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Hippocampal synaptic plasticity and NMDA receptors: a role in information storage?

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SUMMARY

There has recently been renewed interest in the idea that alterations in synaptic efficacy may be the neural basis of information storage. Particular attention has been focused upon long-term potentiation (LTP), a long-lasting, but experimentally induced synaptic change whose physiological properties point to it being a candidate memory mechanism. However, considerations of storage capacity and the possibility of concomitant activity-dependent synaptic depression make it unlikely that individual learning experiences will give rise to gross changes in field potentials similar to those that occur in LTP, even if learning and LTP utilize common neural mechanisms.

One way of investigating the functional significance of LTP is to use selective antagonists of those excitatory amino acid receptors whose activation is essential for its induction. This paper discusses various design requirements for such experiments and reviews work indicating that the *N*-methyl-D-aspartate receptor antagonist AP5 causes a behaviourally selective learning impairment having certain common features to the behavioural profile seen after hippocampal lesions. Two new studies are described whose results show that AP5 has no effect upon the retrieval of previously established memories, and that the dose-response profile of the impairment of spatial learning occurs across a range of extracellular concentrations in hippocampus for which receptor selectivity exists. These experiments show that activation of NMDA receptors is essential for certain kinds of learning.

ABBREVIATIONS

aCSF,	artificial cerebrospinal fluid	EGTA,	ethylene-bis(oxyethylenenitrite)
AP5,	2-amino-5-phosphonopentanoate		tetra-acetic acid
AP7,	2-amino-7-phosphonoheptanoate	EPSP,	excitatory post-synaptic potential
CNQX,	6-cyano-7-nitroquinoxaline-2,3-dione	HPLC,	high performance liquid chromatography
CNS,	central nervous system	i.c.v.,	intracerebroventricular
CPP,	3-(2-carboxypiperazin-4yl)-propyl-1-phosphonic acid	K/Q,	kainate/quisqualate receptor
DEDTC,	di-ethyl-dithiocarbamate	LGN,	lateral geniculate nucleus
DNQX,	6,7-dinitroquinoxaline-2,3-dione	LTP,	long-term potentiation
EAA,	excitatory amino acids	NMDA,	<i>N</i> -methyl-D-aspartate

1. INTRODUCTION

The hypothesis that the physical basis of information storage in the mammalian brain resides in alterations of synaptic efficacy has long been proposed (Cajal 1911; Konorski 1948; Hebb 1949), is central to several neurobiological models of memory (see, for example, Marr 1971) and is widely accepted by many neuroscientists. However, whereas synaptic plasticity is a neurobiological concept referring to the capacity of synapses to alter as a function of injury, development, or patterns of activation, learning and memory are psychological concepts inferred from alterations of behaviour in response to experience. It follows that valid tests of the hypothesis that certain types of synaptic plasticity play a role in information storage require experimental studies to map between two

different levels of analysis. Such work should evaluate whether identified neural mechanisms underlying a particular form of synaptic plasticity are in practice activated during certain kinds of learning, and whether they are necessary for information storage to occur. Theoretical work is also essential to evaluate the computational and neurobiological adequacy of formal models that make use of synaptic plasticity to store information (see, for example, Willshaw & Buckingham, this symposium).

This paper describes experimental work conducted within the spirit of such an enquiry but that falls far short of the ideal. It focuses on only one type of learning (namely spatial navigation) and on only one type of synaptic plasticity (hippocampal long-term potentiation, LTP). However, we defend this limited starting point on the grounds that the physiological

properties of LTP are now reasonably well understood; that its induction can be experimentally controlled by drugs that may be used in controlling the behaviour of animals; and that the type of learning we are investigating, although poorly understood psychologically, is nevertheless known to depend upon the integrity of the hippocampal formation. Thus there are grounds for believing that the role of synaptic plasticity in learning can be evaluated rigorously and the learning paradigm we are using is, neuropsychologically, a reasonable behavioural assay. More specifically, the experiments are intended to test the hypothesis that the underlying neural mechanisms of one form of hippocampal LTP are involved in the storage of information by examining whether the pharmacological blockade of LTP causes a behaviourally specific learning impairment that can be dissociated from sensorimotor side-effects or impairments in retrieval, or both.

2. HIPPOCAMPAL SYNAPTIC PLASTICITY

2.1. *Different forms of synaptic plasticity*

Synaptic plasticity is a very general concept referring to structural and functional alterations of neural efficiency including reactions to injury (e.g. lesion-induced synaptogenesis, (Liu & Chambers 1958)), developmental changes (e.g. synapse elimination (Brown *et al.* 1976)) and alterations induced by specific patterns of neural activity. This latter type of plasticity is our primary concern.

High-frequency activation of presynaptic afferents within hippocampal circuitry results in both short- and long-lasting changes of synaptic efficacy. Facilitation and augmentation refer to changes that are exclusively pre-synaptic and have a timecourse of less than a few seconds (Magleby & Zengel 1976; McNaughton 1983). Post-tetanic potentiation is, as its name implies, also induced by high-frequency stimulation and has a decay timecourse of a few minutes. However, the discovery of a long-lasting or long-term potentiation (Bliss & Lomo 1973) with a decay timecourse that, in chronic preparations, can be as long as days, weeks or even months has, understandably, attracted considerable attention as a phenomenon whose underlying mechanisms could be involved in long-term memory. Long-term potentiation is not the only long-lasting change in neural function known to occur in hippocampus. Others include kindling, an epileptiform discharge induced by repetitive sub-threshold stimulation (Goddard *et al.* 1969); long-lasting decreases in after-hyperpolarizing currents, first identified as correlates of learning in *Hermisenda* (see Alkon 1987), but also found in hippocampus after classical conditioning (Disterhoft *et al.* 1986); and long-term depression including both heterosynaptic depression (Lynch *et al.* 1977) and, more controversially, associative homosynaptic depression (Stanton & Sejnowski, 1989). The remainder of this paper focuses on LTP because several of its physiological properties are suggestive of a memory mechanism.

2.2. *Long-term Potentiation*

Long-term potentiation (LTP) was discovered in the late 1960s and first reported in detail by Bliss & Lomo (1973). The original experiments, conducted by using anaesthetized rabbits, involved the bilateral placement of stimulating electrodes in the perforant path entering the hippocampus and recording electrodes in the molecular layer and hilus of the dentate gyrus. One three-phase experiment began with repetitive low-frequency electrical stimulation (one per 30 s) to evoke field potentials consisting of a mixture of fibre potentials, EPSPs, IPSPs and population spikes. These remained stable throughout the preliminary 'baseline' phase. However, when a brief tetanic burst of stimulation (100 Hz for 2 s) was given unilaterally (phase 2) followed by a return to low-frequency stimulation (phase 3), the field potentials recorded on the side of the brain that had received the high-frequency burst were found to be substantially enhanced relative to their pre-tetanic baseline. Bliss & Lomo (1973) showed that this increased responsiveness consisted of both a synaptic component (LTP) and a reduced threshold for cell firing for a given size of EPSP (E-S potentiation). Both components of the response are long-lasting (Bliss & Gardner-Medwin 1973). Subsequent work in many laboratories has confirmed and expanded upon these original observations by using both *in vivo* and *in vitro* preparations (see Brown *et al.* (1988); Nicoll *et al.* (1988); Deadwyler & Landfield (1988), for recent reviews).

2.3. *Physiological properties of LTP*

Research on LTP has revealed several other physiological properties relevant to a possible role in learning (table 1): (i) it was discovered and occurs prominently in the hippocampus, which has long been implicated in learning and memory (see, for example, Scoville & Milner (1957)); (ii) it can be induced by extremely brief patterns of afferent stimulation, including both single stimuli paired with post-synaptic depolarization and rhythmic bursts of presynaptic activation that mimic naturally occurring patterns of neural activity (Wigström *et al.* 1986; Larson & Lynch 1986; Pavlides *et al.* 1988); (iii) it is induced rapidly, reaching a peak within 30–60 s (McNaughton 1973; Gustafsson *et al.* 1989) but, according to one report, giving rise to post-synaptic changes over a longer time period (Davies *et al.* 1989); (iv) its induction can be associative in the sense that activity at one point on the dendritic tree can influence whether synaptic change will occur at another point (McNaughton *et al.* 1978; Levy & Steward 1979; Barrionuevo & Brown 1983), a property relevant to a possible role in associative learning; (v) it is synapse-specific (Andersen *et al.* 1977), thereby permitting massive information storage capacity and a parallel distributed processing 'style' of computation, and (vi) it saturates upon repeated activation (McNaughton 1983).

However, suggestive as these properties may seem, there are further properties of LTP which complicate hypotheses about its functional significance (table 1):

Table 1. *Properties of LTP relevant to its putative role in learning*

occurs in hippocampus	also occurs in other brain areas, and other brain areas are involved in learning
induced by brief patterns of stimulation	large field-potential changes are not observed naturally following learning
rapid induction	always decays over time
associative	temporal parameters of associative induction more specific than those of conditioning
synapse-specific change	
saturates	

(i) it occurs outside the hippocampus (notably in cortex (Lee 1983; Artola & Singer 1987)), and other brain regions are clearly involved in learning. Thus a preoccupation with hippocampal LTP may be misleading. (ii) Although induced by patterns of stimulation that might occur naturally, there is controversy about whether LTP actually occurs as a correlate of learning. Increases in the population spike in the dentate hilus of rabbits that have undergone nictitating membrane conditioning (Thompson, this symposium), although consistent with an LTP mechanism, may also be caused by changes in excitability induced by some other mechanism. In comparison, only modest changes in the slope function of field potentials are seen in animals that have been given considerable opportunity to explore a complex environment (Sharp *et al.* 1985). Part of the problem is whether one would really expect to see gross changes in a physiologically activated field potential as a consequence of the subtle spatially distributed subset of synaptic changes that might accompany a single learning experience (see Morris (1989*b*) for discussion). (iii) The time-window within which the pre- and post-synaptic events for inducing associative LTP must occur (≈ 100 ms) maps onto the temporal parameters of associative learning (e.g. classical conditioning) poorly. (iv) Once induced, LTP seems to involve a heterogeneous family of synaptic changes with dissociable timecourses lasting a few hours, or several days (referred to as LTP1 and LTP2, respectively (Racine *et al.* 1983)), timecourses that have no obvious parallel in extant behavioural findings. (v) The mechanisms of LTP might not be involved in long-term memory because non-decremental LTP has never been observed.

