DIFFERENTIAL EFFECTS OF CALCIUM AND TETANIC STIMULATION FREQUENCIES ON HIPPOCAMPAL SYNAPTIC POTENTIATION AND DEPRESSION.

By

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B. Sc (Pharm.)., The University of British Columbia, 1981

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Department of Pharmacology & Therapeutics,

Faculty of Medicine,

The University of British Columbia)

We accept this thesis as conforming

to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

April 1985

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ABSTRACT

In the hippocampus, tetanic stimulation of an input results in a long lasting potentiation (LLP) of synaptic transmission involving that input. While high frequency tetanic stimulations are preferred to elicit LLP, low frequency tetanus induces homosynaptic and heterosynaptic depressions. The present investigations were conducted to (1) analyse the characteristics of pulses in orthodromic and antidromic tetanic stimulations and relate them to post-tetanic changes in evoked potentials (2) determine if potentiation and depression co-occur and (3) determine whether an established LLP in one input is subsequently modified by the initiation of LLP in another input (to the same CA1b neurons) or whether LLP can be reversed by homosynaptic and heterosynaptic depressions and lastly (4) determine how interference or enhancements of calcium and potassium fluxes with pharmacological substances related to potentiation and depression.

Experiments were conducted on transversely sectioned rat hippocampal slices. Evoked potentials in subfield CA1b were elicited with stimulations of CA1b axons, commissural (Com), or Schaffer collaterals (Sch). Sch terminal excitability was tested with a stimulating electrode placed in the Sch/CA1b synaptic regions. Recordings were made with microelectrodes positioned in the CA1b cell bodies and/or dendritic regions, and in field CA3.

It was found that potentiation and depressions co-occur. Presynaptic volleys accompanied all tested tetanic trains. Similarly, antidromic trains discharged CA1b neurons continuously but did not cause LLP. Low frequency tetanic trains caused facilitated synchronous discharges of CA1b neurons during significant portions of these trains. In contrast, few if any syn-

chronous discharges followed high frequency tetanus. Yet high frequency tetanus elicited LLP and low frequency tetanus caused homo- and heterosynaptic depressions. An established LLP could be masked but not reversed by homo- and heterosynaptic depressions but this LLP was not interrupted by subsequently induced LLP of a separate input. Iontophoretic L-glutamate on CA1b cell bodies caused depression which was more pronounced if a tetanus was evoked during L-glutamate ejections. The depressions to low frequency tetanus and L-glutamate were counteracted by verapamil. Lastly, barium and 4-aminopyridine potentiations were reversed with washing. Applications of these drugs did not alter Sch terminal excitability. Tetanus induced during the presence of 4-aminopyridine still elicited LLP.

It is concluded that homo- and heterosynaptic depressions are partly due to the accumulation of calcium into the CA1b neurons. The magnitude of calcium entry into presynaptic and postsynaptic regions is governed by the tetanic frequencies evoked. The results are consistent with a presynaptic mediated LLP.

Date: April 29, 1985

B. R. Sastry

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ABBREVIATIONS

AP	Action potential.
Asp	L-aspartate.
CA	Cornu ammonis.
CNS	Central nervous system
Com	Commissural afferents
DG	Dentate gyrus
EPSP	Excitatory postsynaptic potential
GABA	Gamma-aminobutyric acid
GAD	Glutamic acid decarboxylase
IPSP	Inhibitory postsynaptic potential
NMA	N-methyl-DL-aspartate
NMDA	N-methyl-D-aspartate
PP	Perforant pathway
PS	Population spike
PW	Positive wave
Sch	Schaffer collaterals

ACKNOWLEDGEMENTS

I am deeply indebted to my supervisor Dr. Bhagavatula R. Sastry for his encouragement, emotional support and academic guidance. His patience and belief in me afforded me the unique opportunity to make something out of my life. My heartfelt thanks are due to Joanne W. Goh, Dr. Hermina Maretic and Dr. P. Murali Mohan for their friendship and collaboration in some of the experiments in this thesis. I thank Elaine L. Jan and Lisa Heiduschka for their expert assistance in the editing and format line setting of the manuscript, Christian Caritey for developing the slice chambers used in the laboratory.

