., vol 323: pp 132-137.

Harris, E.W., Ganong, A.H., Monaghan, D.T., Watkins, J.C. and Cotman, C.W. (1986)
Action of 3-((+)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP): a new and highly potent antagonist of N-methyl-D-aspartate receptors in the hippocampus. BRAIN RES., vol 382: pp 174-177.

Harris, E.W. and Cotman, C.W. (1986)
Long-term potentiation of guinea pig mossy fibre responses is not blocked by N-Methyl D-aspartate antagonists. NEUROSCI. LETT., vol 70: pp 132-137.

Harris, K.M. and Teyler, T.J. (1984)
Developmental onset of long-lasting potentiation in area CA_1 of the rat hippocampus. J. PHYSIOL., vol 346: pp 27-48.

Hebb, D.O. (1949)
The organization of behaviour. Wiley, New York.

Higashima, M. and Yamamoto, C. (1985)
Two components of long-term potentiation in mossy fibre induced excitation in hippocampus. EXP. NEUROL., vol 90: pp 529-535.

Hoffmann, F., Nastainczyk, W., Rohrkasten, A., Schneider, T. and Sieber, M. (1987)
Regulation of the L-type calcium channel. TRENDS. PHARMACOL.SCI., vol 8: pp 393-398.

Honig, W.K. (1978)
Studies of working memory in pigeons. In: Cognitive processes in animal behaviour (eds) Hulse, S.H., Fowler, H. and Honig, W.K., Hillsdale, New Jersey, Lawrence Erlbaum.

Honore, T., Davies, S.N., Drejer, J., Fletcher, E.J., Jacobsen, P., Lodge, D. and Neilsen, F.E. (1988)
Quinoxalinediones: Potent competitive non-NMDA glutamate receptor antagonists. SCIENCE, vol 241: pp 701-703.

Huang, Y-Y., Wigstrom, H. and Gustafsson, B. (1987)
Facilitated induction of hippocampal long-term potentiation in slices perfused with low concentrations of magnesium. NEUROSCI., vol 22(1): pp 9-16.

Hubel, D.H. and Wiesel, T.N. (1970)
The period of susceptibility to physiological effects of unilateral eye closure in kittens. J. PHYSIOL., vol 206: 419-436.

Hvalby, O., Lacaille, J-C., Hu, G-Y. and Andersen, P. (1987)
Post synaptic long-term potentiation follows coupling of dendritic glutamate application and synaptic activation. EXPERENTIA, vol 43: pp 599-601.

Jarrard, L.E. (1978)
Selective hippocampal lesions: Differential effects on performance by rats of spatial tasks with preoperative versus postoperative training. J. COMP. PHYSIOL. PSYCHOL., vol 92(6): pp 1119-1127.

Jarrard, L.E. (1983)
Selective hippocampal lesions and behaviour: Effects of kainic acid lesions on performance of place and cue tasks. BEHAV. NEUROSCI., vol 97(6): pp 873-889.

Jarrard, L.E., Okaichi, H., Steward, O. and Goldschmidt, R.B. (1984)
On the role of hippocampal connections in the performance of place and cue tasks: Comparisons with damage to hippocampus. BEHAV. NEUROSCI., vol 98(6): pp 946-954.

Jarrard, L.E. (1986)
Selective hippocampal lesions and behaviour: Implications for current research and theorising. In: The Hippocampus, vol 4 (ed) Isaacson, R.L. and Pribram, K.H., Plenum Press.

Jarrard, L.E. (1989)
On the use of ibotenaic acid to lesion selectively different conponents of the hippocampal formation. J. NEUROSCI. METHS., vol 29: pp 251-259.

Jensen, M.L. and Auer, R.N. (1988)
Ketamine fails to protect against ischaemic neuronal necrosis in the rat. BR. J. ANAESTH., vol 61: 206-210.

Johnson, J.W. and Ascher, P. (1987)
Glycine potentiates the NMDA response in cultured mouse brain neurons. NATURE, vol 325: pp 529-531.

Kandel, E.R. (1981)
Environmental determinants of brain architecture and of behaviour: Early experience and learning. In: Principles of neural science (eds) Kandel, E.R. and Schwartz, J.H., Edward Arnold.

Kasamutsu, T., Itakura, T. and Jonsson, G. (1981)
Intracortical spread of exogenous catecholamines: Effective concentration for modifying cortical plasticity. J. PHARMACOL. EXP. THER., vol 217(3): pp 841-850.

Kauer, J.A., Malenka, R.C. and Nicholl, R.A. (1988)
A persistent postsynaptic modification mediates long-term potentiation in the hippocampus. NEURON, vol 1: pp 911-917.

Kauer, J.A. and Nicholl, R.A. (1988)
An APV-resistant non-associative form of long-term potentiation in the rat hippocampus. In: Synaptic plasticity in the hippocampus, (eds) Haas, H.L. and Buzsaki, G., Springer-Verlag. Berlin Heidelberg.

Keith, J.R. and Rudy, J.W. (1990)
Why NMDA receptor-dependent long-term potentiation may not be a mechanism of learning and memory: Reappraisal of the NMDA receptor blockade strategy. In Press.

Kelso, S.R., Ganong, A.H. and Brown, T.H. (1986)
Hebbian synapses in the hippocampus. PROC. NATL. ACAD. SCI. USA., vol 88: pp 5326-5330.

Kemp, J.P., Foster, A,C. and Wong, H.F. (1987)
Non-competitive antagonists of excitatory amino acid receptors. TRENDS. NEUROSCI., vol 10(7): pp 294-298.

Kemp, J.A., Priestley, T. and Woodruff, G.N. (1986)
MK-801, A novel, orally active anticonvulsant, is a potent, non-competitive N-Methyl-D-Aspartate-receptor antagonist. Br. J. Pharm. Proc. Suppl., 89., 353P.

Kesner, R.P., Hardy, J.D. and Novak, J.M. (1983)
Phencyclidine and behaviour: II. Active avoidance learning and radial arm maze performance. PHARMACOL. BIOCHEM. & BEHAV., vol 18: pp 351-356.