Our own view of these problems is that although they must be addressed, none are fatal for the hypothesis. To take one example, the phenomenon of associative long-term depression, LTD (Stanton & Sejnowski 1989) throws new light on the timecourse problems (iv) and (v) above). Non-decremental LTP is not seen in freely moving animals; this may be because the conditions for producing LTD are inadvertently but necessarily produced by naturally occurring patterns of activity. Bearing in mind that LTP is an 'artificial' phenomenon demonstrated by stimulating large numbers of afferent fibres synchronously, its decline over time may be due to a naturally occurring but activity-dependent LTD on small subsets of these fibres rather than a strictly time-dependent decay. Interestingly, Pavlides *et al.* (1988) have recently shown that short tetanic bursts on the trough of the naturally occurring 'theta' wave can cause a depression of synaptic efficacy.

Thus LTP displays several suggestive properties that, although not without problems, are at least suggestive of an underlying memory mechanism. Several research groups have sought to exploit these properties in studies of its functional significance. Barnes (1979) has found correlations between behavioural learning and the decay timecourse of LTP, whereas both McNaughton *et al.* (1986) and Castro *et al.* (1989) have shown that saturation of LTP is associated with an anterograde spatial amnesia. New avenues for exploring the functional significance of LTP have also opened up following the discovery that activation of a sub-type of excitatory amino receptor is necessary for the induction of LTP.

2.4. Role of NMDA receptor activation in induction of LTP

The last few years have seen striking advances in our understanding of excitatory neurotransmission in the CNS made possible through the development of several selective agonists and antagonists of excitatory amino acid (EAA) receptors. Studies making use of techniques such as ligand binding, intracellular recording and patch-clamping have led to our current understanding of there being at least three main classes of receptor. The most widely characterized receptors are called kainate-, quisqualate- and NMDA-preferring receptors, so named after the preferential agonist at each site (Watkins & Evans 1981; Foster & Fagg 1984; Mayer & Westbrook 1987; Watkins & Collingridge 1989). Certain antagonists (e.g. CNQX, DNQX) interact preferentially at kainate and quisqualate receptors, whereas others (e.g. AP5) bind to the NMDA receptor. Neurophysiological studies reveal that in the hippocampus, activation of kainate/quisqualate (K/Q) receptors mediates fast synaptic transmission whereas NMDA receptors are not involved in this component of the synaptic response. This may not be the case in other brain areas (Thomson *et al.* 1985; Salt 1986; Fox *et al.* 1989*b*; Sillito *et al.* 1990). The NMDA-associated ionophore does, however, have the unusual property of responding to post-synaptic depolarization (Nowak *et al.* 1984; Mayer *et al.* 1984). At the resting potential (≈ -80 mV), the NMDA ion channel is blocked by the divalent cation magnesium such that binding of an endogenous transmitter to NMDA receptors is unable to open the channel. However, if the neuron is partially depolarized (e.g. to -35 mV), the magnesium block is released and subsequent activation of NMDA receptors can then open these channels. NMDA receptor ion channels are thus dually regulated, being both ligand- and voltage gated (figure 1). Furthermore, it is now

also known that fast synaptic transmission depends on the passage of Na^+ ions through the K/Q receptor ion-channel, whereas the opening of the NMDA-receptor channel allows the preferential passage of Ca^{2+} ions into postsynaptic dendritic spines (McDermott *et al.* 1986).

A particularly important discovery has been that the selective NMDA antagonist AP5, although having no effect upon normal fast synaptic transmission in hippocampus, blocks the induction of LTP (Collingridge *et al.* 1983). Harris *et al.* (1984) extended this finding by showing that AP5's blockade of LTP was stereospecific; the D-isomer blocked LTP (at an ED_{50} of $\approx 8 \mu\text{M}$) whereas the L-isomer was essentially without effect. The importance of this discovery is that, together with the work on receptor pharmacology, it has led to an understanding of the likely sequence of events occurring during the induction of LTP that, in turn, explains several of its properties outlined in §2.3. Specifically, a high-frequency tetanus results in a sufficiently large and sustained depolarization such that the voltage-dependent block of the NMDA receptor ion channel is released. Ca^{2+} ions, rather than just Na^+ , then enter the post-synaptic spine where they can act upon one or several biochemical processes that may eventually result in the alteration of synaptic efficacy. AP5 blocks LTP by binding competitively to the NMDA receptor recognition site such that, even when the voltage-dependent block of the ion channel is released during a tetanus, the endogenous transmitter (e.g. L-glutamate) cannot gain access to its receptor to open the channel. AP5 is without effect on the expression of LTP because, once induced, LTP involves an alteration of fast synaptic transmission, i.e., the mediation of EPSPs via K/Q receptors.

This brief account of the events occurring during the induction of LTP helps to explain several of its physiological properties. Thus LTP is induced by brief patterns of stimulation because Ca^{2+} can enter post-synaptic dendritic spines during either a short-lasting tetanus or when single pulses of afferent stimulation occur in instantaneous conjunction with post-synaptic depolarization. Long-term potentiation is synapse-specific because the NMDA-associated ion channel is ligand-gated and so, under normal physiological conditions, requires the binding of transmitter released from specific presynaptic terminals. And LTP is associative because induction also requires post-synaptic depolarization caused by neural activation that, under normal conditions, will have been induced elsewhere on the dendrite other than at the site of presynaptic activity. Thus NMDA receptors are 'conjunction devices' endowing neurons with the biophysical capacity to detect the co-occurrence of presynaptic activity with post-synaptic depolarization, i.e. with the capacity to function as 'Hebb-like' synapses (Kelso *et al.* 1986; Wigström *et al.* 1986).

Selective amino acid antagonists, such as DNQX and AP5, are marvellous tools with which to dissect various aspects of excitatory neurotransmission. Newer compounds are also becoming available that act as NMDA-receptor channel blockers (e.g. MK-801, Wong *et al.* (1986)), or on the glycine regulatory site

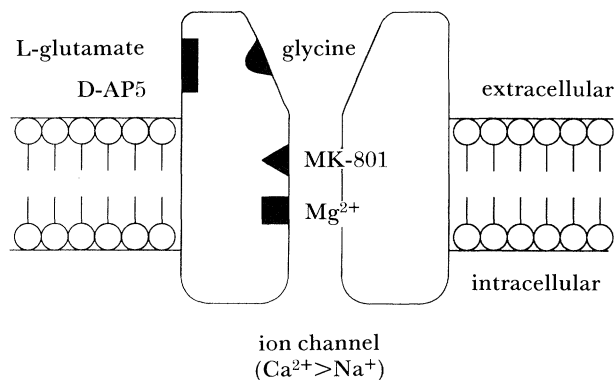


Figure 1. Simplified cartoon of NMDA receptor complex. The receptor protein is associated with an ion-channel, which, when open, is preferentially permeable to Ca^{2+} rather than Na^+ . Ordinarily, the channel is blocked by Mg^{2+} . D-AP5 binds competitively to the receptor recognition site for the endogenous transmitter (e.g. L-glutamate). Channel-opening is modulated by glycine and may be blocked by non-competitive antagonists such as MK-801.

(Johnson & Ascher 1987), which forms part of the NMDA-receptor complex (Kemp *et al.* 1988). One recent example of their use has been in a study concerned with whether the expression of LTP involves an increase in transmitter release, as proposed by Bliss & Lynch (1988), or a selective alteration in the number of postsynaptic receptors (Lynch & Baudry 1984). Muller *et al.* (1988) examined the effects of DNQX and AP5 upon LTP, and also, for purposes of calibration, a phenomenon called 'paired-pulse potentiation' that is known to involve increased transmitter release. By using a stimulation paradigm in which the voltage-dependent block of the NMDA-receptor ion channel was partially released, they found that LTP is associated with an increase in the component of the EPSPs seen in the presence of AP5 (i.e. those caused by activation of K/Q receptors) without any change in the smaller EPSPs induced in the presence of DNQX. This was not because of a lack of sensitivity for detecting changes in the current flowing through NMDA receptors because increases in both the DNQX- and AP5-sensitive components of the EPSP were seen in paired-pulse potentiation. A related finding has been reported by Kauer *et al.* (1988). The implication is that the expression of LTP is more likely to be associated with a selective post-synaptic change, e.g. in the number of K/Q receptors, or the number of ions flowing through the K/Q ionophore with each channel opening, than with a sustained increase in transmitter release. However, somewhat different results have been found by Collingridge (personal communication) & Tsien (1990) who both find that a small component of the increase in the EPSP remains in the presence of low concentrations of CNQX and that this is sensitive to AP5. One complication in analysing the expression of LTP is the finding of Davies *et al.* (1989) that the altered sensitivity to iontophoretic application of the quisqualate agonist AMPA develops only gradually over a period of two hours (see also Horn, this symposium).

3. ROLE OF NMDA RECEPTOR ACTIVATION IN LEARNING AND MEMORY

3.1. Design requirements

In addition to being useful tools for exploring excitatory neurotransmission, selective EAA antagonists can also be used in behaving animals to investigate learning. One straightforward prediction of the hypothesis that the neural mechanisms underlying LTP are involved in information storage is that blockade of NMDA receptor action *in vivo* should cause impairments of learning. However, because NMDA receptors are widely distributed in the CNS, generalized NMDA receptor blockade is likely to have functional consequences over and beyond effects on learning. It follows that, if an NMDA antagonist such as AP5 is used to investigate the role of LTP in learning, it is essential that experiments incorporate the following.