The financial supports of Graduate Student Research Assistantship from the Medical Research Council and Staff Development Fellowship from the University of Zambia are greatly appreciated.

Dedicated to

my father and mother

brothers and sisters

to my first loves

Tiisetso and Sanika Jnr.

Thank you for your patience

and sacrifices

1. INTRODUCTION

Once neuroblasts differentiate into nerve cells, they never divide again to give rise to daughter nerve cells (Gazen, 1970; Jacobson, 1970). In maturity, the only nerve cell development that occurs is in completing genetically determined structural and functional specializations whose phenotypic expressions are depended on their regional location. Hence the nervous system is fully constructed in its nerve cell numbers and connections before it is used (Eccles, 1977). Clearly changes in the performance of the brain during life are not due to the addition of new nerve cells. There must exist, therefore, other mechanisms that mediate processes such as learning and memory.

Most neurobiologists are presently engaged in investigations that are aimed at determining the subtle changes in the chemistry or microstructure and microfunction that mediate such complex functions. In particular, the phenomena of long lasting potentiation (LLP) in the hippocampus has been receiving close scrutiny, for it is thought to be involved in learning and memory (Swanson, Teyler and Thompson, 1983 review). The first detailed account of LLP was given in the early seventies (in vivo: Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973; in vitro: Schwartzkroin and Wester, 1975; Alger and Teyler, 1976) when it was shown that repetitive tetanic stimulations of specific hippocampal inputs led to the development of post-tetanic LLP. The increase in synaptic efficacy could be maintained for days or weeks in vivo. LLP presented itself as a decrease in onset latencies and/or increases in amplitudes of the evoked synaptic potentials. At the intracellular level, LLP was seen as an increased probability in cell discharge.

Over the last decade, it became apparent that LLP could not be elicited with antidromic tetanic stimulations or direct soma (intracellular) depolarizations. While orthodromic tetanic stimulations could elicit LLP, such trains had to be evoked in the presence of extracellular calcium. More importantly, only the tetanised input subsequently exhibited LLP. These studies led to the conclusion that changes associated with LLP development were localized to the synaptic regions (Swanson, Teyler and Thompson, 1983 review).

A relationship between LLP development and tetanic frequencies became apparent when it was noted that (a) high frequency tetanic stimulations most reliably induced LLP of immediate onset (Schwartzkroin and Wester, 1975) whereas (b) low frequency tetanic stimulations usually caused a post-tetanic depression, even of non-tetanized inputs to the same output neurons, and this depression was sometimes followed by a gradually developing LLP in the tetanized input (Dunwiddie and Lynch, 1978; Chirwa, Goh, Maretic and Sastry, 1983). The characteristics of the depression were consistent with a generalised change to the postsynaptic neurons.

While many features of LLP and depression have now been elucidated, the exact mechanisms mediating LLP (or depression) have yet to be determined. Despite the concerted efforts of many investigators, it is still not known whether the changes in LLP are localised to presynaptic or postsynaptic components or involve both regions. The hypothesis of Baudry and Lynch (1980) contended that there was an increase in subsynaptic receptors during LLP. Yet evidence is available that correlates presynaptic alterations with LLP (Skrede and Malthe-Sorenssen, 1981; Sastry, 1982).

Preliminary reports from our laboratory have given results that are consistent with a presynaptic mediated LLP (Chirwa, Goh, Maretic and Sastry, 1983). On the other hand, the results on depression were in accordance with a postsynaptic change. The studies in this thesis further examine the differential effects of calcium and tetanic frequencies on presynaptic and postsynaptic regions and relate these effects to LLP and/or depression. The studies attempted to show the following.

