STUDIES ON SYNAPTIC POTENTIATION IN THE HIPPOCAMPUS

By

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The present investigation was conducted on transversely sectioned rat hippocampal slices to examine the mechanisms involved in synaptic potentiation. Results indicate that long-term potentiation (LTP) induced by input tetanization requires extracellular Ca\(^{++}\), because during the induction of LTP postsynaptic depolarization must accompany presynaptic activity (LTP could be induced by raised K\(^+\) [10 to 80 mM] in Ca\(^{++}\)-free medium). Since LTP (induced by raised K\(^+\)) occurs in the absence of Ca\(^{++}\) and, therefore, presumably in the near absence of transmitter release, N-methyl-D-aspartate (NMDA) receptor activation is not obligatory. Moreover, NMDA receptors appear not to be involved in the CA\(_3\) area. A necessity for both pre- and postsynaptic depolarization also accounts for the need for co-stimulation of afferents for LTP induction. Associative potentiation was found not to require tetanic stimulation of the test input; single pulse activation of the test input (at 0.2 Hz) paired with tetanic trains to a conditioning input (presumably to the same postsynaptic neurones) could produce LTP. A short-term potentiation (STP), which resembled post-tetanic potentiation (PTP) in time course, could be induced in an associative fashion by conditioning tetanic trains (paired with single test stimuli), that were insufficient to produce LTP. In the absence of conditioning stimuli, interruption of a regular 0.2 Hz test input stimulation for 10 minutes disclosed a subsequent potentiation. This potentiation could be distinguished from associative potentiation in that it was not associated with a decrease in
A decrease in presynaptic terminal excitability was characteristic of associative STP and LTP, and followed similar time courses. Since raised K\(^+\) reversed rather than accentuated the decreased excitability, it was concluded that it is not due to Na\(^+\)-inactivation and may be caused by a hyperpolarization which might also lead to an increase in evoked transmitter release. The hypothesis of Baudry and Lynch (1980a) that LTP is due to an increase in glutamate receptors seems unlikely; there was no increase in Na\(^+\)-independent glutamate binding sites (determined by the same method as used by Lynch et al. [1982]) in association with LTP induced by a single brief 400 Hz (200 pulses) input tetanus. A decrease in the uptake of glutamate occurs with tetanic stimulation under conditions where there is no LTP (absence of Ca\(^{++}\) and raised Mg\(^{++}\) and Mn\(^{++}\)) and, therefore, does not appear to be a mechanism producing LTP.

Bhagavatula R. Sastry
(Supervisor)
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<td>ChAT</td>
<td>choline acetyltransferase</td>
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<td>DLH</td>
<td>DL-homocysteate</td>
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<tr>
<td>EDTA</td>
<td>ethylenediamine tetraacetic acid</td>
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<td>EPSP</td>
<td>excitatory postsynaptic potential</td>
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<tr>
<td>GABA</td>
<td>(\gamma)-aminobutyric acid</td>
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<td>GAD</td>
<td>glutamic acid decarboxylase</td>
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<td>5-HT</td>
<td>5-hydroxytryptamine</td>
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<td>IPSP</td>
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<td>long-term potentiation</td>
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<td>N-methyl-D-aspartate</td>
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<td>PTP</td>
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<td>STP</td>
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1 INTRODUCTION

It is well known that long-term potentiation (LTP) of the stratum radiatum stimulation-evoked \( \text{CA}_1 \) population spike can be produced following a high frequency tetanic stimulation to the input fibres (Schwartzkroin and Wester, 1975). The mechanisms responsible for LTP, however, are not entirely clear and there is debate as to whether the phenomenon is due to a pre- or postsynaptic alteration. The present investigation was generally aimed at determining mechanism(s) of LTP and, in view of evidence that LTP is "associative", i.e., cannot be induced by tetanic stimulation of a "weak" input alone (McNaughton, 1982; McNaughton et al., 1978; Robinson and Racine, 1982) was specifically aimed at answering the following questions: 1) is Ca\(^{++}\) directly required for LTP induction?; 2) is tetanic stimulation of a test input really necessary for the induction of LTP?; 3) is LTP sustained by a presynaptic change?; 4) what is the mechanism of the reduced excitability in presynaptic terminal regions that accompanies LTP?; 5) does an increase in glutamate receptors correlate with LTP?; 6) can a decrease in glutamate uptake account for LTP?; 7) are NMDA receptors involved in production of LTP?

It is generally believed that the induction of LTP requires the presence of extracellular Ca\(^{++}\) (Dunwiddie and Lynch, 1979; Wigström et al., 1979); tetanic stimulation of an input during perfusion of hippocampal slices with Ca\(^{++}\)-free medium failed to produce LTP. However, this treatment also succeeded in blocking synaptic transmission. Therefore, it is unclear whether LTP induction was counteracted due to a removal of Ca\(^{++}\) or due to an abolition of synaptic transmission. Studies were, therefore, con-
ducted to examine whether LTP could be induced in Ca\(^{++}\)-free medium if both the presynaptic terminals as well as postsynaptic neurones were depolarized using elevated extracellular K\(^{+}\) concentrations.

That the induction of LTP requires the co-activation of several input fibres is shown by the finding that "associative" LTP can be induced in response to a "weak" test input (which does not exhibit LTP when tetanized), by concurrent tetanization of a presumably separate "strong" input to the recorded neurones (McNaughton, 1982; McNaughton et al., 1978; Robinson and Racine, 1982). This observation led the above authors to think that the common link between the two separate inputs is the postsynaptic neurone and, therefore, that LTP is postsynaptic. However, there is no reason to assume that presynaptic fibres do not communicate with each other. Studies were, therefore, conducted to examine this possibility and it was indeed found that presynaptic fibres in the CA\(_{1}\) region interact with each other, possibly via the postsynaptic neurone. The results prompted further studies to determine if the presynaptic excitability change in Schaffer collateral terminals associated with LTP was induced by associative interactions. Furthermore, experiments were also conducted to examine whether a potentiation of the test EPSP could be elicited by associative conditioning without delivering a tetanic stimulation to the test input. The temporal constraints governing the position of the test and conditioning stimuli for induction of associative potentiation were also investigated.

Since it has been established that a high frequency activation of an input results in PTP and LTP, it was of interest to examine the effects of non-activation of the input. These experiments were conducted by interrupt-
ing the control rate of input stimulation of 0.2 Hz with a "rest" period of 10 minutes. The EPSP as well as the Schaffer collateral terminal excitability were monitored before and after the quiescent period.

A previous report (Sastry, 1982) suggested that a decrease in excitability (reflecting a hyperpolarization) of presynaptic terminals could be a mechanism responsible for LTP. An attempt was, therefore, made to correlate the time course and magnitude of the decrease in presynaptic terminal excitability with the potentiation of the EPSP. The nature of the decrease in Schaffer collateral terminal excitability is not known and several possibilities exist. Experiments were conducted to ascertain if the excitability change could be due to Na\(^+\)-inactivation or hyperpolarization.

N-Methyl-D-aspartate (NMDA) receptors are thought to play a role in the induction of LTP (Collingridge et al., 1983b; Harris et al., 1984). The effects of exogenously applied N-methyl-DL-aspartate (NMDLA) were examined on the CA\(_1\) population spike and Schaffer collateral terminal excitability. Studies were also carried out to ascertain if the terminal excitability change could be induced by localized application (iontophoretically) of NMDLA in the CA\(_1\) apical dendritic zone where these fibres terminate. The reason for localizing the application was to exclude the possibility that an increase in the firing rate of CA\(_3\) neurones during bath application of the amino acid was the reason for producing the presynaptic change. It has been reported that 2-amino-5-phosphonovalerate (APV), a "selective NMDA antagonist" counteracts the induction of LTP (Collingridge et al., 1983b; Harris et al., 1984). To further investigate a presynaptic role in NMDLA-induced potentiation of the population spike, the ability of APV to antagonize the
effects of NMDLA on the CA\textsubscript{1} population spike and Schaffer collateral terminal excitability was examined. All previous studies concerning the NMDA hypothesis were conducted in the CA\textsubscript{1} region of the hippocampus (Collingridge et al., 1983b; Harris et al., 1984). It was, therefore, decided to determine if tetanus-induced LTP of the mossy fibre-CA\textsubscript{3} system involved NMDA receptors by applying APV during the tetanic stimulation. The effects of exogenously applied NMDLA were also examined on the CA\textsubscript{3} population spike.

Baudry and Lynch (1980a) hypothesized that LTP could be accounted for by a postsynaptic mechanism whereby LTP of the synaptically-evoked population response is a consequence of a Ca\textsuperscript{++}-triggered increase in the number of subsynaptic neurotransmitter receptors. The suspected transmitter released by the Schaffer collaterals (which comprise part of the stratum radiatum) is glutamate (Storm-Mathisen, 1977a). It was shown by Wieraszko (1983) that stimulus-evoked uptake of D-aspartate, a marker for glutamatergic terminals, is decreased during LTP. This raises the interesting possibility that a decrease in transmitter uptake could result in a potentiation of the synaptically-evoked response due to a greater availability of the amino acid to subsynaptic receptors. Studies were conducted to 1) examine if an increase in Na\textsuperscript{+}-independent glutamate binding sites (presumed to be receptors by Baudry and Lynch [1980a]) is necessary for the visualization of LTP; 2) investigate the possibility that a decrease in glutamate uptake can be a mechanism for the maintenance of LTP.
2 LITERATURE SURVEY

2.1 Anatomy of the hippocampal formation

The hippocampal formation is a structure that comprises part of the limbic system. It is made up of the hippocampus proper (also called Ammon's horn), the dentate gyrus and most of the subiculum. The highly organized lamellar organization of the hippocampal formation has made this cortical structure a desirable area to perform neurophysiological studies upon. The cell body layer of the hippocampus, if divided into the CA$_1$($a,b,c$), CA$_2$, CA$_3$($a,b,c$) and CA$_4$ regions and the dentate gyrus, is made up of an inferior and superior blade containing its cell body layers (Lorente de Nó, 1934) (Figure 1). These two structures, which are tightly interlocked have the CA$_4$ area of the hippocampus penetrating the hilus (which is the triangular shaped region between the inferior and superior blades) of the dentate gyrus. The predominant cell type in the hippocampus is the pyramidal cell (Blackstad, 1956; Golgi, 1886; Lorente de Nó, 1934), whereas granule cells are found in the dentate gyrus (Golgi, 1886; Lorente de Nó, 1934). A limited number of inhibitory interneurones (basket cells) can be found interspersed in both the pyramidal as well as granule cell layers (Ramón y Cajal, 1968). In a dorsal to ventral order, the layers of the hippocampus-dentate gyrus system are stratum oriens, stratum pyramidale, stratum radiatum, stratum lacunosum, stratum moleculare, stratum granulosum and stratum polymorphpe (Ramón y Cajal, 1911) (Figure 1).
Anatomical illustration of a transverse section of the hippocampal formation. The hippocampal formation consists of the hippocampus (also called Ammon's horn or Cornu Ammonis) which is a C-shaped structure that folds tightly into another C-shaped structure, the dentate gyrus (also called Fascia dentata). The hippocampus is divided into four subfields: CA1, CA2, CA3 and CA4.

Enlargement on right shows layered structure of the hippocampal formation.

PROS – prosubiculum
HF – hippocampal fissure
Sch – Schaffer collateral
Comm – commissural input
MF – mossy fibre
PP – perforant path input
B – basket cell

▲ represent pyramidal cells of the hippocampus
● represent granule cells of the dentate gyrus
2.2 Major afferent supply to the hippocampal formation

2.2.1 Perforant path. Anatomical evidence indicates that the major input to the dentate granule cells arises from the entorhinal cortex and is known as the perforant path (Ramón y Cajal, 1911; Lorente de Nó, 1934). Electrophysiological evidence provided by Lømo (1971) shows large field potentials recorded in the dentate granular cell layer by stimulation in the entorhinal area. The extracellularly recorded excitatory postsynaptic potential (EPSP) is maximal in size and is characterized by a negative wave in stratum moleculare (Andersen et al., 1966b; Lømo, 1971), an observation which is consistent with the anatomical localization of perforant path synapses (Blackstad, 1958; Nafstad, 1967; Hjorth-Simonsen and Jeune, 1972). Although the perforant path innervates primarily the dentate gyrus, it has also been shown with histological techniques that some fibres from this input terminate in the prosubiculum, CA1, CA2, and CA3 areas (Lorente de Nó, 1934).

2.2.2 Alvear path. Another input that arises from the entorhinal area to innervate the hippocampus is the alvear path which courses along the dorsal surface of the hippocampus to terminate in the prosubiculum and CA1a regions (Lorente de Nó, 1934).

2.2.3 Commissural input. The CA3 and CA4 pyramidal cells give rise to axons which form the commissural fibres that course through the ventral commissure (with a few that run through the dorsal commissure) to terminate in the contralateral hippocampus (Andersen, 1960a, 1960b; Blackstad, 1956; Deadwyler et al., 1975; Gottlieb and Cowan, 1973). Anatomical studies have demonstrated that the commissural input, which enters the
hippocampus via the fimbria, innervates all fields of the hippocampus and
the dentate gyrus (Gottlieb and Cowan, 1973; Hjorth-Simonsen and Laurberg,
1977). In support of these histological findings, evidence from electrophys­
iological analysis has shown that stimulation of commissural fibres pro­
duces synaptic excitation of dentate granule cells (Cragg and Hamlyn, 1957)
and the apical as well as basilar dendrites of CA₁ pyramidal neurones
(Andersen, 1960a, 1960b). Degeneration studies conducted by Blackstad
(1958) illustrate a greater number of commissural terminations in the apical
dendrites as compared to the basal dendrites of CA₁ neurones, an observa­
tion that is supported by electrophysiological evidence (Cragg and Hamlyn,
1957).

2.2.4 Septal input. The septo-hippocampal fibres course through the
dorsal as well as the body of the fornix to enter the alveus. This input
terminates primarily in the hilus of the dentate gyrus and stratum oriens
and stratum radiatum of the CA₂ and CA₃ regions. A sparse population of
septo-hippocampal fibres enters the molecular layer of the dentate gyrus,
perhaps with the perforant path (Mellgren and Srebro, 1973). The hippocam­
pal formation receives afferents that originate from the medial as well as
lateral septal areas, the former input terminating in the dorsal hippocampus
while fibres from the latter project to the ventral hippocampus (Siegel and
Tassoni, 1971). It is believed that septo-hippocampal fibres are important
in normal hippocampal functions, especially in the generation of slow wave
or theta electrical activity (Green and Arduini, 1954).

2.2.5 Inputs from the brainstem. Efferents from the brainstem nuclei
such as the locus coeruleus, raphe nuclei and ventral tegmental nuclei form
monosynaptic connections with different regions of the hippocampal forma-
tion. It appears that the locus coeruleus projects to the subiculum, the
CA$_1$ area (Pasquier and Reinoso-Suarez, 1978) and the dentate gyrus
(Blackstad et al., 1967; Lindvall and Björklund, 1974; Loy et al., 1980;
Storm-Mathisen, 1978). Fibres from the raphe nuclei innervate the dentate
gyrus, the subiculum and parts of the hippocampus (Azmitia and Segal, 1978;
Azmitia and Marovitz, 1980; Fuxe and Jonsson, 1974; Köhler and Steinbusch,
1982; Segal, 1980; Steinbusch, 1981) while a projection from the ventral
tegmental area, which is thought to be dopaminergic, terminates primarily in
the dentate area (Simon et al., 1979). Theta activity in the hippocampus is
thought to be influenced by inputs from the locus coeruleus and raphe nuclei
to the septal area and hippocampus (Gray, 1977).

2.2.6 Inputs from the diencephalon. A direct input from the thalamus
innervates the subiculum and CA$_1$ regions of the hippocampus. Fibres
emerge from anterior, posterior and midline nuclei of the thalamus to termi-
nate in the subiculum and CA$_1$ area (Schwerdtfeger, 1984). In humans,
another input from the lateral geniculate nucleus also projects directly to
the hippocampus (Babb et al., 1980).

The hypothalamus has two major inputs to the hippocampal formation,
one project to the subiculum and the other to the dentate gyrus
(Schwerdtfeger, 1984).

2.3 Internal circuitry of the hippocampal formation

Dentate granule cells give rise to short axons called the mossy fibres
which travel in the hilar region to terminate on the dendrites of pyramidal
cells in the CA$_2$, CA$_3$ and CA$_4$ regions of the hippocampus (Blackstad et-
al., 1970; Lorente de Nó, 1934; Ramón y Cajal, 1968) (Figure 1). The mossy fibre bundle that arises from the inferior blade of dentate granule cells terminate a short distance away at the CA$_3$ and CA$_4$ regions of Lorente de Nó (1934). The mossy fibres that arise from the superior blade, however, traverse through CA$_3$ and end in the CA$_2$ region (Blackstad et al., 1970; Lorente de Nó, 1934). According to Hamlyn (1961), the whole field of CA$_3$ is innervated by the mossy fibres which course through and activate the apical dendrites of the pyramidal neurones. These synapses are en-passant so that a single presynaptic volley will normally produce excitation of a number of CA$_3$ neurones (Blackstad and Kjaerheim, 1961). It has been shown both histologically (Hjorth-Simonsen and Jeune, 1972) and physiologically (Lømo, 1971) that mossy fibres exhibit a highly organized parallel lamellar arrangement.

Pyramidal cells of the CA$_3$ region give off axons which leave the hippocampus via the fimbria to cross over to the contralateral hippocampus as commissural fibres (described earlier). These axons branch off before leaving the hippocampus to form the Schaffer collaterals (Schaffer, 1892; Ramón y Cajal, 1911) and cross over the pyramidal cell body layer to terminate in the stratum lacunosum of the CA$_1$ and CA$_2$ areas (Lorente de Nó, 1934) (Figure 1). Electrophysiological evidence supplied by Andersen (1960a) has confirmed the anatomical data above. Powerful synaptic excitation is seen in CA$_1$ neurones upon stimulation of CA$_3$ cells or the Schaffer collateral input and this activation is represented by a large negative wave which is maximal when recorded in the apical dendrites of CA$_1$ neurones.
The axons of CA₁ cells leave the cell body from the basilar side and head dorsally for a short distance before entering the alveus to course along the dorsal surface of the hippocampus and ultimately exit through the fimbria (Lorente de Nó, 1934). Some of these axons divide to form a recurrent collateral which also travels in the alveus but in the opposite direction towards the prosubiculum (Lorente de Nó, 1934; Hjorth-Simonsen, 1973; Chronister and Zornetzer, 1973) (Figure 1).

Sequential activation of neurones in the hippocampus via a trisynaptic loop consisting of 1) perforant path-dentate granule cells; 2) mossy fibres-CA₃ pyramids; 3) Schaffer collaterals-CA₁ pyramids has been described (Andersen et al., 1971) (Figure 1). It was found that the perforant path, mossy fibres, Schaffer collaterals and alvear fibres which constitute the outputs from the entorhinal-dentate-CA₃-CA₁ areas, respectively, are all arranged in parallel bands that are oblique to the longitudinal axis of the hippocampus (Andersen et al., 1971; Lømo, 1971). Therefore, one can expect a transversely sectioned slice of the hippocampal formation to contain an intact trisynaptic loop. This, in fact, has been established electrophysiologically by Skrede and Westgaard (1971) who demonstrated that in transverse slices of 300 to 400 μm thick, all the synaptic inputs described were intact and functional.

2.4 Inhibitory influences in the hippocampal formation

The presence of inhibitory interneurones called basket cells in the hippocampus and the dentate gyrus has been well established (Andersen et al., 1964a, 1964b; Ramón y Cajal, 1911; Spencer and Kandel, 1961c). These cells are thought to mediate recurrent inhibition and, therefore, 1) the
inhibitory postsynaptic potential (IPSP) elicited following afferent excitation has a longer latency than the excitatory postsynaptic potential (EPSP) and 2) antidromic stimulation of CA₃ axons produced either large IPSPs or antidromic invasion of CA₃ neurones followed by an IPSP (Andersen et al., 1964a, 1964b; Spencer and Kandel, 1961). Andersen et al. (1964a) observed that the IPSP was maximum at the cell body layer and was characterized by an extracellular positivity. This suggests that the inhibition is mediated by a hyperpolarization that is generated at or close to the cell body. Since the latency to onset of the IPSP is typically longer than that of the EPSP (when synaptic inputs are stimulated) and also than that of antidromic invasion (when axons of pyramidal cells are stimulated antidromically), it has been postulated that the inhibition is produced by an interneurone which terminates on the cell bodies of pyramidal cells (Andersen et al., 1964a, 1964b). Basket cells exhibit repetitive firing. The latency to onset of the IPSP decreased and the frequency as well as number of discharges of the interneurones (observed as a high frequency ripple on the rising phase of the response) increased with increasing afferent stimulation intensity (Andersen et al., 1964a).

It was suggested that the inhibitory transmitter of the basket cells is γ-aminobutyric acid (GABA) (Andersen, 1975; Andersen et al., 1964a; Curtis et al., 1970). Using specific antisera to glutamic acid decarboxylase (GAD), the enzyme for catalyzing the formation of GABA, it was shown that dense GAD-positive staining was present at the cell bodies of pyramidal and granule cells, corresponding to the location of basket cell terminations (Ribak et al., 1978). The IPSP which is reduced by GABA antagonists (Alger
and Nicoll, 1982; Nicoll and Alger, 1981) is mediated by an increase in Cl$^-$ conductance of the cell (Kandel et al., 1961).

In addition to the classical recurrent inhibition described above, there appears to be feed-forward inhibition in the hippocampus. Andersen (1975) observed that some basket cells discharge with very weak input volleys. In fact, interneurones can be activated with significantly lower stimulus intensities than postsynaptic pyramidal cells and some interneurons may discharge in response to stimulations that are subthreshold to evoke a synaptic response in the pyramidal cells (Buzsáki and Eidelberg, 1981; Buzsáki et al., 1983; Fox and Ranck, 1981; Knowles and Schwartzkroin, 1981). At higher stimulus strengths, it has been shown that interneuronal discharge may precede the onset of the synaptically-induced population spike (Buzsáki and Eidelberg, 1982). It is not known whether feed-forward inhibition, like recurrent inhibition, is mediated by basket cells. Knowles and Schwartzkroin (1981) provide evidence for interneurones which are morphologically dissimilar to the classical basket cells to mediate both recurrent and feed-forward inhibition in the hippocampus.

In addition to the recurrent and feed-forward IPSPs discussed, a long-lasting late hyperpolarizing potential is observed in pyramidal cells following orthodromic but not antidromic stimulation, suggesting a feed-forward mode of activation (Newberry and Nicoll, 1984a). GABA application onto pyramidal cell dendrites elicits an initial depolarization followed by a hyperpolarization of the cell (Alger and Nicoll, 1979). The initial depolarizing phase has been attributed to GABA acting on the dendrites of the pyramidal neurones because it seems to involve a Cl$^-$ conductance and is
blocked by GABA antagonists (Alger and Nicoll, 1979). The late hyperpolarizing potential is believed to be due to an increase in $K^+$ conductance at the dendritic zone that is not dependent on intracellular $Ca^{++}$ and is not blocked by the classical GABA antagonists bicuculline and picrotoxin (Newberry and Nicoll, 1984a). A novel bicuculline-insensitive GABA binding site has been described (Bowery et al., 1979, 1981). To distinguish the classical GABA receptor from this new receptor, the former has been termed the GABA$_A$ receptor and the latter the GABA$_B$ receptor. The GABA$_B$ receptor is thought to be present presynaptically (Bowery et al., 1980, 1981; Olpe et al., 1982) and a selective agonist at this site is baclofen (Bowery et al., 1979, 1980, 1981). The late hyperpolarizing potential of Newberry and Nicoll (1984a) was suggested to be due to activation of dendritic GABA$_B$ receptors because an application of baclofen generates a similar type of potential (Newberry and Nicoll, 1984b). Baclofen has been shown to inhibit excitatory transmission in the hippocampus, an effect that has been attributed to a blockade of excitatory transmitter release (Olpe et al., 1982). Since axo-axonal synapses in the hippocampus have never been demonstrated, the relevance of the above results to physiological GABAergic presynaptic inhibition is not clear at present.

2.5 Interpretation of waveforms in the hippocampus

Since all recordings done in the present study were extracellular, I shall limit myself to a discussion of these responses. A unique and very useful feature of the CA$_1$ region of the hippocampus is that two distinctly separate sets of input fibres can activate the same population of pyramidal cells. Stimulation of stratum radiatum selectively activates the apical
dendrites and stimulation of stratum oriens results in excitation of the basilar dendrites (Figure 1). At a particular recording site, an extracellular negativity usually represents either a local depolarization or a distant hyperpolarization and, conversely, a positive waveform indicates a local hyperpolarization or a distant depolarization, depending on the source-sink relationship of current flow. Upon stimulation of stratum radiatum (which includes the Schaffer collaterals), an EPSP is generated at the apical dendrites of CA1 neurones. A recording electrode placed at the source of the EPSP (100–200 µm from cell body layer) will recognize a negatively directed waveform (Figure 2C) due to a mixture of fields caused by depolarization of the apical dendrites and hyperpolarization of the soma by the IPSP. On the other hand, if the recording site is the CA1 pyramidal cell body layer, the EPSP will reverse direction and manifest itself as a positive response (Figure 2B) because this region is the sink for depolarizing current generated at the dendrites and the source for a hyperpolarizing IPSP. As we move the recording electrode towards the basal dendrites, we find a positively directed EPSP with a smaller magnitude than that recorded in the cell body layer (Figure 2A). When the stimulation strength is increased to evoke a synchronous population spike (whose size is proportional to the number of postsynaptic neurones that are activated to discharge action potentials), its direction is negative in the cell body layer (Figure 2E) and basal dendrites (Figure 2D) but is positive in the apical dendrites (Figure 2F). Note that the latency to onset of the spike is shortest with the cell body recording (Figure 2E), suggesting that the site for action potential generation in CA1 neurones is at or close to the soma. Similar
Waveforms recorded in the CA1 region evoked by stimulation of input fibres in stratum radiatum. Records in A, B and C are extracellular population EPSPs recorded in the basilar dendrites, cell body layer and apical dendrites, respectively. Upon increasing the stimulus intensity, population spikes can be elicited (D, E and F). Note that the population spike is positive at the apical dendrites and negative at the cell body and basilar dendrites. Each response is an average of 4 consecutive sweeps. Records A and D, B and E and C and F were obtained from 3 different experiments. Negativity is downwards.
observations were documented by Andersen (1960a).