- (i). Neurophysiological observations. To check that the drug is actually impairing or blocking LTP *in vivo*.
- (ii). Control procedures. To dissociate primary and secondary effects of the drug.

The first of these requirements is straightforward, although it is remarkable how few behavioural studies of NMDA antagonists have included neurophysiological measures. The disquiet is more than academic because non-competitive NMDA antagonists, such as MK-801, may not block LTP *in vivo* if the attempt to induce it is made within the same short interval after drug administration as typically used in behavioural studies between drug injection and behavioural testing (e.g. 30 min). Halliwell & Morris (1987) found no change in either the magnitude or decay timecourse of LTP when tetanic stimulation was given 20 min after a 1 mg kg⁻¹ i.p. injection of MK-801 in urethane anaesthetized rats. A more detailed study by Abraham & Mason (1988), but also in anaesthetized animals, did find blockade of LTP but only when stimulation was given 150 min after i.p. injections at the equally high dose of 1 mg kg⁻¹. *In vitro* observations by Coan *et al.* (1987) confirmed that use dependency, characteristic of MK-801's action as an NMDA-receptor channel blocker (Wong *et al.* 1986), may take up to two hours to develop fully in hippocampal slices. Until this channel block is fully developed, there is no reason why sufficient Ca²⁺ should not pass through the channel during brief periods of presynaptic activation coupled with post-synaptic depolarization. Interestingly, there is evidence suggestive of use-dependency in learning paradigms (Clineschmidt *et al.* 1982), but its occurrence is complicated by the fact that agonist activation will surely vary across brain regions. Thus MK-801 may induce a rapid channel block in, for example, globus pallidus (where baseline cell firing rates are high) but have much slower effects in the CA1 region of the hippocampus (where they are low). The spirit if not the detail of this idea may help us understand why MK-801 causes its pronounced behavioural syndrome (ataxia, head-bobbing) within 15 min but fails to block LTP until 2 h have elapsed since drug-injection. In our view, the convenience of non-competitive antagonists in crossing the blood-brain barrier is offset by this ambiguity concerning

their capacity to block hippocampal LTP *in vivo*. Notwithstanding their use by several behavioural-research groups (Robinson *et al.* 1989; Mondadori *et al.* 1989; Whishaw & Auer 1989) there are clearly obstacles to basing firm conclusions about the role of LTP in learning on experiments that use non-competitive antagonists.

The second design requirement – the need for control procedures – divides into two logically separate issues. First, it is essential to investigate whether ‘side-effects’ caused by drug action outside the hippocampus could be responsible for the apparent impairment of hippocampally mediated learning. Secondly, there may be effects of neurophysiological function within hippocampus in addition to the impairment of LTP. Both of these points follow from a crucial logical point (which we have stressed repeatedly in previous papers (Morris *et al.* 1986*a*; McNaughton & Morris 1987; Morris 1989*a, b*; Morris *et al.* 1989*b*) namely, the drug-induced blockade of LTP and its impairment of learning are each dependent consequences of a single independent treatment. It is logically fallacious to presume, in the absence of further evidence, that one dependent consequence (e.g. a blockade of LTP) is necessarily the cause of the other (e.g. an impairment of learning).

Effects outside hippocampus: it is known that AP5 can cause sensorimotor effects outside the hippocampus upon sensory transduction (Cahusac *et al.* 1985; Salt 1986) motor control and motor patterning (Dale & Roberts 1984; Klockgether *et al.* 1986; Dale 1989), muscle flaccidity (Turski *et al.* 1985) and supra-segmental reflexes (Davies & Watkins 1983), any or all of which could give rise to a behavioural profile of impairment deceptively like that caused by hippocampal lesions. Exactly how these sensorimotor disturbances would mimic hippocampal disruption is unclear because hippocampctomized rats can see and move around properly, and they have both normal musculature and normal spinal reflexes. However, the mere possibility that a complex profile of extra-hippocampal disturbance might mimic the effects of a lesion should always be borne in mind.

Effects within hippocampus: AP5 can also cause effects within the hippocampus other than on LTP induction, including a decrease in hippocampal ‘theta’ activity (Leung & Desborough 1988), reductions in hippocampal excitability (Errington *et al.* 1987; Abraham & Mason 1988), and truncation of normal complex-spike firing (Abraham & Kairiss 1988). Even more than the sensorimotor ‘side-effects’ listed above, these drug-induced alterations of normal hippocampal function might be expected to cause behavioural disturbances similar to those produced by partial or complete hippocampal lesions. It should be noted, however, that in Leung & Desborough’s (1988) study, the reduction in hippocampal theta was only apparent at their highest dose when 100 nanomoles of AP5 was infused (i.c.v.) over 3 min, a rate of infusion 60-times faster than our 20 nmol h⁻¹ chronic infusion protocol. Furthermore, they reported that at this dose, their animals ‘became ataxic and assumed an unsteady gait with the abdomen held near the floor during walking.’ Such behaviour is never seen in our animals chronically

treated with AP5 at doses up to those sufficient to block LTP completely.

The pharmacological method of investigating the role of LTP in learning is, therefore, fraught with several interpretative difficulties. In the experiments described below, we have endeavoured to address some of these problems, some directly, other indirectly. The cardinal features of our strategy have been as follows.

(i) The use of a variety of neuropsychologically dissociable tasks, to search for behavioural specificity.

(ii) The conduct of both neurophysiological and behavioural measures whenever possible, sometimes in the same animal.

(iii) The precise measurement of drug levels in the CNS by using both whole-tissue and extracellular sampling techniques together with HPLC fluorometric methods, to afford comparison with *in vitro* studies of NMDA receptors and a sound basis for comparing successive behavioural studies.

3.2. Behavioural and anatomical specificity

Morris *et al.* (1986a) showed that chronic intraventricular infusion of 40 mM D,L-AP5, a dose shown to be sufficient to block LTP *in vivo*, caused an impairment of spatial learning in the water-maze while leaving visual discrimination learning (tested in the same apparatus) intact (figure 2). This profile of impairment is similar to, although less severe than, that caused by hippocampal lesions (O'Keefe & Nadel 1978; Morris *et al.* 1986b). The behavioural specificity was also borne out in Tonkiss *et al.*'s (1988) discovery that AP5 impairs DRL performance (a non-spatial operant schedule involving differential reinforcement of low rates of an operant response for food reward) in a

qualitatively similar way to that caused by hippocampal lesions, whereas the anatomical specificity is supported by observations that local, intrahippocampal infusions of nanomolar quantities of AP5 cause a dose-dependent impairment of both LTP *in vivo* and place-navigation (Morris *et al.* 1989b; two doses tested only). It is tempting to interpret these results as supporting the general hypothesis that activation of the neural mechanisms responsible for hippocampal LTP is necessary for those types of learning that require the integrity of hippocampal function but unnecessary for other types of learning. However, although consistent with this general hypothesis, it would be premature to draw so bold a conclusion on this evidence alone for the reasons outlined in §3.1 above.

First, as pointed out by Goddard (1986), the behavioural specificity seen after i.c.v. infusions might arise from differential drug diffusion in the brain. Given that intracerebral drug concentrations have been reported to decline with distance from the infusion cannula (Urquhart *et al.* 1984; Kleinschmidt *et al.* 1987), the latter's proximity to hippocampus but greater distance from, for example, visual cortex, may have resulted in higher and more effective drug concentrations in hippocampus. We have addressed this problem by directly measuring whole-tissue levels of AP5 in various brain regions, and by conducting a second visual discrimination experiment in which the drug was infused bilaterally (20 mM D,L-AP5, 0.5 μ l h⁻¹ from each of two minipumps) into a cortical area just anterior to the visual cortex (Occ2 (Zilles 1985) we did not infuse into the visual cortex itself because of the 500 μ m gliosis that can develop around an indwelling cannular). The results showed a remarkably uniform distribution of AP5 throughout the rodent forebrain

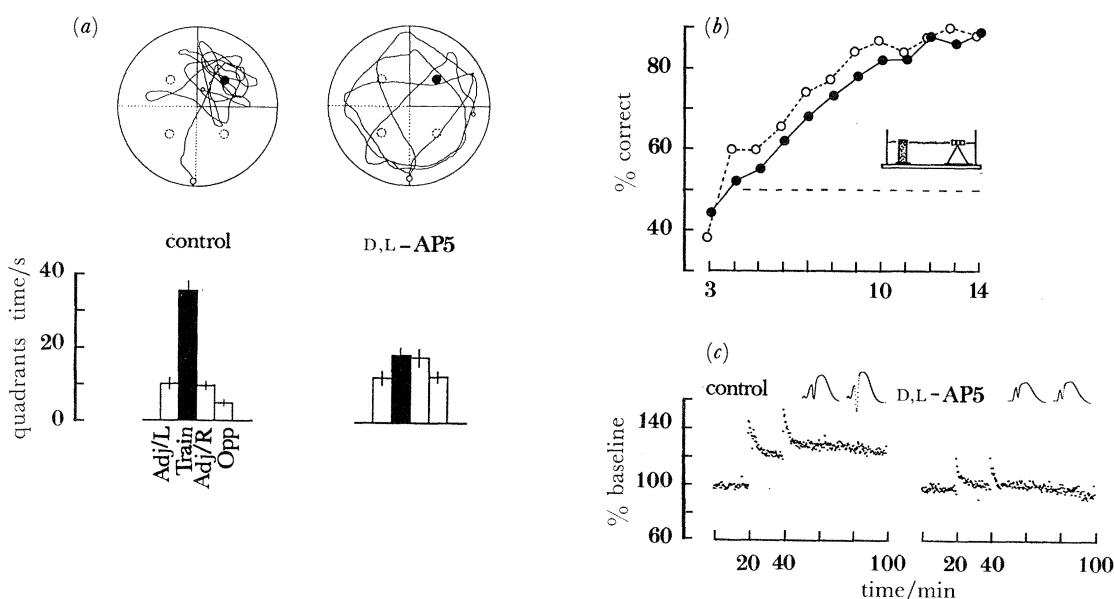


Figure 2. Intraventricular infusion of AP5 impairs spatial learning. Rats were implanted with an i.c.v. cannula connected to osmotic minipumps chronically delivering 40 mM D,L-AP5 at 0.5 μ l h⁻¹ over a period of up to 14 days. (a) Spatial learning. Paths taken during a 60 s transfer test (platform absent) after 15 trials of training over five days. The former position of the platform is shown as (●). (b) Visual discrimination. Choice performance on a two-platform visual-discrimination task (inset) over 12 days of training (10 trials per day); (○), control; (●), D,L-AP5. (c) LTP. Mean normalized early-rising slope values of field-potentials evoked in the hilus of the dentate gyrus after perforant path stimulation. Brief tetanic stimulation was given at the 20 and 40 min points.