1. The net influx of calcium into presynaptic and postsynaptic regions was a function of the tetanic stimulation frequency.
2. Calcium entry into presynaptic regions mediated LLP development.
3. Calcium entry into postsynaptic regions mediated depressions.
4. Presynaptic and postsynaptic effects of calcium could selectively be dissected out with pharmacological substances.

All the studies in this thesis were conducted on the rat hippocampal slice in vitro.

2. BASIC MORPHOLOGY OF THE HIPPOCAMPAL FORMATION

2.1 Introduction

The bulk of the information on the hippocampal formation presented here comes from the classic studies of Cajal (1911) and Lorente De Nó (1934). Hence the description and naming of parts of the hippocampal formation used in this thesis are consistent with the schemes employed by Cajal (1911) and Lorente De Nó (1934). However, some sections have been updated and/or clarified based on recent detailed studies such as those of Blackstad (1959), White (1959) and Swanson, Wyss and Cowan (1978).

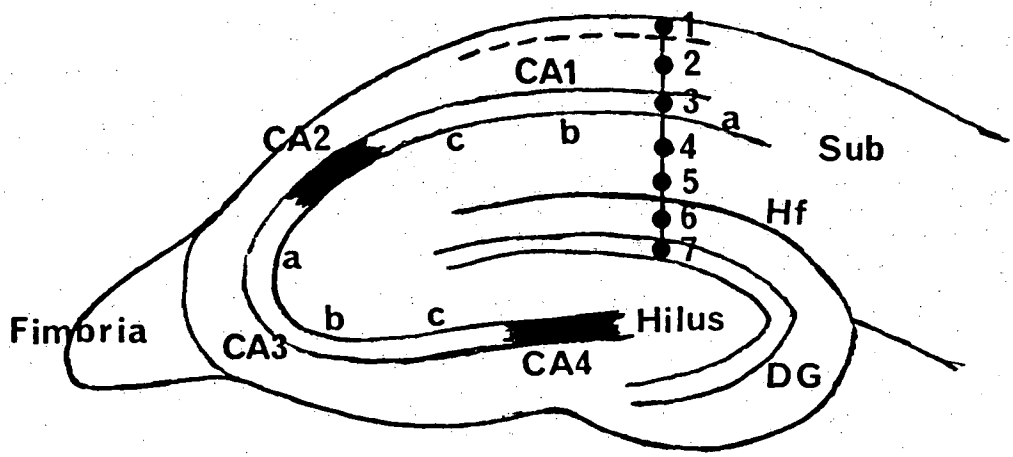
2.2 The hippocampal region

The cortex is often divided into the allocortex or the isocortex. During ontogenic development, the isocortex separates from the cortical mantle. The allocortex however does not cleave but remains as an S-shaped infolding of the cortical mantle (Filimonoff, 1947). This mantle layer is further folded into a C-shape along the inferior horn of the lateral ventricle and lies ventral and medial to the rhinal fissure. The cortex immediate to the allocortex is denoted as the periallocortex. The hippocampal formation is comprised of the allocortex and the periallocortex (Chronister and White, 1975; Teyler and Discenna, 1984).

The allocortex is subdivided into the hippocampus proper, the dentate gyrus (which together are denoted as the 'hippocampus') and much of the subiculum (Lorente De Nó, 1934; Swanson, Wyss and Cowan, 1978; Teyler and DiScenna, 1984). Parts of the subiculum lying adjacent to the presubiculum are usually assigned to the periallocortex (Lorente De Nó, 1934). The periallocortex itself is largely composed of the presubiculum (area 27), the area retrosplenalis e (area 29e), the parasubiculum (area 49) and the entorhinal region (area 28) (Brodman, 1909; Lorente De Nó, 1934; Blackstad, 1956; Chronister and White, 1975).