King, A.E., Cherubini, E. and Ben-Ari, Y. (1989)
N-Methyl-D-a-aspartate induces recurrent synchronised burst activity in immature hippocampal CA_3 neurones *in vitro*. DEV. BRAIN RES., vol 46: pp 1-8.

Kleckner, N.W. and Dingledine, R. (1988)
Requirements for glycine in activation of NMDA receptors expressed in xenopus oocytes. SCIENCE, vol 238: pp 835-837.

Kleinschmidt, A., Bear, M.F. and Singer, W. (1987)
Blockade of NMDA receptors disrupts experience dependent plasticity in kitten striate cortex. SCIENCE, vol 238: pp 355-358.

Klockgether, T., Schwartz, M., Turski, L. and Sontag, K-H. (1986)
The rat ventromedial thalamic nucleus and motor control: Role of N-Methyl-D-Aspartate-mediated excitation, GABAergic inhibition, and muscarinic transmission. J. NEUROSCI., vol 6(6): pp 1702-1711.

Koek, W., Woods, J.H. and Ornstein, P. (1987)
A simple and rapid method for assessing similarities among directly observable behavioural effects of drugs: PCP-like effects of 2-Amino-5-phosphonvalerate in rats. PSYCHOPHARMACOL. vol 91: pp 297-304.

Komatsu, Y., Toyama, K., Maeda, J. and Sakaguchi, H. (1981)
Long-term potentiation investigated in a slice preparation of striate cortex of young kittens. NEUROSCI. LETT., vol 26: pp 269-274.

Kudo, N. and Yamada,T. (1987)
N-Methyl-D-Aspartate-induced locomotor activity in a spinal cord-hindlimb muscles preparation of the new-born rat studied *in vitro*. NEUROSCI. LETT., vol 75: pp 43-48.

Kuhnt, U., Mihaly, A. and Joo, F. (1985)
Increased binding of calcium in the hippocampal slice during long term potentiation. NEUROSCI. LETT., vol 53: pp 149-154.

LaRoche, S., Doyere, V. and Bloch, V. (1989)
Linear relation between magnitude of long-term potentiation in the dentate gyrus and associative learning in the rat. A demonstration using commissural inhibition and local infusion of an N-Methyl-D-Aspartate receptor antagonist. NEUROSCI., vol 28(2): pp 375-386.

Larson, J. and Lynch, G. (1986)
Induction of synaptic potentiation in hippocampus by patterned stimulation involves two events. SCIENCE, vol 232: pp 985-988.

Larson, J. and Lynch, G. (1988)
Role of N-Methyl-D-aspartate receptors in the induction of synaptic potentiation by burst stimulation patterned after the hippocampal theta rhythm. BRAIN RES., vol 441: pp 111-118.

Lashley, K. S. (1950)
In Search of the engram. SYMP. SOC. EXP. BIOL., vol 4: pp 454-482.

Lee, K., Schottler, F., Oliver, M. and Lynch, G. (1980)
Brief bursts of high frequency stimulation produces two types of structural changes in rat hippocampus. J. NEUROPHYSIOL. vol 44: pp 247-258.

Lee, K.S. (1982)
Sustained enhancement of evoked potentials following brief high frequency stimulation of the cerebral cortex *in vitro*. BRAIN RES., vol 239: pp 617-623.

Lee, K.S. (1983)
Co-operativity among afferents for the induction of long-term potentiation in the CA_1 region of the hippocampus. J. NEUROSCI., vol 3: pp 1369-1372.

Leung, L-W.S. and Desborough, K.A. (1988)
APV, an N-methyl-D-Aspartate receptor antagonist, blocks the hippocampal theta rhythm in behaving rats. BRAIN RES., vol 463: pp 148-152.

Levy, W.B. and Steward, O. (1979)
Synapses as associative memory elements in the hippocampal formation. BRAIN RES., vol 175: pp 233-245.

Levy, W.B. and Steward, O. (1983)
Temporal contiguity requirements for long term-associative potentiation/depression in the hippocampus. NEUROSCI., vol 8(4): pp 791-797.

Lindroth, P., Hamberger, A. and Sandberg, M. (1985)
Liquid chromatographic determination of amino acids after recolumn fluorescence derivitisation. In: Neuromethods, 3: Amino Acids, (eds) Boulton, A.A., Baker, G.B. and Wood, J.D., Humana Press, Clifton, New Jersey.

Liu, C-N. and Chambers, W.W. (1958)
Intraspinal sprouting of dorsal root axons. ARCHIVES NEUROL. PSYCHIAT., vol 79: pp 46-61.

Lodge, D., Aram, J.A., Church, J., Davies, S.N., Martin, D., Millar, J. and Zeman, S. (1988)
Sigma opiates and excitatory amino acids. In: Excitatory amino acids in health and disease, (ed) Lodge, D., John Wiley and Sons Ltd.

Lomo, T. (1966)
Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. ACTA PHYSIOL. SCAND., vol 68: (Suppl) 277: 128.

Lomo, T. (1971)
Patterns of activation in a monosynaptic cortical pathway: The perforant path input to the dentate area of the hippocampus. EXP. BRAIN RES., vol 12: pp 18-45.

Lucas, D.R. and Newhouse, J.P. (1957)
The toxic effects of sodium L-glutamate on the inner layers of the retina. ARHC. OPTHAL., N.Y., vol: 58: pp 193-201.

Lynch, G.S., Dunwiddie, T. and Gribkoff, V. (1977)
Hetrosynaptic depression: A post synaptic correlate of LTP. NATURE, vol 266: pp 737-739.

Lynch, G., Larson, J., Kelso, S., Barrionuevo, G. and Schotter, F. (1983)
Intracellular injections of EGTA blocks induction of hippocampal LTP. NATURE, vol 305: pp 719-721.

MacDermott, A.B., Mayer, M.L., Westbrook, G.L., Smith, S.H. and Barker, J.L. (1986)
NMDA Receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurons. NATURE, vol 231: pp 519-522.

MacDermott, A.B. and Dale, N. (1987)
Receptors, ion channels and synaptic potentials underlying the integrative actions of excitatory amino acids. TRENDS NEUROSCI., vol 10(7): pp 280-284.