after i.c.v. infusions whereas, with intracortical infusion, concentration only showed an appreciable decline at distances greater than approximately 8 mm (Butcher *et al.* 1990). Furthermore, the rate of visual-discrimination learning was unaffected by intracortical infusion of AP5 despite a measured whole-tissue concentration in visual cortex comparable to that which, in hippocampus, is associated with impairments of place learning. The issue raised by Goddard (1986) was an important and legitimate one but we believe that these results render it less tenable as an explanation of behavioural specificity.

Secondly, the apparent behavioural specificity reflects only a 'single dissociation'. No 'double dissociation', such as an impairment of visual discrimination but not spatial learning following some other drug or lesion treatment, has been either sought or found (see Weiskrantz 1968; Shallice 1989). The most immediate worry that this fosters is that the dissociation may reflect nothing more than task difficulty. However, the visual discrimination task is learned more slowly by normal rats than either the standard place-navigation task (*ca.* 120 trials *vs.* 20 trials, respectively) or a two-platform spatial discrimination task whose motivational, reinforcement and sensorimotor demands are equated to those of the visual discrimination task (120 against 50 trials, (Morris *et al.* 1986*b*)). Thus AP5 impairs the easier rather than the operationally more difficult task (*cf.* Mondadori *et al.* 1989).

However, there are deeper issues at stake. The failure to find any effects of a potent competitive NMDA antagonist upon cortically mediated visual-discrimination learning is in contrast to the marked impairment of ocular dominance reversal following reverse suture after monocular rearing in kittens (Kleinschmidt *et al.* 1987). Bear *et al.* (1987) and Singer (1990) have interpreted this and other related findings as evidence that activation of cortical NMDA receptors is essential for self-organization in the developing nervous system. But what of their role in the adult animal? If our visual discrimination findings are reliable, they imply that mechanisms other than NMDA-receptor mediated plasticity must be responsible for storing information about the association of a visual cue with reward or non-reward. A second implication is that, at doses sufficient to block LTP and impair spatial learning (but not necessarily at higher doses), chronic AP5 infusion cannot be causing a gross sensory impairment. In the water-maze, AP5-treated rats can see well enough to head towards a visible target, and they can discriminate a grey platform from one painted with black-and-white stripes of roughly equivalent luminosity. In contrast, studies of the effects of iontophoretic application of NMDA antagonists to LGN and visual-cortex neurons point to participation of NMDA receptors in the normal throughput of information on the geniculo-striate pathway and in contrast sensitivity (Sillito *et al.* 1990; Fox *et al.* 1989*b*; Daw *et al.* 1990). The inconsistency between these and our own behavioural findings may be partly due to dose dependency. Rats who, at sacrifice, are found to have substantially higher whole-tissue drug concen-

trations than those sufficient to block LTP do show abnormalities of behaviour consistent with a partial loss of vision (we have not attempted any formal testing). However, the inconsistency may also be due to our use of a relatively unsophisticated visual discrimination task. A more subtle task, such as one involving discrimination between stripes of differing spatial frequency, or of lower contrast discriminability, may show performance (as against learning) impairments at cerebral concentrations equivalent to those that impair spatial learning. The question would then arise of whether the apparent spatial-learning impairment might actually be due to the AP5-treated animals having difficulty seeing distal cues (but see §3.5).

3.3. *Sensorimotor side-effects*

Rats given chronic i.c.v. infusion of AP5 at rates of up to 15 nmol h⁻¹ (30 mM D-AP5, 0.5 µl h⁻¹) show remarkably normal behaviour. They are certainly not ataxic, appear to show normal exploratory movements, can swim normally (with appropriate forelimb inhibition) and, in the water-maze, use the hidden platform as a refuge in much the same way as untreated rats. However, it is also true that their range of movements is not entirely normal. Some rats show a slight muscle flaccidity, a slower righting reflex, or have difficulty in holding onto the top of a wire cage when it is tilted from horizontal to vertical. In the first of our water-maze experiments (Morris *et al.* 1986*a*), a few rats fell off the hidden platform after a 'wet-dog' shake early in training. This abnormality diminished over days and, we now know, is only inevitable in animals whose hippocampal-tissue AP5 concentration is very high.

Clearly these disturbances could be contributing to the AP5-induced impairment of some hippocampally mediated tasks (spatial learning, DRL performance) although for others, such as odour-potentiated taste aversion (Crooks *et al.* 1989), the proposal strains credibility. For example, rats that fall off the escape platform in the water-maze (and normal rats do this too) might come to find the platform aversive. As escape is the only source of reinforcement in the task, learning might then proceed more slowly, not because of a direct participation of NMDA receptors in spatial learning but, secondarily, because the incentive to learn was diminished. This possibility has been ruled out following the accidental observation during some pilot work that the behaviour of falling-off the platform is greatly diminished by pretraining before administering the drug. Morris (1989*a*, expt 3) examined this formally by looking at the effects of non-spatial pretraining upon the AP5-induced impairment of place-navigation. Rats were first trained (as normals) to use an escape platform as a refuge, under conditions that prevented spatial learning: by successively hiding the platform in different locations over trials within a water-maze surrounded by black curtains that occluded sight of distal cues. Escape performance remained random during this pretraining but the rats did learn to climb onto the platform whenever they

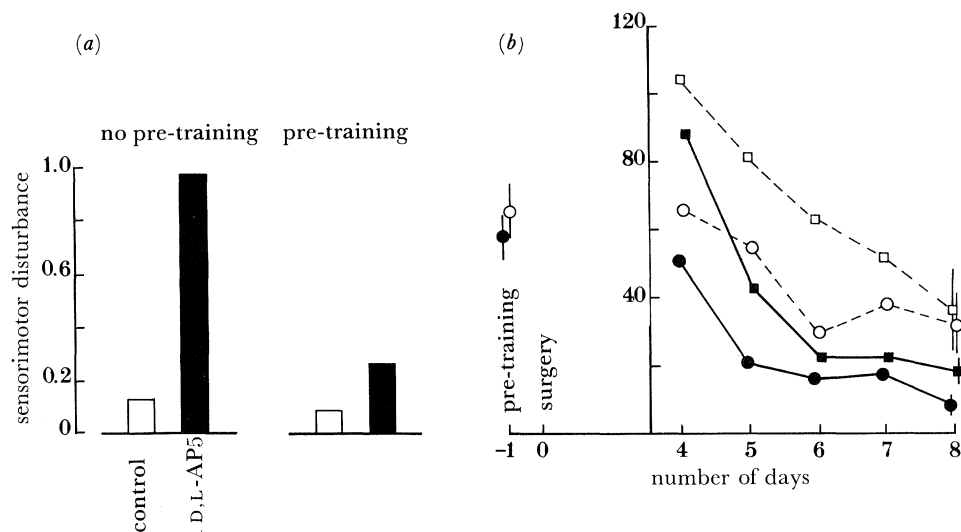


Figure 3. The spatial learning impairment can be partly dissociated from a gross sensorimotor disturbance. (a) Non-spatial pre-training caused a reduction in falls off the platform during the drug phase. (b) The extent of the AP5-induced learning impairment was unaffected by non-spatial pretraining. Escape latencies were lower at the outset of the drug phase in pretrained rats but the AP5/aCSF difference was maintained; (●), control/P; (■), control/NP; (○), AP5/P; (□), AP5/NP.

bumped into it. Following surgery to implant minipumps containing AP5 or aCSF in these and other naive rats, spatial training was instituted by withdrawing the curtains and putting the platform in a fixed location (i.e. a protocol identical to that of the original 1986 study). Pre-training caused a striking reduction in the number of occasions rats fell off the platform but the drug-induced place-navigation impairment remained (figure 3). The analysis of variance showed no statistical interaction between the drug-induced learning impairment and pretraining (the F-ratio was less than 1). The reason for this somewhat unexpected result is that rats have to learn that the hidden platform is a refuge and that AP5 interferes with this learning process as well as that of learning where the platform is located. Thus delaying the administration of AP5 until after the rats have learned to use the escape platform diminishes the sensorimotor disturbance. The irony is that the 'sensorimotor disturbance' turns out to be a secondary consequence of a failure to learn rather than a viable explanation of the apparent learning impairment. Non-spatial pre-training is now used routinely in all our AP5 experiments and the procedure works well except in animals receiving very high drug doses (in whom the sensorimotor disturbances become so pronounced that the rats are virtually untestable).