2.6  **Electrophysiology of hippocampal pyramidal neurones**

2.6.1  **Firing patterns and membrane properties.** Characteristics of neurones in the hippocampus have been well documented *in vivo* (Kandel and Spencer, 1961; Kandel *et al.*, 1961; Spencer and Kandel, 1961a, 1961b). The pyramidal neurone has a soma, two distinct dendritic systems (apical and basilar) and an axon that courses in the alveus. Spike generation in these cells following various modes of activation is similar to that seen in motorneurones where the rising phase of the action potential shows a small inflection. Excitatory and inhibitory postsynaptic potentials were graded and it was concluded that depolarization is related to excitation and hyperpolarization is related to inhibition. In addition, stimulation of axons antidromically produces a marked inhibition of the neurone through recurrent collaterals (Kandel *et al.*, 1961). Spontaneous activity in pyramidal cells consisted of either single spikes or bursts of action potentials. Single spikes or bursts were associated with a post-spike potential which lasted roughly 20 ms and was depolarizing. It appears that these after-potentials were additive and the magnitude corresponded to the number of spikes in a burst. However, the time constant of decay was independent of the duration of bursts. A burst is self-limiting and the membrane repolarizes even in the presence of a long depolarizing pulse. A short depolarizing current pulse, however, can trigger a burst which fails to terminate immediately upon cessation of the stimulation, perhaps because the summation of the depolarizing after potentials can sustain repetitive firing (Kandel and Spencer, 1961). The depolarizing after potential could be mediated by a
slow voltage-dependent Ca$^{++}$ current (Johnston et al., 1980). The firing level for an action potential was constant for different methods of excitation. Membrane properties determined for these neurones included time constant (9.9 ms), rheobase for intracellular stimulation (1-5 X 10$^{-10}$ A) and total neurone resistance (around 13 MΩ) (Spencer and Kandel, 1961a). In some neurones, there appeared to be what was termed a fast prepotential which was a small potential that preceded the somatic spike. It was established that this fast prepotential was spike activity generated within the neurone from which it was recorded. Since the potential was so small, it was concluded that it was generated at some site distant from the soma. Antidromic activation of the cell failed to evoke any fast prepotentials so it is unlikely that they arose from axons. It was suggested that the prepotentials represented dendritic spikes and may have some role in impulse generation (Spencer and Kandel, 1961b).

With the advent of the hippocampal slice preparation, Schwartzkroin (1975, 1977) has shown that virtually all the properties of hippocampal neurones in-vitro are similar to those found in-vivo, suggesting that cells in the slice do not become abnormal following the slicing procedure. Therefore, it is reasonable to believe that data obtained from a slice preparation is a true reflection of the hippocampal system in intact animals.

2.6.2 Synaptic activation. The excitatory synaptic contacts on pyramidal cells are located almost exclusively on dendritic spines (Andersen et al., 1966b). On the other hand, inhibitory interneurones terminate predominantly at or near the somatic area. An estimate of the size of the EPSP produced by activation of a single presynaptic fibre is 0.1 mV and the dis-
charge threshold for the pyramidal cell is about 6 mV (Andersen and Langmoen, 1981). The usual resting membrane potential of these pyramidal cells was reported to be around -60 mV (Andersen et al., 1980a). When two separate inputs to the same pyramidal cell dendrite were stimulated simultaneously, the EPSPs summated in a linear fashion. Similarly, when IPSPs were evoked, they also added algebraically to the summated EPSPs (Andersen and Langmoen, 1981). These observations suggest that roughly 60 synapses have to be activated simultaneously to synaptically drive a pyramidal neuron to discharge. Since these cells are thought to have 10,000 synaptic contacts (Hamlyn, 1963), it appears that activation of less than one percent of these synapses is necessary to evoke a spike.

2.6.3 Afterhyperpolarization following synaptic activation and repetitive firing. Burst firing in hippocampal neurones induced in a variety of ways is accompanied by a post-activation hyperpolarization of the cell which can last as long as several minutes (Alger and Nicoll, 1980; Gustafsson and Wigström, 1981; Hablitz, 1981; Hotson and Prince, 1980; Nicoll and Alger, 1981; Schwartzkroin and Stafstrom, 1980; Segal, 1981). The nature of the hyperpolarization has been claimed to be due to various factors. Segal (1981) feels that a Na⁺-pump can be responsible for this process following repetitive activation of neurones by the excitatory agent glutamate. Others believe that a prolonged Ca²⁺-activated K⁺ conductance can play a role (Alger and Nicoll, 1980; Hotson and Prince, 1980; Nicoll and Alger, 1981; Schwartzkroin and Stafstrom, 1980). Recently, it was demonstrated that a prolonged synaptically driven IPSP could in part account for the hyperpolarization following synaptic activation of pyramidal cells (Newberry and
Nicoll, 1984a, 1984b). This late feed-forward IPSP is thought to be mediated by GABA acting on \( \text{GABA}_B \) receptors leading to an increase in a \( K^+ \) conductance whose activation is independent of \( Ca^{++} \). This potential is not sensitive to classical GABA antagonists like bicuculline and picrotoxin and may represent an EGTA-resistant burst afterhyperpolarization (Alger and Nicoll, 1980; Hablitz, 1981; Schwartzkroin and Stafstrom, 1980). Since it has been reported that the afterhyperpolarization is made up of more than one component (Gustafsson and Wigstrom, 1981; Schwartzkroin and Stafstrom, 1980), it is possible that two or all three of the processes that have been discussed are involved. Bear in mind, however, that the late IPSP is only seen following synaptic but not antidromic or direct activation of pyramidal cells.

2.6.4 Ephaptic interactions: A synchronous extracellular field potential in the CA \(_1\) area can influence the membrane potentials of surrounding pyramidal cells that are not included in the directly stimulated population. These so called "ephaptic" interactions that are generated by transient extracellular electrical fields have been implicated in synchronizing and facilitating neural discharges in the hippocampus (Richardson et al., 1984; Taylor and Dudek, 1982; Turner et al., 1984). It has been postulated that ephaptic coupling provides a positive feedback for excitation of neurones so that an increase in the field potential would mean an enhancement in ephaptic interactions and, hence an amplification of the population response further (Turner et al., 1984).

In addition to ephaptic interactions, there are other ways in which hippocampal cells can "communicate". It has been demonstrated physiologi-
cally and anatomically that electrotonic coupling exists between pyramidal cells and granule cells, probably through gap junctions (MacVicar and Dudek, 1981, 1982). According to these authors, such a coupling could result in synchronization of rhythmic activity as well as epileptic discharges in a population of cells. Changes in the extracellular concentration of ions could result in excitability changes in neurones. Depolarization of the cellular membrane of quiescent neurones could be achieved during neuronal activity of other cells in the same area by an elevation of extracellular $K^+$ or a fall in extracellular $Ca^{++}$ concentrations. It has been shown both in vivo and in vitro using ion-sensitive electrodes that repetitive stimulation of inputs in the hippocampus leads to a marked elevation in extracellular $K^+$ and a depletion of $Ca^{++}$ (Benninger et al., 1980; Fritz and Gardner-Medwin, 1976; Krnjević et al., 1980, 1982). Although studies to date regarding ephaptic interactions and electrotonic coupling were done on neuronal somata, there is no reason to exclude the possibility that such processes also play a role presynaptically to modulate transmitter release. Weight and Erulkar (1976) reported that repetitive postsynaptic action potentials can alter transmitter release presynaptically, possibly through an increase in extracellular $K^+$ concentrations.

2.7 Transmitter candidates in the hippocampal formation

Before a compound is accepted to be a chemical neurotransmitter, it is generally agreed upon that certain criteria must be met (Werman, 1966). 1) The agent, when applied onto postsynaptic neurones, produces an action that is identical to that of synaptically released transmitter. 2) During stimulation of the presynaptic fibres, the substance is released from the termi-
3) The substance is released from axon terminals in a Ca\textsuperscript{++}-dependent manner. 4) Drugs which interact with the endogenous transmitter should also interact with the exogenously applied suspected transmitter in an identical manner. 5) The substance must be present in the releasing neurones. 6) The releasing neurone must have enzymes for the synthesis and release of the suspected transmitter. 7) Various precursors and intermediate compounds for the synthesis of the substance should be present. 8) Inactivating mechanisms such as enzymatic breakdown processes or uptake mechanisms could be present. These criteria may be useful to assess the suitability of any substance as a transmitter candidate at a given synapse. However, it has to be borne in mind that these are guidelines and not necessarily rigid rules because there may be exceptions. For instance, criteria 2) and 4) may not be satisfied if available detection methods are not sufficiently sensitive to monitor low concentrations of transmitter or if extensive inactivation occurs following release. The detection of acetylcholine in brain and spinal cord superfusates has only been successful following blockade of cholinesterase (Kanai and Szerb, 1965; Kuno and Rudomin, 1965; Mitchell and Phillis, 1962; Mitchell, 1963; Szerb, 1963). Criterion 1) is also not without exception as it is possible that the nature of the response to a particular transmitter is time and/or concentration dependent. For example, an excitatory agent when applied exogenously could produce a depolarization blockade or desensitization of receptors if applied at a high concentration or for a prolonged period. Under physiological conditions, inactivating mechanisms may terminate the action of released transmitter rapidly so that the phase of depolarization blockade or desensitization may not be appa-
rent. In addition, it is possible that a given compound has dissimilar effects at different regions of the neurone. For example, GABA has been shown to hyperpolarize neuronal somata but depolarize dendrites of hippocampal cells (Alger and Nicoll, 1979; Langmoen et al., 1978). In spite of some inadequacies, however, the criteria for identification of transmitters have generally proven to be quite useful.

2.7.1 Excitatory amino acids. Glutamate and aspartate are the most likely candidates to serve as neurotransmitters in major excitatory pathways of the hippocampus (Storm-Mathisen, 1977a). Nadler et al. (1976) found that glutamate and aspartate were released in a Ca\(^{++}\)-dependent manner following K\(^{+}\)-induced depolarization of hippocampal fragments. Electrical stimulation of the perforant path resulted in an increase in endogenous glutamate release (Crawford and Connor, 1973; White et al., 1977) and lesions of this pathway caused reduced glutamate release (Nadler et al., 1976, 1978). Contrasting results, however, were presented by Di Lauro et al. (1981) who claim that aspartate is the major transmitter of the perforant path fibres. Their argument was that studies done by Nadler et al. (1976, 1978) allowed sufficient time for the regeneration of aspartergic terminals prior to conducting their assays. Following interruption of the commissural input, the release of aspartate but not glutamate was significantly reduced (Nadler et al., 1978). Kainic acid-induced destruction of CA\(_3\) cells (which give rise to Schaffer collaterals and commissural fibres) results in a decrease in both aspartate and glutamate in the dorsal hippocampus (Fonnum and Walaas, 1978). Autoradiographic studies using \(^3\)H-glutamate indicate that the label is concentrated in the inner one third of the dentate area,
stratum oriens and stratum radiatum (where commissural and Schaffer fibres terminate) and in the hilar region of the dentate, where mossy fibres are found (Iversen and Storm-Mathisien, 1976; Storm-Mathisien and Iversen, 1979). Destruction of commissural and Schaffer collateral fibres led to a marked decrease in glutamate uptake (the main inactivating mechanism for glutamate) from the CA\textsubscript{1} area (Storm-Mathisien, 1977b). D-Aspartate has been thought to be a marker for both glutamate and aspartate terminals (Balcar and Johnston, 1972; Davies and Johnston, 1976; Roberts and Watkins, 1975) but appears to selectively favour glutamate terminals (Malthe-Sørensen et al., 1979). Loaded D-aspartate was released in a Ca\textsuperscript{++}-dependent manner following stimulation of commissural and Schaffer fibres in the hippocampal slice (Malthe-Sørensen et al., 1979). Uptake studies have to be viewed with caution because, firstly, the high affinity uptake system does not distinguish between glutamate and aspartate as both amino acids utilize the same carrier (Balcar and Johnston, 1972; Roberts and Watkins, 1975; Young et al., 1974) and, secondly, endogenous and added glutamate are localized in different synaptosomal compartments (Kvamme, 1981) so that experiments using exogenously loaded glutamate or aspartate may give misleading results. The synthesis of releasable glutamate can be brought about by loading slices with its precursors glucose and glutamine (Hamberger et al., 1978, 1979a, 1979b). Electrophysiological studies on hippocampal neurones demonstrate a powerful excitation of pyramidal cells by glutamate and aspartate (Dudar, 1974; Schwartzkroin and Andersen, 1975; Zanotto and Heinemann, 1983) with the most sensitive areas located at the dendrites where excitatory inputs terminate. The evidence presented strongly supports the possibility that an
acidic amino acid is the neurotransmitter in the perforant path, commissural input and Schaffer collateral input. However, it is not possible at this point to be certain whether glutamate and/or aspartate are involved in these pathways.

In contrast to the inputs discussed, the other major afferent system, the mossy fibres (arising from dentate granule cells) does not seem to utilize either glutamate or aspartate as a transmitter (Nadler et al., 1978; White et al., 1979). The report by Crawford and Connor (1973) claims that mossy fibres release glutamate. Their evidence is based on collection of evoked release of endogenous glutamate by stimulation in the entorhinal cortex. Such stimulation would activate the trisynaptic loop consisting of perforant path, mossy fibres and Schaffer collaterals. Therefore, the validity of such an assumption is highly questionable because a selective activation of the mossy fibres using this mode of stimulation is impossible. The transmitter of the mossy fibres remains largely unknown but it is interesting that granule cells and mossy fibres showed dense staining for dynorphin (McGinty et al., 1983).

Electrophysiological and pharmacological studies support the presence of multiple receptors for glutamate in the hippocampus (Collingridge et al., 1983a; Fagni et al., 1983; Koerner and Cotman, 1982). These have been characterized by Fagni et al. (1983) and are divided into four subtypes: 1) the NMDA receptor which is activated by N-methyl-D-aspartate and exhibits desensitization; 2) the kainate receptor which is activated by kainic acid and does not desensitize; 3) the G2 receptor which is activated by glutamate and aspartate and shows desensitization; 4) the G1 receptor which is acti-
vated by DL-homocysteate and does not desensitize. Bear in mind that these four subtypes are glutamate receptors and are, therefore, activated to varying degrees by this amino acid. Each subtype is identified by the most potent agonist at that particular receptor. On the basis of electrophysiological studies, the above authors postulate that the Gl receptor is the synaptic receptor.

2.7.2 Acetylcholine. Denervation studies have demonstrated that acetylcholinesterase (AChE) and choline acetyltransferase (ChAT) (the enzymes responsible for the breakdown and synthesis of acetylcholine [ACh], respectively) disappear almost entirely from the hippocampal region after interruption of the fimbrial fibres or lesions in the septum (Lewis et al., 1967; Mellgren and Srebro, 1973; Shute and Lewis, 1961). Fibres showing accumulation of AChE have been traced from the hippocampus back to the medial septum (Lewis and Shute, 1967). The densest innervation of cholinergic fibres occurs in stratum oriens in the CA3 region, the hilus of the dentate and to a lesser extent, the dentate molecular layer (Crutcher et al., 1981; Kimura et al., 1981; Lynch et al., 1978; Storm-Mathisen, 1977a). Spontaneous as well as stimulus-evoked (by stimulation of medial septum) release of ACh has been collected from the hippocampus (Dudar, 1975; Smith, 1972). No evoked release of ACh is seen with stimulation of the lateral septal nucleus, contralateral hippocampus or caudate nucleus and the evoked release mentioned above is dependent on the integrity of the septo-hippocampal projection. Electrophysiological and pharmacological studies have also supported the cholinergic nature of the septo-hippocampal pathway (Brücke et al., 1963; Green and Arduini, 1954; Stumpf, 1965). When applied iontophore-
tically, ACh usually produces a slow excitation in hippocampal cells that is thought to be mediated by activation of muscarinic receptors (Biscoe and Straughan, 1966; Bland et al., 1974; Herz and Nacimiento, 1965; Salmoiraghi and Stefanis, 1965; Stefanis, 1964; Steiner, 1968).

2.7.3 \( \gamma \)-Aminobutyric acid. The well characterized basket cells, which are inhibitory interneurones in the hippocampus, are thought to utilize \( \gamma \)-aminobutyric acid (GABA) as their neurotransmitter (Andersen, 1975; Storm-Mathisen, 1977a). \(^3\)H-GABA localization and glutamic acid decarboxylase (GAD, an enzyme for the synthesis of GABA) activity were measured in hippocampus (Iversen and Bloom, 1972; Ribak et al., 1978; Storm-Mathisen, 1972, 1976). The lack of reduction in GAD activity and GABA uptake after lesions of afferent pathways suggests that GABA-containing cells are intrinsic to the hippocampal formation (Nadler et al., 1974; Storm-Mathisen, 1972, 1975; Storm-Mathisen and Fonnum, 1972; Storm-Mathisen and Guldberg, 1974). These biochemical findings are consistent with electrophysiological studies which show that interneurones mediate the inhibition of pyramidal cells (Andersen et al., 1964a, 1964b). Physiological and pharmacological data indicate that pyramidal cells are inhibited by iontophoretically applied GABA and the response to this agent as well as IPSPs are blocked by bicuculline, a GABA antagonist (Biscoe and Straughan, 1966; Curtis et al., 1970; Stefanis, 1964). The sensitivity to GABA appears to be greatest at the cell bodies, corresponding with the termination of GABAergic nerve terminals (Schwartzkroin et al., 1974).

2.7.4 Aromatic amines. In the rat, noradrenaline (NA) containing nerve terminals and axons are diffusely distributed in the hippocampus but
are concentrated in the hilus of the dentate and to a lesser extent in the CA\textsubscript{3} and CA\textsubscript{1} regions (Blackstad et al., 1967). The afferent fibres enter the hippocampal formation via the fimbria, the fornix, the cingulum bundle and the amygdaloid area (Fuxe, 1965; Fuxe et al., 1969; Lindvall and Björklund, 1974; Ungerstedt, 1971). Following lesions in the locus coeruleus and NA containing pathways ascending from the locus coeruleus, extensive reduction of NA and NA markers were observed in the hippocampal region (Anden et al., 1966; Lindvall and Björklund, 1974; Thierry et al., 1973; Ungerstedt, 1971). It was also found that dopamine-β-hydroxylase, a synthesizing enzyme for NA, was drastically reduced after destruction of the locus coeruleus (Ross and Reis, 1974). Therefore, it appears that the major or, perhaps, only noradrenergic input to the hippocampus arises from the locus coeruleus and there are no intrinsic NA neurones in the hippocampal formation. The effect of iontophoretically applied NA on hippocampal neurones is inhibitory (Biscoe and Straughan, 1966; Herz and Nacimiento, 1965; Salmoiraghi and Stefanis, 1965; Stefanis, 1964) and appears to mimic the action of stimulation of the locus coeruleus in producing inhibition (Segal and Bloom, 1974a, 1974b). This inhibition was shown to be different from recurrent basket cell inhibition and was not blocked by bicuculline (Storm-Mathisen, 1977a).

The serotonergic projection to the hippocampus arises from the medial and dorsal raphe nuclei (Azmitia and Segal, 1978; Moore and Halaris, 1975). Although this input is distributed diffusely throughout the hippocampus, it appears to be concentrated in the stratum moleculare and the subiculum (Fuxe et al., 1970; Fuxe and Jonsson, 1974). Serotonin (5-HT) containing axons
invade the hippocampus via the fimbria and the cingulum bundle (Björklund et al., 1973; Fuxe, 1965; Moore and Halaris, 1975). Following destruction of the raphe nuclei, 5-HT, 5-HT uptake and tryptophan-5-hydroxylase (a synthesizing enzyme for 5-HT) decrease drastically in hippocampus and various forebrain regions (Kuhar et al., 1972). As with NA, iontophoretically applied 5-HT also causes inhibition of hippocampal pyramidal cells (Biscoe and Straughan, 1966; Herz and Nacimiento, 1965; Salmoiraghi and Stefanis, 1965; Segal, 1975; Stefanis, 1964). Electrical stimulation in the raphe nuclei appears to produce an inhibition of prolonged time course in pyramidal cells (Segal, 1975). The inhibition produced by raphe stimulation was enhanced by the 5-HT uptake blocker p-chlorophenylalanine and inhibited by the tryptophan-5-hydroxylase inhibitor p-chlorophenylimipramine (Segal, 1975, 1976). These data support the existence of a raphe-hippocampal inhibitory pathway using 5-HT as a neurotransmitter.

2.8 Long-term synaptic potentiation in hippocampus

The phenomenon of long-term potentiation (LTP) of synaptic transmission can be elicited in various regions of the intact hippocampus (Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973; Douglas and Goddard, 1975) as well as in vitro hippocampal slices (Alger and Teyler, 1976; Andersen et al., 1977; Lynch et al., 1976; Schwartzkroin and Wester, 1975; Yamamoto and Chujo, 1978) (Figure 3). The increase in synaptic efficacy during LTP can be observed as an enhancement in the size of the population spike or the population EPSP, a reduction in the latency to onset of the population spike, an increase in the rate of rise and amplitude of the intracellular EPSP or a decrease in threshold for synaptically evoking an all-or-none
Figure 3

Tetanus-induced long-term potentiation (LTP) of synaptically-driven CA$_1$ responses. Records in the top row show extracellular population spikes recorded in the CA$_1$ cell body layer and records in the bottom row are population EPSPs monitored at the apical dendritic zone. Both sets of responses were evoked by stimulation of stratum radiatum. On the extreme left are control responses. Subsequent to a 400 Hz, 200 pulses tetanus delivered to stratum radiatum, the post-tetanic responses were followed for 60 minutes. Records shown are for 1, 15, 30 and 60 minutes post-tetanus. Negativity is downwards.
action potential in a CA₁ cell. The induction of LTP is believed to be Ca²⁺-dependent and cannot be elicited if the tetanic stimulation is delivered in Ca²⁺-free medium (Dunwiddie and Lynch, 1979; Wigström et al., 1979). Furthermore, a Ca²⁺ agonist, Sr²⁺, can support both synaptic transmission and LTP in the hippocampal slice (Wigström and Swann, 1980). A transient elevation of extracellular Ca²⁺ can produce a post-application LTP-like state (Turner et al., 1982), a condition that is associated with a prolonged increase in intracellular Ca²⁺ content (Baimbridge and Miller, 1981). In the CA₁ region, LTP has been demonstrated to be input specific (i.e., it can be observed only in a previously tetanized but not a non-tetanized input impinging on the same population of CA₁ neurones) (Andersen et al., 1977; Lynch et al., 1976). In contrast to the CA₁ area, LTP in the CA₃ region appears not to exhibit input selectivity (Misgeld et al., 1979; Yamamoto and Chujo, 1978). The induction of LTP requires a cooperativity of input fibres, that is, a sufficient number of presynaptic fibres has to be activated. A "weak" subsynaptic response that is evoked by stimulation of the input with low stimulus intensity will usually exhibit marginal or no LTP following a tetanus to the same input. However, if the tetanus of the "weak" input is either paired with a tetanus to another "strong" separate input (to the same population of postsynaptic neurones) or the stimulus intensity is increased during the tetanus to recruit more fibres, associative LTP of the "weak" input results (Barrionuevo and Brown, 1983; Lee 1983a; McNaughton, 1982; McNaughton et al., 1978; Robinson and Racine, 1982). It is believed by some investigators that the mechanisms underlying LTP of the population spike and the population EPSP are dissimi-
lar because a potentiation of one can be observed without any apparent change in the other (Bliss et al., 1983; Bliss and Lømo, 1973). In addition, it was observed that the time courses of decay for LTP of the population spike and population EPSP are not necessarily the same (Douglas and Goddard, 1975). Since all the studies above were conducted on extracellular field potentials, caution has to be exercised in interpreting the results. An extracellular recording at the cell body layer of the CA1 region will yield a positively directed population EPSP that is interrupted by a negatively going population spike. The reason for this is that the EPSP is generated by a synaptically-induced depolarization of the dendrites whereas the site for spike initiation is close to the soma (Andersen et al., 1980a). Obviously, these two fields of opposing polarity overlap and it is possible that an increase in size of one will result in an apparent diminution of the other. Furthermore, any changes in the IPSP which is a positive potential when recorded at the cell body layer may cause a change in the size of the EPSP if their time courses overlap. Therefore, with these uncertainties, firm conclusions regarding the differences between LTP of the population spike and population EPSP cannot be reached without intracellular studies.