2.3 The hippocampus

The hippocampus is a bilaterally symmetrical structure, each consisting of two interdigitating archicortical fields termed cornu ammonis and the dentate gyrus (see fig. 1). The cornu ammonis and the dentate gyrus contain densely packed sheets of cells, the pyramids and the granule cells, which are the principal cell type of their respective fields. In each field are a variety of interneurons, with their dendrites intermingled among those of the principal cell types.



Layers

1. Alveus.
2. Stratum oriens.
3. Stratum pyramidale.
4. Stratum radiatum.
5. CA1 Stratum moleculare.
6. DG Stratum moleculare.
7. Stratum granulosum.

Fig. 1. General morphology of the hippocampal formation. The dots along the vertical line give the approximate positions of the layers above. Abbreviations: CA, cornu ammonis; DG. dentate gyrus; Hf, hippocampal fissure; Sub., subiculum.

2.4 Dentate gyrus

In the dentate gyrus, granule cells exist in a single layer termed the stratum granulosum. Between the stratum granulosum and the hippocampal fissure is the second dentate gyrus layer denoted as the stratum moleculare. The granular cells have their apical dendrites oriented toward the hippocampal fissure but within the stratum moleculare.

The dentate gyrus curves into a V- and/or U-shape around the latter part of the cornu ammonis field. In this dentate gyrus curvature, the blade or side which is adjacent to the subiculum and the initial parts of the cornu ammonis is denoted as the suprapyramidal region. The other dentate gyrus blade, which is intraventricular, is termed as the infrapyramidal region (Swanson, Wyss and Cowan, 1978).

2.5 Hilus and CA4 region

Within the dentate gyrus concavity and close to its apex is the hilus region. Some authors have considered the hilus as the third layer of the dentate gyrus (Cajal, 1911; Blackstad, 1956). But within the hilus, the boundaries between the dentate gyrus and the cornu ammonis are not readily discernible (Amaral, 1978; Swanson, Wyss and Cowan, 1978). In fact this region is extremely variable in appearance across species.

According to Lorente De Nó (1934) the cornu ammonis on approaching the hilus bends on itself, first upwards (first blade) and then downwards (second blade). These deflections are least developed in rodents and increase in complexity in the rabbit, monkey and man, respectively. A significant number of cells between the first blade and the cornu ammonis presumably belong to the cornu ammonis field. Yet the cells between the second blade and the granular layer send their axons to the dentate gyrus (see later sections). Parts of the hilus region, therefore, seem to belong

to the dentate gyrus and yet other sections are associated with the cornu ammonis. The hilar region therefore can be viewed as being a structural transition zone from the dentate gyrus into the cornu ammonis. There are pyramidal cells that stream out of this hilar region, and this latter part comprises the area CA4 of Lorente De Nó, (1934).

2.6 Cornu ammonis

The pyramids of the cornu ammonis field are arranged in the stratum pyramidale layer. The other layers of the cornu ammonis field include the alveus, which is next to the epithelium of the lateral ventricle. Between the alveus and the stratum pyramidale lies the stratum oriens. Next to the stratum pyramidale, but on the opposite side to the stratum oriens, are found the stratum lucidum, stratum radiatum, stratum lacunosum and stratum moleculare, in that order.

The extent of development of the different layers in the cornu ammonis varies among species. In rodents, for example, the division between stratum radiatum and stratum lacunosum is somewhat artificial. Consequently, in rodents, the stratum radiatum and stratum lacunosum are often described together (Lorente De Nó, 1934). Similarly the stratum pyramidale and the stratum lucidum are described together.

The boundaries of the cornu ammonis itself follow the length of the stratum radiatum. Using methods such as reduced silver impregnation, the stratum radiatum is revealed as a dense fiberplexus above the pyramidal cell layer (Lorente De Nó, 1934). At the dentate gyrus, the cornu ammonis ends where this dense fiberplexus (which is the stratum radiatum) abruptly ceases within the hilus/CA4 regions.