The dose dependency of the sensorimotor disturbances induced by both competitive and non-competitive antagonists is a fitting reminder that great care must be exercised in using these drugs to investigate learning. We had hoped to do some work with MK-801 (the minipump surgery necessary for AP5 infusions being both expensive and time consuming) but our experience of this drug's pronounced sensorimotor abnormalities (ataxia, head-bobbing) at doses near to the ED_{50} values for its anticonvulsant effectiveness (ca. 0.3 mg kg^{-1} ; Tricklebank *et al.* 1989) led to our

abandoning this approach. It is still not clear whether the disturbances induced by competitive antagonists, such as AP5, AP7 and CPP, are qualitatively dissimilar but we do know that they are minimal at doses useful for investigating hippocampal-dependent learning.

3.4. Dose-response analysis

Over the past two years, we have conducted a detailed dose-response analysis of the effects of AP5 upon spatial learning and LTP *in vivo*. Although preliminary reports of this experiment have appeared elsewhere (see, for example, Morris *et al.* 1989b), the description below is the first comprehensive summary of the results.

There were two main reasons for conducting a dose-response analysis. First, the hypothesis that activation of the neural mechanisms responsible for LTP is required for normal hippocampal-dependent learning would be disproved if conditions were found in which LTP was completely blocked but learning proceeded normally†. Conversely, finding parallel, overlapping dose-response functions would put the original observations of an AP5-induced impairment of spatial learning on a firmer footing. Secondly, measurements of both whole-tissue and extracellular AP5 in the CNS of animals subject to both behavioural and physiological procedures would provide an excellent opportunity to assess the specificity of AP5's mode of action *in vivo* in comparison with its now well understood action *in vitro*. Our reason for taking both measures of AP5 concentration was because we already

† Note that there is an asymmetry about this prediction. The hypothesis is silent about the opposite finding: a behavioural impairment in the absence of any blockade of LTP. The neural mechanisms underlying LTP are no more the be-all and end-all of learning among advocates of the hypothesis than they are among its critics.

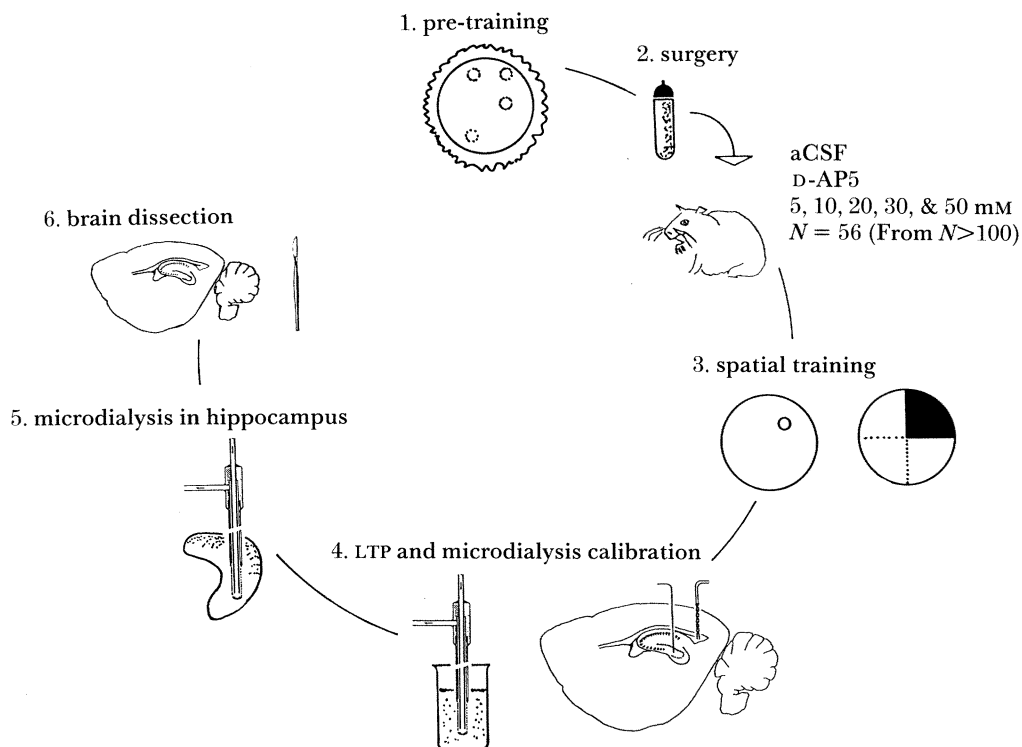


Figure 4. Plan of dose-response experiment: non-spatial pretraining, 12 trials, curtains around the pool, platform moved between trials. Surgery: minipumps containing aCSF or D-AP5 (5, 13, 20, 30, 40 and 50 mM), chronic pumping rate $0.5 \mu\text{l h}^{-1}$. Spatial training: 30 trials, curtains open, platform in a fixed counterbalanced location for individual animals and removed for the 60 s transfer test, all paths tracked by using an automatic image-analysing system. Attempted LTP induction: rats given urethane anaesthesia 1.5 g kg^{-1} , bipolar stimulating electrode in angular bundle and recording electrode in dentate hilus, field potentials amplified and monitored on-line by using an 11/23 computer with the early-rising slope calculated by linear regression, low-frequency stimulation = 0.1 Hz, tetanus = 3 trains of 33 pulses at 400 Hz given at least 45 min after assumption of stable low-frequency baseline, potentials monitored for a further 60 min, input/output functions (0–9 V) taken before and after the main series. The microdialysis probes were calibrated by using $5 \mu\text{M}$ D-AP5 during this electrophysiology phase. Microdialysis: the probe was positioned in hippocampus, aCSF perfused at $1.5 \mu\text{l min}^{-1}$, six samples at 20 min per sample. Dissection: samples taken from left and right hippocampus; HPLC was used to analyse AP5 content from both extracellular and whole-tissue samples, AP5 being separated from other amino acids and detected as a fluorescent derivative following precolumn derivitization with *o*-phthalaldehyde (the separation procedure involved a gradient elution between two buffers (buffer A, 50 mM sodium dihydrogen phosphate, pH 5.14, +2.5% tetrahydrofuran; buffer B, methanol + 1.25% tetrahydrofuran) and a $5 \mu\text{m}$ C-18 Nucleosil column (250 mm \times 4.6 mm), calibration by injection of 50 pmole D-AP5, data handling by

suspected that the whole tissue level was substantially higher than the effective extracellular concentration.

The general plan of the experiment is shown in figure 4. The behavioural protocol began with three days of non-spatial pretraining. The rats were then given surgery for implanting minipumps containing either aCSF or various concentrations of D-AP5, or given a sham operation (i.e. no minipump). Beginning three days later, all rats were given five days of spatial training followed, on the last day, by a transfer test. On the next day, or any of the succeeding three days, an attempt was made to induce hippocampal LTP *in vivo* (under urethane anaesthesia). Following this, and while still under anaesthesia, the extracellular fluid in hippocampus was sampled for two hours by using microdialysis probes. Finally, each animal was killed, their brains removed and various brain regions dissected for whole-tissue measurements of AP5 and endogenous excitatory amino acids by using HPLC.

It did not always prove possible to collect the complete set of data from all animals. However, out of a total of more than 100 rats that began non-spatial pretraining, 56 rats completed all phases. All correlational analyses were conducted by using individual animal scores but it proved convenient to subdivide the rats into groups on the basis of either their whole tissue or extracellular AP5 concentration. Thus groups were created whose whole-tissue AP5 concentration in hippocampus was 0 (i.e. aCSF and control rats), between 0.01 and 0.10, 0.11 to 0.20, 0.21 to 0.30, and, finally, above $0.31 \text{ nmol ng}^{-1}$ wet mass. This sorting process immediately enabled us to identify the nine animals with very high AP5 concentrations as including all of the eight animals who showed persistent sensorimotor abnormalities of the kind discussed above. All data from these nine 'very-high-dose' animals were disregarded in subsequent data analyses.

Analyses of the remaining 47 animals showed a dose-

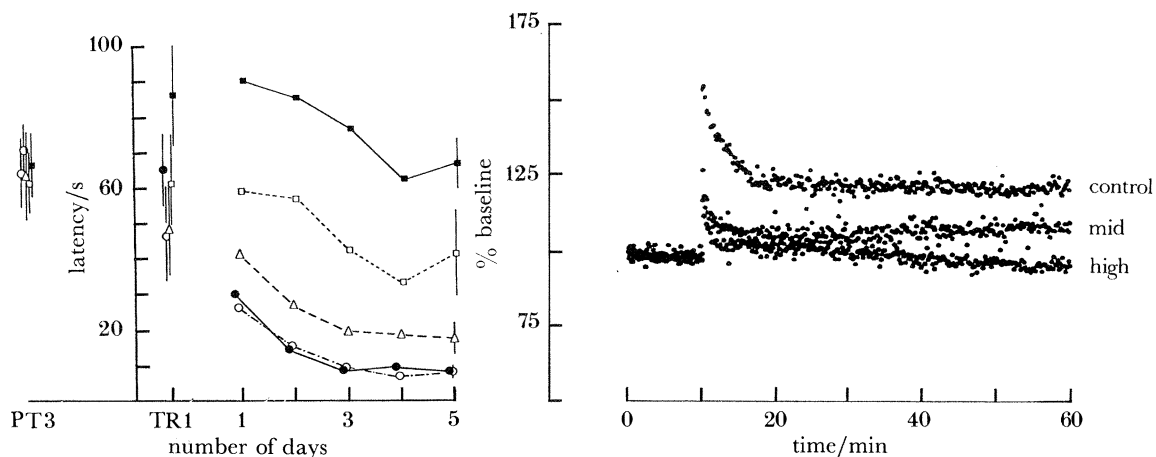


Figure 5. (a) Escape latency (s) on the last day of non-spatial pretraining (PT3), the 1st trial of spatial training (TR1) and throughout the five days of the spatial learning phase. Rats were divided into groups on the basis of whole-tissue AP5 content (control, aCSF rats (●); low, 0.01–0.10 (○); mid, 0.11–0.20 (△); high, 0.21–0.30 (□); very-high, > 0.31 (■), all in nanomoles per milligram wet mass). A dose–response relation is apparent, with the ‘very-high group’ performing worse than at the end of pretraining; only these rats showed any signs of a sensorimotor impairment. (b) LTP as a percentage of the base line early-rising slope of the field-potential. Only three groups are shown for the sake of clarity. The ‘low group’ was indistinguishable from ‘controls’, whereas the ‘very-high group’ was like the ‘high group’ in showing no LTP.