Early experiments involving hippocampal lesions and ablations have led many investigators to agree that the hippocampus is in some way involved in learning and memory (Best and Best, 1976; Coleman and Lindsley, 1977; Green, 1964; Isaacson, 1974; O'Keefe, 1983; O'Keefe and Nadel, 1978; Olds et al., 1972; Penfield and Milner, 1958; Scoville and Milner, 1957; Segal and Olds, 1973). However, there is disagreement as to what kind of memories the
hippocampus may be responsible for. Consistent with this hypothesis, it was shown that the firing rates of hippocampal neurones gradually increased during the course of classical conditioning of the rabbit nictitating membrane response (Berger and Thompson, 1978; Berger et al., 1976). It was originally proposed by Hebb (1949) that memory involves an increase in synaptic transmission and that the information is stored as a result of long-lasting changes in some synaptic properties. Therefore, it was concluded that LTP in the hippocampus could be a physiological mechanism for learning and memory at the cellular level (Berger and Thompson, 1978; Chung, 1977; Teyler, 1976). Evidence for this claim was provided by Berger (1984) who showed that prior induction of LTP in the hippocampus of intact animals by tetanically stimulating an input results in a faster rate of classical conditioning when compared to untetanized control animals. If LTP is, indeed, linked to learning, then as an animal learns a task, one should see an increase in the size of the synaptically evoked population response. This appears not to be true because the amplitudes of the population EPSP as well as the population spike induced by perforant path stimulation were measured before and after classical conditioning and neither was found to be increased after learning of the task (Laroche, 1985). Some evidence questions whether the hippocampus is even involved in learning and memory. Rats with large bilateral lesions of the hippocampus were still able to learn and remember tasks as well as undamaged controls if the lesions were carried out in stages (Isseroff et al., 1976; Stein et al., 1969). Much of the research that is used to implicate the hippocampus in memory processes utilizes lesion techniques which inadvertently cause extensive damage to other brain
areas and fibres that pass through this structure. In a recent study that used neurotoxins to selectively destroy hippocampal neurones without damaging fibres-of-passage or afferents to the hippocampus, it was demonstrated that under such conditions, the performance of complex memory tasks was minimally affected (Jarrard, 1983, 1985). In the same studies, aspiration of the hippocampus and interruption of the main afferent and efferent pathways resulted in markedly poorer performance on the same tasks (Jarrard, 1985).

In light of the contradicting evidence, it is not possible to say with certainty that the hippocampus is involved in learning and memory, much less speculate on the role of hippocampal LTP in these processes. The elucidation of the mechanisms responsible for LTP, whether it has any relevance to learning and memory, will provide substantial information with regard to understanding the plastic properties of synapses in the central nervous system. LTP is not specific to the hippocampus as it has been shown to be present in the neocortex (Kasamatsu et al., 1981; Lee, 1983b), mammalian sympathetic ganglion (Brown and McAfee, 1982; Koyano et al., 1985), abdominal ganglion of *Aplysia* (Castellucci and Kandel, 1976; Castellucci et al., 1970) and crayfish neuromuscular junction (Baxter et al., 1985). It was established in some systems (crayfish neuromuscular junction, abdominal ganglion of *Aplysia* and rat sympathetic ganglion) using the method of quantal analysis that LTP could be accounted for by a presynaptic mechanism through increased transmitter release (Baxter et al., 1985; Briggs et al., 1985; Castellucci and Kandel, 1976; Koyano et al., 1985). It was also reported that LTP of the septo-hippocampal input to CA3 neurones involved
an increase in quantal content but there was no change in the quantal unit. Furthermore, the same authors showed that there was no change in the sensitivity of subsynaptic receptors on CA$_3$ neurones to applied acetylcholine, the transmitter released by the septo-hippocampal input (Voronin, 1980, 1983). Mellgren and Srebro (1973) reported that the septo-hippocampal projection to the hippocampus terminates in stratum oriens and stratum radiatum of CA$_3$ neurones. Therefore, these synaptic contacts on the CA$_3$ pyramidal cells could be located at some distance from the soma. In the studies of Voronin (1980, 1983) recordings were done in CA$_3$ cell bodies, so it is possible that increases in the quantal unit did occur at the dendrites but were not recognized at the soma due to decrement of the potentials during electrotonic propagation to the recording site. Perhaps, a better system to conduct quantal analysis upon is the mossy fibre-CA$_3$ synapse where it has been demonstrated that the excitatory synapses terminate very close to the soma (Blackstad et al., 1970; Blackstad and Kjaerheim, 1961; Brown and Johnston, 1983; Johnston and Brown, 1983; Hamlyn, 1961; Hamlyn, 1962; Lorente de Nó, 1934). Therefore, the locus for LTP in the hippocampus is not clear. Since there is evidence for the involvement of both pre- and postsynaptic elements in LTP of intrinsic hippocampal systems, the general consensus is that both components play a role.

The following observations suggest that LTP is presynaptic. 1) LTP in the CA$_1$ area is input specific (Andersen et al., 1977; Lynch et al., 1976). 2) High frequency tetanic stimulations that produce minimal synchronous discharge of the postsynaptic neurones are more favourable for inducing LTP whereas lower frequency trains which produce frequency facilitation tend
to result in homo- and heterosynaptic depression (Bliss and Lømo, 1973; Dunwiddie and Lynch, 1978; Sastry et al., 1984a). 3) Passive membrane properties of CA\textsubscript{1} neurones such as resting membrane potential, input resistance and time constant were unaltered during LTP (Andersen et al., 1980b; Barrionuevo and Brown, 1983). 4) Synchronous postsynaptic discharge is not required during the tetanic stimulation to successfully induce LTP (Wigström et al., 1982). The most obvious interpretation of points 1) to 4) is that LTP is presynaptic, but another possible explanation is that a postsynaptic change which occurs at the dendritic region is responsible. 5) More definitive evidence for a presynaptic involvement is provided by several laboratories. These investigators demonstrate that stimulus-evoked release of both exogenously loaded as well as endogenous neurotransmitter is increased during LTP (Bliss et al., 1985; Dolphin et al., 1982; Skrede and Malthe-Sørensen, 1981). 6) A decrease in presynaptic terminal excitability is associated with LTP and this excitability change is not seen at non-terminal axonal regions (Sastry, 1982).

The following evidence has been presented in support of a postsynaptic locus for LTP. 1) The number of Na\textsuperscript{+}-independent glutamate binding sites (presumed by the authors to be subsynaptic receptors although pharmacological and physiological evidence is lacking) is increased following tetanic stimulations to an input (Baudry and Lynch, 1980a). 2) Intracellular injections of EGTA into CA\textsubscript{1} cells blocked the induction of LTP (LTP induction is thought to be Ca\textsuperscript{++}-dependent) (Lynch et al., 1983). 3) The associative nature for the induction of LTP (where a presumably separate input can "cooperate" with the test input to facilitate production of LTP in the lat-
ter) has convinced many investigators that the common link and, therefore, locus for LTP is the postsynaptic neurone (Douglas et al., 1982; McNaughton, 1982; McNaughton et al., 1978; Robinson and Racine, 1982). A subsequent investigation has shown, however, that the cooperativity among afferents does not correlate with enhanced postsynaptic discharge during conditioning, but rather could be due to interactions among presynaptic fibres (Lee, 1983a). 4) Changes in inhibitory processes (presumably postsynaptic inhibition) are thought to play a role in LTP. Unlike the situation in the CA1 region, LTP in the CA3 region is not input specific and the size of the EPSP appears to be inversely related to the magnitude of the IPSP in CA3 neurones (Misgeld et al., 1979; Yamamoto and Chujo, 1978). It has been shown recently, however, that a decrease in inhibition is not responsible for LTP in the CA3 region (Griffith et al., 1986). In the dentate gyrus, enhanced inhibition of granule cells during afferent tetanization succeeded in blocking the induction of LTP (Douglas et al., 1982). Furthermore, blockade of inhibition using GABA antagonists facilitated induction of LTP in the CA1 area (Wigström and Gustafsson, 1983). Although it appears that the induction of LTP may be enhanced by the blockade of presumed postsynaptic inhibition, the maintenance of the phenomenon, at least in the CA1 region is not dependent on inhibitory processes (Haas and Rose, 1982, 1984). 5) Anatomical changes in dendritic morphology have been suggested as possible postsynaptic mechanisms for LTP. A swelling of dentate granule cell spines was reported following tetanic stimulations to the perforant path (Fifkova and Van Harreveld, 1977; Van Harreveld and Fifkova, 1975). These authors postulated that this prolonged change can account for the
increase in synaptic efficacy during LTP. This explanation is not entirely plausible because it was shown earlier that swelling of spines accompanies spreading depression (Van Harreveld and Khattab, 1967). Furthermore, the authors used a tetanic stimulus frequency (30 Hz) that could produce frequency facilitation during the conditioning train and a following post-tetanic homo- and heterosynaptic depression of synaptic transmission (Dunwiddie and Lynch, 1978; Sastry et al., 1984a). It was demonstrated in a later study (Lee et al., 1980) that a higher stimulus frequency (100 Hz), which is more favourable for eliciting LTP, failed to produce any swelling of spines. A recent report further disproves the spine swelling theory by showing that there is no correlation between spine swelling and LTP (Chang and Greenough, 1984). The brief bursts of high frequency stimuli in the study of Lee et al. (1980) produced a rearrangement of synapses to result in an increase in the density of dendritic shaft synapses and a decrease in spine variability that is thought to improve synaptic transmission. Using computer simulated models, it was deduced that various alterations in dendritic spines can be conducive to synaptic amplification (Horwitz, 1981; Miller et al., 1985).

Several hypotheses have been presented regarding the locus and cellular mechanisms that may be responsible for LTP. 1) Baudry and Lynch (1980a) suggested that an increase in the number of subsynaptic neurotransmitter (glutamate) receptors can account for LTP. A tetanic stimulation of an input presumably results in activation of the postsynaptic membrane to allow Ca\(^{++}\) influx. This increase in intracellular Ca\(^{++}\) then activates a protease which then unmask new glutamate receptors (Baudry and Lynch, 1980b;
Baudry et al., 1981a, 1981b; Vargas and Costa, 1981). Evidence that has been reported demonstrates an increase in Na\(^+\)-independent glutamate binding sites following tetanic stimulations in hippocampal slices (Baudry et al., 1980; Lynch et al., 1982). This increase in binding sites, which may or may not be receptors, appears to be irreversible (Baudry et al., 1983) and is inducible by elevated Ca\(^{++}\) concentrations (Baudry and Lynch, 1979).

2) Changes in dendritic spine morphology have also been postulated to play a role in the synaptic enhancement observed during LTP. Based on anatomical and computer simulated data, several investigators have concluded that certain changes in the shape and size of dendritic spines and the rearrangement of synaptic contacts can contribute to facilitated synaptic transmission (Desmond and Levy, 1981, 1984; Lee et al., 1980; Horwitz, 1981; Miller et al., 1985). As discussed previously, the hypothesis regarding spine swelling (Fifkova and Van Harreveld, 1977; Van Harreveld and Fifkova, 1975) appears not to be a feasible one.

3) The contribution of inhibitory processes to the induction and maintenance of LTP has been investigated. It was postulated that the generation of LTP is facilitated if the conditioning tetanus is delivered during a blockade of inhibition with GABA antagonists (Wigström and Gustafsson, 1983). However, it appears that the maintenance of LTP in the CA\(_1\) area is not dependent on a reduction in the orthodromically-evoked or recurrent IPSP. In contrast, LTP in the CA\(_3\) region is not input specific and is accompanied by a diminution of the IPSP (Misgeld et al., 1979; Yamamoto and Chujo, 1978). Apparently, in the CA\(_3\) region, the size of the EPSP during LTP is inversely related to the magnitude of the IPSP.

4) The stimulus-evoked uptake of D-\(^3\)H-aspartic acid (a
marker for glutamatergic nerve terminals) is decreased following high frequency tetanic stimulations to an input (Wieraszko, 1983). This observation led to the hypothesis that a reduction in the uptake of neurotransmitter could account for LTP because of greater availability of the substance to the subsynaptic receptors. Although this may be true, it is known that a blockade of transmitter uptake does not necessarily result in enhanced synaptic transmission (Curtis et al., 1976). It is possible that uptake mechanisms are activated with a slower time course than synaptic transmission so that the interaction with subsynaptic receptors occurs before the transmitter is eliminated. 5) Presynaptic terminal alterations resulting in an increased release of transmitter has been thought to occur during LTP. It has been demonstrated that evoked release of loaded $^3$H-aspartate is enhanced following tetanic stimulation of inputs in stratum radiatum (Skrede and Malthe-Sørensen, 1981). The increase in stimulus-evoked endogenous glutamate release parallels that of LTP in the dentate region after tetanic stimulation of the perforant path (Bliss et al., 1985; Dolphin et al., 1982). The excitability of presynaptic terminals is reduced during LTP (Sastry, 1982). This excitability change has a similar time course to LTP and, therefore, may play a role in this potentiation. It was suggested that a hyperpolarization of the presynaptic terminals would result in a reduced excitability as measured by Wall's technique (Wall, 1958; Wall and Johnson, 1958) and an increase in the action potential height (Eccles and Krnjević, 1959a, 1959b; Lloyd, 1949) which brings about an enhancement in the evoked release of transmitter (Hubbard and Willis, 1962; Takeuchi and Takeuchi, 1962). 6) N-Methyl-D-aspartate (NMDA) receptor activation is thought to be
necessary for LTP induction (Collingridge et al., 1983b; Harris et al., 1984). 2-Amino-5-phosphonovalerate (APV), reportedly a specific NMDA receptor antagonist (Collingridge et al., 1983a) counteracts the development of LTP when the tetanus is given during application of the drug (Collingridge et al., 1983b; Harris et al., 1984). Iontophoretic application of NMDA at the apical synaptic zone of CA1 neurones produced a post-application potentiation of the population EPSP evoked by stimulation of stratum radiatum (Collingridge et al., 1983b). A prolonged negative wave that is recorded in the synaptic region during tetanic stimulation of the input is thought to be mediated by an activation of NMDA receptors because it can be reduced by application of APV (Wigström and Gustafsson, 1984). This negative wave may be important for LTP induction because a blockade of inhibition with picrotoxin during tetanic stimulation produces an enhancement of the wave as well as facilitated LTP development following the treatment. These authors suggested that the dendritic depolarization achieved by NMDA receptor activation during tetanization is facilitated during a reduction in postsynaptic inhibition and that it is this depolarization which produces optimal conditions for initiating LTP. 7) Neuroleptic drugs which are calmodulin antagonists impair the induction of LTP (Finn et al., 1980; Mody et al., 1984) raising the possibility that this Ca²⁺ buffering protein is involved in LTP. 8) Selective depletion of noradrenaline and 5-HT in rats results in a reduction in tetanus-induced LTP, suggesting that these monoamines play a role in this phenomenon (Bliss et al., 1981, 1983). Furthermore, it was shown that applied noradrenaline results in an LTP-like phenomenon in the dentate gyrus (Neuman and Harley, 1983). 9) LTP could not be
elicited in cells injected intracellularly with the K$^+$ channel blocker CsCl (Haas and Rose, 1984). Therefore, a reduction of a potassium conductance could be responsible for LTP. 10) A recent report (Malenka et al., 1986) provides evidence for protein kinase C (a Ca$^{++}$-dependent phospholipid kinase which is selectively activated by phorbol esters) involvement in the induction of LTP.

2.9 Post-tetanic potentiation

Post-tetanic potentiation (PTP) of synaptically transmitted responses (Eccles and Krnjević, 1959a, 1959b; Lloyd, 1949) which has a time course of several minutes (1-4) differs from LTP in that the latter phenomenon is characterized by a much longer duration. The suggestion that PTP in the CNS is generated presynaptically has been with us since Lloyd (1949) supplied evidence in favour of this hypothesis. He observed that PTP in the spinal cord was input specific and there was no potentiation of non-tetanized heterosynaptic afferents that were known to terminate on the same postsynaptic motoneurones. Furthermore, a tetanus of afferents impinging on a certain population of motor horn cells resulted in no potentiation but rather a depression of the antidromic compound action potential evoked by stimulation of the axons of these cells in the ventral root. A hyperpolarization of afferent fibres could be responsible for PTP because changes in the intensity and duration of the potentiation are closely associated with a positive after-potential that is recorded extracellularly from the tetanized fibres (Lloyd, 1949). This idea was later corroborated by the findings of Eccles and Krnjević (1959b) who recorded intracellularly in primary afferents in the spinal cord and observed a period of hyperpolarization of these fibres.
following their tetanization. The excitability of primary afferent projections was also found to be decreased post-tetanically and this change parallels the degree of synaptic potentiation (Wall and Johnson, 1958), an observation that is in accordance with a hyperpolarization of the fibres. The presynaptic axonal spike height appears to be increased during the period of hyperpolarization that accompanies PTP and artificial anodal polarization of the spinal dorsal roots also produces a similar effect (Eccles and Krnjević, 1959b). However, attempts to alter postsynaptic potentials by polarizing presynaptic dorsal root fibres failed, the explanation given by the above authors being that the site of the polarizing electrode was too far from the terminals to produce adequate electrotonic changes in the membrane potential at the nerve endings. In studies conducted to examine the relationship between presynaptic spike size and postsynaptic potentials, it was discovered in the squid giant synapse that small presynaptic changes had a very profound postsynaptic effect (Hagiwara and Tasaki, 1958). An enhancement of the presynaptic spike height is accompanied by a facilitation of evoked transmitter release (Hubbard and Willis, 1962; Takeuchi and Takeuchi, 1962). Perhaps, the most convincing evidence in support of a presynaptic locus for PTP is supplied through quantal analysis where it was concluded that the number of quanta of evoked transmitter is increased whereas the quantal size remains unchanged (del Castillo and Katz, 1954).

Similar to the situation found in the spinal cord, neuromuscular junction, squid giant synapse and numerous other systems, the hippocampus also exhibits PTP. There is general agreement that the properties and requirements for induction of PTP and LTP in this system are not the same
(Dunwiddie and Lynch, 1979; McNaughton, 1982). Since the evidence in support of a presynaptic locus for PTP is fairly strong in other tissues, it is assumed by many that this phenomenon in the hippocampus is no different.

3 METHODS

3.1 Preparation of slices

Transversely sectioned hippocampal slices (500 μm thick) were obtained from male Wistar rats (75-125 g). The animal was initially anaesthetized with a mixture of halothane (2%) and oxygen. During this process, an ice pack was placed beneath the rat to lower its body temperature (rectal temperature prior to surgery was 31–32°C) so that the viability of the slices could be increased. The skin covering the top of the head was cut with a scalpel in an anterior to posterior direction to expose the skull bones. The plates of the skull and the dura mater covering the brain were carefully removed and the brain was severed from the spinal cord at the pontine level. The optic nerves were also severed and the brain was taken out and quickly drenched with cold (4°C) standard medium (constitution given in the next section) to reduce metabolic rate and oxygen requirements. The hippocampi from one or both hemispheres were dissected free and transverse slices of this structure were obtained using a McIlwain tissue chopper. To separate and arrange the slices, the sliced hippocampus was placed in a petridish filled with cold standard medium which was oxygenated with carbogen (95% O₂, 5% CO₂). The slices were sandwiched between two nylon meshes to minimize movement during the experiments and quickly transferred
to the slice chamber (see Figure 4 for diagram of chamber). The whole procedure from the start of surgery until the time the slices were inserted into the bath usually took 3 minutes or less. All experiments conducted were on slices submerged in the perfusing medium and temperature was maintained at 32 ± 0.5°C. The perfusing medium was constantly bubbled with carbogen and an extra line carrying humidified carbogen was also fed to the atmosphere above the slices. During the equilibration period (one hour) before commencement of experiments, the bath was maintained at room temperature and the chamber opening was covered with a piece of parafilm to ensure that the air above the slices was saturated with oxygen. The flow rate of the medium was maintained at 3 ml/min. Only one slice per animal was used if an experiment involved using media other than the standard medium. In any event, no slices older than four hours were utilized because of possible effects of deterioration even though they usually survived for more than 12 hours.

3.2 Constitution of perfusing media

The pH of all media were maintained at 7.4 while bubbled with carbogen (95% O₂, 5% CO₂). Unless otherwise specified, experiments were conducted in standard medium.

Standard medium: 120 mM NaCl, 3.1 mM KCl, 1.3 mM Na₂HPO₄, 26 mM NaHCO₃, 2 mM CaCl₂, 2 mM MgCl₂, 10 mM dextrose.

Ca²⁺-free standard medium: same as standard medium except CaCl₂ was omitted, 0.5 or 1 mM MnCl₂ was added and MgCl₂ was increased to 3.5 or 3 mM, respectively, to compensate for divalent ion content.
Figure 4

Diagram of slice chamber and perfusion system for maintaining hippocampal slices. AB: aluminium block; Co: extra carbogen line; Gn: ground lead; GW: ground wire; HE: heating element; LM: lines for medium; LN: lower nylon platform; Mf: manifold; Mn: manipulator; Nz: nozzle; O1: outlet for medium; SC: slice chamber; SL: suction line; SP: sensor probe; UN: upper nylon platform.
Picrotoxin medium: 120 mM NaCl, 3.1 mM KCl, 26 mM NaHCO₃, 4 mM CaCl₂, 4 mM MgCl₂, 10 mM dextrose, 10 μM picrotoxin. The divalent cation concentration was increased as compared to the standard medium to minimize epileptiform activity that can be induced by picrotoxin. NaH₂PO₄ was omitted because of problems with the solubility of the increased amount of CaCl₂. This change did not significantly affect the buffering capacity of the medium and it was maintained at pH 7.4 while being bubbled with carbogen. Picrotoxin medium was used in the associative LTP experiments and in experiments where a facilitated LTP was desired because it was reported (Wigström and Gustafsson, 1983) that GABA antagonists facilitated the induction of LTP.

Ca²⁺-free (Mn²⁺) picrotoxin medium: same as picrotoxin medium except CaCl₂ was omitted and MnCl₂ (1 mM) was added. MgCl₂ was increased to 7 mM to maintain the divalent cation concentration.

Picrotoxin-EDTA medium: same as picrotoxin medium except 200 μM EDTA (ethylenediaminetetraacetic acid, pH 7.4) was added.

Ca²⁺-free (Mn²⁺) picrotoxin-EDTA medium: same as Ca²⁺-free (Mn²⁺) picrotoxin medium except 200 μM EDTA was added.

Ca²⁺-free (Co²⁺) picrotoxin-EDTA medium: same as above except 1 mM CoCl₂ was used instead of 1 mM MnCl₂.

High K⁺ media: NaCl was reduced by the appropriate amount when KCl was increased to maintain a constant osmolarity in control and test media.

Other drug-containing media: NMDLA, NMLA, NMDA, L-glutamate (Sigma Chemicals) and DL-APV (Cambridge Research Biochemicals) were added to standard medium from concentrated stock solutions. The final volume
of drug-containing medium was altered by less than 0.5% using this method.

3.3 Recording systems

Recording microelectrodes (fibre-filled borosilicate glass, OD 1.5 mm, ID 1.0 mM, Frederick Haer and Co., tip 1 μm, filled with 4 M NaCl, resistance 1–2 MΩ) were pulled using a Narashige microelectrode puller. The signals recorded by the micropipette or by a NaCl-filled barrel of an iontophoresis electrode were amplified by either a DAM-5A (World Precision Instruments) or a Neurolog (Medical Systems Corp.) amplifier and were displayed on a DATA 6000 (Data Precision) waveform analyzer. Records of evoked responses were averaged (4–8 sweeps) by the DATA 6000 unit and were plotted on paper by a Hewlett-Packard 7470A graphics plotter. To record population spikes or population EPSPs, the recording electrode was positioned either in the cell body layer of the CA₁ or CA₃ areas or the apical dendritic region of the CA₁ neurones (100–200 μm from cell body layer) (Figure 5). The all-or-none action potentials in single CA₃ cells were monitored by placing the recording electrode in the CA₃ cell body layer (Figure 6).

3.4 Stimulating systems

A Grass S88 stimulator was used to deliver current pulses through one or two channels using Grass PSIU6 constant current stimulus isolation units. The isolation units were connected to bipolar concentric metal stimulating electrodes (SNEX 100, Rhodes Electronics, resistance 1–2 MΩ) for evoking population responses. Population spikes and EPSPs in the CA₁ area were evoked by stimulation of stratum oriens or radiatum and population spikes in the CA₃ region were evoked by stimulation of the mossy fibres.
Positioning of stimulating and recording electrodes for synaptically-evoked population responses. Concentric bipolar metal electrodes were used for stimulation and fibre-filled glass micropipettes (tip 1-2 μm, filled with 4 M NaCl) were used for recording. To evoke synaptic responses in the CA1 region, stratum oriens or stratum radiatum were stimulated at 0.2 Hz. Extracellular population spikes were recorded in the CA1 cell body layer and population EPSPs at the apical dendritic zone. CA3 population spikes were monitored with a microelectrode placed in the CA3 cell body layer and were evoked by stimulation of the mossy fibres (MF) at 0.2 Hz. The population responses were all evoked using negative stimulus pulses delivered through a constant current unit.
Monopolar glass electrodes (similar to the recording electrodes), monopolar tungsten electrodes or a NaCl-filled barrel of a 7 barrel iontophoresis electrode were used for stimulation and measuring threshold for activation of Schaffer collateral terminal regions of single CA₃ cells (Figure 6). Control stimulation frequencies used to evoke synaptic and antidromic responses were 0.1-0.2 Hz. In experiments where both the stratum oriens and stratum radiatum were used, they were evoked alternately at 5 second intervals such that the frequency of stimulation of each input was 0.1 Hz. The polarity of the pulses used for stimulation was always negative.