The boundary between the cornu ammonis and the subiculum is relatively well defined and occurs where the stratum radiatum disappears. In this

region, the pyramidal cells are not clustered together but exist in a single layer. The main regions of the hippocampal formation are illustrated in figure 1.

2.7 Fields and subfields of the cornu ammonis

The fields in the cornu ammonis (CA) are delineated on the basis of structural features of the primary cell types in the region. For instance, two cells with similar axonal apparatus but differing in dendritic distributions are taken to represent two distinct cell types (Lorente De Nó, 1934). Alternatively, if the dendrites are similar but the axonal ramifications are disimilar, then such cells are not equivalent.

In the CA, the pyramids are the primary cell types. Hence the various fields and subfield classifications are based on the general morphology of the pyramids in each area. Other structural features such as size, presence and/or absence of dendritic spines are also used to describe the subfields. The cornu ammonis is divided into four main fields namely CA1, CA2, CA3 and CA4 (see fig. 1). Field CA4 has been described in section 2.5 above.

The CA3 pyramids are large with ascending shafts (apical dendrites) that do not have side branches in the stratum radiatum but branch out upon reaching the stratum moleculare (Lorente De Nó, 1934). The initial parts of these apical dendrites possess thick spines, which are in contact with the terminals of mossy fibers from the dentate gyrus granule cells.

Field CA3 is further subdivided into CA3a, CA3b and CA3c. Subfield CA3c is adjacent to CA4 and its pyramidal cells have thick spines on both apical and basal dendrites. Presumably only CA3c pyramids are simultaneously innervated by mossy fibers of the infrapyramidal and suprapyramidal DG (granule cells) blades (Lorente De Nó, 1934).

Subfield CA3b consists of mixed pyramids; and some 50% of these have Schaffer collaterals (see later sections). In contrast, the pyramids in CA3a (unlike CA3b and CA3c) do not give Schaffer collaterals according to Lorente De Nó (1934). Most of the CA3a pyramids send out myelinated axons that give out one or two myelinated collaterals that ascend to the stratum radiatum where they form an associational pathway running within the stratum radiatum of CA3 to CA1b. This powerful association pathway has yet to be fully characterized.

Next to subfield CA3a is the field CA2. Some authors consider CA2 as a small, transitional zone between CA3 and CA1 (Swanson, Wyss and Cowan, 1978). Though the CA2 pyramids are large (i.e., similar to those of CA3), their dendrites lack thick spines and are without mossy fiber inputs (Lorente De Nó, 1934; Haug, 1974; Swanson, Wyss and Cowan, 1978). Unlike CA3 pyramids, the apical dendrites of CA2 pyramids begin to divide immediately after leaving the cell body layer. CA2 pyramids do not give Schaffer collaterals.

Even though CA1 is subdivided into CA1a, CA1b and CA1c, these divisions seem to be more clear in primates than in rodents (Lorente De Nó, 1934). The onset of CA1c from CA2 area is characterized by the presence of smaller pyramidal cells. Their dendrites are fine, with numerous side branches.

Ca1b has the smallest pyramids in the whole cornu ammonis. The Schaffer collaterals of CA3 and CA4 cease at the limit of CA1b and CA1a. Subfield CA1a itself has a mixed primary cell population that comprises, in part, of subiculum cells.

2.8 Interneurons

The pyramidal cells in the CA and the granule cells in the DG make up 96-98% of the neuropil of the hippocampus (Buzsáki, 1984). However, other types of neurons are distributed within these regions.

In the stratum granulosum layer can be found a variety of interneurons (i.e., the basket cells of Lorente De Nó, 1934; Cajal, 1911). On the basis of their shape, the classification of these interneurons include pyramidal, horizontal, fusiform, inverted fusiform and multipolar (Ribak and Seress, 1934; Buzsáki, 1984). The dendrites of these interneurons have little or no spines (Amaral, 1978; Ribak and Seress, 1983; Buzsáki, 1984). Their axons form an extensive plexus in the granule cells and inner stratum moleculare layers that subsequently form synapses on the cell body and/or proximal dendrites of granule cells.