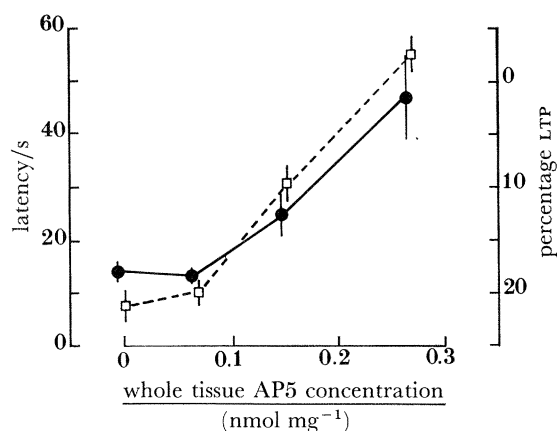


Figure 6. A plot of mean escape latency across the spatial learning trials and of mean percentage LTP 40 min after the tetanus against hippocampal whole-tissue AP5 in brain (at sacrifice). Note inversion of LTP *y*-axis; (●), latency; (□), LTP.

related impairment of both spatial learning and LTP (figure 5). Mean escape latency (averaged across the 30 spatial trials) increased as a function of whole-tissue content whereas the amount of LTP that could be evoked declined. The relationships between AP5 content and both percentage LTP and mean escape latency were highly significant correlations (figure 6).

The correlations did, however, indicate that the whole-tissue AP5 content was very high, disturbingly high if these values are any reflection of the effective AP5 concentration at NMDA receptors. The first clue that this was not the case lay in the electrophysiological data. This revealed the surprising finding that LTP could still be induced *in vivo* at a hippocampal tissue concentration that causes a complete blockade *in vitro* by using bath-applied AP5 at the same concentration (Harris *et al.* 1984). For example, the mid-level whole-

tissue concentration, 0.11–0.20 nmol mg⁻¹ wet mass (mean = 0.15; i.e. $\approx 150 \mu\text{M}$) was associated with 10% LTP (figure 6). Might it be that the action of AP5 *in vivo* is radically different from its action *in vitro*? Such a possibility seemed unlikely, but the mere possibility brings us to the reasons why we examined both whole-tissue AP5 and the effective extracellular concentration by using dialysis.

Whole-tissue AP5 provides a measure of the total amount of AP5 remaining in hippocampus at sacrifice. However, it does not indicate in which of several ‘compartments’ the drug is located; in plasma, cells or the interstitial space. To meet the second of our aims for this experiment, the comparison with *in vitro* studies of NMDA receptors, we needed to know the effective extracellular concentration. The analysis proceeded in a series of steps. First, we calibrated the percentage recovery of AP5 from our microdialysis probes by using a solution of known concentration (a value of 6.1% was obtained). Secondly, we checked the dialysis perfusate was at steady-state (figure 7a) by using values obtained from later time points in the collection period (i.e. the 40 min period beginning 60 min after starting perfusion). Thirdly, we conducted a regression analysis of dialysis perfusate against whole-tissue values of AP5 (figure 7b). The slope of this line, when percentage recovery is taken into consideration, indicates that the effective extracellular concentration is approximately 30-times lower than the whole tissue concentration across a wide dose range. We therefore repeated the correlation analyses using extracellular concentration rather than whole tissue content and found, for example, that the correlation with mean escape latency rose to a value of $r = 0.84$ that, in addition to being highly significant (d.f. 55, $p < 0.001$), implies that extracellular concentration accounts for 71% of the variability in escape latency. Furthermore, having obtained a measure of extra-

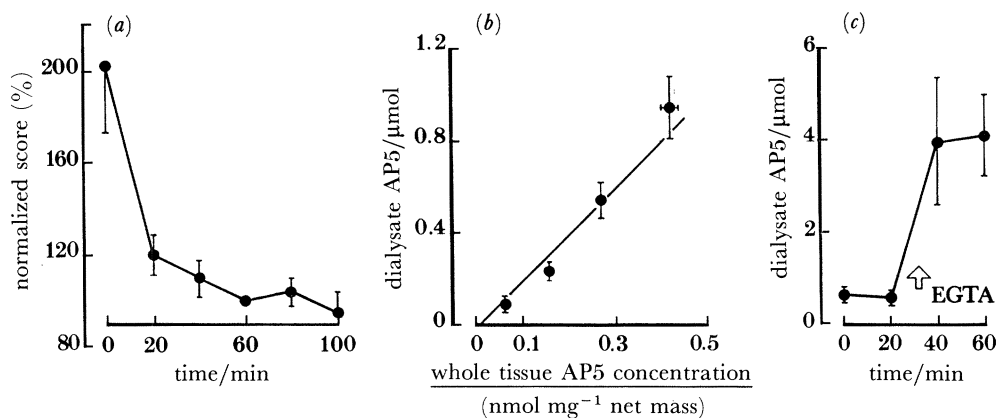


Figure 7. (a) Recovery timecourse for microdialysis perfusate from 0–120 in 20 min samples. The high initial value probably reflects tissue-damage. The mean values for the 60 and 80 min time periods were used in all subsequent calculations (b) Relation between dialysate AP5 concentration and whole-tissue concentration. The calculated recovery rate from the microdialysis cannulae was 6.1% and thus the ratio of whole-tissue to effective extracellular concentration is *ca.* 30:1. (c) perfusion of 20 mM EGTA in Ca²⁺-free aCSF caused massive release of AP5 indicating that at least part of the difference between the whole-tissue and extracellular concentrations can be accounted for in terms of a Ca²⁺-dependent 'trapping' mechanism.

cellular concentration, we were finally in a position to compare our results with those of *in vitro* studies†. Figure 8 shows a comparison with two pertinent experiments: Monaghan *et al.*'s (1988) data on the displacement of D-[³H]AP5 by D-AP5 in a ligand binding assay, and Harris *et al.*'s (1984) data on AP5-induced impairment of LTP in the *in vitro* hippocampal slice. Our learning data has been normalized by comparing, across a range of AP5 extracellular concentrations, the savings in spatial escape latency relative to that of controls. The LTP *in vivo* data have also been normalized. Plotting the results in this way points to a remarkable parallel: the impairment in spatial learning occurs across exactly the same dose range as the AP5-induced blockade of both LTP *in vivo* and LTP *in vitro* and each of these three functions is, as anticipated, slightly to the right of the ligand binding results (these having been collected in the absence of L-glutamate).

But the story does not end there. The 30-fold discrepancy between the whole-tissue and extracellular concentrations implies that there must be some 'trapping' of AP5 rendering it inaccessible to the microdialysis probes and, presumably, also to NMDA receptors. As this discrepancy between these two AP5 concentrations began to emerge through the series of replications, we naturally wondered about the fate of the AP5 remaining in brain that was unavailable to receptors. Biochemical experiments conducted in collaboration with M. Kessler and R. Griffiths established that D-AP5 does not inhibit the uptake of L-[³H]-glutamate on the high-affinity glutamate uptake carrier, nor is D-[³H]AP5 itself taken up. However, following a suggestion of J. C. Watkins, we added

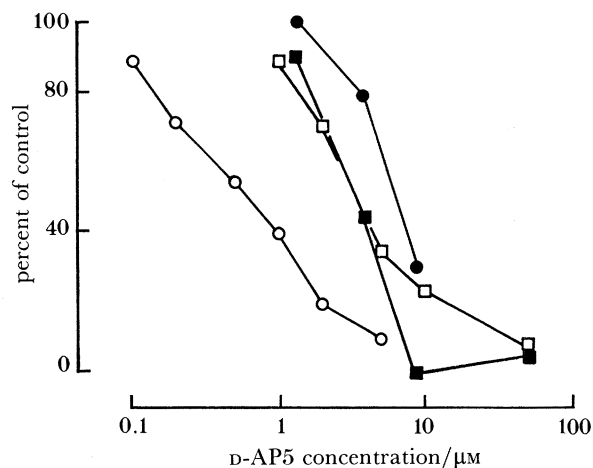


Figure 8. Comparison of spatial learning and LTP *in vivo* with *in vitro* binding and LTP data from published studies. Spatial learning was calculated as the normalized savings between the overall mean escape latency during the 30 spatial training trials and the latency on the last day of pretraining (PT3 in figure 5); LTP *in vivo* was calculated as the percentage of that shown by the control group; (●), spatial learning; (■), LTP *in vivo*; (□), LTP *in vitro* (Harris *et al.* 1984); (○), [³H] D-AP5 binding (Monaghan *et al.* 1988).

20 mM EGTA to the infusion medium of the microdialysis probes and found a dramatic, eightfold increase in AP5 concentration in the perfusate lasting at least 40 min (figure 7c). This finding suggests that a significant proportion of the whole-tissue AP5 is 'trapped' in extracellular space by a Ca²⁺-dependent mechanism.

In summary, parallel dose–response functions were obtained for both spatial learning and LTP. Importantly, we could find no intracerebral concentration of AP5 capable of blocking LTP completely but that was without effect on learning. At sacrifice, most of the infused AP5 had escaped the brain but, of that left, over 96% was 'trapped' and inaccessible to NMDA receptors. Nevertheless, direct measurement of the residual extracellular concentration of AP5 indicated

† We appreciate that the method used to convert dialysate into extracellular concentration (*in vitro* bath recovery) does not precisely reflect *in vivo* recovery (Benveniste 1989). However, the calibration procedure provides the closest approximation to *in vivo* conditions available without resorting to intricate mathematical modelling.

that the concentration sufficient to block LTP *in vivo* and severely impair learning corresponded to data obtained from *in vitro* studies of NMDA receptor function. These findings provide striking support for the hypothesis that learning dependent upon normal hippocampal function involves activation of hippocampal NMDA receptors.