3.5 **Excitability testing of Schaffer collateral terminal regions**

The method employed for excitability testing of presynaptic terminals is a modification of a procedure first described by Wall (1958). A recording electrode was placed in the CA₃ cell body layer to record an extracellular all-or-none spike in a CA₃ neurone and a monopolar stimulating electrode was placed at the apical dendritic area of CA₁ neurones where the Schaffer collaterals terminate (Figure 6). The amount of injected current required to activate a CA₃ cell was determined using negative pulses delivered through the stimulating electrode. The position of the stimulating electrode was determined by moving it vertically into the slice to a location where the threshold for activation of the cell was minimal. The threshold is taken as the amount of current that is required to discharge an action potential in the cell in an all-or-none fashion in 50% of the trials. Stimulation strengths required to fire the CA₃ neurone ranged from 3-12 µA (0.05-0.2 ms duration). Control thresholds were monitored for at least 15 minutes to ensure stability before commencing an experiment. In
Positioning of stimulating and recording electrodes for excitability testing of the Schaffer collateral terminal regions of single CA3 cells. The extracellular recording electrode which was placed in the cell body layer to record all-or-none action potentials in single CA3 cells was a fibre-filled glass micropipette (tip 1-2 Μm, filled with 4 M NaCl). The stimulating electrode which was positioned in the apical dendritic region of CA1 (where the Schaffer collaterals terminate) was either a glass micropipette similar to the recording electrode, a monopolar tungsten electrode or a 4 M NaCl-filled barrel of a 7 barrel iontophoresis electrode. The current pulses used for stimulation were delivered by a constant current unit and were always negative in polarity. The threshold for activation of the test Schaffer collateral terminal regions was taken as the amount of current required to discharge an action potential in the recorded CA3 neurone in 50 percent of the trials.
each experiment, the test fibre was determined to be antidromic by infusing Ca$^{++}$-free medium (containing 1 mM Mn$^{++}$, 3 mM Mg$^{++}$) for at least 10 minutes.

3.6 Iontophoretic applications of drugs

Iontophoretic applications of drugs were done using a 6 channel Neurophore BH2 iontophoresis unit (Medical Systems Corp.). NMDLA, L-glutamate and DL-homocysteate (DLH) were applied iontophoretically, the former two compounds in the CA$_1$ apical dendritic area and the latter agent in the CA$_3$ cell body layer. The appropriate drug was filled in 3 side barrels and 4 M NaCl in the remaining barrels of a 7 barrel micropipette (tip 2-3 μm). The NaCl-containing barrels were used for recording, stimulating or current balancing. The concentrations of the NMDLA, L-glutamate and DLH (Sigma Chemicals) solutions were 100 mM each and all were ejected from their barrels by the passing of a negative charge. Backing currents (10-15 nA, positive charge) were applied to retain the drugs in their barrels and both backing currents as well as iontophoresis of drug were automatically current balanced. The effects of injected current were examined by passing negative charge of the same magnitude as that used for drug application through NaCl-filled barrels of the 7 barrel electrode.

3.7 Iontophoretic DLH-experiments

DL-Homocysteate (DLH, 20-100 nA, 3 min) was applied at the CA$_3$ cell body layer to produce a transient increase in the firing rate of these neurones. The threshold for antidromic activation of single CA$_3$ cells by stimulation at the Schaffer collateral terminal regions was monitored before and after DLH application in standard medium (Figure 7A). In some experi-
Experimental arrangement for monitoring excitability changes in the Schaffer collateral terminal regions following a high frequency activation of CA3 cell bodies by an iontophoretic application of DL-homocysteate (DLH). The barrel iontophoresis electrode was positioned in the CA3 cell body layer. The central barrel (filled with 4 M NaCl) was used for recording the all-or-none CA3 action potential. Three side barrels were filled with 4 M NaCl and were used for passing backing currents (10-15 nA) and current balancing during iontophoresis. The remaining 3 side barrels were filled with 100 mM DLH which was applied by passing a negative charge through these barrels. DLH (20-100 nA, 3 min) was applied in standard medium and Ca^{++}-free (containing 1 mM Mn^{++}) medium. The stimulating electrode was either a monopolar glass micropipette (tip 1-2 μm, filled with 4 M NaCl) or a monopolar tungsten electrode and was placed at the CA3 apical dendritic zone where the Schaffer collaterals terminate. Threshold for activation of the CA3 neurone by stimulation at the Schaffer collateral terminals was determined as the amount of current (negative pulses) needed to evoke the CA3 cell to discharge an action potential.
ments, the effects of DLH applied in Ca\textsuperscript{++}-free medium were examined on the antidromic threshold. In these cases, the perfusing medium was switched from standard medium to one containing no Ca\textsuperscript{++} (with 1 mM Mn\textsuperscript{++}, 3 mM Mg\textsuperscript{++}) for 5 minutes. DLH was iontophoresed during the last 3 minutes of this perfusion. The magnitude of the negative charge used for ejecting DLH was determined individually for each experiment, using a sustained increase in the firing rate of CA\textsubscript{3} cells as the desirable objective. Upon termination of DLH application in these experiments, threshold for the CA\textsubscript{3} cell was monitored for 30 min. DLH was again applied for 3 minutes but in the standard Ca\textsuperscript{++}-containing medium. The post-treatment threshold followed for an additional 30 min. To eliminate the possibility that the 5 minute exposure to Ca\textsuperscript{++}-free medium alone affected the Schaffer collateral terminal excitability, in some experiments the threshold was monitored during and for 15 minutes following such an exposure.

The recording/iontophoresis electrode was thought to be at the cell bodies of the CA\textsubscript{3} neurones for several reasons. Firstly, the electrode was placed in the CA\textsubscript{3} cell body layer under visual control with the aid of a microscope. Secondly, the firing rate of the recorded neurone was increased during DLH application, regardless of whether it was given in the presence or absence of Ca\textsuperscript{++}. This observation suggests a direct effect on the recorded neurone. Furthermore, neuronal somata rather than axons respond to amino acid application with an increase in firing rate. The stimulating electrode was thought to activate the Schaffer collaterals because cells could still be evoked antidromically during Ca\textsuperscript{++}-free medium perfusion. Cells that could not be evoked by such a stimulation during Ca\textsuperscript{++}-
free medium infusion, perhaps because synapses were involved between stimulating and recording sites, were not included in the results.

3.8 APV, NMDA, NMLA and NMDLA experiments

NMDLA (10-100 nA, 1-2 min) was iontophoresed onto the apical dendritic area of CA₁ (100-200 μm from cell body layer) by passing a negative charge through the electrode barrel containing the drug solution. The CA₁ population spike evoked by stratum radiatum stimulation was monitored before, during and after the drug application using a separate recording electrode placed in the cell body layer. It was assumed that NMDLA application was affecting the population of neurones recorded from if the appearance of the population spike was altered during the iontophoresis.

NMDLA (25-400 μM, 1-2 min), NMDA (50 μM, 2 min), NMLA (50 μM, 2 min) and DL-APV (25-100 μM, 4 min) were added to either standard medium or Ca²⁺-free (1 mM Mn²⁺, 3 mM Mg²⁺) medium and were applied to the whole bath. In experiments where NMDLA was applied during APV infusion, the amino acid was initiated 2 minutes after APV application began. To ensure that the population spike was abolished by Ca²⁺-free medium application before treatment with NMDLA, the former was infused for 3 minutes before commencement of NMDLA application. When tetanic stimulations were delivered to either the mossy fibre or stratum radiatum inputs during APV infusion, it was given 30 seconds before termination of the drug application. The effects of NMDLA were examined on the stratum radiatum-CA₁ and mossy fibre-CA₃ population spikes as well as Schaffer collateral terminal excitability. Because of technical difficulties in stimulating and recording, the effects of drug application on the CA₁ population spike and the Schaffer
collateral terminal excitability were done separately. In Figures 13A, 13B, 15A, 15B, 16A and 16B, six individual sets of experiments were conducted.

3.9 Presynaptic interaction experiments

Schaffer collateral terminal excitability was determined as described previously (Figure 6). The threshold for activation of a CA3 cell by stimulation at the Schaffer collateral terminals was determined as the amount of current needed to discharge the cell in 50% of consecutive attempts. The influence of the activation of other fibres on the excitability of the experimental fibre was determined by using a separate conditioning bipolar electrode placed 100-200 μm from the test stimulating electrode (Figure 8). The conditioning stimulation consisted of 5 or 10 pulses at 100 Hz delivered every five seconds. The intensity of the conditioning stimulation was varied between 60-120 μA and the duration of each of the pulses was held constant at 0.2 ms. For each experiment, it was determined that the test fibre was not discharging in response to the conditioning stimulation. In some additional experiments, the conditioning stimulation was delivered through the same electrode used for measuring the test threshold (Figure 9). The stimulus in these experiments consisted of 1, 5 or 10 pulses at 100 Hz delivered every 5 seconds. Furthermore, the stimulation intensities were also varied between 4-80 μA (duration of each pulse was 0.1 or 0.2 ms). For both sets of experiments, the interstimulus interval between the conditioning and test stimuli was varied between 10 and 300 ms. Ca++-dependence of the conditioning effect was also examined by conducting the same experiments in Ca++-free medium (containing 1 mM Mn++, 3 mM Mg++). The Ca++-free medium application also verified that the evoked spikes were anti-
Experimental arrangement for observing the effects of activity in other presynaptic fibres on the excitability of the test Schaffer collateral terminal. The recording electrode (glass, tip 1-2 μm, filled with 4 M NaCl) was placed in the CA3 cell body layer to monitor all-or-none action potentials in single CA3 cells. The test stimulating electrode (S2) which was either a glass micropipette similar to the recording electrode or a monopolar tungsten metal electrode was positioned in the apical dendrites of the CA1 area to monitor the threshold for antidromic activation of the test CA3 cell from its terminal regions. The conditioning (S1) electrode was a concentric bipolar metal electrode placed in another region of the CA1 apical dendrites (100-200 μm away from the test S2 electrode). It was established that the conditioning (5 or 10 pulses at 100 Hz) did not activate the test cell to discharge an action potential (see record at bottom of figure, negativity is down). The test threshold (S2) was determined at conditioning (S1)-test (S2) interstimulus intervals between 10-300 ms.
Experimental arrangement for monitoring excitability changes in the Schaffer collateral terminal induced by activity in the same and other nearby fibres. The recording electrode was placed in the CA3 cell body layer to record all-or-none action potentials in single CA3 cells. The conditioning/test stimulating electrode was placed in the CA1 apical dendritic area to determine the threshold for activation of the test Schaffer collateral and to deliver the conditioning stimulation (1, 5 or 10 pulses at 100 Hz). The threshold for the test cell was determined at conditioning (S1)-test (S2) interstimulus intervals between 10-300 ms. The records at the bottom of the figure show the all-or-none action potential in the test CA3 cell evoked by suprathreshold conditioning stimulation (S1, 1 pulse) followed by a test (S2) stimulation. Test threshold (S2) was determined as the amount of current required to fire the test cell in 50 percent of the attempts. Negativity is down.
dromic. The possibility of GABAergic presynaptic inhibition being responsible for the post-conditioning excitability changes was examined by conducting the experiments in the presence of 100 μM picrotoxin.

The excitability changes in the terminal of the experimental fibre produced by conditioning stimulation in stratum radiatum could be brought about by an activity-induced elevation in extracellular K⁺ content. This possibility was examined by raising the K⁺ concentration in the standard bathing medium from 3.1 mM to 4.5, 6 and 12 mM. In these experiments, NaCl was reduced by the appropriate amount to eliminate the possibility of any osmotic changes that may occur. The media containing elevated K⁺ were each infused for 1 minute and the washout time between each dose was 20 minutes. The order of the concentrations used was from the lowest to the highest. Antidromic threshold for the fibre was determined at 30 seconds during the application of the elevated K⁺ medium. Another explanation for the presynaptic interactions could be that released transmitter acts on the Schaffer collateral terminals to produce the observed excitability changes. This possibility was examined by using varying doses of L-glutamate (the suspected transmitter of the Schaffer collaterals) in the perfusing standard medium (0.2-8 mM). Each glutamate application was 1 min in duration and the time between each dose was 20 min. Starting with the lowest dose first, threshold was determined at 30 seconds after initiation of each application.

3.10 Associative-short-term potentiation (STP) and LTP experiments

These experiments were conducted in picrotoxin-containing medium to minimize inhibitory influences as well as to facilitate the induction of associative potentiation (Wigström and Gustafsson, 1983). To examine
associative conditioning effect on the population EPSP, a test bipolar stimulating electrode was positioned in stratum radiatum to evoke a "weak" population EPSP (200-600 µV) that was recorded at the apical dendritic area of CA$_1$. The conditioning bipolar stimulating electrode was placed either in another region of stratum radiatum, in the stratum oriens or in the alveus (Figure 10). "Strong" responses were evoked through the conditioning electrode: stratum radiatum EPSP was 1-3 mV; stratum oriens response consisted of a population EPSP (2-5 mV) that was interrupted by a population spike (0.5-1 mV); alvear stimulation resulted in an antidromic compound action potential of CA$_1$ cells (3-7 mV). All of these responses were recorded through the electrode placed at the CA$_1$ apical dendritic region. Paired pulse facilitation to the second stimulus pulse was observed when the test "weak" EPSP was stimulated twice with an interstimulus interval of 50 ms (Figure 10, inset a). To test for overlap between the "weak" and "strong" inputs, the "strong" input was stimulated 50 ms before the test "weak" input. If there was a facilitation of the "weak" input, it was assumed that there was overlap whereas no facilitation or a slight depression suggested no common fibres between the two inputs (Figure 10, inset b). The presence of picrotoxin in the bathing medium rules out the possibility that GABA-mediated inhibition could play a role in masking or suppressing paired pulse facilitation (unless it is due to activation of GABA$_B$ receptors). The conditioning stimulation consisted of 1, 5 or 10 trains (100 Hz, 10 pulses in each train, one train every 5 seconds). Each conditioning train was either delivered alone (unpaired, with no concurrent activation of the test input) or paired with a single stimulation of the test
Experimental arrangement for associative induction of short-term (STP) and long-term potentiation (LTP) of the CA1 population EPSP. The test population EPSP (weak response, 200-600 μV) was evoked by stimulation of stratum radiatum (S2) and recorded in the CA1 apical dendritic region. Conditioning stimulating (S1) electrodes which were used to evoke strong responses were placed either in stratum radiatum, stratum oriens or the alveus and were used for delivering 1, 5 or 10 trains (10 pulses in each train at 100 Hz, one train every 5 seconds). The conditioning trains were either paired with one single stimulation of the test (S2) input during each train or were not paired with the test input and given alone. To establish the separateness of the test (S2) and conditioning (S1) inputs, paired pulse experiments were conducted. Inset a shows paired pulse facilitation if the test input (S2) is stimulated twice in succession at an interstimulus interval of 50 ms. Inset b shows a slight suppression of the test EPSP (S2) by a prior stimulation (preceded S2 by 50 ms) of a heterosynaptic stratum radiatum input (S1), suggesting no overlap of the test and conditioning inputs. In all experiments (radiatum, oriens or alvear conditioning), the relationship between the test and conditioning inputs were examined in a similar fashion. The two inputs were considered to be separate only if the test EPSP was either not potentiated or slightly depressed using the paradigm shown in inset b. Negativity is downward in records. Calibration bars in inset b apply to both insets a and b.
input. The temporal relationship between conditioning and test inputs for the induction of associative potentiation was determined by varying the interstimulus interval between the onset of the conditioning train and activation of the test stimulus (-100 to +100 ms). For these experiments, the conditioning input was stratum oriens and the number of conditioning trains was fixed at five. Except for the temporal relationship experiments, when the conditioning stimulation was paired with the test EPSP, the latter was always evoked at 1 ms after the onset of each conditioning train. The control stimulus frequency for both the conditioning and test inputs was 0.1 Hz throughout the experiment (except when conditioning was given). Each was stimulated with a single pulse through their respective electrodes and it was arranged such that the two stimuli would alternate at 5 second intervals.

To examine if any presynaptic excitability changes accompanied STP and LTP induced by the associative paradigm described above, the threshold (determined as the amount of current required to activate the cell to discharge an action potential in 1-2 of 3 consecutive attempts) for antidromic activation of single CA3 neurones by stimulation at the Schaffer collateral terminal regions was determined before and after the unpaired and paired conditioning trains (test stimulus was given at 1 ms after onset of each conditioning train) (Figure 11). The conditioning input was either stratum radiatum or stratum oriens and it was confirmed that the unpaired conditioning trains did not activate the test CA3 neurone. As in the experiments with the EPSP above, the conditioning stimulus consisted of 1, 5 or 10 trains (each train delivered every five seconds, 10 pulses at 100 Hz in each train).
Figure 11

Experimental arrangement for the associative induction of Schaffer collateral terminal excitability alterations using conditioning stimulations that lead to associative STP and LTP of the population EPSP. A recording micro-electrode was placed in the CA3 cell body layer to monitor an all-or-none action potential in the test CA3 cell (see inset, negativity is downwards). The test stimulating electrode (S2) was positioned at the terminal regions of the Schaffer collaterals and was used for determining the threshold for antidromic activation of the test cell. The conditioning bipolar electrode (S1) was placed either in stratum oriens or stratum radiatum and was used to deliver 1, 5 or 10 conditioning trains (10 pulses at 100 Hz in each train, one train every 5 seconds). Conditioning trains were either delivered unpaired (with no concurrent activation of the test cell) or paired (at 1 ms following the onset of each train) with stimulation of the test cell. It was confirmed that during pairing, the stimulation used to evoke the test fibre (S2) was suprathreshold for action potential discharge.
3.11 Experiments involving interruption of input stimulation

As with other experiments conducted, the control frequency of stimulation to evoke a population EPSP was 0.2 Hz. The stimulating electrode was placed in stratum radiatum and the recording electrode at the CA$_4$ apical dendrites. After establishing stable controls for at least 30 minutes, the stimulation was stopped for 10 minutes and reinstated following this period. The size of the population EPSP was monitored for 30 minutes after such a treatment. To examine if any presynaptic excitability changes were associated with the alterations in the EPSP, the threshold for antidromic activation of Schaffer collateral terminals was determined before and for 30 minutes after a 10 minute "rest" period. Control threshold was stable for at least 15 minutes before the stimulation was interrupted and stimulus strength during the control and following the quiescent period was maintained at a suprathreshold level for action potential discharge (except when threshold measurements were being taken).

3.12 Elevated K$^+$ experiments

When K$^+$ was increased in the medium, the Na$^+$ content was reduced to maintain a constant molar concentration of these two ions combined. The K$^+$ concentration was increased from 3.1 mM to 20 mM in standard medium. The effects of a 10 minute exposure of the slices to high K$^+$ medium on the stratum radiatum-induced EPSP in the CA$_4$ apical dendrites and the Schaffer collateral terminal excitability were examined. A potentiation of the EPSP as well as a decrease in excitability of the Schaffer collateral terminals were observed following the exposure to high K$^+$ medium. These observations were similar to those seen during LTP (Sastry, 1982). It was of
interest to ascertain whether this decrease in terminal excitability was a result of Na\(^+\)-inactivation or hyperpolarization. Therefore, during the post-application period when the Schaffer collateral antidromic threshold was elevated (at 7-10 minutes post-application), a medium containing a slightly elevated (4.5 mM) K\(^+\) concentration (which was known in previous experiments to produce a decrease in control threshold) was infused for 1 minute and threshold determined at 30 seconds during the perfusion.

In subsequent experiments where elevated K\(^+\) doses were applied, picrotoxin-EDTA medium and Ca\(^{++}\)-free (Mn\(^{++}\) or Co\(^{++}\)) picrotoxin-EDTA medium were used as control and test media, respectively. The first reason for this choice is that picrotoxin facilitates LTP induction. If potentiation was present following Ca\(^{++}\)-free medium application, it would have been more visible if the magnitude of this potentiation was greater, especially if Co\(^{++}\) or Mn\(^{++}\) was not completely eliminated during the washout period. Secondly, the EDTA was added to facilitate the washout of Co\(^{++}\) and Mn\(^{++}\). Therefore, the entire experiment was conducted with the picrotoxin-EDTA medium as the control perfusing medium. Apical dendritic EPSPs were recorded in response to stratum radiatum stimulation. Dose response curves for elevated K\(^+\) concentrations were obtained in both control medium (5-80 mM K\(^+\)) as well as Ca\(^{++}\)-free medium (10-80 mM K\(^+\)) containing either 1 mM Mn\(^{++}\) or 1 mM Co\(^{++}\). Starting with the lowest dose, the K\(^+\) applications were given in control and Ca\(^{++}\)-free media alternately and the interval between termination of one application and commencement of the next was 30 minutes. In some experiments, for the same concentration of K\(^+\), the application in Ca\(^{++}\)-free medium preceded the application in control
medium and in others, the order was reversed. Two separate series of experiments were conducted for Mn\(^{++}\)-containing and Co\(^{++}\)-containing Ca\(^{++}\)-free media. Before each K\(^{+}\) application (whether it was given in control or Ca\(^{++}\)-free medium), the appropriate Ca\(^{++}\)-free medium was infused for 4 minutes to abolish the synaptic response and to wash out the Ca\(^{++}\) present in the control medium. The elevated K\(^{+}\) medium (in control or appropriate Ca\(^{++}\)-free medium) was then applied for 3 minutes before returning to Ca\(^{++}\)-free medium for 2 minutes (to wash out excess K\(^{+}\)) and eventually back to control medium. The Ca\(^{++}\)-free medium application before and after high K\(^{+}\) in Ca\(^{++}\)-free medium was to minimize any interaction with the Ca\(^{++}\) contained in control medium. The same treatment was applied to elevated K\(^{+}\) application in control medium so that all factors except for the Ca\(^{++}\) content during K\(^{+}\) exposure would be identical. To ascertain if exposure of the slices to Ca\(^{++}\)-free medium alone would produce any post-application changes in the population EPSP, a 10 minute infusion of this medium (containing Mn\(^{++}\) or Co\(^{++}\) in their respective experiments) was given and post-treatment records taken for 30 minutes.

3.13 \(^3\)H-Glutamate-binding studies

Tetanic stimulations were delivered through a metal bipolar electrode to produce either a post-tetanic LTP (400 Hz, 200 pulses) or homosynaptic depression (20 Hz, 600 pulses) of the CA\(_1\) neuronal response. The stimulation strength for the tetanic stimulations was fixed (150 \(\mu\)A, 0.2 ms duration, negative pulses). In these experiments, two sets of 5 slices from the same animal were used. One set was tetanized with either the low or the high frequency stimulation and the other set, which was placed in the same
bath, were not tetanized and were utilized as controls. The five slices in the experimental group were tetanized individually and the CA\(_1\) population spike was recorded in the cell body layer of the last slice to be tetanized. The post-tetanic response was monitored for 10 minutes to ensure that either LTP or homosynaptic depression was present after the appropriate tetanus before the slices were removed for Na\(^+\)-independent \(^3\)H-glutamate binding determinations. An increase in these binding sites was observed at 7 and 30 minutes post-tetanus (Baudry et al., 1980). In experiments which involved infusion of media other than the standard medium, the control and experimental sets of slices were placed in separate slice chambers. Cl\(^-\)-free medium (all Cl\(^-\) in the medium was replaced with nitrate) was infused for 5 minutes. The CA\(_1\) population spike in response to stratum radiatum stimulation was recorded in one slice of the treated group prior to, during and for 10 minutes following exposure to this medium. The treated and untreated groups of slices were then removed for Na\(^+\)-independent binding determinations. The result from each test group was paired with the result from its own control group of slices.

The synaptic membrane preparation was obtained using the method of Enna and Snyder (1975). Each group of 5 slices was homogenized (using a glass homogenizer with a teflon pestle) in 1 ml of 0.32 M sucrose by hand (20 strokes). The homogenate was then centrifuged at 1000X g for 10 minutes. The supernatant was collected, diluted fivefold with 0.32 M sucrose and recentrifuged at 14,000X g for 20 minutes. The pellet obtained (P\(_2\) fraction) was resuspended in 2 ml of cold distilled water and allowed to incubate for 40 minutes. This suspension was then centrifuged at 7000X g
for 20 minutes after which the supernatant was discarded and the pellet washed gently with cold distilled water in order to collect only the upper layer. This suspension was centrifuged at 40,000X g for 30 minutes and the supernatant was discarded. The pellet was resuspended by sonication in cold distilled water and recentrifuged at 40,000X g for 30 minutes. The final pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.4) by sonication (about 0.5-1.0 mg protein/ml).

The Na\textsuperscript{+}-independent \textsuperscript{3}H-glutamate binding assay was conducted in triplicate. Aliquots of the above synaptic membrane preparation (0.1 ml) were preincubated at 30°C for 10 minutes. Following this period, 0.1 ml of L-\textsuperscript{3}H-glutamate (Amersham, 35 Ci/mmol, final concentration 100 nM) was added. After fifteen minutes, the binding was terminated by addition of 3 ml cold buffer (50 mM Tris-HCl, pH 7.4). Each suspension was filtered under vacuum suction through a millipore filter (0.45 \textmu m pore size). The tube was rinsed with 3 ml cold buffer which was also poured over the filter. The filters were finally washed with 4 ml cold Tris-HCl buffer and allowed to drain with suction on for 5 minutes. The filtration procedure lasted less than 15 seconds and it was ensured in each experiment that the time taken to filter both the test sample and its paired control were comparable. Blanks consisted of 0.1 ml Tris-HCl buffer (50 mM, pH 7.4) without tissue and were treated in a similar fashion as the synaptic membrane preparation. Each filter was placed into a scintillation vial containing 10 ml of aqueous scintillation cocktail (ACS, Amersham). The tritium content of each sample was counted at 30% efficiency with a Packard liquid scintillation counter. According to Lynch et al. (1982), if the above assay was conducted in the
presence of 100 μM cold glutamate, the binding was not greater than that obtained for the blanks. Therefore, in this study, as with theirs, specific binding was determined as total counts for each sample minus blanks.