Similarly, interneurons classified as fusiform, stellate, spheroid or unipolar are distributed in the hilar/CA4 regions (Amaral, 1978). Their dendrites lack spines and these interneurons possess locally arborizing axons (Buzsáki, 1984). Within the CA fields are found large interneurons of the following variety; bipolar, fusiform, triangular, polygonal, horizontal and Golgi type II (Ribak, Vaugh and Saito, 1978). In addition, small fusiform and ovoid interneurons have been identified in the CA fields.

3. ENTORHINAL CORTEX, DG AND CA MAJOR AFFERENTS

3.1 The perforant path

Figure 2 illustrates the major afferents of the hippocampus. The medial and lateral parts of the ipsilateral entorhinal cortex send out fibers termed perforant path (PP) that form synapses on the spines of the dentate gyrus granule cell dendrites in the middle and outer thirds of the DG molecular layer (Hjorth-Simonsen, 1973; Matthews, Cotman and Lynch, 1976). Even the contralateral entorhinal cortex sends out a small fraction of its perforant path fibers to each hippocampal DG area (Zimmer and Hjorth-Simonsen, 1975).

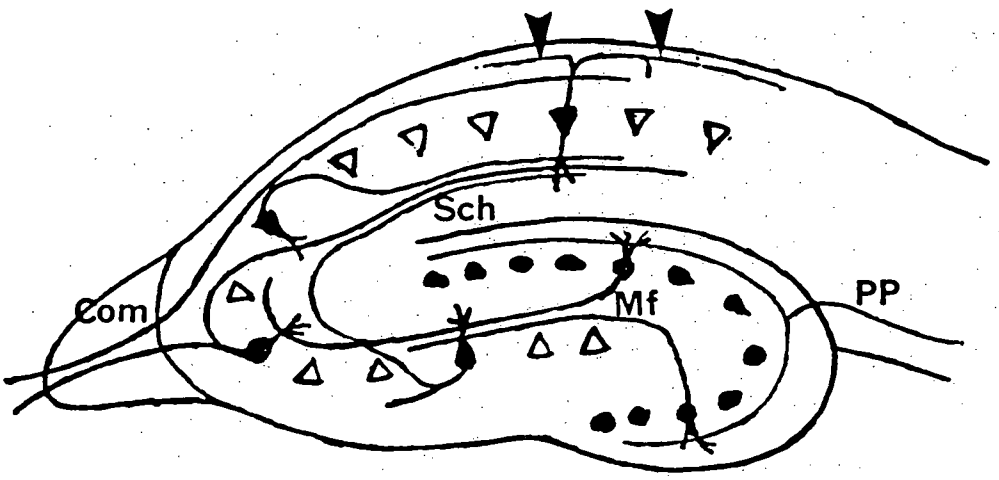


Fig. 2. The major afferent systems in the hippocampus. Abbreviations denote: PP, perforant pathway; Mf, mossy fibers; Com, commissural afferents; Sch, Schaffer collaterals. The arrows in diagram show the branches of the CA1 axons in the alveus.

In rodents, the PP projection is perpendicular to the main axis of the hippocampal formation. Some divergence has been observed in the lateral PP projection, where a small zone in the entorhinal cortex actually innervates a much wider zone within the DG (Wyss, 1981).

Though not completely characterized, evidence is available that has demonstrated a direct PP projection to the CA fields (Gottlieb and Cowan, 1972; Steward, 1976). It is also known that some DG interneurons project their dendrites into the termination zone of the PP (Ribak and Seress, 1983). It is probable that some PP fibers make contact with the dendrites of interneurons.