MacDonald, J.F., Porietis, A.V. and Wojtowicz, J.M. (1982)
L-Aspartic acid induces a region of negative slope conductances in the current-voltage relationship of cultured spinal cord neurons. BRAIN RES., vol 237: pp 248-253.

Manilow, R. and Miller, J.P. (1986)
Post synaptic hyperpolerisation during conditioning reversibly blocks induction of LTP. NATURE, vol 320: pp 529-530.

Mattson, M.P, Dou P. and Kater, S.B. (1988)
Outgrowth-regulated actions of glutamate in isolated hippocampal pyramidal neurons. J. NEUROSCI., vol 8(6): pp 2087-20100.

Mayer, M.L., Westbrook, G.L. and Guthrie, P.B. (1984)
Voltage dependent block by magnesiun of NMDA responses in spinal cord neurones. NATURE, vol 309: pp 261-263.

McClellan, A.D. and Farel, P.B. (1985)
Pharmacological activation of locomotor patterns in larval and adult frog spinal cords. BRAIN RES., vol 332: pp 119-130.

McDonald, J.W., Johnston, M.V. and Young, A.B. (1989)
Ontogeny of the receptors comprising the NMDA receptor channel complex. SOC. NEUROSCI. ABST., 86.3.

McDonald, J.W. and Johnston, M.V. (1990)
Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. BRAIN RES. REV., vol 15: pp 41-70.

McLennan, H. (1982)
Receptors for the excitatory amino acids in the mammalian central nervous system. PROGS. NEUROBIOL., vol 20: pp 251-271.

McNamara, J.O., Russell, R.D., Rigsbee, L. and Bonhaus, D.W. (1988)
Anticonvulsant and antieleptogenic actions of MK-801 in the kindling and electroshock models. NEUROPHARM., Vol 27: pp 563-568.

McNaughton, B.L., Douglas, R.M. and Goddard, G.V. (1978)
Synaptic enhancement in fascia dentata: co-operativity among co-active afferents. BRAIN RES., vol 157: pp 277-293.

McNaughton, B.L. (1983)
Activity dependent modulation of hippocampal synaptic efficacy: Some implications of memory processing. In: Neurobiology of the hippocampus (ed) Siefert, W. Academic Press. Lond.

McNaughton, B.L., Barnes, C.A., Rao, G., Baldwin, J. and Rasmussen, M. (1986)
Long-term enhancement of hippocampal synaptic transmission and the acquisition of spatial information. J. NEUROSCI., vol 6(2): pp 563-571.

McNaughton, N. and Miller, J.J. (1986)
Collateral specific long-term potentiation of the output field of CA_3 of hippocampus in the rat. EXP BRAIN RES., vol 62: pp 250-258.

Melchers, B.P.C., Pennartz, C.M.A., Wadman, W.J. and Lopez Da Silva, F.H. (1988)
Quantitative correlation between tetanus induced decreases in extracellular Ca^{++} and LTP. BRAIN RES., vol 454: pp 1-10.

Meldrum, B.S., Croucher, M.J., Badman, G. and Collins, J.F. (1983)
Antiepileptic actions of excitatory amino acid antagonists in the photosensitive baboon, *papio papio*. NEUROSCI. LETT., vol 39: 101-104.

Meldrum, B.S. (1984)
Amino acid neurotransmitters and new approaches to anticonvulsant drugs. EPILEPSIA., vol 25: pp S140-149.

Meldrum, B.S. (1988a)
Historical introduction. In: Current problems in epilepsy: Anatomy of eliptogenesis, (eds) Meldrum, B.S., Ferrendelli, J.A. and Weiser, H.G. John Libby (Lond).

Meldrum, B.S. (1988b)
In vivo and *in vitro* models of epilepsy and their relevanceto man In: Current problems in epilepsy: Anatomy of eliptogenesis, (eds) Meldrum, B.S., Ferrendelli, J.A. and Weiser, H.G. John Libby (Lond).

Meldrum, B.S. (1989)
Competitive antagonists as drug. In: The NMDA receptor, (eds) Watkins, J.C. and Collingridge, G.L., Oxford univ. Press.

Menzel, E.W. (1978)
Cognitive mapping in chimpanzees. In: Cognitive aspects of animal behaviour, (eds) Hulse, S.H., Fowler, H.F. and Honig, W.K. Hillsdale, New Jersey, Lawrence Erlbaum.

Miller, R.J. (1987)
Multiple calcium channels and neuronal function. SCIENCE, vol 235: pp 46-52.

Minota, S., Miyazaki, T., Wang, H-Y., Read, H.L. and Dunn. N.J. (1989)
Glycine potentiates NMDA receptors in rat hippocampal CA_1 neurons. NEUROSCI. LETT., vol 100: pp 237-242.

Misgeld, U., Sarvey, J.M. and Klee, M.R. (1979)
Hetrosynaptic post activation in hippocampal CA_3 neurons: Long-term changes in the post-synaptic potentials. EXP. BRAIN RES., vol 37: pp 217-229.

Monaghan, D.T., Holets, V.R., Toy, D.W. and Cotman, C.W. (1983)
Anatomical distributions of four pharmacologically distinct ^3H- L-glutamate binding sites. NATURE, vol 306: pp 176-179.

Monaghan, D.T. and Cotman, C.W. (1985)
Distribution of N-Methyl-D-Aspartate-sensitive L-[^3H] glutamate binding sites in rat brain. J. NEUROSCI., vol 5(11): pp 2909-2919.

Monaghan, D.T. and Cotman, C.W. (1986)
Identification and properties of N-methyl-D-aspartate receptors in rat brain synaptic plasma membranes. PROC. NATL. ACAD. SCI. USA., vol 83: pp 7532-7536.

Monaghan, D.T., Olverman, H.J., Nguyen, L., Watkins J.C. and Cotman, C.W. (1988)
Two classes of N-Methyl-D-aspartate recognition sites: Differential distribution and differential regulation by glycine. PROC. NATL. ACAD. SCI. USA., vol 85: pp 9836-9840.