3.14 \(^3\)H-Glutamate accumulation studies

As with the Na\(^+\)-independent \(^3\)H-glutamate binding studies, two sets of 5 slices were used for each experiment and LTP or homosynaptic depression was produced using either a 400 Hz, 200 pulses or 20 Hz, 600 pulses tetanic stimulation, respectively, of stratum radiatum (150 μA, 0.2 ms duration, negative pulses). In some experiments, the Ca\(^{++}\)-dependence of tetanus-induced uptake changes was examined by delivering either the low or high frequency tetanus to each of the 5 experimental slices between 5 to 10 minutes of a 10 minute infusion of Ca\(^{++}\)-free medium (contains 1 mM Mn\(^{++}\), 3 mM Mg\(^{++}\)). At the same time, in a separate slice chamber, the same tetanus was delivered to each slice in the control group that was being perfused with standard Ca\(^{++}\)-containing medium. Both sets of slices were removed simultaneously for \(^3\)H-glutamate uptake measurements 10 minutes following tetanus of the last slice. Data from each test group was paired with results from its own control group for analysis.

Immediately upon their removal from the slice chamber, each group of 5 slices was incubated in standard medium (which has 147.3 mM Na\(^+\)) containing \(^3\)H-glutamate (Amersham, 35 Ci/mmol, final concentration 1 μM) to determine \(^3\)H-glutamate accumulation into whole slices (which was presumed to be due to uptake). Each group of slices was incubated for 10 minutes at 32°C with 0.5 ml of the above radioactive solution. Then, the slices were washed with 10 ml cold (4°C) buffer (50 mM Tris-HCl, pH 7.4) under suction
through a nitrocellulose filter (Millipore, pore size 0.45 μm). The vacuum was kept on for 2 minutes following the wash to eliminate excess liquid. The slices were then homogenized by hand with a glass-teflon homogenizer (20 strokes). An aliquot (0.1 ml) of the homogenate was added to a vial containing 10 ml aqueous scintillation cocktail (ACS, Amersham) to be counted by the liquid scintillation counter.

3.15 Protein determination

Protein analysis was conducted for both the Na⁺-independent binding as well as uptake studies using the method of Lowry et al. (1951) with bovine serum albumin as the standard. All samples were analyzed in triplicate and a standard curve was constructed for each experiment. 100 μl each of standard stock solutions containing 20, 40, 60, 80 and 100 μg protein/100 ml as well as 10 μl aliquots of the samples to be analyzed were added to separate test tubes kept on ice. The volume in each test tube was made up to 0.5 ml with distilled water and blanks consisted of 0.5 ml distilled water. 5 ml of Lowry's Solution A (2% Na₂CO₃, 0.02% KNa tartrate, 0.4% NaOH) containing 1% CuSO₄ was added to each tube. All samples were vortexed and left to stand at room temperature for 10 minutes. Following this incubation, 0.5 ml Folin-Ciocalteu phenol reagent (Sigma Chemicals) which was diluted 1:1 with distilled water was added. Tubes were vortexed again and the reaction was allowed to proceed for an additional 25 minutes. The protein content for the standard curve and test samples were determined by measuring the absorption of each solution at 750 nm using a Beckman ACTA C2 spectrophotometer. The standard curve of absorption vs concentration of protein was constructed and the unknown protein content in each sample
extrapolated from its absorption. Since only 10 µl of the sample was used (so that the concentration would fall within the range of the standard curve) and 100 µl of each of the standards was used for determining the curve, the actual protein concentration for the test sample expressed as mg protein/ml will be 10X the value obtained from reading off the curve. Radioactivity due to \(^3\)H-glutamate in the binding and uptake studies was corrected appropriately for protein content in the samples.

4 RESULTS

4.1 Effects of DLH application at the CA\(_3\) cell body on Schaffer collateral terminal excitability

DLH application on CA\(_3\) cell bodies (20-100 nA, 3 min) resulted in an increase in firing rate of these neurones (up to 100 Hz). The increase in action potential discharge was seen both in the presence (n = 19) as well as absence (n = 9) of Ca\(^{++}\) (1 mM Mn\(^{++}\), 3 mM Mg\(^{++}\)) in the bathing medium. The rate of cell firing promptly returned to control levels following termination of the DLH application. Subsequent to DLH application (the agent was applied during perfusion with standard medium), the response could not be recorded in the majority of cases (14 of 19 cells) for about 2 minutes post-treatment. Perhaps, prolonged DLH application produced shunting of the spike at the CA\(_3\) cell somata. Upon recovery, the threshold for spike generation from the Schaffer collateral terminals was increased as compared to control. In most cases, the initial increase in the threshold fell slightly at about 7 to 8 minutes post-treatment (but was still at a level
higher than control). At roughly 10-12 minutes post-drug, the threshold started to increase again and maintained at a new level for at least 15 minutes (Figure 12, Table 1).

The antidromic threshold was unaltered during or following an application of Ca\textsuperscript{++}-free (Mn\textsuperscript{++}-containing) medium (Table 1). As with the case of DLH application during application of standard medium, the same application in Ca\textsuperscript{++}-free medium resulted in a 2 minute post-treatment suppression of the response. However, the prolonged increase in threshold produced by application in Ca\textsuperscript{++}-containing medium was not observed.

4.2 Effects of bath application of NMDLA on the CA\textsubscript{1} population spike and Schaffer collateral terminal excitability

NMDLA (100 \textmu M, 2 min) application to the whole bath resulted in a total abolition of the CA\textsubscript{1} population spike during and immediately following the drug perfusion. This depression was succeeded by a potentiation of the response 5-7 minutes after termination of NMDLA application (population spike as a % of pre-drug control: 200 ± 15 SEM at 20 min post-NMDLA, n = 8) (Figure 13A). The CA\textsubscript{3} antidromic spike resulting from Schaffer collateral terminal region stimulation could not be evoked (presumably because the action potential in the CA\textsubscript{3} neuronal soma-dendritic region was shunted) using 5 to 10 times control threshold during and for 5 to 7 minutes following the same NMDLA application. Subsequent to this period of refractoriness, the threshold was sustained at a level above control (threshold as a % of pre-drug control: 151 ± 5 SEM at 20 min post-NMDLA, n = 8) (Figure 13B). The size of the population spike as well as the CA\textsubscript{3} antidromic threshold increase usually returned to control levels in 30-40 minutes. The
Figure 12

Effect of DL-homocysteate (DLH, 100 nA, 3 min) on the threshold for antidromic activation of a CA3 neurone, in the presence and absence of extracellular Ca++. The Ca++-free (contained 1 mM Mn++, 3 mM Mg++) medium was applied for 5 minutes and DLH was iontophoresed during the last 3 minutes of the application. Inset A shows the arrangement of the electrodes for stimulation, recording and iontophoresis. Inset B shows the antidromic response from a single CA3 neurone. Note the increase in threshold after application of DLH in standard (2 mM Ca++, 2 mM Mg++) medium and no change following application in Ca++-free medium. Threshold was measured using 0.2 ms negative pulses (evoked at 0.2 Hz, used a constant current unit) by increasing the stimulation until the cell responded with an all-or-none action potential in about 50 percent of the trials.
Table 1.

A. Effects of DLH applied on CA3 neuronal somata on the threshold for antidromic activation of the neurone at Schaffer collaterals in the CA1b area.

<table>
<thead>
<tr>
<th></th>
<th>15 min post-DLH (% of control threshold)</th>
<th>30 min post-DLH (% of control threshold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLH applied in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>normal medium</td>
<td>143.79 ± 3.50 (n = 19)\textsuperscript{a}</td>
<td>143.84 ± 3.49 (n = 19)\textsuperscript{a}</td>
</tr>
<tr>
<td></td>
<td>Range: 115 - 165</td>
<td>Range: 117 - 167</td>
</tr>
<tr>
<td>DLH applied in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}-free medium (1.0 mM Mn\textsuperscript{2+})</td>
<td>101.22 ± 1.61 (n = 9)\textsuperscript{b}</td>
<td>100.22 ± 1.13 (n = 9)\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>Range: 96 - 110</td>
<td>Range: 97 - 108</td>
</tr>
</tbody>
</table>

B. Effect of exposure to Ca\textsuperscript{2+}-free (1.0 mM Mn\textsuperscript{2+}) medium for 5 min on the threshold.

<table>
<thead>
<tr>
<th></th>
<th>2 min in Ca\textsuperscript{2+}-free (DLH was applied later) (% of control threshold)</th>
<th>4 min in Ca\textsuperscript{2+}-free (DLH was not applied) (% of control threshold)</th>
<th>15 min post Ca\textsuperscript{2+}-free (DLH was not applied) (% of control threshold)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>98.77 ± 2.38 (n = 9)\textsuperscript{b}</td>
<td>99.60 ± 1.92 (n = 10)\textsuperscript{c}</td>
<td>99.30 ± 1.66 (n = 10)\textsuperscript{c}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Both sets of data were from the same 19 experiments; \textsuperscript{b}all data were from the same 9 experiments; \textsuperscript{c}both sets were from the same 10 experiments; \textsuperscript{d}all values given are mean ± SEM. See text for more details.
Figure 13

Effects of N-methyl-DL-aspartate (NMDLA, 100 μM, 2 min) applied to the bath on the CA1 population spike and the threshold for antidromic activation of single CA3 cells from the terminal regions of Schaffer collaterals. The potentiating action of NMDLA on the population spike is shown in A. Insets show a control CA1 population spike on the left and a potentiated response at 20 minutes post-NMDLA application on the right. Calibration bars in the right record apply to both responses. An increase in the antidromic threshold of a single Schaffer collateral terminal is seen following termination of the above application (B). The upward arrows indicate the commencement of NMDLA application and the downward arrows show time of termination of the drug. Results shown in A and B are from two separate experiments.
NMDLA dose of 100 μM was chosen after doing dose-response studies (25-400 μM) and it seemed to be the concentration that produced the most consistent and reliable results, although similar observations were made with the other doses. It has to be borne in mind that the flow rate of the medium, the concentration of NMDLA and the time of exposure of the slices to the drug are critical for observation of a potentiation of the population spike. The depression of the response without any subsequent potentiation is more frequently produced by application of this amino acid and it is, therefore, important to titrate the dose and duration of application for each individual experimental arrangement. Some experiments were done using 50 μM NMDA or 50 μM NMLA instead of NMDLA. The results for the effects of NMDA on the population spike (212 ± 19% SEM of pre-drug control at 20 min post-NMDA, n = 8) and Schaffer collateral threshold change (158 ± 7% SEM of pre-drug control at 20 min post-NMDA, n = 8) were comparable to those obtained using NMDLA. However, NMLA had no significant effect on the CA1 population spike at this concentration (n = 4).

4.3 Effects of bath-application of NMDLA on the CA3 population spike

As in the case of the Schaffer collateral-CA1 population spike, an application of NMDLA (100 μM, 2 min) to the bath resulted in a complete suppression of the mossy fibre-CA3 population spike during and for 5 to 7 minutes following drug application. Following this period of refractoriness, the population spike recovered to a magnitude below that of pre-drug control (population spike as a % of pre-drug control: 77 ± 10 SEM at 20 min post-NMDLA, n = 10) (Figure 14). In a few cases (2 of 10 expts.), there was a very brief potentiation of the response at 4-5 min post-drug but this
Figure 14

Effects of bath application of NMDLA (100 μM, 2 min) on the mossy fibre-CA3 population spike. Horizontal bar above the graph shows duration of NMDLA application. Each point on graph represents mean ± SEM (n = 10).
increase quickly subsided in 2-3 min and the response was maintained at a
depressed level thereafter.

4.4 Effects of bath application of NMDLA in the presence of Ca\textsuperscript{++}-free
medium on the CA\textsubscript{1} population spike and Schaffer collateral terminal excitability

If NMDLA was applied during an exposure of the slice to a Ca\textsuperscript{++}-free
(1 mM Mn\textsuperscript{++}, 3 mM Mg\textsuperscript{++}) medium, the potentiation could not be induced
(population spike as a % of pre-drug control: 98 ± 5 SEM at 20 min post-
NMDLA, n = 8) (Figure 15A). Since the induction of the potentiation of the
population spike is blocked by Ca\textsuperscript{++}-free medium, the associated decrease
in excitability of Schaffer collateral terminals, if it plays a role in this
potentiation, should also be counteracted by this treatment. Consistent
with this idea, the application of NMDLA during infusion with Ca\textsuperscript{++}-free
medium abolishes the increase in Schaffer collateral terminal threshold
(threshold as a % of pre-drug control: 100 ± 4 SEM at 20 min post-NMDLA, n
= 9) (Figure 15B).

4.5 Effects of bath application of NMDLA in the presence of APV on the
CA\textsubscript{1} population spike and Schaffer collateral terminal excitability

When NMDLA (100 μM, 2 min) was applied in the presence of APV (100 μM,
4 min) in the bathing medium, neither the initial depression (population
spike as a % of pre-drug control: 89 ± 6 SEM at 3 min post-NMDLA, n = 8)
nor the potentiation (population spike as a % of pre-drug control: 102 ± 5
SEM at 20 min post-NMDLA, n = 8) of the population spike produced by NMDLA
could be observed (Figure 16A). The same treatment also prevented the
increase in the Schaffer collateral threshold increase induced by NMDLA
Figure 15

Blockade of the effects of NMDLA (100 μM, 2 min) on the CA3 population spike and the threshold for antidromic activation of a single CA3 cell from the terminal regions of Schaffer collaterals by its application in the presence of Ca^{++}-free medium (Mn^{++} 1 mM, Mg^{++} 3 mM). The induction of both the potentiation of the population spike (A) as well as the increase in Schaffer collateral antidromic threshold (B) are blocked. The upward arrows indicate the commencement of NMDLA application and the downward arrows show time of termination of the drug. Horizontal bars above graphs show duration of application of Ca^{++}-free medium. Results shown in A and B are from two separate experiments.
Blockade of the effects of NMDLA (100 μM, 2 min) on the CA₁ population spike and the Schaffer collateral antidromic threshold by concurrently administered APV (100 μM, 4 min). The results for the CA₁ population spike are shown in A and the results for the Schaffer collateral antidromic threshold in B. The upward arrows indicate the commencement of NMDLA application and the downward arrows show time of termination of the drug. Horizontal bars above graphs show duration of application of APV. Results shown in A and B are from two separate experiments.
(threshold as a % of pre-drug control: 98 ± 5 SEM at 20 min post-NMDLA, n = 8) (Figure 16B).

4.6 Effects of bath application of APV on tetanus-induced LTP of the CA₁ population spike

When a tetanic stimulation (400 Hz, 200 pulses) was delivered to the stratum radiatum input during the last 30 seconds of APV application (100 µM, 4 min) to the bath, LTP could not be induced (population spike as a % of control: 88 ± 6 SEM at 20 min post-tetanus, n = 8). However, when the same tetanic stimulation was given in standard medium without APV 30 minutes after the first tetanus, LTP could be reliably elicited (population spike as a % of control: 183 ± 10 SEM at 20 min post-tetanus, n = 8) (Figure 17A). Lower doses of APV (25 µM, 4 min) were also effective in blocking the induction of LTP (n = 4). It should be noted that APV (25 or 100 µM) itself frequently produced a slight suppression of the CA₁ population spike (82 ± 3% SEM of control at 1 min in APV, 7 of 10 expts.) and in 4 of 10 experiments produced a post-application potentiation of the response (125 ± 4% SEM of control at 10 minutes post-APV).

4.7 Effects of bath application of APV on tetanus-induced LTP of the CA₃ population spike

In contrast to the results obtained in the CA₁ region, APV had no significant effect on either the control CA₃ population spike or the induction of LTP in this area. Following a tetanic stimulation (400 Hz, 200 pulses) to the mossy fibre input during infusion with APV (100 µM, 4 min), LTP could be induced (population spike as a % of control: 164 ± 7 SEM at 20 min post-tetanus, n = 8) (Figure 17B). The magnitude of LTP obtained for
Figure 17

Effects of APV (100 μM, 4 min) on the induction of LTP in the stratum radiatum-CA1 and mossy fibre-CA3 population spikes. The results for the CA1 population spike are shown in A and those for the CA3 population spike are shown in B. The arrows in the graphs indicate times at which tetanic stimulations (400 Hz, 200 pulses) were delivered to the stratum radiatum (A) or mossy fibre (B) inputs. Horizontal bars above the graphs show duration of application of APV. The results shown are from two separate experiments.
the mossy fibre-CA$_3$ system in the absence of APV treatment in a separate set of experiments was not significantly different (p > 0.2) from that obtained when the tetanus was given in the presence of APV (population spike as a % of control: 161 ± 10 SEM at 20 min post-tetanus, n = 8).

4.8 Iontophoretic applications of NMDLA at the CA$_1$ apical-dendritic zone

Since an application of NMDLA to the bath produced a potentiation of the Schaffer collateral-CA$_1$ population spike, it was of interest to examine if this potentiation could be a result of changes at the synaptic level. Furthermore, an application of NMDLA to the bath could be mimicking a tetanic stimulation to stratum radiatum by inducing an increase in the firing rate of CA$_3$ cells. Therefore, it was decided to apply NMDLA locally to the apical dendritic area of CA$_1$. An application of NMDLA (100 µM, 1-2 min) at the apical dendritic zone of CA$_1$ produced a rapid depression of the population spike during its iontophoresis (population spike as a % of control: 10 ± 4 SEM at 3 min post-NMDLA, n = 8). The response recovered over 5-6 minutes following termination of the drug. After this period of recovery, there was a potentiation of the population spike (160 ± 4% SEM of pre-drug control at 20 min post-NMDLA, n = 6) which gradually recovered to control size in 30-40 minutes. These findings are in support of those reported earlier by Collingridge et al. (1983b). Low doses (10-40 nA) of NMDLA at the synaptic zone were sufficient to produce the initial depression without any subsequent potentiation (n = 8) but higher doses (100 nA) were required to elicit the potentiation of the population spike (n = 6). When the effects of iontophoretic application at the CA$_1$ apical dendritic/synaptic zone were examined on the threshold for antidromic activation of
single Schaffer collateral terminals, it was found that NMDLA (100 nA, 1-2 min) induced a slight drop (10-15%) in threshold during the drug ejection for a brief period of about 10 seconds but was succeeded by a long-lasting post-application elevation of the threshold (146 ± 6% SEM of pre-drug control at 20 min post-NMDLA, n = 8) (Figure 18). As in the case of inducing potentiation of the response by the NMDLA application in the synaptic zone, a low dose of the amino acid (10-50 nA) failed to reliably induce the presynaptic change whereas a higher dose (100 nA) was more consistent in producing this effect. At no time during or after the iontophoretic application of NMDLA on the Schaffer collateral terminals did the CA3 cell become unevokable by stimulation at the terminals. This observation suggests that NMDLA perfusion, which produces a period of refractoriness of the CA3 cell during and for 5-7 minutes following the application, probably does not act on the terminals to render the cell unevokable but, rather, may be causing some change in the CA3 cell body so that it cannot sustain an action potential.

4.9 Interactions among presynaptic terminals in the CA1 region

4.9.1 Conditioning through a separate electrode. The experimental arrangement is shown in Table 2A. A conditioning tetanus (S1) of 5 pulses at 100 Hz resulted, in the majority of cases, in a decrease in the test Schaffer collateral terminal threshold (S2) for 10-300 ms post-conditioning (Table 2B). An increase in the conditioned threshold was observed in one experiment at the interstimulus delay of 300 ms (179% of unconditioned control, 1 of 13 slices). As the intensity of the conditioning (S1) stimulation was increased, the conditioned threshold (S2) of the fibre was de-
Figure 18

NMDLA-induced increase in the threshold for activation of a single Schaffer collateral from its terminal regions. NMDLA (100 nA, 1 min) was applied iontophoretically at the apical dendritic/synaptic area of CA1. The upward arrow indicates commencement and the downward arrow shows termination of the ejection. Inset A illustrates the experimental arrangement. Inset B is a record of the all-or-none action potential recorded from one cell in the CA3 cell body layer evoked by stimulation at the terminal regions of the Schaffer collaterals. Negativity is down.
Table 2. EFFECTS OF VARIOUS CONDITIONING STIMULATIONS ON THE THRESHOLD FOR ACTIVATION OF SINGLE SCHAFFER COLLATERAL TERMINALS

<table>
<thead>
<tr>
<th>S1-S2 Delay (ms)</th>
<th>10</th>
<th>50</th>
<th>100</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. Decrease in S2 Range</td>
<td>72-95</td>
<td>77-94</td>
<td>81-99</td>
<td>91-102</td>
</tr>
<tr>
<td>threshold (%)</td>
<td>Mean±SEM</td>
<td>87.5±2.0</td>
<td>85.2±1.5</td>
<td>90.4±2.0</td>
</tr>
<tr>
<td>uncond. control</td>
<td>n</td>
<td>13</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>C. S2 threshold at</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1-S2 delay</td>
<td>Range</td>
<td>86-98</td>
<td>81-98</td>
<td>77-95</td>
</tr>
<tr>
<td>50 ms (%)</td>
<td>Mean±SEM</td>
<td>93.7±2.1</td>
<td>91.4±2.3</td>
<td>87.5±2.7</td>
</tr>
<tr>
<td>uncond. control</td>
<td>n</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
</tbody>
</table>

E. Decrease in S2 Range | 65-99 | 73-93 | 79-101 | 92-105 |
| threshold (%) | Mean±SEM | 85.0±1.6 | 85.1±0.9 | 90.7±0.9 | 99.2±0.5 |
| uncond. control | n | 27 | 29 | 29 | 25 |
| F. S2 threshold at | | | | |
| S1-S2 delay | Range | 96-101 | 75-96 | 67-92 | 69-89 |
| 50 ms (%) | Mean±SEM | 98.6±0.4 | 86.7±1.4 | 80.4±2.0 | 76.7±1.8 |
| uncond. control | n | 17 | 17 | 15 | 15 |

A. Experimental set-up to illustrate positioning of stimulating and recording electrodes for the data shown in B and C. Conditioning (S1) stimulus consisted of 5 pulses at 100 Hz. B. The threshold for antidromic activation of a single Schaffer collateral in response to the test (S2) stimulus was determined at conditioning intervals ranging from 10-300 ms. C. The stimulation strength of S1 was progressively increased and S2 threshold was determined at a conditioning interval of 50 ms. D. Experimental set-up for the data shown in E and F. Conditioning (S1) stimulus consisted of 1 pulse. E. S2 threshold was determined at interstimulus intervals ranging from 10-300 ms following a suprathreshold (for spike generation) S1 stimulus. F. As in C above.

* p < 0.01 when compared to unconditioned control using paired t-test. ** The difference between the two sets of data denoted by this letter is significant to p < 0.02. ***The difference between the two sets of data denoted by this letter is significant to p < 0.01.
creased in a graded manner (Table 2C). In contrast, when the number of pulses in the conditioning train was increased from 5 to 10 (frequency of the train was kept constant at 100 Hz), there were more instances where the S2 threshold was increased instead of decreased (244 ± 31% SEM of control at 50 ms interstimulus interval, 3 of 7 expts; 230 ± 17% SEM of control at 300 ms interstimulus interval, 5 of 7 expts.). The results are summarized in Table 3. To examine the Ca ++-dependence of the conditioning effect on the test threshold, Ca ++-free medium (containing 1 mM Mn ++, 3 mM Mg ++) was infused for 10 minutes before repeating the experiment at an interstimulus interval of 50 ms. The results show that the decrease in conditioned (S2) threshold produced by the conditioning (S1) was counteracted (Table 4).

4.9.2 Conditioning through the test electrode. The experimental arrangement is shown in Table 2D. The conditioning consisted of one pulse that was suprathreshold for action potential discharge in the test fibre (except in experiments where the number of conditioning pulses was increased). Essentially, the results obtained for this series of experiments were similar to those using a separate electrode for conditioning. The conditioned threshold (S2) following the conditioning stimulus (S1) was usually decreased for 10-300 ms (Table 2E) but in two instances exhibited increases at the interstimulus interval of 300 ms (172-288% of unconditioned control, 2 of 27 slices). When the strength of the conditioning stimulus was increased, there was a graded decrease in the conditioned threshold (Table 2F). When the number of conditioning pulses was increased to 10 (given at a frequency of 100 Hz), however, there was a tendency towards an increase rather than a decrease in threshold (210 ± 16% SEM of unconditioned control
### Table 3. Effect of Increasing the Number of Pulses in Each Conditioning Train on Schaffer Collateral Terminal Excitability.

<table>
<thead>
<tr>
<th>Number of conditioning (S1) pulses at 100 Hz</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>S2 threshold at S1-S2 delay of 50 ms (% of unconditioned control)</td>
<td>none</td>
<td>188-294</td>
</tr>
<tr>
<td>S2 threshold at S1-S2 delay of 300 ms (% of unconditioned control)</td>
<td>179</td>
<td>183-278</td>
</tr>
</tbody>
</table>

$S1 = $ five or ten pulses at 100 Hz
Table 4. Effect of Ca\textsuperscript{++}-Free Medium Exposure on the Conditioned Threshold.