3.5. Storage or retrieval?

If blocking NMDA receptors impairs spatial learning, is this due to an encoding deficit, faster forgetting, impaired retrieval or to interruption of some other psychological process involved in memory? We have begun the task of dissecting which subset of memory processes is impaired with an experiment specifically comparing storage with retrieval.

A clue to the nature of the impairment is provided by the neuropharmacological studies summarized in §2.4 indicating that activation of NMDA receptors is required for the induction of LTP but not its expression or maintenance, and that the increase in the EPSP may consist exclusively of a component sensitive to the non-NMDA antagonists CNQX and DNQX (Collingridge *et al.* 1983; Kauer *et al.* 1988; Muller *et al.* 1988; cf. Tsien 1990). In a behavioural parallel to these findings, the following experiment indicates that AP5 has no effect upon the retrieval of previously learned information at a dose that, in the same animals, impairs new learning.

The effect of a drug upon retrieval is usually investigated by training animals in its absence but later testing them in its presence. This design suffers from the weakness that the lack of an effect upon retrieval is revealed solely as a null result. Accordingly, we extended the usual design by including other groups required, at the time we introduced the drug, to learn a reversal of the task trained initially. This extended

design permits a more rigorous examination of dissociable sub-processes of memory by providing both a second index of retrieval and confirmation that the drug is behaviourally effective in impairing learning.

Rats were given *initial training* in the water-maze as normal animals with the hidden platform in either the NE (half the animals) or SW quadrant of the water-maze (26 trials, last four days at 1 trial per day). The good performance shown over the final four trials indicated that information about the platform's location was well represented and easily retrieved from long-term memory. The animals were then divided into four groups for surgery (implantation of minipumps). Two groups received aCSF, whereas two others received D-AP5 at a minipump concentration of 30 mM, this being the concentration calculated, from the previous study, as just sufficient to block LTP completely. Beginning three days later, retraining in the presence of the drug was conducted at 1 trial per day. Two groups (AP5-same and aCSF-same) were retrained with the platform in the same place it had occupied during initial training; groups AP5-different and aCSF-different had the platform moved to the opposite location (i.e. SW instead of NE and vice versa). At the end of behavioural training, the rats were sacrificed, their brains removed and samples from various brain regions dissected for subsequent analysis of AP5 whole tissue content using HPLC.

During initial training, all rats learned to approach the escape platform with short escape latencies and relatively direct paths. On the crucial first trial following surgery (i.e. before any effects of retraining), rats in groups different swam persistently in the former location of the platform (figure 9). Consequently, their mean escape latency was very high (82 ± 10 s) with some animals not finding the platform in its new location within the maximum permitted swim time of 120 s. Importantly, on this first trial, the AP5-different

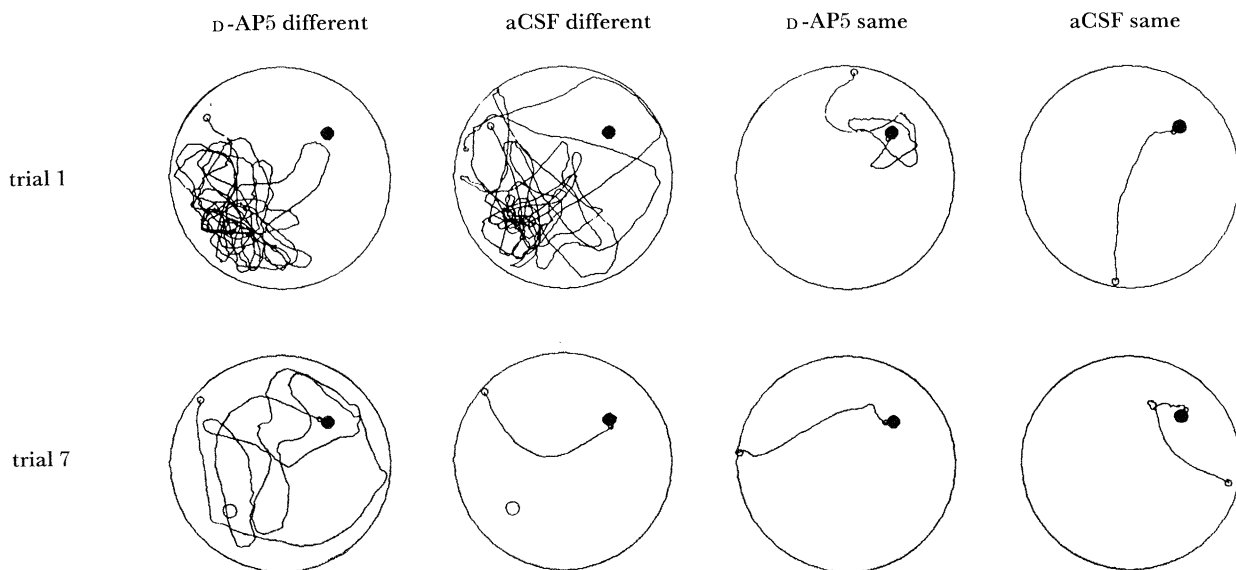


Figure 9. Storage against retrieval study. Paths taken by representative animals on the 1st and 7th trials of the drug-phase. The two 'different groups' rats were originally trained to find the hidden platform in SW. Both show good memory of this former location on trial 1 but only the 'aCSF-different' rat learns to approach the new platform location accurately over the course of reversal training. The two rats of the 'same groups' both approach the originally learned platform location without difficulty.

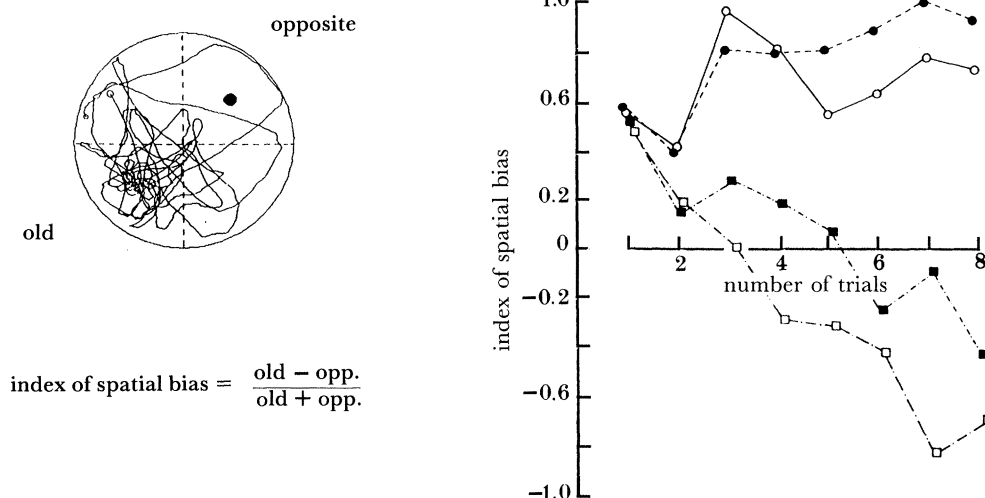


Figure 10. Index of spatial bias. All groups begin with an equivalent bias to the originally trained platform location. The index moves gradually negative for 'groups different' as reversal proceeds with 'group aCSF-different' learning significantly faster; (■), D-AP5 different; (□), aCSF different; (●), D-AP5 same; (○), aCSF same.

and aCSF-different rats did not differ. The AP5-same and aCSF-same groups did not differ on this trial either, these rats swimming directly to the platform's original training location. However they escaped relatively rapidly (latency = 15 ± 5 s) because the platform was still in the same place. To obtain a measure of performance that would be independent of escape latency, an 'index of spatial bias' was calculated on the basis of time spent in two areas of the pool. This index measures the tendency of animals to revisit the old training quadrant versus their tendency to visit the opposite one (i.e. the new quadrant for groups different) and was calculated according to the formula:

$$\text{index of spatial bias} = \frac{\text{old} - \text{opposite}}{\text{old} + \text{opposite}}$$

This index can vary from +1.0 (all time spent in training quadrant) to -1.0 (all time in opposite quadrant) and should be relatively insensitive to escape latency. Figure 10 shows the results of this analysis. Note that the two aCSF groups showed an index of +0.55 on trial 1, irrespective of their widely differing escape latencies. The AP5 groups also showed an equivalent spatial bias. It follows that AP5 cannot be impairing retrieval.

Retraining continued over eight trials and, by the end of this phase, groups AP5-same, aCSF-same and aCSF-different were all escaping via relatively direct paths with short escape latencies (figure 9). However, rats in group AP5-different failed to learn the new platform location well and continued to take circuitous routes. Their poor performance was also reflected in a significantly slower decline and reversal of the index of spatial bias than that of group aCSF-different.

The analysis of hippocampal AP5 content revealed an overall mean whole tissue content of $0.28 \text{ nmol mg}^{-1}$ wet mass, which did not differ between the two AP5 groups. Although we did not examine LTP *in vivo*, the drug infusion protocol was identical to that used in the dose-response study described above. Accordingly,

we can extrapolate from the regression function relating whole-tissue content and LTP (figure 6) to ascertain that, at this concentration, LTP *in vivo* would have been just completely blocked. The intracerebral AP5 concentration was not so high as to cause gross sensorimotor disturbances and, as it happens, none were observed.