Monaghan, D.T., Bridges, R.J. and Cotman, C.W. (1989)
The excitatory amino acid receptors: Their classes, pharmacology, and distinct properties in the function of the central nervous system. ANN. REV. PHARMACOL. TOXICOL., vol 29: pp 365-402

Monaghan, D.T. and Cotman, C.W. (1989)
Regional variations in NMDA receptor properties. In: The NMDA Receptor, (eds) Watkins, J.C. and Collingridge, G.L., Oxford University Press.

Mondadori, C., Weiskrantz, L., Buerki, H., Petschke, F. and Fagg, G.E. (1989)
NMDA receptor antagonists can enhance or impair learning performance in animals. EXP. BRAIN RES., vol 75: pp 449-456.

Morris, R.G.M. (1981)
Spatial localisation does not depend on the presence of local cues. LEARNING MOTIVATION, vol 12: pp 239-260.

Morris, R.G.M., Garrud, P., Rawlins, J.N.P. and O'Keefe, J. (1982)
Place navigation impaired in rats with hippocampal lesions. NATURE, vol 297: pp 681-683.

Morris, R.G.M. (1983)
An attempt to dissociate "Spatial-Mapping" and "Working Memory" theories of hippocampal function. In: Neurobiology of the Hippocampus, (ed) Siefert, W., Academic Press, London.

Morris, R.G.M. and Hagan, J.J. (1983)
Hippocampal electrical activity and ballistic movement: pp 321-331. In: Neurobiology of the hippocampus, (ed) Siefert, W., Academic Press.

Morris, R.G.M. (1984)
Development of a watermaze procedure for studying spatial learning in rats. J. NEUROSCI. METHODS, vol 11: pp 47-60.

Morris, R.G.M., Andersen, E., Lynch, G.S. and Baudry, M. (1986)
Selective impairment of learning and blockade of long-term potentiation by an N-Methyl-D-aspartate receptor antagonist, APV. NATURE, vol 319: pp 774-776.

Morris, R.G.M. (1989)
Synaptic plasticity and learning: Selective impairment of learning in rats and blockade of long-term potentiation *in vivo* by the N-Methyl-D-Aspartate receptor antagonist, AP5. J.NEUROSCI., vol 9(9): pp 3040-3057.

Muller, D., Joly, M. and Lynch. G. (1988)
Contributions of quisqualate and NMDA receptors to the induction and expression of LTP. SCIENCE, vol 242: pp 1694-1697.

Nadel, L. and MacDonald, L. (1980)
Hippocampus: Cognitive map or working memory? BEHAV. NEURAL BIOL., vol 29: pp 405-409.

Nowak, L., Bregestovski, P. and Ascher, P. (1984)
Magnesium gates glutamate-activated channels in mouse central neurones. NATURE, vol 307: pp 462-465.

Oades, R.D. (1981)
Impairment of search behaviour in rats after haloperidol treatment, hippocampal or neocortical damage suggests a mesocorticolimbic role in cognition. BIOL. PSYCHOL., vol 12: pp 77-85.

O'Keefe, J. and Dostrovsky, J. (1971)
The hippocampus as a spatial map. Preliminary evidence from unit activity in the freely moving rat. BRAIN RES., vol 34: pp 171-175.

O'Keefe, J., Nadel, L., Keigthley, S. and Kill, D. (1975)
Fornix lesions selectively abolish place learning in the rat. EXP. NEUROL., vol 48: pp 152-166.

O'Keefe, J. (1976)
Place units in the hippocampus of the freely moving rat. EXP. NEUROL., vol 51: pp 78-109.

O'Keefe, J. and Conway, D.H. (1978)
Hippocampal place units in the freely moving rat: Why they fire when they fire. EXP. BRAIN RES., vol 31: pp 573-590.

O'Keefe, J. and Nadel, L. (1978)
The hippocampus as a cognitive Map. Oxford University Press.

O'Keefe, J. and Conway, D.H. (1980)
On the trail of the hippocampal engram. PHYSIOL. PSHYCHOL., vol 8(2): 229-238.

O'Keefe, J. (1983)
Spatial memory within and without the hippocampal system. In: Neurobiology of the hippocampus, (ed) Seifert, W., Academic Press.

Olney, J.W., Ho, O.C. and Rhee, V. (1971)
Cytotoxic effects of acidic and sulphur containing amino acid on the infant mouse central nervous system. EXP. BRAIN RES., vol 14: pp 61-76.

Olney, J.W. (1978)
Neurotoxicity of excitatory amino acids. In: Kainic acid as a tool in neurobiology, (eds) McGreer, E.G., Olney, J.W. and McGreer, P.J., Raven Press. New York.

Olney, J.W., LaBruyere, J., Collins, F.J. and Curry, K. (1981)
D-aminophosphonovalerate is 100-fold more powerful that D-(α)-amino adipate in blocking N-methyl aspartate neurotoxicity. BRAIN RES., vol 221: pp 207-210.

Olton, D.S. and Samuelson, R.J. (1976)
Remembrance of things past: Spatial memory in rats. J. EXP. PSYCHOL. ANIMAL. BEHAV. PROC., vol 2(2) pp 97-115.

Olton, D.S. (1978)
Characteristics of spatial memory. In: Cognitive processes in animal behaviour, (eds) Hulse, S.H., Fowler, H. and Honig, W.K., Hillsdale, New Jersey, Lawrence Erlbaum.

Olton, D.S., Walker, J.A. and Gage, F.H. (1978)
Hippocampal connections and spatial discrimination. BRAIN RES., vol 139: pp 295-308.

Olton, D.S., Becker, J.T. and Handleman, G.E. (1979)
Hippocampus, space and memory. BEHAV. BRAIN SCI., vol 2: pp 313-365

Olton, D.S. and Papas, B.C. (1979)
Spatial memory and hippocampal function. NEUROPSYCHOLOGIA, vol 17: pp 669-682.

Olton, D.S. and Feustle, W.A. (1981)
Hippocampal function required for non-spatial working memory. EXP. BRAIN RES., vol 41: pp 380-389.

Olton, D.S. (1983)
Memory functions and the Hippocampus. In: Neurobiology of the Hippocampus, (ed) Siefert, W., Academic Press, London.

Olverman, H.J., Jones, A.W. and Watkins, J.C. (1984)
L-Glutamate has higher affinity than other amino acids for [^{3}H]-D-AP5 binding sites in rat brain membranes. NATURE, vol 307: pp 460-462.