<table>
<thead>
<tr>
<th>S1-S2 Delay (ms)</th>
<th>Range</th>
<th>50</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S1-S2 Delay (ms)</td>
<td>Range</td>
<td>73-95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S2 threshold</td>
<td>Mean ± SEM</td>
<td>84.7±1.8*</td>
</tr>
<tr>
<td>(% of control)</td>
<td>n</td>
<td>8</td>
</tr>
<tr>
<td>10 min in Ca-free medium (with 1mM Mn, 3mM Mg)(% of control)</td>
<td>Range</td>
<td>87-102</td>
</tr>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>98.8±2.0*</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>8</td>
</tr>
</tbody>
</table>

* The difference between the two sets of data denoted by this symbol is significant to p < 0.01.
at 50 ms conditioning interval, 7 of 8 slices). The conditioning effect on the Schaffer collateral threshold was also determined to be Ca\(^{++}\)-dependent because the decrease in conditioned threshold (S2) was counteracted in Ca\(^{++}\)-free medium (98 ± 2 % SEM of unconditioned control at 50 ms conditioning interval, n = 10). Upon statistical analysis using the paired t-test, results obtained in Ca\(^{++}\)-free medium were not significantly different from unconditioned controls (p > 0.2) but were significant (p < 0.01) when compared to corresponding conditioned thresholds (at 50 ms interstimulus interval) of the same fibres when the experiment was conducted in standard Ca\(^{++}\)-containing medium. To examine if GABAergic presynaptic inhibition plays a role in the presynaptic interactions, the experiment was repeated in the presence of 100 µM picrotoxin (added to standard medium). The results suggest that conventional presynaptic inhibition is not involved (Figure 19, n = 4).

4.10 Elevated extracellular K\(^{+}\) and glutamate on the Schaffer collateral antidromic threshold

In most cases, the threshold for the test fibre was decreased with the lowest concentration (4.5 mM) of elevated K\(^{+}\) (76 ± 4% SEM of control at 30 seconds during K\(^{+}\) application, 5 of 6 expts.) and was increased in all experiments at the highest concentration (12 mM) (242 ± 28% SEM of control at 30 seconds during K\(^{+}\) application, 7 of 7 expts.). At the intermediate dose (6 mM), however, both effects could be seen (81 ± 7% SEM of control at 30 seconds during K\(^{+}\) application, 2 of 7 expts.; 207 ± 30% SEM of control at 30 seconds during K\(^{+}\) application, 5 of 7 expts.; when all the results obtained for 6 mM K\(^{+}\) application were pooled, the value was not significantly different from control). These results are summarized in Table 5. The lower concentrations of glutamate (0.2-0.5 mM)
Failure of picrotoxin (100 μM) to counteract the increase in Schaffer collateral terminal excitability produced by paired pulse stimulation. The conditioning stimulus pulse was always suprathreshold for action potential discharge in the test cell. The test threshold was measured between 5-200 ms following the conditioning pulse. The curve with filled circles illustrates results obtained for the experiment conducted in standard medium and the graph with filled diamonds represents the results of the same experiment repeated in the presence of 100 μM picrotoxin in standard medium. Each point on graph is mean ± SEM (n = 4).
Table 5. Effects of Raising Extracellular K\(^+\) Concentration on the Schaffer Collateral Terminal Excitability.

<table>
<thead>
<tr>
<th>Concentration of K(^+) in medium</th>
<th>4.5</th>
<th>6</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in threshold</td>
<td>64 - 89</td>
<td>74 - 88</td>
<td></td>
</tr>
<tr>
<td>(% of pre-treatment control)</td>
<td>5 of 6 expts.</td>
<td>2 of 7 expts.</td>
<td></td>
</tr>
<tr>
<td>Increase in threshold</td>
<td>122 - 315</td>
<td>155 - 400</td>
<td></td>
</tr>
<tr>
<td>(% of pre-treatment control)</td>
<td>5 of 7 expts.</td>
<td>7 of 7 expts.</td>
<td></td>
</tr>
</tbody>
</table>
produced no change whereas higher concentrations used (1-6 mM) resulted in decreases in threshold (Table 6). Doses higher than 6 mM could not be used for bath application because of shunting of the spike at the CA3 cell body. When this occurred, the response could not be recorded. To overcome this problem, glutamate was iontophoresed at the stimulating site (200 nA, 3 min). Results obtained were similar to those of bath application (threshold as a % of pre-drug control: 70 ± 4 SEM at 1 min during glutamate application, n = 19; 58 ± 4 SEM at 3 min during glutamate application, n = 19). With the bath application of glutamate, it appears that the firing rate of CA3 cells is increased at concentrations of 200 μM (n = 6) whereas in most cases, the Schaffer collateral threshold excitability is not affected until the concentration attains a minimum of 1 mM (n = 12).

4.11  Associative short-term potentiation (STP) and LTP

4.11.1 Stratum radiatum conditioning. Conditioning trains delivered to stratum radiatum that were not paired with the test EPSP (1, 5 or 10 trains, 10 pulses at 100 Hz in each train, one train every 5 seconds) often resulted in a depression of the test EPSP (85 ± 4% SEM of control at 60 seconds post-10 train unpaired tetanus of radiatum, 7 of 8 expts.) (Figure 20A). However, if the test EPSP was evoked once (at 1 ms following the onset of conditioning train) during each conditioning train, a short-term potentiation (STP, population EPSP as a % of control at 60 seconds post-10 train paired conditioning by radiatum: 184 ± 8 SEM, 6 of 8 expts.) followed by LTP (population EPSP as a % of control at 15 min post-10 train paired conditioning by radiatum: 162 ± 5 SEM, 6 of 8 expts.) of the test response could be observed. Usually, with 1 to 5 paired trains, STP, which lasted 2
Table 6. Effects of Bath Application of Glutamate on Schaffer Collateral Terminal Excitability.

<table>
<thead>
<tr>
<th>Glutamate concentration</th>
<th>0.5 mM</th>
<th>1.0 mM</th>
<th>2.0 mM</th>
<th>4.0 mM</th>
<th>6.0 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schaffer collateral threshold as a % of pre-drug control</td>
<td>99 ± 1.5</td>
<td>96 ± 0.8</td>
<td>94 ± 1.5</td>
<td>90 ± 2.2</td>
<td>89 ± 2.3*</td>
</tr>
<tr>
<td>n</td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

*Values are expressed as mean ± SEM.
Figure 20

Associative induction of STP, LTP and the reduction in the Schaffer collateral terminal excitability. (A) The schematic diagram on the left illustrates the experimental arrangement. A bipolar test stimulating electrode (S₂) was positioned in the stratum radiatum and a bipolar conditioning stimulating electrode (S₁) was positioned in another area of the stratum radiatum. A recording microelectrode (containing 4 M NaCl) was positioned in the apical dendritic area of CA₁ neurones to monitor the test EPSP evoked at 0.2 Hz (stimulation strength was adjusted to obtain a response between 300-600 µV). The conditioning stimulation strength was adjusted to evoke a population EPSP of 1-3 mV in size. If a twin stimulation of S₂ (50 ms interval) resulted in a facilitation of the second population EPSP (see inset, left) and if a stimulation of S₁ preceding S₂ stimulation by 50 ms resulted in no facilitation of the second population EPSP (see inset, right), then the S₁ and S₂ stimulations were presumed to activate separate input fibres. In all experiments, the effect of unpaired conditioning trains (UC; i.e., the test stimulation was off during the conditioning; each conditioning train contained 10 pulses at 100 Hz) and of paired conditioning trains (PC; i.e., the test stimulation was on 1 ms after the onset of each train) were examined on the test population EPSP. During the first 3 minutes after UC or PC, the response was monitored every 15 seconds and at all other times at 30 second intervals. The graph on the right shows results from one experiment. Note STP after 1 and 5 PCs, and LTP after 10 PCs.

(B) Effects of the conditioning on the excitability of the terminal region of a Schaffer collateral. A monopolar test stimulating electrode (S₂) was positioned in the apical dendritic area of the CA₁ neurones to activate (0.2 ms negative pulses, 3-10 µA, 0.2 Hz) the terminal regions of Schaffer collaterals so that antidromic all-or-none action potentials (see inset) could be recorded from the CA₃ cell bodies. A conditioning stimulation electrode (S₁) was positioned in the stratum radiatum and the unpaired (UC) and paired (PC) conditioning trains were applied as described in A. It was confirmed that the conditioning stimulation did not activate the test Schaffer collateral. During the PC, the stimulation strength to antidromically activate the test Schaffer collateral was increased to 2 times control to make sure that the fibre was activated during PC. A similar activation of the test fibre without the presence of the conditioning produced no changes in the excitability of the test fibre (results not shown). The amount of current required to produce an all-or-none action potential was taken as that which induced a spike in 1-2 of 3 consecutive attempts. In the graph to the right of the schematic diagram, recordings taken at 30 second intervals were plotted. Note that 1 and 5 PCs induced a 3 minute decrease while 10 PCs induced a prolonged decrease in the excitability of the test fibre terminal.

Results in (A) and (B) were from different experiments.
AMOUNT OF CURRENT TO ACTIVATE SCHAFER COLLATERAL TERMINAL AS A % OF CONTROL

TEST POPULATION EPSP AS A % OF CONTROL

TIME (min)

-100  0  10  20  30  40  50  60
to 3 minutes could be induced repeatedly without observing of LTP whereas increasing the number of paired trains to 10 resulted in STP followed by LTP (Figure 20A). The induction of LTP appeared not to be an all-or-none phenomenon but, rather, a graded one. LTP could usually be induced using between 5 to 10 paired trains and the magnitude as well as duration of the potentiation increased with an increasing number of paired trains. The results from experiments using 10 paired trains were quantified because this paradigm appeared to be the optimal one for producing maximal LTP and further increasing the number of trains often produced a depression rather than facilitation of the test EPSP.

The excitability of the Schaffer collateral terminals was determined following 1, 5 or 10 paired and unpaired conditioning trains to stratum radiatum (Figure 20B). It was confirmed that the test neurone was not activated by the conditioning trains. The unpaired conditioning trains did not produce any significant change in the test threshold (98 ± 3% SEM of control at 60 seconds post-5 unpaired trains, 7 of 7 expts.; 99 ± 2% SEM of control at 60 seconds post-10 unpaired trains, 7 of 7 expts.). Paired conditioning trains, however (amount of current to stimulate test cell was set at a suprathreshold level for action potential discharge, pairing occurred at 1 ms following onset of each conditioning train), resulted in a post-conditioning decrease in excitability, which is reflected by an increase in threshold (threshold as a % of control at 60 seconds post-5 paired trains: 185 ± 6 SEM, 6 of 7 fibres). Increasing the number of paired conditioning trains eventually led to a long-lasting decrease in the Schaffer collateral terminal excitability (threshold as a % of control at 15 min post-10 paired trains: 154 ± 5 SEM, 5 of 6 fibres) (Figure 20B).
4.11.2 Stratum oriens-conditioning. Unpaired conditioning trains (10 pulses at 100 Hz, one train every 5 seconds) delivered to stratum oriens produced a post-tetanic depression of the test stratum radiatum-induced population EPSP (Figure 21). When the conditioning trains were paired with the test EPSP (at 1 ms after the onset of each train), a post-conditioning potentiation could be seen (population EPSP as a % of control at 60 seconds post-5 paired trains: 121 ± 4 SEM, 5 of 5 expts.). The temporal relationship between the test EPSP and the conditioning train for the induction of STP was determined. In order to elicit STP, the test stimulation could not precede the onset of the conditioning train by greater than 50 ms or fall more than 80-90 ms after the beginning of the conditioning tetanus (Figure 21).

The decrease in excitability of Schaffer collateral terminals observed with stratum radiatum conditioning was also seen following stratum oriens conditioning (threshold as a % of control at 60 seconds post-5 paired trains by oriens: 138 ± 6 SEM, 5 of 6 expts.). As before, unpaired conditioning trains did not produce any threshold changes (threshold as a % of control at 60 seconds post-5 unpaired trains of oriens: 99 ± 3 SEM, 6 of 6 expts.)

4.11.3 Alveus-conditioning. Conditioning stimulation to the alveus produced similar effects to stratum radiatum or stratum oriens conditioning on the test EPSP (Table 7).

4.12 Effects of a transient interruption of input stimulation on the EPSP and Schaffer collateral terminal excitability

Control stimulation frequency of stratum radiatum was 0.2 Hz. A 10 minute period of non-stimulation of this input resulted in a potentiation of
The limits of the temporal relationship between conditioning and test stimuli for the induction of associative potentiation. The test EPSP was evoked by stimulation of stratum radiatum and the conditioning was achieved through stimulation of stratum oriens (5 trains, 10 pulses in each train at 100 Hz, one train every 5 seconds). One test stimulus was paired with each of the five conditioning trains at every interstimulus interval examined. The conditioning-test interval was varied between -100 to +100 ms. A negative delay indicates that the test stimulus preceded the onset of the conditioning train and a positive delay indicates the time at which the test population EPSP was evoked following the onset of the conditioning tetanus. Each point on the graph (filled circles) represents mean ± SEM of the test population EPSP magnitude measured at 1 minute post-5 paired trains. The one point represented by the filled diamond shows a significant depression of the test EPSP at 1 minute post-5 unpaired trains of stratum oriens (i.e., test EPSP was not evoked during conditioning). n is shown in parentheses above each point on the graph.
Table 7. Post-Conditioning Potentiation Induced by Pairing Tetanic Trains of the Alveus with a Single Stimulation of the Test Input.

<table>
<thead>
<tr>
<th>Time post-paired conditioned tetanus</th>
<th>60 s</th>
<th>15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test population EPSP</td>
<td>146 ± 7 SEM</td>
<td>133 ± 5 SEM</td>
</tr>
<tr>
<td>as a % of control</td>
<td>n = 6</td>
<td>n = 4</td>
</tr>
</tbody>
</table>
the CA1 population EPSP which lasted 15 to 20 minutes upon return to the control stimulation (Figure 22A). To ascertain if this potentiation was associated with any changes in Schaffer collateral terminal excitability, the threshold for antidromic activation of CA3 cells (Figure 6) was monitored before and after the 10 minute "rest" period. Results indicate that the potentiation of the EPSP is not accompanied by any excitability changes of the Schaffer collateral terminals (Figure 22B).

4.13 Effects of elevated extracellular K+ on the EPSP and Schaffer collateral terminal excitability

An exposure of the slices to a medium containing 20 mM K+ for 10 minutes resulted in an initial potentiation of the response (at 30 seconds to 1 minute during application) followed by a rapid suppression that lasted for the duration of the application and for about 1 minute following its termination. This period of non-responsiveness was succeeded by a potentiation of the population EPSP that lasted roughly 20 minutes (Figure 23A). The same elevated K+ exposure caused an initial drop in the Schaffer collateral terminal threshold during the application at a time that corresponded to the increase in the EPSP size. This increase in excitability lasted for about 30 seconds and very soon after, the fibre was not evokable with stimulation strengths of 3 to 5 times control. As with the EPSP, the spike in the CA3 cell did not begin to recover until 1 minute after return to standard medium. The initial phase of recovery was characterized by a delay to onset of the antidromic spike of 2-3 ms but this effect was short-lived as the action potential regained its original pre-drug latency in 1-2 minutes, presumably because the excess K+ was eliminated. The
Figure 22

Effects of a 10 minute interruption of input stimulation on the magnitude of the test population EPSP and Schaffer collateral terminal excitability. The control rate of stratum radiatum stimulation to evoke a population EPSP was 0.2 Hz. Following an interruption of this control stimulus frequency with a 10 minute "rest" period, the EPSP was potentiated (A). This potentiation was not associated with any changes in Schaffer collateral terminal excitability (B). The bars above the graphs show the time at which the input was not stimulated and each point on graphs represent mean ± SEM (n = 10 for each set of experiments).
Effects of elevated extracellular K\(^+\) (20 mM, 5 min) on the CA\(_1\) population EPSP and Schaffer collateral terminal excitability. Following the high K\(^+\) exposure, the population EPSP was potentiated (A) and this potentiation was associated with an increase in the Schaffer collateral terminal threshold (i.e., a decrease in excitability) (B). The bars in the graphs represent the duration of 20 mM K\(^+\)-containing medium exposure to the whole bath. Each point on graphs is mean ± SEM (n = 10 for each set of experiments).
post-treatment threshold was at a level above control for about 20 minutes (Figure 23B).

Since high K\(^+\) treatment results in a post-application potentiation of the EPSP and, like LTP, is associated with a decrease in presynaptic terminal excitability, these two potentiations may share a common mechanism. The presynaptic excitability change could be due to several factors and it was of interest to determine if either Na\(^+\)-inactivation or a hyperpolarization could account for it. It was determined in previous experiments (Table 5) that an elevation of K\(^+\) in the bathing medium from 3.1 mM to 4.5 mM produces an increase in excitability (reflected as a decrease in threshold) of Schaffer collateral terminals. If the prolonged decrease in excitability of Schaffer collateral terminals which is associated with the potentiation of the EPSP is due to Na\(^+\)-inactivation, one would expect the depolarizing effect of raising extracellular K\(^+\) to produce more inactivation and, hence, a further elevation of the threshold. In contrast, if the excitability decrease is a result of a hyperpolarization of the terminals, then it is reasonable to assume that the K\(^+\)-induced depolarization will cause a decrease in threshold. The results show a fall in the threshold by treatment of the slice with 4.5 mM K\(^+\)-containing medium during the period of decreased excitability (threshold as a % of pre-K\(^+\) control at 30 seconds during 4.5 mM K\(^+\) perfusion: 73 ± 7 SEM, 8 of 10 expts.). This observation, although not confirming that the decrease in excitability is due to hyperpolarization, certainly supports this premise and rules out the possibility of Na\(^+\)-inactivation as a mechanism.
4.14 Ca\(^{++}\)-dependence of K\(^{+}\)-induced potentiation of the EPSP

In preliminary studies using standard medium, it was found that synaptically-driven responses never recovered to control size following exposure of the slices to Ca\(^{++}\)-free (Mn\(^{++}\) or Co\(^{++}\)-containing) media, presumably because these Ca\(^{++}\)-antagonists were not totally eliminated. Therefore, in this series of experiments, picrotoxin (10 µM) and EDTA (200 µM) were added to all media, the former to facilitate LTP induction (Wigström and Gustafsson, 1983) and the latter to hasten the removal of Mn\(^{++}\) and Co\(^{++}\). Elevation of extracellular K\(^{+}\) (10-80 mM) during perfusion with Ca\(^{++}\)-free (Mn\(^{++}\)) medium resulted in a dose-dependent potentiation of the population EPSP at 15 minutes following termination of the application. The same treatment (5-80 mM K\(^{+}\)) when administered in control Ca\(^{++}\)-containing medium, failed to produce any potentiation at 15 minutes post-treatment (enhancement of the response was seen, however, prior to 15 minutes but this did not appear to be sustained). Rather, the response exhibited a tendency towards depression, especially with the higher doses (Figure 24). Exposure of the slices to a 9 minute infusion of Ca\(^{++}\)-free (Mn\(^{++}\)) medium (with normal K\(^{+}\) concentration of 3.1 mM) resulted in a slight post-treatment potentiation of the response (population EPSP as a % of control at 15 min post-Mn\(^{++}\): 111 ± 3 SEM, n = 8). To establish that the K\(^{+}\)-induced potentiation was due to the Ca\(^{++}\)-blocking effect of Mn\(^{++}\) and not due to a property peculiar to that ion, the experiment was conducted with Ca\(^{++}\)-free medium containing 1 mM Co\(^{++}\). Similar results were obtained with medium containing this Ca\(^{++}\)-antagonist at a high K\(^{+}\) dose (80 mM) (population EPSP as a % of control at 15 min post-Co\(^{++}\): 142 ± 14 SEM, n = 5)
The post-treatment potentiation and depression of the population EPSP induced by application of elevated $K^+$ in the absence (1 mM Mn$^{++}$, 7 mM Mg$^{++}$) and presence (4 mM Ca$^{++}$, 4 mM Mg$^{++}$) of extracellular Ca$^{++}$, respectively. The graph for $K^+$ exposure in Ca$^{++}$-free medium is shown by the filled diamonds and the graph for $K^+$ application in Ca$^{++}$-containing standard medium is shown by the filled circles. The extracellular $K^+$ was increased from 3.1 to 5-80 mM in Ca$^{++}$-containing medium and 10-80 mM in Ca$^{++}$-free medium. All $K^+$ applications (3 minutes duration) were preceded by (4 minutes) and followed by (2 minutes) infusions of Ca$^{++}$-free (1 mM Mn$^{++}$, 7 mM Mg$^{++}$) medium. Note that there is a definite increase in the population EPSP size for increasing $K^+$ concentrations when applied in Ca$^{++}$-free medium and, although not statistically significant, a trend towards a decrease with increasing $K^+$ concentrations applied in Ca$^{++}$-containing medium. Each point on graph represents mean ± SEM of population EPSP size measured at 15 minutes post-exposure to elevated $K^+$. $n$ is shown by the number in parentheses above each point.
but lower K+ concentrations (10-40 mM) failed to produce consistent potentiation of the EPSP. Furthermore, Ca++-free (Co++) application for 9 minutes (containing normal K+ of 3.1 mM) also failed to produce the post-application potentiation exhibited following Ca++-free (Mn++) administration (population EPSP as a % of control at 15 min post Co++: 93 ± 3 SEM, n = 4). The recovery rate of the EPSP appeared to be much faster following Mn++ than Co++ application. Perhaps, EDTA chelates Mn++ better than Co++ and, therefore, preferentially facilitates the washout of the former. If Co++ is not eliminated rapidly and completely, it is not surprising that post-application potentiation of the EPSP may not be observed. However, if the potentiation is sufficiently large to overcome the depression caused by the residual Ca++-antagonist, then one may be able to see it.

4.15 Na+-independent-3H-glutamate binding

Following a high frequency (400 Hz) tetanic stimulation to stratum radiatum, LTP of the population spike is present. However, there seems to be no change in the Na+-independent 3H-glutamate binding (presumed to be due to glutamate receptors by Baudry and Lynch [1980a]) at 10 minutes post-tetanus (Table 8). However, when a low frequency (20 Hz) tetanus is delivered to the input, a post-tetanic homosynaptic depression of the population spike accompanied by an increase in the 3H-glutamate binding results (Table 8). Similarly, a depression of the population spike ten minutes following a transient exposure of slices to Cl−-free medium is associated with an increase in binding (Table 9). Note that the population spike is markedly potentiated during infusion with Cl−-free medium but
Table 8. Na⁺-Independent $^3$H-Glutamate Binding Following Tetanic Stimulation of Stratum Radiatum.

<table>
<thead>
<tr>
<th></th>
<th>10 min post-20 Hz, 600 pulses</th>
<th>10 min post-400 Hz, 200 pulses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population spike</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( %of control)</td>
<td>Range</td>
<td>Mean ± SEM</td>
</tr>
<tr>
<td></td>
<td>34 - 59</td>
<td>210 - 335</td>
</tr>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>42 ± 5*</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>10 slices</td>
</tr>
</tbody>
</table>

Na⁺-Independent

$^3$H-glutamate binding ( % of unstimulated control) | Range     | Mean ± SEM | n |
|                                                     | 110 - 135 | 121 ± 2*   | 10 groups† |
|                                                     | 92 - 103  | 98 ± 2     | 10 groups† |

* p < 0.01 when compared to untetanized control using paired t-test.
† Each group has 5 slices.
Table 9. Na⁺-Independent \(^3\)H-Glutamate Binding Following A Transient Exposure to Cl⁻-Free Medium.

<table>
<thead>
<tr>
<th></th>
<th>4 min in Cl⁻-free medium</th>
<th>10 min post-Cl⁻-free medium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population spike</td>
<td>Range</td>
<td>205 - 456</td>
</tr>
<tr>
<td>(% of pre-treatment</td>
<td>Mean ± SEM</td>
<td>356 ± 3*</td>
</tr>
<tr>
<td>control)</td>
<td>n</td>
<td>10 slices</td>
</tr>
</tbody>
</table>

\(^3\)H-Glutamate binding following exposure to Cl⁻-free medium (% of untreated control)

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>123 - 144</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SEM</td>
<td>131 ± 2⁺</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10 groups⁺</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.01 when compared to pre-treatment control using paired t-test.

⁺ p < 0.01 when compared to untreated control using paired t-test.

⁺⁺ Each group has 5 slices.
that this increase is not sustained following the termination of the application (Table 9).

In some experiments (n = 4 slices), the stimulating site was kept constant and the recording electrode was moved to various positions between the CA_2 and CA_1a regions along the cell body layer of the slice. It was discovered that responses of substantial size could be detected from all the recording sites. This observation suggests that postsynaptic activation due to the present method of stimulation is extensive and it is probably unnecessary to use multiple stimulating sites to activate an "adequate" number of input fibres as was done by Lynch et al. (1982). Furthermore, the demonstration of a reliable increase in $^3$H-glutamate binding following a low frequency tetanus but not following a high frequency tetanus (although the stimulating electrode, stimulation sites and stimulation strengths were the same in both cases), indicates that the lack of change in binding following the high frequency tetanus was not due to an inadequate activation of input fibres.

4.16 $^3$H-Glutamate accumulation into whole slices

$^3$H-Glutamate accumulation into slices following a high frequency (400 Hz) LTP-inducing tetanus of stratum radiatum is decreased at 10 minutes post-tetanus (Table 10). Wieraszko (1983) and I, in this study, presumed that the accumulation of radioactivity into slices reflects the activity of a Na⁺-dependent glutamate uptake process. Since the induction of LTP appears to be Ca^{++}-dependent (Dunwiddie and Lynch, 1979; Wigström et al., 1979), I decided to examine if the reduction in presumed uptake can account for LTP by delivering the high frequency tetanus in the absence of extracel-
Table 10. $^3$H-Glutamate Accumulation Into Slices Following A High Frequency (400 Hz, 200 pulses) Tetanic Stimulation of Stratum Radiatum.