Taken together, these data imply that at an intracerebral concentration sufficient to block LTP *in vivo*, AP5 impairs storage but not retrieval. Quite apart from the parallel to the neuropharmacological findings referred to above, this result has implications for one of the alternative accounts of this series of experiments: the possibility of a drug-induced disturbance of sensorimotor control. We have earlier ruled out the possibility that AP5 causes a gross sensory disturbance on the basis of the successful learning of visual discrimination tasks by drug treated rats, but we noted that this finding left open the possibility of a more subtle disturbance affecting sight of distal cues. However, the present findings (that AP5 treated rats were equally spatially biased as aCSF rats on the first trial) suggest that distal cues can be seen equally well by AP5-treated rats.

4. CONCLUSIONS

The discovery of the NMDA receptor and its role in one type of activity-dependent synaptic plasticity is one of the most important recent developments in neuroscience. Its property of being both ligand- and voltage-gated explains several of the physiological properties of hippocampal LTP, notably the associativity requirement of induction and that its expression is synapse-specific. Both properties are clearly relevant to the possible role of NMDA receptors in learning. The central finding of the experiments described above is that blocking this receptor pharmacologically also causes an anterograde learning impairment for at least one of the several types of learning known to depend

on the integrity of the hippocampus. It is worth emphasizing that this result was in no way a foregone conclusion; part of its force is that NMDA antagonists might have had no effect on hippocampal-dependent learning despite causing a complete blockade of LTP.

This main finding has several theoretical and clinical implications. However, before turning to these, a word of caution is appropriate. The history of research on the physical basis of memory is littered with false leads (e.g. work on RNA-synthesis and 'memory molecules', see Byrne *et al.* (1966)) and, in the particular case of systemic pharmacological work, inappropriate inferences drawn from poorly controlled experiments (e.g. work on the 'cholinergic hypothesis' of memory (see Collerton 1986; Hagan & Morris 1988)). Conscious of this background, we make no apology for our emphasis on the theoretically uninteresting but experimentally crucial need for control experiments at every step of the analysis. These include explicit attempts to dissociate primary and secondary effects of drug action, checking that AP5 does actually block LTP *in vivo* in the same animals as those subject to behavioural training, and precise measurement of interstitial drug levels *in vivo*. Such strictures offer no guarantee of avoiding inappropriate inferences, but we hope they have helped us from being led astray. More specifically, the present paper refers to experiments whose results indicate that (i) one of the sensorimotor side-effects of AP5 can be statistically dissociated from the drug-induced impairment of spatial learning; (ii) AP5-treated rats can see proximal and distal cues sufficiently well to learn a visual discrimination task at normal rate and to retrieve spatial information as well as untreated rats; and (iii) the drug-induced impairment of spatial learning occurs across an equivalent range of interstitial drug concentrations in hippocampus as that responsible for blocking hippocampal LTP *in vitro*. However, many more control experiments are required, particularly studies exploring the other effects of NMDA antagonists within hippocampus in addition to their blockade of LTP.

Leaving these problems aside, what are the theoretical implications of the present results? First, they raise the possibility that at least some of the neural mechanisms of LTP are activated during certain kinds of learning and play a causal role in information processing or storage or both. If this is true, under what circumstances does NMDA-receptor activation normally occur? Secondly, why does the hippocampus possess at least two different kinds of long-lasting synaptic plasticity and how do these interact? Thirdly, can these findings contribute information useful for the development of formal 'neural-network' models of hippocampal function?

With respect to the first issue, it is important to appreciate that NMDA receptors are not 'learning receptors'. Their function cannot be adequately described in psychological terms, the appropriate level of discourse being pharmacological. NMDA receptors are a molecular device for detecting the conjunction of presynaptic activity and post-synaptic depolarization, and for signalling this detection by means of a different ionic signal (namely Ca^{2+}) to that used ordinarily to

mediate fast EPSPs (Na^+). Although this pharmacological property is potentially relevant to the psychological processes of associative learning, such as the detection of contiguity, it is important to appreciate that 'detection' is not the same as 'representation'. Thus although activation of NMDA receptors may help us understand aspects of associative learning (e.g. how the nervous system detects that one stimulus occurs in association with another), a fuller understanding will depend critically upon how stimulus information is represented neurally and into what circuitry these receptors are embedded (see McNaughton (1989)). In other circuits, or at different stages of development, NMDA receptors perform a myriad of different functional roles through this same pharmacological property. Thus activation of NMDA receptors during development seems to play a role in experience-dependent self-organization (Kleinschmidt *et al.* 1987; Cline *et al.* 1987; Bear *et al.* 1987; Singer 1990; Horn, this symposium), whereas, in the adult spinal cord, their activation is involved in the integration of supra-segmental reflexes (Davies & Watkins 1983). In *Xenopus* embryos, activation of NMDA receptors provides a convenient way of turning on a Ca^{2+} -dependent K^+ current, which repolarizes neurons and thus stabilizes the rhythmic activation characteristic of swimming (see Dale (1989)).

With respect to studies of learning, blockade of NMDA receptors seems to cause a behaviourally selective impairment. In addition to effects on spatial learning, competitive NMDA antagonists cause impairments of DRL schedules and odour-potentiated taste-aversion (Tonkis *et al.* 1988; Crooks *et al.* 1989 (chronic infusion)), passive avoidance and delayed conditional discrimination (Danysz & Wroblewski, 1989; Tan *et al.* 1989 (i.p. injection)) and fear-potentiated startle (Miserindino *et al.* 1990 (intra-amygdala infusion)). They cause no significant impairment of visual or olfactory discrimination learning at short ITIs (although there may be an impairment at long ITIs (Staubli *et al.* 1989 (chronic infusion))), nor, when injected intracerebrally post-trial, do they significantly impair step-through avoidance learning (Mondadori *et al.* 1989). In one case, analysis of the impairment reveals that it may be secondary to a deficit in stimulus discriminability (Tan *et al.* 1989) whereas in another case, relevant control experiments show no change in the processing of conditional or unconditional stimuli (Miserindino *et al.* 1989). There are also reports of correlations between the number of NMDA receptors and certain types of learning (McCabe & Horn 1988; Wenk *et al.* 1989). In our view, it is still too early to draw any clear theoretical picture concerning the role of NMDA receptor activation in information processing or storage (or both) from the profile of results obtained so far.

However, if hippocampal NMDA receptors are activated during a subset of hippocampal-dependent types of learning, their activation may, at least partially, be modulated by hippocampal rhythmic slow activity (RSA or 'theta'). The hippocampal theta rhythm is a prominent pattern of 7–10 Hz electroencephalographic activity that, in rodents and other

species, accompanies stimulus processing and certain types of movement (Robinson 1980). Many previous efforts to understand its psychological function have been of limited success but the discovery of the voltage dependence of the NMDA receptor iontophore suggests a new hypothesis. Specifically, we propose that the rhythmical fluctuations of voltage characteristic of hippocampal theta play a role in regulating the probability that afferent input to the hippocampus from layers II and III of entorhinal cortex (Witter *et al.* 1989) can trigger NMDA receptor channel opening. Neural activity arriving on the crest of the theta rhythm would have a higher probability of activating the channel than input timed to arrive on the trough of the theta wave. Pavlides *et al.* (1988) have recently reported results confirming this prediction, and also indicating that activity on the trough of the theta wave may cause a depression of synaptic efficacy (see Stanton & Sejnowski (1989)). Larson & Lynch's (1986) earlier work on 'primed-burst' potentiation showed that very short bursts of activity at the theta frequency can induce LTP, whereas Abraham & Kairiss (1988) have found that NMDA antagonists 'truncate' the complex-spike (burst) firing characteristic of certain hippocampal neurons. Further, our proposal implies that, in rodents, information is presented to the hippocampal matrix no faster than at intervals of ≈ 150 ms. This timing would be commensurate with the need to allow sufficient time (i) for separate neural inputs to disparate parts of a cell's dendritic tree to be 'associate' via the relatively slow NMDA-dependent Ca^{2+} current and (ii) for subsequent cellular repolarization.

With respect to the important third and fourth issues, we can do little more than stress the need for neuropharmacological and behavioural experiments addressing the mechanisms and functional significance of the non-associative, AP5-independent form of hippocampal LTP shown at mossy-fibre terminals onto CA3 pyramidal cell dendrites (Harris & Cotman 1986; Nicoll *et al.* 1988). Lesion studies indicate that the retention of reference- and working-memory in the radial maze survives neurotoxin induced damage to CA3 neurons (Jarrard 1986). However, this leaves open the possibility that CA3 plays an important role in acquisition. Further, with respect to relatively short-term memory, Frederickson *et al.* (1990) have shown that chelation of mossy-fibre bouton zinc by DEDTC does impair a delayed matching-to-place (working-memory) procedure in the water-maze (the zinc content of mossy fibres is the highest in the rodent forebrain and there is evidence that it is co-released with the endogenous transmitter at these synapses). A critical theoretical issue is why the hippocampal network possesses two distinct forms of synaptic plasticity, with apparently different synaptic learning rules, and why these occur at the terminals of afferents having radically different connection probabilities onto a common set of principal neurons. Rolls' (1989) proposal that such an arrangement would be useful for constructing 'episodic' memories deserves to be followed up with formal analysis. However, until more is known about the psychological function of area CA3, indeed of the hippocampus as a whole, the task of

building 'realistic' neural-network models is probably overambitious. Some progress can, however, be made by examining 'simplifying models' (Willshaw & Buckingham, this symposium).

Finally, there is great interest in the clinical potential of NMDA antagonists as anticonvulsants and for their capacity to limit brain damage in stroke (Meldrum 1985; Iversen *et al.* 1989). That these drugs might also cause memory impairments in humans need not limit their usefulness when administered acutely (e.g. as soon as possible after a stroke), but may be more problematic if they had to be taken regularly (e.g. as anticonvulsants). At the very least, such a possibility points to the need for neuropsychological testing of cognitive function to accompany trials of these compounds' clinical effectiveness.

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