Panakhova, E., Buresova, O. and Bures, J. (1984)
Persistence od spatial memory in the Morris water tank task. INT. J. PSYCHOPHYSIOL., vol 2: pp 5-10.

Park, K.C., Nehls, D.G., Graham, D.I., Teasdale, G.N. and McCulloch, J. (1988)
The glutamate antagonist MK-801 reduces focal ischaemic brain damage in the rat. ANN. NEUROL., vol 24: pp 543-551.

Park, C.K., Nehls, D.G., Graham, D.I., Teasdale G.M. and McCulloch, J. (1988)
Focal ischaemia in the cat: Treatment with the glutamate antagonist MK-801 after induction of ischaemia. J. CEREB. BLOOD FLOW METAB., vol 8: pp 757-762.

Patel, S. (1988)
Chemically-induced limbic seizures. In: Current problems in epilepsy: Anatomy of eliptogenesis, (eds) Meldrum, B.S., Ferrendelli, J.A. and Weiser, H.G. John Libby (Lond).

Paxinos, G. and Watson, C. (1982)
The rat brain in stereotaxic co-ordinates. Academic Press.

Pearce, I.A., Cambray-Deakon, M.A. and Burgoyone, R.D. (1987)
Glutamate acting on NMDA receptors stimulate neurite outgrowth from cerebellar granule cells. F.E.B.S. LETT., vol 223: pp 143-147.

Perkins, M.N., Collins, J.F. and Stone, T.W. (1982)
Isomers of 2-Amino-7-phosphonoheptanoic acid as antagonists of neuronal excitants. NEUROSCI LETT., vol 32: pp 65-68.

Peterson, D.W., Collins, J.F. and Bradford. H.F. (1983)
The kindling amygdala model of epilepsy: Anticonvulsant action of amino acid antagonists. BRAIN RES., vol 275: pp 169-172.

Poon, M.L.T. (1980)
Induction of swimming in lamprey by L-Dopa and amino acids. J.COMP PHYSIOL., vol 136: pp 337-344.

Pulsinelli, W.A., Brierley, J.B. and Plum, F. (1982)
Temporal profile of neuronal damage in a model of transient forebrain ischaemia. ANN. NEUROL., vol 11: pp 491-498.

Racine, R.J., Milgram, N.W. and Hafner, S. (1983)
Long-term potentiation phenomena in the rat limbic forebrain. BRAIN RES., vol 260: pp 217-231.

Ramirez, O.A., Orsinger, O.A. and Carrer, H.F. (1988)
Differential threshold for long-term potentiation in the hippocampus of rats with inborn high or low learning capacity. NEUROSCI. LETT., vol 92: pp 275-279.

Ramirez, O.A. and Carrer, H.F. (1989)
Correlation between threshold to induce long-term potentiation in the hippocampus and performance in a shuttle box avoidance response in rats. NEUROSCI. LETT., vol 104: pp 152-156.

Rasmussen, M., Barnes, C.A. and McNaughton, B.L. (1989)
A Systematic test of cognitive mapping, working-memory, and temporal discontiguity theories of hippocampal function. PSYCHOBIOL. vol 17: pp 335-348.

Rawlins, J.N.P. (1985)
Association across time: The hippocampus as a temporary memory store. BEHAV. BRAIN SCI., vol 8: pp 479-496.

Reyman, K.G., Malish, R., Schulzeck, K., Brodemann, R., Ott, T. and Matthies, H. (1985)
The duration of long-term potentiation in the CA_1 region of the hippocampal slice preparation. BRAIN RES. BULL., vol 15: pp 249-255.

Reymann, K.G., Matthies, H.K., Frey, U. and Vorobyev, V.S. (1986)
Calcium induced LTP in the hippocampal slice: Characterisation of the time course and conditions. BRAIN RES. BULL., vol 17: pp 291-296.

Robinson, G.B. (1986)
Enhanced long-term potentiation induced in rat dentate gyrus by co-activation of septal and entorhinal inputs: Temporal constraints. BRAIN RES., vol 379: 56-62.

Robinson, Jr., G.S., Crooks, Jr., G.B., Shinkman, P.G. and Gallagher, M. (1989)
Behavioural effects of MK-801 mimic deficits associated with hippocampal damage. PSYCHOBIOL., vol 17(2): pp 156-164.

Rose, G.M. and Dunwiddie, T.V. (1986)
Induction of hippocampal long-term potentiation using physiological patterned stimmulation. NEUROSCI. LETT., vol 69: pp 244-248.

Rothman, S. (1984)
Synaptic release of excitatory amino acid neurotransmitters mediates anoxic neuronal death. J. NEUROSCI., vol 4(7): pp 1884-1891.

Rothman, S.M., Thurston, J.H. and Hauhart, R.E. (1987)
Delay neurotoxity of excitatory amino acids *in vitro*. NEUROSCI., vol 22(2): pp 471-480.

Rothman, S.M. and Olney, J.W. (1987)
Excitotoxicity and the NMDA receptor. TRENDS NEUROSCI., vol 10(7): pp 299-302.

Rudell, A.P., Fox, S.E. and Ranck, Jr., J.B. (1980)
Hippocampal excitability phase-locked to theta rhythm in walking rats. EXP. NEUROL., vol 68: pp 87-96.

Rutherich, H., Matthies, H. and Ott, T. (1982)
Long-term changes in synaptic excitability of hippocampal cell populations as a result of training. In: Neuronal plasticity and memory formation (eds) Marsan, C.A. and Matthies, H.

Sakimoto, T., Porter, L.L. and Asanuma, H. (1986)
Long-lasting potentiation in the motor cortex in cats. SOC. NEUROSCI. ABS. 12: 259.

Salt, T.E. (1986)
Mediation of thalamic sensory input by both NMDA receptors and non-NMDA receptors. NATURE, vol 322: pp 263-265.

Salt, T.E. (1987)
Excitatory amino acid receptors and synaptic transmission in the rat ventrobasal thalamus. J. PHYSIOL., vol 391: pp 499-510.