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Mean ± SEM</th>
<th>n</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. $^3$H-Glutamate accumulation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>into slices (% of unstimulated control)</td>
<td>25 - 80</td>
<td>60 ± 5*</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td><strong>B. $^3$H-Glutamate accumulation following tetanus in Ca-free (1 mM Mn, 3 mM Mg) medium</strong></td>
<td>16 - 86</td>
<td>53 ± 8†</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

In A, $^3$H-glutamate accumulation into tetanized slices was expressed as a % of accumulation into untetanized control slices. In B, $^3$H-glutamate accumulation into slices tetanized in Ca$^{++}$-free medium was expressed as a % of accumulation into slices tetanized in normal medium.

* $p < 0.01$ when compared to unstimulated control using paired t-test.
† $p < 0.01$ when compared to control tetanized in normal medium using paired t-test.
‡ Each group has 5 slices.
lular Ca^{++} (medium contained 1 mM Mn^{++}, 3 mM Mg^{++}). The results obtained from this experiment indicate that there is even a further reduction in the uptake when the tetanus is given in Ca^{++}-free medium (Table 10).

When a low frequency (20 Hz) tetanus is given to the stratum radiatum input in the presence of standard medium, there is in most cases an increase in \(^{3}\text{H}\)-glutamate accumulation (210 ± 23\% SEM of unstimulated controls at 10 minutes post-tetanus, 7 of 12 groups of 5 slices each). There was no change in 1 of 12 and a decrease in 4 of 12 groups (68 ± 10\% SEM of unstimulated controls at 10 minutes post-tetanus).

5 DISCUSSION

5.1 Ca^{++}-dependence of LTP

It is widely believed that the induction of LTP requires the presence of extracellular Ca^{++} (Dunwiddie and Lynch, 1979; Wigström et al., 1979). The evidence provided by the two studies quoted above involve delivering tetanic stimulations to an input while synaptic transmission is blocked by the removal of Ca^{++} from the bathing medium. It has been established in the present study on the associative induction of LTP that a postsynaptic activation is required during input stimulation to successfully elicit the potentiation (Figure 20). Therefore, in the studies of Dunwiddie and Lynch (1979) and Wigström et al. (1979), LTP induction may have been counteracted because of lack of postsynaptic depolarization during the tetanic stimulation (due to blockade of evoked transmitter release during perfusion with
Ca\(^{++}\)-free medium) and not, as the authors claim, due to Ca\(^{++}\)-dependence. The experiments in the present study involving elevated K\(^+\) in Ca\(^{++}\)-free medium (Figure 24) support the hypothesis that LTP induction does not require Ca\(^{++}\). The K\(^+\) applied to the whole bath produces depolarization of both the presynaptic terminal and the postsynaptic neurones to fulfill the requirements for triggering LTP. In contrast to the potentiation of the EPSP following treatment of the slice with high K\(^+\) in Ca\(^{++}\)-free medium, the same doses of K\(^+\) applied in Ca\(^{++}\)-containing medium appear to produce a depression (Figure 24). It was previously suggested that homo- and heterosynaptic depression is a postsynaptic phenomenon that is brought about by Ca\(^{++}\) influx into CA\(_1\) neurones (Chirwa et al., 1983; Sastry et al., 1984a). It is possible that the progressive depression seen following administration of increasing K\(^+\) concentrations in the presence of Ca\(^{++}\) is similar to that observed during tetanus-induced homo- and heterosynaptic depression. Perhaps, the induction of LTP simply requires concurrent pre- and postsynaptic depolarization to activate a voltage-sensitive process that is independent of Ca\(^{++}\). The observation that elevated K\(^+\) application in the absence of extracellular Ca\(^{++}\) results in a greater post-treatment potentiation of the EPSP than its application in the presence of Ca\(^{++}\) may be explained simply by postulating that the Ca\(^{++}\)-induced depression of the EPSP masks or overshadows any potentiation that may have developed. Alternatively, the presence of Ca\(^{++}\) may actually retard the development of LTP itself and it is possible to induce this phenomenon in standard Ca\(^{++}\)-containing medium, not because of the availability of Ca\(^{++}\) but rather, in spite of it.
A transient elevation of extracellular Ca\(^{++}\) from 2 to 4 mM results in a post application potentiation of the CA\(^1\) population spike and EPSP (Turner et al., 1982). The authors, who suggest that this potentiation is similar to that seen during LTP also show that there is a prolonged increase in the Ca\(^{++}\) levels in the slices following such a treatment (Baimbridge and Miller, 1981). The potentiating effect of Ca\(^{++}\) in their studies may have been due to slow elimination of the ion from the slices. It is possible that Ca\(^{++}\) produced charge screening of membranes or persisted in the tissue to result in increased transmitter release. The observed effects may, therefore, be due to the residual Ca\(^{++}\) rather than a Ca\(^{++}\)-triggered alteration which in itself does not require elevated Ca\(^{++}\) for maintenance.

Their observations on the magnitude of the CA\(^1\) antidromic population spike due to alvear stimulation and on the responsiveness of CA\(^1\) neurones to iontophoretically applied glutamate suggests that increased levels of the ion are present up to 30 minutes post-treatment. Furthermore, intracellular Ca\(^{++}\) concentrations can still be higher than control at 60 minutes post-treatment. The treatment used by Turner et al. (1982) did not always produce a depression of the synaptic response, although they reported that this occasionally occurred. Their study differs from the present one in that they raised extracellular Ca\(^{++}\) and not extracellular K\(^+\). As mentioned previously, they raised extracellular Ca\(^{++}\) from 2 to 4 mM whereas the Ca\(^{++}\) concentration in the standard medium used in my study was 4 mM. Perhaps, Ca\(^{++}\) entry into postsynaptic neurones to produce the depression is greatly facilitated by activation of voltage-sensitive channels. This could explain the apparent discrepancy between their results and the observations reported here.
The slight potentiation of the population EPSP observed following exposure of the slice to Ca\(^{++}\)-free (Mn\(^{++}\)-containing) medium with "normal" K\(^+\) concentration (3.1 mM) resembles in magnitude and time course that seen after interruption of control input stimulation (discussed in a later section). Since synaptic transmission is blocked during the Ca\(^{++}\)-free medium perfusion, it would be quite analogous to the situation of non-stimulation. Perhaps, these two potentiations share similar mechanisms.

5.2 NMDA receptor involvement in LTP

Since Collingridge et al. (1983b) suggested that NMDA receptors are involved in the induction of LTP, NMDLA and the purported NMDA "antagonist", APV, were used in the present study to examine this possibility. There were essentially no differences between the actions of NMDLA and NMDA on the stratum radiatum-CA\(_1\) neuronal system so it is probably safe to assume that the results obtained for NMDA would have been similar to those obtained using NMDLA here.

Bath application as well as iontophoretic application at the CA\(_1\) apical synaptic zone of NMDLA or NMDA induced a post-application potentiation of the population spike as well as a decrease in the excitability of Schaffer collateral terminal regions, suggesting that the Schaffer collateral-CA\(_1\) neuronal synaptic zone is a site of action for the amino acid.

NMDLA application in the absence of extracellular Ca\(^{++}\) (medium contains 1 mM Mn\(^{++}\), 3 mM Mg\(^{++}\)) resulted in no post-application potentiation of the population spike and also no increase in the Schaffer collateral antidromic threshold. The most obvious explanation is, of course, that the induction of these changes is Ca\(^{++}\)-dependent. However, other
considerations have to be taken into account. It was reported that elevated Mg\(^{++}\) concentrations produce a voltage-dependent block of ion channels activated by NMDA (Mayer et al., 1984; Nowak et al., 1984). By raising the Mg\(^{++}\) concentration in the extracellular medium from 2 mM in standard medium to 3 mM in Ca\(^{++}\)-free medium, one could produce a greater degree of blockade of the NMDA receptor-coupled channel. In addition, Mn\(^{++}\) also interferes with the NMDA-induced conductance increase (Dingledine, 1983a, 1983b). Apparently, the NMDA-induced depolarization of neurones does not result from an increase in Ca\(^{++}\) conductance (Mayer et al., 1984; Nowak et al., 1984) because Cd\(^{++}\) does not interfere with the inward current. As postulated previously, NMDA-induced potentiation, like elevated K\(^{+}\)-induced potentiation could be triggered by some Ca\(^{++}\)-independent voltage-sensitive process. If this is the case, then activation of NMDA receptors per se is not necessary for the potentiation and the amino acid could just serve as an agent that produces the necessary depolarization.

As evidenced by the different NMDLA doses (iontophoretic) required to elicit depression (low doses) compared with potentiation of the population spike and the associated decrease in presynaptic terminal excitability (larger doses), it seems that the early depression can be more easily produced. Perhaps, there are either a fewer number of NMDA-preferring receptors present or the affinity of these receptors may be different on the terminals than on CA\(_1\) neurones so that higher doses of NMDLA may be required to activate a sufficient number to trigger events leading to potentiation. Another explanation is that the postsynaptic depolarization produced by NMDLA when coupled with presynaptic depolarization (as in the case
of elevated K⁺ medium exposure) or input activation induces the potentiation of the population spike and the associated presynaptic excitability change.

Based on the present results, it is proposed that either presynaptic NMDA receptors can mediate an LTP-like state or activation of postsynaptic NMDA receptors results in sufficient postsynaptic depolarization to trigger the associative interactions leading to the presynaptic change. This proposal regarding a presynaptic mechanism for NMDLA-induced potentiation is supported by Lynch et al. (1985). The decrease in presynaptic terminal excitability that is associated with LTP may bring about an enhancement of stimulus-evoked transmitter release. The exact mechanism of this presynaptic change has yet to be determined. The possibilities that exist to produce a decrease in excitability include a hyperpolarization, Na⁺-inactivation, an increase in capacitance, an increase in resting conductance or an increase in membrane resistance associated with a hyperpolarization. Of the above suggestions, only a hyperpolarization or an enhancement of membrane resistance resulting in a hyperpolarization would be conducive for increasing transmitter release from presynaptic terminals. Evidence provided (discussed later) suggests that Na⁺-inactivation is not responsible for the presynaptic change and supports the hyperpolarization theory. Although the present results suggest a presynaptic component in NMDLA-induced potentiation of the CA₁ population spike, a possible additional postsynaptic involvement cannot be excluded. It is possible that the trigger for the long-lasting change in synaptic efficacy occurs at the subsynaptic level and this event then somehow influences the presynaptic terminal to result in the
sustained increase in transmission. Conversely, the opposite could be true in that a presynaptic trigger causes a long-lasting postsynaptic alteration to maintain LTP.

It was hypothesized (Wigström and Gustafsson, 1984; Wigström et al., 1985) that a prolonged extracellular negativity (in the order of 100 ms) recorded in the apical dendrites of CA1 neurones due to input activation plays a role in LTP induction because picrotoxin which facilitates LTP development (Wigström and Gustafsson, 1983) also potentiates this wave. They suggested that this wave is a result of activation of NMDA receptors because APV appears to counteract it. Collingridge (1985) hypothesized that the voltage-dependent block of NMDA receptors by Mg$^{++}$ during normal synaptic transmission is reduced during depolarization resulting from tetanic stimulation of the input. This temporary "unblocking" of the NMDA system can then trigger changes leading to LTP. It has been established that the induction of synaptic potentiation does not require extracellular Ca$^{++}$. If this wave is involved in LTP induction, then it is probably not due to a Ca$^{++}$ conductance. The ability of acetylcholine to produce a slow excitation of hippocampal neurones and neocortical neurones due to activation of muscarinic receptors has been reported (Biscoe and Straughan, 1966; Krnjević et al., 1980; Krnjević and Phillis, 1963; Krnjević and Ropert, 1981; Straughan, 1975). The sensitivity of the negative wave to muscarinic antagonists like atropine should be examined. Furthermore, it will be interesting to investigate the effect of muscarinic agonists and antagonists on the development of LTP.
The observed effects of APV on applied NMDLA in the CA\textsubscript{1} region suggests that the "antagonist" counteracted the actions produced by the amino acid. It has been reported that D-APV is a selective "antagonist" at NMDA receptors in the hippocampus (Collingridge et al., 1983a, 1983b, 1984; Harris et al., 1984). According to Collingridge et al. (1984), D-APV does not affect the size of the synaptically evoked response whereas L-APV suppresses it. However, a recent report (King and Dingledine, 1985) suggests that D-APV may first activate and then block NMDA receptors. A partial agonist property of either the D- or the L- isomer of APV could explain why the drug suppresses the population spike, albeit weakly, during application and also why a post-application potentiation of the population spike occasionally results (Sastry et al., 1984b). These effects are also seen with the agonists NMDLA and glutamate (Chirwa et al., 1984). Furthermore, it has been observed that a tetanic stimulation delivered to stratum radiatum during application of the agonist, glutamate, produces a profound suppression of the population spike (Chirwa et al., 1984). Similarly, a tetanus given in the presence of APV also produces a depression of the population spike (Sastry et al., 1984b). It would have been preferable to use D-APV in the present studies but, unfortunately, I did not have access to this drug. The term "antagonist" when applied to APV has to be used with caution because the agent could very well be a partial agonist. In a previous study, it was reported that LTP could not be seen if tetanic stimulation of stratum radiatum was given during iontophoretic application of APV in the apical dendritic area of CA\textsubscript{1} neurones and that a weak LTP could, in fact, be observed if verapamil was applied along with APV (Sastry et al., 1984b).
LTP induced in the presence of verapamil appears to be quantitatively larger than in control situations (Sastry et al., 1984a). Presuming that iontophoretic application of APV did not reach all sites of generation of LTP, it is possible that verapamil potentiated LTP at these sites.

The results concerning the action of APV on the induction of LTP confirm earlier reports (Collingridge et al., 1983b; Harris et al., 1984) that this drug blocks the development of tetanus-induced LTP in the CA1 region (Figure 17). It is interesting, however, that 25 µM APV was effective in counteracting the induction of LTP in the CA1 region but 100 µM was insufficient to do so in the CA3 area. Furthermore, the observation that NMDLA application does not produce a post-treatment potentiation of the CA3 population spike suggests different mechanisms for LTP in the CA1 and CA3 areas.

Based on reports in literature, it appears reasonable to suggest that LTP in the CA1 region is mediated by NMDA receptors. However, one has to bear in mind that there are certain inconsistencies between the characteristics of tetanus-induced LTP and NMDLA-induced potentiation of the population spike. For instance, a profound depression of the population spike following NMDLA application always occurs prior to any observable potentiation. In contrast, LTP induced by high frequency tetanic stimulation does not exhibit any appreciable post-tetanic depression (Sastry et al., 1984a). It is possible that the effects caused by NMDLA are dose-dependent and careful adjustment of the concentration could, conceivably, result in a state that more closely resembles that obtained during LTP. NMDLA could have produced a profound shunting of the spike due to excessive depolarization. Other
arguments against the NMDA theory are supplied by the results obtained with elevated $K^+$ applications in $Ca^{++}$-free medium (Figure 24). Firstly, the $Ca^{++}$-free medium in these experiments contained 7 mM $Mg^{++}$ and 1 mM $Mn^{++}$ which probably results in a more effective blockade of NMDA receptor channels than $Ca^{++}$-free medium used in the NMDLA experiments (contained 3 mM $Mg^{++}$, 1 mM $Mn^{++}$) and yet the former treatment resulted in a potentiation of the synaptic response whereas the latter did not. Secondly, assuming that NMDA receptor activation takes place following transmitter (glutamate) release, it is improbable that NMDA receptors are involved in elevated $K^+$-induced potentiation because the absence of $Ca^{++}$ in the medium would necessarily mean a severely reduced or an abolition of both evoked and resting release of neurotransmitter.

All previous studies in support of the NMDA theory for LTP were conducted in the $CA_1$ region (Collingridge et al., 1983b; Harris et al., 1984). The results in the present study indicating that the induction of LTP in the $CA_3$ region is not counteracted by APV and the failure of NMDLA to produce a potentiation of the $CA_3$ population spike suggest that the NMDA theory is not viable here either.

In view of the arguments against the involvement of NMDA receptors in LTP, it is unclear why APV blocks tetanus-induced LTP in the $CA_1$ area. Perhaps, this drug has effects other than NMDA receptor blockade which can account for the observed results. In conclusion: 1) NMDLA produces a potentiation of the population spike, an effect that is associated with and has a similar time course to a reduction in the presynaptic Schaffer collateral terminal excitability; 2) both the potentiation and the presynaptic
excitability change are blocked if NMDLA is applied in Ca\(^{++}\)-free medium; 3) the actions of NMDLA on the CA\(_1\) population spike and Schaffer collateral terminal are counteracted by APV; 4) the induction of LTP produced by a tetanic stimulation to stratum radiatum in the CA\(_1\) region but not the mossy fibres in the CA\(_3\) area is blocked by APV; 5) NMDLA does not produce a potentiation of the CA\(_3\) population spike. In the present study direct evidence is provided for a presynaptic change during NMDLA-induced potentiation of the CA\(_1\) neuronal response. It is proposed that this potentiation produced by NMDLA is a result of a voltage-sensitive process which triggers a sustained increase in transmitter release from Schaffer collateral terminals.

5.3 **Interactions among presynaptic terminals in the CA\(_1\) region**

The results indicate that presynaptic fibres in the CA\(_1\) apical dendritic region "communicate" with each other (Table 2). Since the test fibre does not have to be activated to see a threshold change, the interaction is not necessarily a post-spike phenomenon in the test fibre but, rather, could be an indirect interaction with surrounding fibres. The results obtained using two different experimental paradigms (i.e., separate conditioning electrode to activate other fibres or conditioning delivered through the test electrode to activate the test fibre and other surrounding fibres) were essentially similar, suggesting that the mechanisms for mediating excitability changes in the test fibre, whether or not it was discharging during conditioning were the same. The decreases in conditioned threshold were more marked with increasing stimulus strengths. However, when the stimulation intensity was kept constant and the number of conditioning pulses was
increased, the test threshold exhibited a tendency towards an increase rather than a further decrease (Table 2, Table 3). This apparently paradoxical observation can be explained if we postulate that the interaction among fibres is due to an activity-induced elevation in extracellular K$^+$. Table 5 illustrates the effects of various concentrations of extracellular K$^+$ on the Schaffer collateral terminal threshold. The lowest dose produces predominantly a decrease in threshold whereas the highest dose results in an increase in threshold. Perhaps, progressive increases in extracellular K$^+$ levels result in a graded depolarization of fibres until a certain point where further elevation of the ion produces a depolarization-induced decrease in excitability of the terminals (e.g., Na$^+$-inactivation or hyperpolarization due to activation of a Na$^+$-pump). The most intense depolarization resulting from the K$^+$ buildup should be at an area closest to the site of release and the depolarizing effect should diminish as one moves away from this region. Repetitive activation of the same fibres by increasing the number of conditioning pulses could result in a marked elevation of K$^+$ locally at the stimulating site. However, increasing the stimulus intensity would tend to activate more fibres over a wide area and, presumably, the K$^+$ buildup is not as great. This explanation would be consistent with the observed effects of elevated K$^+$ on the Schaffer collateral threshold. If released K$^+$ is the cause for the observed presynaptic excitability changes, the releasing source for this ion is unknown. The possibilities include the presynaptic fibres themselves, postsynaptic CA$_1$ neurones or glial cells (MacVicar, 1984).
The time course for the increase in terminal excitability following conditioning is remarkably similar to that of GABA-mediated primary afferent depolarization in the spinal cord (Eccles, 1964; Eccles et al., 1963a, 1963b). To speculate that GABAergic presynaptic inhibition may be responsible for these hippocampal interactions is an attractive proposition but it is unlikely because 100 μM picrotoxin does not counteract this process (Figure 19) and also presynaptic inhibition has not been demonstrated in the hippocampus. Even if the conventional type of "presynaptic inhibition" involving interneurones may not be present, there is no reason to rule out other modes of transmitter-mediated inhibition. For instance, it is possible that transmitter liberated by neuronal discharge acts directly on the terminals of the active as well as other passive fibres nearby to alter their excitability.

As mentioned above, an alternate hypothesis for the cause of the activity-induced Schaffer collateral terminal excitability changes is that neurotransmitter released by neuronal discharges acts on the active as well as surrounding passive fibres. The results for the experiments involving bath application and iontophoretic application of glutamate suggest that this is, indeed a possibility. It is puzzling, however, that at all doses used, the amino acid produced an increase in excitability of the presynaptic terminals and decreases in excitability were never seen. Perhaps, the concentrations of glutamate used were not as high as concentrations that would be attained in the synaptic cleft during synaptic transmission. It is also possible that the transmitter-induced decrease in excitability has a very short time course and precedes the decrease in excitability or the
receptors involved are those that desensitize (e.g., NMDA receptors [Fagni et al., 1983; Murali Mohan and Sastry, 1985]). Using the experimental methods in the present study, the earliest time at which threshold was determined was at 30 seconds following initiation of the glutamate application. If the duration of the glutamate-induced increase in threshold was in the order of milliseconds to several seconds, then the paradigm used would not have recognized it. The prolonged negative wave of Wigström et al. (1985) evoked by input stimulation which has a time course of roughly 100 ms may be responsible for the decreases in threshold of Schaffer collateral terminals seen following conditioning. Since the wave is thought to be due to NMDA receptor activation, there is a requirement for transmitter release to occur before it can be elicited. There may be a depolarization in the terminal that is set-up due to presynaptic NMDA receptor activation and it will be interesting to see if APV blocks the effects of conditioning on the Schaffer collateral terminal excitability.

Omission of extracellular Ca\(^{++}\) counteracts the decrease in conditioned threshold (Table 4). The reason for this is not entirely clear. Firstly, it is possible that the process is Ca\(^{++}\)-dependent. Secondly, it could be that synaptic transmission, or at least postsynaptic depolarization, is required. Removing Ca\(^{++}\) from the bathing medium could have blocked several processes: 1) K\(^{+}\) release from the postsynaptic cell due to synaptic transmission (which could be a major source of the increase in extracellular concentration of the ion); 2) Ca\(^{++}\)-dependent K\(^{+}\) conductance from pre- or postsynaptic elements (Krnjević and Lisiewicz, 1972; Sastry, 1979a) or glial cells (MacVicar, 1984); 3) glutamate release from
presynaptic terminals so that there is no feedback of the amino acid on these sites.

5.4 Associative STP and LTP

The associative nature of LTP has been established in the hippocampus (Barrionuevo and Brown, 1983; McNaughton, 1982; McNaughton et al., 1978; Robinson and Racine, 1982). In the previous section, it was established that presynaptic terminals in the CA1 region "communicate" with each other. Perhaps, these interactions could be involved in the associative induction of STP and LTP. The results obtained indicate that both STP and LTP can be induced without a tetanic stimulation to the test input but it is essential that an activation of the test input occurs during tetanic stimulation of a heterosynaptic input or during an antidromic tetanus of the postsynaptic cells.

It is widely believed that post-tetanic potentiation (PTP) in various systems, including the hippocampus is a presynaptic phenomenon (del Castillo and Katz, 1954; Eccles and Krnjević, 1959b; Hubbard and Willis, 1962; Lloyd, 1949; Magleby and Zengel, 1975, 1976; McNaughton, 1982; Takeuchi and Takeuchi, 1962; Wall and Johnson, 1958). During PTP in the spinal cord, the presynaptic terminal excitability is decreased (Wall and Johnson, 1958) and this change has been attributed to a hyperpolarization (Eccles and Krnjević, 1959b; Sastry, 1979a, Wall and Johnson, 1958). In the present study, the presynaptic terminal excitability is decreased not only during LTP but also during STP (which may be the same phenomenon as PTP). The close association of the decrease in presynaptic excitability with the potentiation of the EPSP (Table 20) suggests a cause and effect relationship. Therefore, it is
not unreasonable to postulate that both STP and LTP are quantitatively dis-
similar but share the same mechanisms of induction.

Both the presynaptic excitability change as well as the potentiation
of the EPSP are absent following unpaired conditioning, but are seen follow-
ing paired conditioning trains. This finding suggests that the locus res-
ponsible for the potentiation is the presynaptic test input but the
mechanism to trigger this change is postsynaptic. It is possible that input
activation has to be paired with sufficient postsynaptic depolarization to
produce the observed potentiation. Consistent with this idea, picrotoxin,
which blocks postsynaptic inhibition (and causes a depolarization of the
postsynaptic cell), facilitates the induction of LTP (Wigström and
Gustafsson, 1983). Reducing postsynaptic depolarization during tetanic
stimulation of the input by iontophoretic application of GABA, tetrodotoxin
or pentobarbital onto CA1 cell bodies (Scharfman and Sarvey, 1985) or by
injecting intracellular hyperpolarizing currents (Malinow and Miller, 1986)
resulted in a blockade of LTP development. The communication between pre-
synaptic terminals and postsynaptic neurones could be achieved through
ephaptic interactions, electrotonic coupling by gap junctions, a buildup in
extracellular K+ or some other agent. Since it has been determined that
the induction of LTP does not require extracellular Ca++, the likelihood
that released transmitter plays a role in the interaction is minimal.

Activation of the presynaptic fibre could elicit a "subliminal facilita-
tory process" in the terminal which cannot trigger changes leading to
facilitated transmitter release in itself but is capable of doing so if it
is paired with adequate postsynaptic depolarization. Sastry et al. (1986)
has demonstrated that pairing intracellular depolarizing pulses in CA₁ neurones with activation of stratum radiatum can produce LTP of the stratum radiatum-CA₁ population EPSP. The prolonged dendritic negativity that occurs following input stimulation (Wigström and Gustafsson, 1984; Wigström et al., 1985) could be the "subliminal process" in the presynaptic terminals or it could be the conditioning postsynaptic depolarization. The duration of the negative wave is several times longer than that of the population EPSP. The increase in excitability of Schaffer collateral terminals caused by conditioning of other fibres is roughly 200 ms in duration (Table 2) and could very well be due to this same process.