3.2 The DG mossy fibers

The DG granule cells give out narrow bands of axons termed mossy fibers that extend transversely across field CA3 (Blackstad, Brink, Hem and Jeune, 1970). There is little overlap of the mossy fibers from granule cells at adjoining levels. Mossy fibers are highly laminated, and form synapses on the spines of proximal dendrites of CA3 pyramids. The mossy fibers of granule cells in the infrapyramidal DG blade innervate CA3c. Granule cells in the suprapyramidal DG blade send out mossy fibers across the entire CA3 field (Lorente De Nó, 1934; Haug, 1974; Swanson, Wyss and Cowan, 1978).

A small fraction of mossy fibers synapse with cells in the hilar region (Blackstad and Kjaerheim, 1961) and interneurons in field CA3 (Tombol, Babosa, Hajdu and Somogyi, 1979; Misgeld, Sarvey and Klee, 1979).

3.3 The associational and commissural projections from hilar/CA4, CA3 and CA2 fields

Hilar cells send out associational and commissural fibers which terminate on the inner third of the DG molecular layer. As is true for most of the major afferents in the hippocampus, it is not known exactly whether hil-

ar projections terminate on DG granule cells and/or just make contact with the DG interneurons (Swanson, Sawchenko and Cowan, 1981).

CA4 and some CA3 pyramids send out axons to the ipsilateral and contralateral CA3 fields (Gottlieb and Cowan, 1973). These fibers establish synaptic contacts with CA3 cell dendrites in the stratum oriens and the stratum radiatum layers (Swanson, 1973). CA3 and CA4 axons also constitute a commissural projection to the contralateral CA1 field which synapse with CA1 cell dendrites mostly in the stratum oriens (Schaffer, 1892; Laurberg and Sorensen, 1981).

Lastly, from the CA3 and CA4 pyramids thick axons, branch out several collaterals. Some of these collaterals are short and end within the CA3 stratum oriens and/or between the local CA3 pyramids. There are also thick and longer collaterals from axons of CA4, CA3c and some CA3b pyramids (Lorente De Nó, 1934) that cross the stratum pyramidale and the stratum radiatum layers and then enter the stratum lacunosum (still considered as stratum radiatum in rodents) where they constitute myelinated horizontal fibers termed Schaffer collaterals (Sch). The Schaffer collaterals innervate the apical dendrites of CA1 pyramids.

According to Lorente De Nó, (1934) CA2 pyramids and most of CA3c do not give out Schaffer collaterals. Instead, CA2 pyramids send out axons from which branch out thick collaterals which cross through the stratum pyramidale and enter the stratum radiatum, where they constitute a strong longitudinal (axial) association pathway (up to CA1b). In addition, the CA2 pyramidal cell axons form horizontal collaterals and travel within the stratum oriens towards the subiculum and entorhinal cortex.

CA1 pyramids send out axons that (a) branch out within the stratum oriens (b) ascend and ramify in the stratum radiatum and/or (c) have collaterals that reach and travel in the alveus. These CA1 axonal collaterals in the alveus exit out via the fimbria, and some go to the subiculum and areas beyond the entorhinal cortex. Some CA1 axon collaterals distribute back to CA1 and CA2 pyramidal layers. But these CA1 axons are not in contact with the pyramids of CA3 or CA4, nor the DG granule cells.

CA1 axons project out of the hippocampus to other brain regions such as the lateral septal nuclei and prefrontal cortex (Swanson and Cowan, 1977; Swanson, 1981).

3.4 Interneurons

Evidence in the literature indicates that most of the major inputs to the hippocampus simultaneously innervate interneurons and principal cells. The primary physiological function of these interneurons is to cause inhibition of the principal cells in each hippocampal field (Kandel, Spencer and Brinley, 1961; Andersen, Eccles and Løyning, 1963; Storm-Mathisen, 1977; Ribak, Vaugh and Saito, 1978; Seress and Ribak, 1983). A variety of other putative transmitters and neuromodulators are contained in some interneurons (Buzsáki, 1984 review).