Sastry, B.R., Goh, J.W. and Auyeung, A. (1986)
Associative induction of post-tetanic and long-term potentiation in CA_1 neurons of rat hippocampus. SCIENCE, vol 232: pp 988-990.

Sato, K., Morimoto, K. and Okamoto, M. (1988)
Anticonvulsant action of a non-competitive antagonist of NMDA receptors (MK-801) in the kindling model of epilepsy. BRAIN RES., vol 463: pp 12-20.

Schenk, F. and Morris, R.G.M. (1985)
Dissociation between components of spatial memory in rats after recovery from the effects of retrohippocampal lesions. EXP. BRAIN RES., vol 58: pp 11-28.

Schliebs, R., Kullman, E. and Bigl, V. (1986)
Development of glutamate binding sites in the visual structures of the rat brain. Effect of visual pattern deprivation. BIOMED. BIOCHIM. ACTA., vol 4: pp 495-506.

Scoville, W.B. and Milner, B. (1975)
Loss of recent memory after bilateral hippocampal lesions. J. NEUROL. NEUROSURG. PSYCHIAT., vol 20: pp 11-21.

Scwartzkroin, P.A. and Wester, K. (1975)
Long lasting facilitation of a synaptic potential following tetanisation in the *in vitro* hippocampal slice. BRAIN RES., vol 89: pp 107-119.

Segal, M. and Landis, S. (1974)
Afferents to the hippocampus of the rat studied with the method of retrograde transport of horseradish peroxidase. BRAIN RES., vol 78: pp 1-15.

Seisjo, B.K. and Bengtsson, F. (1989)
Calcium fluxes, calcium antagonists, and calcium related pathology of brain ischaemia, hypoglycemia and spreading depression: A unifying hypothesis. J. CEREB. BLOOD FLOW METAB., vol 9: pp 127-140.

Shapiro, M.L. and Caramanos, Z. (1990)
NMDA antagonist MK-801 impairs acquisition but not performance of spatial working and reference memory. NEUROSCI. ABS: 185.9.

Sharp, P.E., McNaughton, B.L. and Barnes, C.A. (1985)
Enhancement of hippocampal field potentials in rats exposed to a novel, complex environment. BRAIN RES., vol 339: pp 361-365.

Sharp, P.E., Barnes,C.A. and McNaughton, B.L. (1987)
Effects of aging on environmental modification of hippocampal evoked responses. BEHAV. NEUROSCI. vol 101(2): pp 170-178.

Sharp, P.E., McNaughton, B.L. and Barnes,C.A. (1989)
Exploration-depentent modulation of evoked responses in fascia dentate. 1: Fundamental observations and time course. PSYCHOBIOLOL., vol 17(3): pp 257-269.

Skelton, R.W., Scarth, A.S., Wilkie, D.M., Miller, J.J. and Phillips, A.G. (1987)
Long-term increases in dentate granule cell responsivity accompanying operant conditioning. J. NEUROSCI. vol 7(10): pp 3081-3087.

Slater, N.T., Stelzer, A. and Galvan, M. (1985)
Kindling-like stimulus patterns induce epileptiform discharges in the guinea-pig *in vitro* hippocampus. NEUROSCI. LETT., vol 60: pp 25-31.

Smith, M.L. and Milner, B. (1981)
The role of the right hippocampus in recall of of spatial location. NEUROPSYCHOLOGICIA, vol 19: pp 781-793.

Smith, M.L., Auer, R.N. and Seisjo, B.K. (1984)
The density and distribution of ischaemic brain injury in the rat following injury 2-10 min. of forebrain ischaemia. ACTA NEUROPATH (BERL)., vol 64: 319-332.

Squires, L.R. and Cohen, N.T. (1984)
Human memory and amnesia. In: Neurobiology of learning and Memory (ed) McGaugh, J.L. and Lynch, G., Guildford Press.

Stasheef, S.F., Bragdon, A.C. and Wilson, W.A. (1985)
Induction of epileptiform activity in the hippocampal slice by trains of electrical stimuli. BRAIN RES., vol 344: pp 296-302.

Staubli, U. and Lynch, G. (1987)
Stable hippocampal LTP elicited by "theta" pattern stimulation. BRAIN RES., vol 435: pp 227-234.

Staubli, U., Thibault, O., DiLorenzo, M. and Lynch, G. (1989)
Antagonism of NMDA receptors impairs acquisition but not retention of olfactory memory. BEHAV. NEUROSCI., vol 103(1): pp 54-60.

Stein, S.G. and Schild, C.P. (1988)
In situ application of APV, an NMDA receptor antagonist, onto the spinal cord reduces the excitability of specific cutaneous reflexes in the turtle. SOC. NEUROSCI., 9.9: pp 13.

Stringer, J.L., Greenfield, L.J., Hackett, J.T. and Guyenet, P.G. (1983)
Blockade of long-term potentiation by phencyclidine and opiates in the hippocampus *in vivo* and *in vitro*. BRAIN RES., vol 280: pp 127-138.

Stripling, J.S. and Patneau, D.K. (1985)
Selective long term changes in the pyriform cortex. SOC. NEUROSCI. ABS. 10: 76.

Sugiyama, H., Ito, I. and Hirono, C. (1987)
A new type of glutamate receptor linked to inositol phospholipid metabolism. NATURE, vol 325: pp 531-533.

Sutherland, R.J., Kolb, B. and Whishaw, I.Q. (1982)
Spatial Mapping: Definitive disruption by hippocampal or medial frontal cortical damage in the rat. NEUROSCI. LETT., vol 31: pp 271-276.

Sutherland, R.J., Whishaw, I.Q. and Kolb, B. (1983)
A behavioural analysis of spatial localisation following electrolytic, kainate-or cholchicine-induced damage to the hippocampal formation in the rat. BEHAV. BRAIN RES., vol 7: pp 133-153.

Sutherland, R.J. (1985)
The navigating hippocampus: An individual medley of movement, space, and memory. In: Electrical activity and the acrhicortex, (eds) Buzsaki, G. and Vanderwolf, C.H., Hungarian academy of science.

Sutherland, R.J., Whishaw, I.Q. and Kolb, B. (1988)
Contributions of cingulate cortex to two forms of spatial leaning and memory. J. NEUROSCI., vol 8(6): pp 1863-1872.