In order to induce STP in the stratum radiatum EPSP by pairing it with the stratum oriens conditioning train, the test EPSP has to be evoked between 50 ms before and 80 ms after the onset of the conditioning train (Figure 21). I propose that STP can only be elicited if the "subliminal process" in the test presynaptic terminal overlaps the depolarization of the postsynaptic neurones caused by the conditioning tetanus train. Assuming that the "subliminal process" is about 100 ms in duration, the observation that associative STP can be produced if the test EPSP evoked at 50 ms prior to the onset of the conditioning train is easily explained. The population EPSP evoked by a single stratum oriens stimulation has a time course of roughly 20 ms. It is possible that a conditioning train delivered to stratum oriens (10 pulses at 100 Hz) will produce spatial and temporal summation of successive EPSPs to result in a summated EPSP of larger size and prolonged time course. Perhaps, this summated EPSP provides the adequate postsynaptic depolarization that lasts in the order of 80-100 ms to account
for the observation that STP can be elicited even though the stratum radiatum test input is stimulated 80 ms following the onset of the stratum oriens conditioning train. Figure 25 is a schematic illustration of this hypothesis. Bear in mind that the temporal requirements for induction of STP were determined using a synaptic input as the conditioning stimulus. In this case, even though the time course of the stratum oriens EPSP is prolonged by the tetanus, it will inevitably decay to baseline eventually. If one could maintain an adequate level of depolarization of the postsynaptic neurones (e.g., by intracellular current injection), perhaps, it will be possible to extend the temporal limit indefinitely so that the determining factor for the time course is the duration of the depolarization. Of course, this would be possible only in the case of the test stimulus occurring following onset of the conditioning stimulation as the duration of the "subliminal process" will determine the limits for the opposite paradigm (when the test stimulus precedes the conditioning).

5.5 Presynaptic involvement in LTP

It has been demonstrated that LTP is associated with an increase in evoked neurotransmitter release (Bliss et al., 1985; Dolphin et al., 1982; Skrede and Malthe-Sørensen, 1981). LTP of the perforant path-granule cell synapse is associated with a reduction in the presynaptic terminal excitability (Sastry, 1982). In the present study, the relationship between potentiation of the synaptic response and the presynaptic excitability change is further examined. A previous study has shown that application of an excitatory agent locally on the cell bodies of CA₁ neurones produces a post-application potentiation of the CA₁ population spike (Goh and Sastry,
Figure 25

Schematic illustration of the hypothetical mechanism responsible for determining the temporal requirements for induction of associative STP. The top trace is a record of the summated EPSPs due to the stratum oriens conditioning train. The bottom records denoted by A and B represent the "subliminal process" in the presynaptic terminal for the induction of associative STP. The position of the waves in A and B show the maximal intervals (before and after the onset of the conditioning train, respectively) at which the "subliminal process" can occur for successful induction of associative STP.
1983). The experiments involving high frequency activation of CA3 cells by iontophoresis of DLH onto CA3 cell bodies (which mimics a tetanic stimulation to the Schaffer collaterals) produced a decrease in the Schaffer collateral terminal excitability. In previous experiments (Sastry, 1982), a tetanic stimulation was delivered through the stimulating electrode and it was possible that electrode properties were altered to result in erroneous interpretations of threshold changes. The present study rules out this possibility because no high frequency stimulation was given through the electrode used for monitoring threshold. It is interesting that DLH application in Ca\textsuperscript{++}-free medium did not produce any prolonged changes in presynaptic excitability. One could invoke Ca\textsuperscript{++}-dependence, but in view of the findings that LTP induction does not require Ca\textsuperscript{++} but does require concurrent postsynaptic depolarization (discussed earlier), this observation is easily explained. Obviously, in the absence of synaptic transmission during infusion of Ca\textsuperscript{++}-free medium, there is no activation of the postsynaptic cells. Therefore, if the decrease in presynaptic terminal excitability is to be responsible for LTP, it should not be inducible in Ca\textsuperscript{++}-free medium using the experimental paradigm above.

The experiments involving elevated extracellular K\textsuperscript{+} effects on the population EPSP and Schaffer collateral threshold (Figure 23) illustrate the close relationship with regard to magnitude and time course of potentiation of the EPSP and decrease in Schaffer collateral terminal excitability. Therefore, it is believed that the presynaptic excitability change is somehow responsible for the potentiation of the EPSP. As postulated earlier, the increased extracellular K\textsuperscript{+} probably produces a depolarization in both
pre- and postsynaptic elements to trigger long-term changes in the properties of the presynaptic terminal which eventually translates to increased transmitter release. The nature of the presynaptic excitability change is largely unknown. The present studies, however, have eliminated the possibility of Na\(^+\)-inactivation because an elevation of extracellular K\(^+\) (which produces a depolarization) during the phase of increased threshold (i.e., decreased excitability) results in a decrease in the threshold rather than a further increase.

There are several possible mechanisms that can account for the presynaptic change leading to LTP. It is generally thought that post-tetanic potentiation (PTP) of the monosynaptic reflex in the spinal cord is mediated by a hyperpolarization of the primary afferent terminals (Eccles and Krnjević, 1959b; Lloyd, 1949; Sastry, 1979a; Wall and Johnson, 1958). The reduction in excitability of presynaptic terminals that is associated with LTP (Sastry, 1982) may be a reflection of a hyperpolarization (Wall, 1958) of these terminals. Short-term post-tetanic potentiation and LTP in the hippocampus appear to be two separate phenomena whose properties and requirements for induction are dissimilar (Dunwiddie and Lynch, 1979; McNaughton, 1982). It is possible that the events leading to the development of PTP and LTP are different. However, they may both still be explained by a common end result, that is, a hyperpolarization of the terminals. If LTP, like PTP, is sustained by a hyperpolarization of the terminals, then this presynaptic change would have to increase transmitter release. It has been reported that a hyperpolarization of presynaptic terminals leads to an increase in the size of the action potential (Eccles and
Krnjević, 1959a, 1959b; Lloyd, 1949) which in turn results in an enhancement of evoked release of transmitter (Hubbard and Willis, 1962; Takeuchi and Takeuchi, 1962). The underlying mechanism for generating a hyperpolarization could be the activation of an electrogenic Na\(^+\)-pump (McDougal and Osborn, 1976), an increase in resting potassium conductance or some other unknown cause. However, an increase in K\(^+\) conductance may not result in increased transmitter release. It has been reported that glutamate, the suspected transmitter between Schaffer collaterals and CA\(_1\) neurones (Storm-Mathisen, 1977a) when applied on hippocampal neurones, produces a post-application hyperpolarization of neuronal somata that is brought about by an increase in Na\(^+\)-pump activity (Segal, 1981). Perhaps, the transmitter released during a tetanic stimulation acts on the presynaptic terminal to enhance the activity of a Na\(^+\)-pump located here.

Aside from a hyperpolarization of the presynaptic terminals, an increase in transmitter release during LTP may be accomplished by other long-lasting alterations. An increase in the resting membrane resistance associated with a hyperpolarization can produce a larger spike in the terminal. In primary afferent terminals in the spinal cord, it appears that the driving force for Cl\(^-\) ions is in an outward (depolarizing) direction (Curtis et al., 1977; Eccles et al, 1963b; Gmelin, 1978). If a similar situation exists in the hippocampal presynaptic terminals, then a blockade of a resting Cl\(^-\) conductance could account for increased membrane resistance accompanied by a hyperpolarization. For this hypothesis to be viable, it is necessary to assume that the neuronal membrane is freely permeable to Cl\(^-\) ions during steady state conditions. It follows, therefore, that in
order to maintain a gradient for Cl\textsuperscript{−} between the inside and outside of the terminal, there has to be some energy requiring process at play, possibly a Cl\textsuperscript{−}-pump, that moves the ion in an inward direction (Nishi et al., 1974).

It has been demonstrated that Ca\textsuperscript{++} influx in the presynaptic terminal occurs during an action potential in primary afferent fibres (Sastry, 1979c). Presuming that a Ca\textsuperscript{++} component exists in the terminals of hippocampal neurones, an enhancement or prolongation of this component during LTP can result in an increase in evoked transmitter release due to greater Ca\textsuperscript{++} entry per impulse. A similar mechanism was suggested for PTP (Sastry, 1979c). In fact, it is possible that a blockade of the rectifying potassium conductance that follows an action potential can account for a more prolonged Ca\textsuperscript{++} component in the spike at the presynaptic terminal (Sastry, 1979c).

An increase in the synthesis and/or the presence of more transmitter in the releasable pool can lead to enhanced transmitter release. Although it has been determined that during LTP of the CA	extsubscript{1} population spike produced by a tetanic stimulation of the Schaffer collateral input, the resting synthesis of glutamate is not altered (Benjamin et al., 1983), it has been reported by others (Corradetti et al., 1983) that stimulus-evoked release of this amino acid is accompanied by an increase in neosynthesis. Since there is an enhancement of evoked transmitter release during LTP (Skrede and Malthe-Sørrensen, 1981), perhaps the activity of the synthesis process is increased proportionally during evoked activity to compensate for the diminution of the releasable amino acid pool. If this is the case, one has to postulate that at least some, if not all of the additional transmitter pro-
duced by an electrical activation of the input during LTP, is available for release in subsequent stimulations. Besides increasing synthesis, another possibility for increasing transmitter release is to facilitate the transfer of transmitter from the storage pool to the releasable pool so that a new equilibrium level is maintained between the two due to an increase in the rate constant for this process. The rate of transmitter synthesis has to be greater than the above transfer rate so that the storage pool will not be eventually depleted.

It has been suggested that increased methylation of presynaptic components can lead to increased transmitter release due to an altered membrane fluidity (Benjamin et al., 1984). Perhaps, an increase in methylation of the presynaptic terminal can account for LTP. Alternatively, the observed change can be occurring at the level of the postsynaptic neurone to result in homo- and heterosynaptic depression. Whether an increase in methylation is responsible for LTP or homo- and heterosynaptic depression should be examined. A recent report provides evidence for the involvement of a Ca^{++}-dependent kinase (protein kinase C) in the induction of LTP (Malenka et al., 1986). Phorbol esters, which selectively activate protein kinase C, can produce a post-application potentiation of the synaptic response. Their results suggest that the locus of the phorbol ester's actions is presynaptic to facilitate transmitter release.

Other possible presynaptic changes that can be responsible for LTP include an increase in the number of presynaptic boutons, an increased safety factor along the axon and its branches so that the probability of action potential propagation to the terminals is enhanced, an increase in
the conduction velocity of presynaptic fibres to cause a more synchronous
release of transmitter resulting in a better summation of the EPSP, an
increase in the size of the presynaptic bouton so that transmitter released
has a possibility of activating more surface area subsynaptically, a rear-
rangement of synapses to facilitate transmission and modulation of either
transmitter release or the postsynaptic response by liberation of an unknown
substance due to input activation (e.g., noradrenaline). A reduction in the
synaptic gap, if present during LTP, can lead to an increase in the concen-
tration of the released transmitter in the cleft leading to an enhancement
of the subsynaptic response.

5.6 Potentiation of the EPSP following interruption of input stimulation

A stable control EPSP in the CA₁ area can be obtained by stimulating
stratum radiatum at a frequency of 0.2 Hz. When this stimulation is inter-
rupted for 10 minutes and then reinstated, the resulting EPSP is potentiated
and recovers over 10 minutes to a level that is comparable to the pre-quies-
cent period. This observation suggests that the control stimulation itself
results in some process that suppresses the EPSP. Since the potentiation of
the EPSP is not accompanied by any changes in the presynaptic Schaffer col-
lateral terminal excitability, the mechanism underlying this phenomenon may
not be the same as that responsible for LTP. The potentiation produced by
transiently interrupting the input stimulation, however, is quantitatively
smaller than that observed following tetanus-induced, elevated K⁺-induced
or NMDLA-induced enhancement of population responses (Figures 3, 13, 22 and
23). Perhaps, the decrease in excitability of the presynaptic terminals was
present but could not be detected reliably with the experimental method due
to its small magnitude. In view of the necessity of test input activation
to induce LTP in that pathway using the associative paradigm (Figure 20), it
is unlikely that the mechanism underlying the potentiation following an interruption of input stimulation is similar to that seen following activation of the afferent fibres. Of course, this interpretation does not preclude a presynaptic mechanism, but it does suggest an alternate one. Perhaps, transmitter release is facilitated through a greater availability in the releasable pool. This may arise from increased transmitter synthesis or a mobilization of neurotransmitter from the storage pool to the releasable pool. In addition, there may be a gradual depletion of transmitter due to depletion using the control stimulus frequency of 0.2 Hz. At some time, following the initiation of stimulation, the supply vs demand of neurotransmitter could be in equilibrium so no further reduction in transmitter output results. Presumably, the EPSP eventually settles to a stable level with a smaller magnitude than at the beginning of stimulation.

Postsynaptic mechanisms could also play a role in the potentiation of the EPSP induced by non-stimulation. It is possible that there is a tonic suppression of the EPSP during input stimulation due to desensitization of NMDA receptors (Fagni et al., 1983; Murali Mohan and Sastry, 1985) (perhaps, there is an NMDA component in the EPSP) or due to a homosynaptic depression caused by Ca$^{++}$ influx into CA$_1$ neurones (Chirwa et al., 1983; Sastry et al., 1984a). It is possible that the response adapts to a particular frequency and that an interruption of this entraining leads to an unsettling of the response which takes about 10 minutes to reequilibrate.

5.7 $^{3}$H-Glutamate-binding-and-uptake-studies

Baudry and Lynch (1980a) hypothesized that LTP is due to an increase in the number of subsynaptic glutamate receptors. It is apparent from the
Na⁺-independent binding results that an increase in the number of these binding sites is not a necessity for the observation of LTP (Table 8). Similarly, Ba⁺⁺ which was reported not to produce an increase in glutamate binding (Baudry and Lynch, 1979), has been shown to be capable of inducing a post-application potentiation of the population spike that lasted for prolonged periods of time (Ca⁺⁺ in normal medium was substituted with Ba⁺⁺) (Maretic et al., 1984). In contrast, it appears that there is an increase in glutamate binding associated with a depression of the population spike. The stimulation parameters for producing kindled burst discharges in hippocampal neurones (Douglas and Goddard, 1975; Goddard et al., 1969; Savage et al., 1982) are quite similar to the tetanic stimulations used to induce homosynaptic and heterosynaptic depressions of the population spike (Sastry et al., 1984a). Perhaps, the increase in glutamate binding sites observed with kindling (Savage et al., 1982) is similar to that seen accompanying homosynaptic depression. Since homosynaptic depression and LTP can co-occur (Sastry et al., 1984a) the observation of one or the other of these phenomena will be determined by their relative magnitudes. I have shown in the present study that a single 400 Hz tetanus consisting of 200 pulses, which produces minimal homosynaptic depression (Sastry et al., 1984a), is capable of producing LTP of the population spike with no associated increase in glutamate binding. In the studies of Baudry et al. (1980) and Lynch et al. (1982), the stimulations used to produce LTP were several trains of tetani at 100-300 Hz. Although they could observe LTP with such stimulation parameters, it is possible that the activation of postsynaptic neurones due to the repetitive trains of tetani produced an underlying depression that was
overshadowed by LTP. It is possible that the increase in glutamate binding observed by them, therefore, was associated with the homosynaptic depression rather than LTP. Although Baudry and Lynch (1980a) presume that the glutamate binding sites are receptors, they show no physiological or pharmacological evidence to support their proposal. It was suggested by the same authors (Baudry et al., 1980) that the affinity of the glutamate binding sites did not change following the induction of LTP. The dissociation constant ($K_d$) for high affinity $\text{Na}^+$-independent binding was reported to be 750 nM (Baudry and Lynch, 1981). Based on this observation, one would suspect that the binding sites are not receptors because concentrations of hundreds of $\mu$M glutamate are required to produce any measurable physiological response. On the other hand, it is possible that alterations in binding properties due to the biochemical procedures occurred. Furthermore, a recent paper by Garthwaite (1985) implicates cellular uptake of glutamate in disguising the true potency of this amino acid on receptors. Perhaps, in intact live tissue, the apparent potency of glutamate is much lower than in isolated membrane preparations because the uptake mechanism would be present in the former but not the latter instance. If an increase in the number of subsynaptic receptors is responsible for LTP, then one has to postulate that the existing receptors are fully occupied during synaptic transmission so that the postsynaptic response for each given neurone is always maximal when activated. Presuming that the neurotransmitter released is in excess of that required for synaptic transmission, then an increase in the number of subsynaptic receptors would necessarily mean a corresponding increase in the quantal unit. Quantal analysis of LTP in various other systems has shown
that quantal content is increased with no change in the quantal unit (Baxter et al., 1985; Briggs et al., 1985; Koyano et al., 1985). Perhaps, the same situation applies to the stratum radiatum-CA$_1$ system in the hippocampus. Exposure of hippocampal membranes to elevated Ca$^{++}$ concentrations (Baudry and Lynch, 1979) as well as low frequency tetanic stimulation of hippocampal slices, which causes Ca$^{++}$ influx into CA$_1$ cells (Chirwa et al., 1983), produce an increase in Na$^+$-independent glutamate binding. On the other hand, a high frequency tetanus does not cause as much elevation in CA$_1$ intracellular Ca$^{++}$ (Chirwa et al., 1983) and there is also no corresponding increase in glutamate binding (Table 8). Therefore, it is possible that the increase in glutamate binding sites is a Ca$^{++}$-mediated event in the CA$_1$ neurones. Recent observations (Murali Mohan and Sastry, 1985) suggest that there is a Ca$^{++}$-dependent desensitization and supersensitivity of glutamate receptors in CA$_1$ neurones. The increase in glutamate binding seen in the present study and those of others (Baudry and Lynch, 1979; Baudry et al., 1980; Lynch et al., 1982) may be due to an increase in glutamate receptors responsible for the Ca$^{++}$-dependent increase in responsiveness of CA$_1$ neurones to glutamate seen by Murali Mohan and Sastry (1985). Since these "receptors" are extrasynaptic (Fagni et al., 1983; Murali Mohan and Sastry, 1985), it is questionable whether they play a role in normal synaptic transmission. If they are, indeed, functional extrasynaptic receptors, then the action of exogenous drugs on these receptors will vary depending on the level of the Ca$^{++}$-mediated changes. The increase in the number of receptors to be associated with a depression rather than an increase in the response of CA$_1$ neurones to the inputs is similar to the
situation in denervation supersensitivity where the number of receptors appear to increase because of a disuse of the synapses.

Baudry et al. (1980) reported that the Na\(^+\)-dependent uptake system did not change following tetanic stimulations. In a previous publication (Sastry and Goh, 1984), it was presumed that these authors were correct and, therefore, we did not account for any changes in Na\(^+\)-dependent uptake to influence the results for Na\(^+\)-independent binding. As can be seen in the present study, tetanic stimulations to an input do, in fact, change the uptake of glutamate drastically. Furthermore, Wieraszko (1983) reported that a high frequency tetanus of the Schaffer collaterals produces a decrease in stimulus-evoked uptake of the neurotransmitter. In the present study, it is demonstrated that the "resting" glutamate uptake is decreased. Since LTP was associated with a decrease in the uptake of glutamate, it was of interest to examine if this could be a mechanism for LTP. This possibility was ruled out because unlike LTP, the induction of this uptake change could not be blocked by delivering the tetanic stimulation during infusion of Ca\(^{++}\)-free medium. In fact, there was a further reduction in the uptake, the reason for which is unknown. The reduction in uptake appears to be attributable to elements other than the postsynaptic cell. However the increase in the uptake could be due to an activation of postsynaptic neurones because there is a further decrease in uptake due to a 400 Hz tetanus given in Ca\(^{++}\)-free medium (which abolished synaptic transmission) as compared to a 400 Hz tetanus given in normal medium. Also, in the majority of cases following a 20 Hz tetanus (which produces frequency facilitation of the population spike during the tetanus [Sastry et al., 1984a]), an increase
in uptake results. There may be a balance between the increase and decrease in the uptake following the 20 Hz tetanus so that the more predominant effect of the two should be seen. Perhaps, this is why in some instances the uptake was increased and in others decreased following the 20 Hz tetanus. It is possible that the changes in the uptake are not peculiar to different elements but are a function of the tetanus frequency, high frequency favourable to induce a decreased uptake and a low frequency to induce an increased uptake. It may also be that the induction of the increase in uptake is Ca\(^{++}\)-dependent. Since a decrease in the uptake does not appear to increase synaptic transmission, perhaps this uptake alteration is located nonsynaptically and does not affect synaptically released glutamate. It was reported by Baudry and Lynch (1981) that Na\(^{+}\)-dependent uptake is saturable with a dissociation constant of 2.4 \(\mu\)M. If the concentration of the neurotransmitter released during synaptic transmission markedly exceeds that required for saturation of this system, then a slight decrease or increase in the uptake should have minimal implications for synaptic transmission. In this connection, it was shown by Curtis et al. (1976) that nipecotic acid, an uptake blocker for GABA, failed to enhance GABAergic inhibition of Purkinje cells. The significance of the changes in the uptake of transmitters following tetanic stimulations is unclear at present. Moreover, since \(^3\)H-glutamate accumulation into whole slices was being measured, it is uncertain as to how much of this accumulation was through specific glutamate uptake systems. It was shown by Wieraszko (1983) that stimulus-evoked uptake is decreased following a high frequency (100 Hz) tetanus. This uptake change was evident at stimulus frequencies between 1 and 20 Hz and
was not significantly different from non-tetanized controls at both of these extremes. Therefore, one would expect that there be no change in stimulus-evoked uptake following induction of LTP at test frequencies of < 1 Hz or > 20 Hz. Since in studies of LTP, the normal stimulation frequency used to evoke the control population spike is 0.1-0.2 Hz, it is probable that stimulus-evoked uptake is not altered during LTP using these stimulation parameters.

5.8 Physiological significance of studies on field potentials

A synchronous activation of a population of input fibres to evoke a population spike or a population EPSP in the postsynaptic neurones is probably not a common occurrence under physiological conditions. One may think that the studies on LTP as conducted in the present investigation are just an anomaly created in a laboratory setting and are irrelevant for natural processes. This may not be entirely true because it has been shown that high frequency asynchronous activity in CA3 neurones (which can be seen in physiological situations) results in LTP of the CA1 population spike (Goh and Sastry, 1983). Even though the population spike is used for quantitative purposes in these experiments, it is not difficult to imagine that a transient increase in the firing rate of presynaptic cells due to activation of a sensory input causes a subsequent increase in synaptic transmission at each of the activated synapses. In fact, according to Andersen and Langmoen (1981), only 60 synapses have to be activated simultaneously to discharge an action potential in a pyramidal cell. This roughly corresponds to 60 presynaptic fibres because an all-or-none EPSP evoked by stimulation of a single fibre has a magnitude of roughly 0.1 mV (Andersen and Langmoen,
1981). Perhaps, after LTP is induced, activation of a fewer number of synapses can cause the cell to discharge. It is quite plausible that 60 synapses can be activated simultaneously because each postsynaptic pyramidal cell is thought to have 10,000 synaptic contacts (Hamlyn, 1963).

Since some evidence has given me reason to doubt the involvement of the hippocampus and LTP in conscious learning and memory (Isseroff et al., 1976; Jarrard, 1983, 1985; Laroche, 1985; Stein et al., 1969), there is no reason to exclude the possibility that LTP is responsible for "subconscious" memory. As mentioned earlier, LTP has been observed in other systems (sympathetic ganglia, neocortex and crayfish neuromuscular junction), some of which cannot be involved in learning and memory as we know it (i.e., the facilitation of synaptic transmission is not contingent upon conscious awareness and thought processes). The "memory" in such systems could improve functioning of the organism at a subconscious level. For instance, LTP at the crustacean neuromuscular junction could result in facilitated reflexes for muscle contraction and LTP at autonomic ganglia could result in more efficient autonomic control in the organism. It may be possible that one could "learn" to improve their muscular reflexes by training. Perhaps, the mechanism for voluntary control of autonomic functions via the phenomenon of biofeedback is through LTP of autonomic ganglia.

6 CONCLUSIONS

1. The induction of LTP is not Ca$^{++}$-dependent but does appear to involve voltage-sensitive processes.
2. There is a requirement for depolarization or activation of the presynaptic terminals in the presence of adequate postsynaptic depolarization to successfully elicit LTP.

3. Both STP and LTP can be induced through an associative conditioning paradigm that does not involve prior tetanization of the test input. The temporal constraints governing the induction of associative STP of the test stratum radiatum input by conditioning delivered through the stratum oriens input were determined. To induce associative STP, it is necessary to activate the test input not more than 50 ms before or 80 ms after the onset of the conditioning train.

4. Since STP and LTP are consistently associated with a decrease in presynaptic terminal excitability, perhaps the presynaptic change is responsible for increasing evoked transmitter release leading to potentiation of the EPSP.

5. Interactions among presynaptic fibre terminations in the CA1 region may play a role in the associative induction of STP and LTP.

6. The mechanism underlying the potentiation of the EPSP due to interruption of input stimulation differs from that responsible for STP and LTP.

7. It is unlikely that NMDA receptors are involved in the induction of LTP.

8. An increase in the number of subsynaptic glutamate receptors is not a prerequisite for the observation of LTP.

9. A decrease in the uptake of neurotransmitter is not a mechanism for LTP.
10. The evidence provided strongly supports a presynaptic locus for STP and LTP. However, an additional postsynaptic mechanism cannot be excluded.

7 REFERENCES


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