Sutherland, R.J. and Rudy, J.W. (1989)
Configural association theory: The role of the hippocampal formation in learning, memory and amnesia. PSYCHOBIOL., vol 17(2): pp 129-144.

Sutula, T. and Steward, O. (1986)
Quantitive analysis of synaptic potentiation during kindling of the perforant path. J. NEUROPHYS., vol 56(3): pp 732-746.

Swann, J.W. and Brady, R.J. (1984)
Penicillin-induced epileptogenesis in immature rat CA_3 hippocampal pyramidal cells. DEV. BRAIN RES., vol 12: pp 243-254.

Swan, J.H., Evans, and Meldrum, B.S. (1988)
Long-term development of selective neuronal loss and the mechanism of protection by 2-Amino-7- phosophonoheptanoate in rat model of incomplete forebrain ischaemia. J. CEREB. BLOOD FLOW METAB., vol 8: pp 64-78.

Swanson, L.W., Wyss, J.M. and Cowan, W.M. (1978)
An autoradiographic study of the organisation of intrahippocampal association pathways in the rat. J. COMP. NEUROL., vol 181: pp 681-716.

Swanson, L. (1983)
Hippocampus and the concept of the limbic system. In: Neurobiology of the Hippocampus, (ed) Siefert, W. Academic Press, Lond.

Tang, A.H. and Ho, P.M. (1988)
Both competitive and non-competitive antagonists of N-Methyl-D-Aspartic acid disrupt brightness discrimination in rats. EUR. J. PHARMACOL., vol 151: pp 143-146.

Teyler, T.J. and Discenna, P. (1986)
The hippocampal memory indexing theory. BEHAV. NEUROSCI., vol 100(2): 147-154.

Teyler, T.J. and Discenna, P. (1987)
Long-term potentiation. ANN. REV. NEUROSCI., vol 10: pp 131-161.

Thompson, R.F. and Spencer, W.A. (1966)
Habituation: A model phenomenon for the study of neural substrates of behaviour. PSYCHOL REV., vol 73: pp 16-43.

Tolman, E.C. (1948)
Cognitive maps in rats and men. PSYCHOL. REV., vol 40: pp 60-70.

Tonkiss, J., Morris, R.G.M. and Rawlins, J.N.P. (1988)
Intra-ventricular infusion of the NMDA antagonist AP5 impairs performance on a non-spatial operant DRL task in the rat. EXP. BRAIN RES., vol 73: pp 181-188.

Tremblay, E., Roisin, M.P., Represa, A., Charriaut-Marlangue, C. and Ben-Ari, Y. (1988)
Transient increased density of NMDA binding sites in the developing rat hippocampus. BRAIN RES., vol 461: 393-396.

Tricklebank, M.D., Singh, L., Oles, R.J., Wong, E.H.F. and Iversen, S.D. (1987)
A role for receptors of N-Methyl-D-aspartic acid in the discriminitive stimulus properties of phencyclidine. EUR. J. PHARMAOCL., vol 141: pp 497-501.

Tricklebank, M.D., Singh, L., Oles, R.J., Preston, C. and Iversen, S.D. (1989)
The behavioural effects of MK-801: A comparison with antagonists acting non-competitively and competitively at the NMDA receptor. EUR. J. PHARMACOL., vol 167: pp 127-135.

Tsumoto, T., Hagihara, K., Sato, H. and Hata, Y. (1987)
NMDA receptors in the visual cortex of young kittens are more effective than those of adult cats. NATURE, vol 327: pp 513-514.

Turner, R.W., Baimbridge, K.G. and Miller, J.J. (1982)
Calcium induced long-term potentiation in the hippocampus. NEUROSCI., vol 7(6): pp 1411-1416.

Turski, L., Schwartz, M., Turski, W.A., Klockgether, T., Sontag, K-H. and Collins, J.F. (1985a)
Muscle relaxant action of excitatory amino acid antagonists. NEUROSCI. LETT., vol 53: pp 321-326.

Turski, L, Schwartz, M., Klockgether, T. and Sontag, K-H. (1985b)
Substantia nigra: A site of action of muscle relaxant drugs. SOC. NEUROSCI. ABSTR.,11, 1162.

Turski, L, Klockgether, T., Sontag, K-H., Herrling, P.L. and Watkins, J.C. (1987)
Muscle relaxant and anticonvulsant activity of 3-((+)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, a novel N-Methyl-D-Aspartate antagonist, in rodents. NEUROSCI. LETT., vol 73: pp 143-148.

Ungerstedt, U. and Pycock, C.H. (1974)
Functional correlates of dopamine neurotransmission. BULL SCHWEIZ. AKAD. MED., vol 30: pp 44-55.

Ungerstedt, U. (1984)
Measurement of neurotransmitter release by intracranial dialysis. In: Measurement of neurotransmitter release *in vivo*, (ed) Marsden, C.A. John Wiley and Sons, Chichester.

Vanderwolf, C.H. (1969)
Hippocampal electrical activity and voluntary movement in the rat. ELECTROENCEPHAL. CLIN. NEUROPHYSIOL., vol 26: pp 407-418.

Vanderwolf, C.H. (1975)
Neocortical and hippocampal activation in relation to behaviour: effects of atropine, eserine, phenothiozines and amphetamine. J. COMP. PHYSIOL. PSYCHOL., vol 88(1): pp 300-323.

Vanderwolf, C.H. and Leung, L-W.S. (1983)
Hippocampal rhythmic slow activity: A brief history and the effects of entorhinal lesions and phencyclidine. In: Neurobiology of the Hippocampus, (ed) Siefert, W., Academic Press, Lond.

Van Harrenveld, A. and Fifkova, E. (1975)
Swelling of dendritic spines in the fascia dentata after stimulation of the perforant path as a mechanism of post-tetanic potentiation. EXP. NEUROL., vol 49: pp 736-749.

Vezzani, A., Wu, H-Q., Moneta, E. and Samanin, R. (1988)
Role of the N-Methyl-D-Aspartate-type receptors in the development and maintenance of hippocampal kindling in rats. NEUROSCI. LETT., vol 87: pp 63-68.

Vincent, J.P., Kartalovski, B., Geneste, P., Kamenka, J.M. and Lazdunski, M. (1979)
Interaction of phencyclidine ("angel dust") with a specific receptor in rat brain membranes. PROC. NATL. ACAD. SCI. USA., vol 76(9): pp 4678-4682.

Wallen, P. and Williams, T. (1984)
Fictive locomotion in the lamprey spinal cord *in vitro* compared with swimming in the intact and spinal animal. J. PHYSIOL., vol 347: pp 225-239.

Ward, L, Mason, S.E. and Abraham, W.C. (1990)
Effects of the NMDA antagonists, CPP and MK-801 on the radial arm maze performance in rats. PHARMACOL BIOCHEM BEHAV., vol 35: pp 785-790.

Watkins, J.C. and Evans, R.H. (1981)
Excitatory amino acid transmitters. ANN. REV. PHARMACOL. TOXICOL., vol 21: pp 165-204.

Watkins, J.C. and Olverman, H.J. (1988)
Structural requirements for activation and blockade of EAA receptors. In: Excitatory amino acids in health and disease (ed) Lodge, D., John Wiley and Sons Ltd

Watkins, J.C. (1989)
The NMDA receptor concept: origins and development. In: The NMDA Receptor, (eds) Watkins, J.C. and Collingridge, G.L., Oxford University Press, Oxford.

Watkins, J.C., Krogsgaard-Larsen, P. and Honore, T. (1990)
Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. TRENDS PHARMACOL. SCI., vol 11: pp 25-33.

Weilich, T. (1985)
Hypoglycemia-induced neuronal damage prevented by an N-Methyl-D-Aspartate antagonist. SCIENCE, vol 230: pp 681-683.

Weiloch, T., Gustafsson, I. and Westerberg, E. (1988)
Effects of the non-competitive NMDA receptor antagonist, MK-801 on ischaemic hypoglycemic brain damage. In: Frontiers of excitatory amino acid research, (eds) Turski, L., Lehmann, J. and Cavalheiro, E.A., Alan R. Liss, New York.

Wenzel, J. and Matthies, H. (1985)
Morphological changes in the hippocampal formation accompanying memory formation and long-term potentiation. In: Memory systems and the brain, (eds) Weinberger, N., McGaugh, J. and Lynch, G. Guildford Press, New York.

Whishaw, I.Q. and Vanderwolf, C.H. (1973)
Hippocampal EEG and behaviour: Changes in amplitude and frequency of RSA (theta rhythm) associated with spontaneous and leared movement patterns in rats and cats. BEHAV. BIOL., vol 8: pp 461: 484.

Whishaw, I.Q. and Auer, R.N. (1989)
Immediate and long lasting effects of MK-801 on motor activity, spatial navigation in a swimming pool and EEG in the rat. PSYCHOPHARM., vol 98: pp 500-507.

Wigstrom, H. and Gustafsson, B. (1983)
Facilitated induction of hippocampal long lasting potentiation during blockade of inhibition. NATURE, vol 301: pp 603-604.

Wigstrom, H. and Gustafsson, B. (1985a)
Facilitation of hippocampal long lasting potentiation by GABA antagonists. ACTA PHYSIOL. SCAND., vol 125: pp 159-172.

Wigstrom, H. and Gustafsson, B. (1985b)
On long-lasting potentiation in the hippocampus: A proposed mechanism for its dependence on co-incident pre-and postsynaptic activity. ACTA PHYSIOL. SCAND. vol 123: pp 519-522.

Wigstrom, H., Gustafsson, B., Huang, Y-Y. and Abraham, W.C. (1986)
Hippocampal long-term potentiation is induced by pairing single afferent volleys with intracellularly injected depolarising current pulses. ACTA. PHYSIOL. SCAN., vol 126: 317-319.

Willetts, J. and Balster, B.L. (1988)
The discriminative stimulus effects of N-Methyl-D-Aspatate antagonists in phencyclidine-trained rats. NEUROPHARMACOL., vol 27 (12): pp 1249-1256.

Willetts, J. and Balster, B.L. (1989)
Effects of competitive and noncompetitive N-Methyl-D-Aspartate (NMDA) antagonists in rats trained to discriminate NMDA from saline. J. PHARMACOL. EXP. THER., vol 251(2): pp 627-633.

Wilson, W.A., Stasheff, S., Schwartzwelder, S., Clark, S., Anderson, W.W. and Lewis, D. (1989)
The NMDA receptor in epilepsy. In: The NMDA receptor, (eds) Watkins, J.C. and Collingridge, G.L., Oxford University Press.

Winocur, G. (1980)
The hippocampus and utilisation. PHYSIOL. PSYCHOL., vol 8(2): pp 280-288.

Winson, J. (1978)
Loss of hippocampal theta rhythm results in spatial memory deficit in the rat. SCIENCE, vol 201: pp 160-163.

Witter, M.P., Groenewegen, H.T., Lopez da Silva, F.M. and Lohman, A.H.M. (1989)
Functional organisation of the extrinsic and intrinsic circuitry of the parahippocampal region. PROGS. NEUROBIOL., vol 33: pp 161-253

Wozniak, D.F., Olney, J.W., Kettinger III, L., Price, M. and Miller, H.P. (1990)
Behavioural effects of MK-801 in the rat. PSYCHOPHARM., vol 101: pp 47-56.

Yamamoto, C. and Chujo, T. (1978)
Long-term potentiation in thin hippocampal sections studied by intracellular and extracellular recordings. EXP. NEUROL., vol 58: pp 242-250.

Yamamoto, C., Matsumoto, K. and Takagi, M. (1980)
Potentiation of excitatory post synaptic potentials during and after repetitive stimulation in thin hippocampal slices. EXP. BRAIN RES. vol 38: pp 469-477.

Yamamoto, C. and Sawada, S. (1981)
Important factors in the induction of long-term potentiation in thin hippocampal sections. EXP NEUROL., vol 74: pp 122-130.

Young, A.B. and Fagg, G.E. (1990)
Excitatory amino acid receptors in the brain: Membrane binding and receptor autoradiographic approaches. TRENDS PHARMACOL. SCI., vol 11: pp 126-133.