Though it is likely that some interneurons in fact excite the principal cells, the overwhelming evidence presently implicates interneurons as mediating inhibition (Andersen, Eccles and Løyning, 1964; Andersen,1975; Turner and Schwartzkroin, 1981; Fox and Ranck, 1981; Finch, Nowlin and Babb, 1983). Both recurrent and feed-forward inhibition have been demonstrated (Buzsáki, 1984 review).

3.5 Summary

The hippocampal formation can be viewed as consisting of adjoining cortical bands (DG, CA3, CA1 and subiculum) that are folded along their longitudinal axis. Axons from one cortical band (e.g., DG) are in parallel arrangement and cross the border to innervate the next strip at right angles (Andersen, 1983). Each major pathway ends on a limited part of the dendritic tree of the recipient cells in a particular field (this is afferent lamination). In general, the segregation of different synapses are typically arranged as follows (a) excitatory cells are confined to dendritic spines whereas (b) inhibitory interneurons terminate on the soma, initial axon and/or initial parts of dendrites (Andersen, Blackstad and Lømo, 1966).

Other than the inputs discussed in the preceding sections, there exists a multitude of pathways that project to the hippocampal formation (Buzsáki, 1984). These have not been discussed here since detailed information is presently not available. Lastly, much is known about the organization of the longer intrahippocampal pathways, e.g., mossy fibers, Schaffer collaterals, etc. However, details on (a) their specific connections on individual neurons and/or (b) the exact connections with the many classes of interneurons remain to be elucidated.

4. FURTHER MORPHOLOGICAL CHARACTERISTICS OF FIELD CA1

The hippocampus has been well defined anatomically and physiologically and therefore serves as a convenient model for the elucidation of central nervous function and structure. Most of the experimental data presented in this thesis were obtained from subfield CA1b of the rat hippocampus. For that reason much of the discussion in the following sections pertain to the CA1 pyramids.

Each CA1 pyramidal soma is typically oval and oriented such that the long axis is vertical to the alvear surface. On average the soma is about 40 by 20 microns. Its basal dendrites exit from the soma in a bush-like fashion with irregular branches that divide repeatedly. The apical dendrites extend outward, in parallel, towards the hippocampal fissure. Then at intervals, thinner secondary dendrites emerge out of the apical dendritic shafts. These smaller dendritic branches are inundated with numerous spines. Relatively fewer spines are located on the primary dendritic shaft.

Andersen, Silfvenius, Sundberg and Sveen (1980) used electron micrographs prepared from ultrathin sections of guinea-pig hippocampus to further analyse the fiber orientation on pyramids in field CA1 that golgi impregnation studies had previously shown to be in parallel with the pyramidal layer (Golgi, 1886; Schaffer, 1892; Cajal, 1911). In addition, the electron microscopic studies of Westrum and Blackstad (1962) had revealed that at intervals these parallel afferents in the strata oriens/radiatum sent out en passage boutons that made contact with the CA1 dendrites. In this scheme each fiber makes a series of synaptic contacts on the dendrites of numerous cells as the axon procedes along its transverse trajectory.

Andersen, Silfvenius, Sundberg and Sveen (1980) found that the number of synapses possessing specialised contacts with the dendritic spines was uniform (about 42 per 100 µm) across the main parts of the strata oriens and radiatum. In these regions, close to 95% of all synaptic contacts terminated on asymmetric dendritic spines and the rest were in direct contact with the dendrites. Towards the ends of the dendrites, however, the number of spine-contacting synapses decreased and there was an increase of about 30% in the number of synapses in direct contact with the dendrites (Ander-

sen, Silfvenius, Sundburg and Sveen, 1980). Similarly a high distribution of direct contact spines was observed in the soma area, i.e., stratum pyramidale and their immediate regions.

5. CA1 FIELD ELECTROPHYSIOLOGY

5.1 Electrical properties of CA1 pyramids

Schwartzkroin (1975; 1977) used guinea pig hippocampus *in vitro* to characterize the passive membrane properties of CA1 pyramidal neurons and found them to be similar to those of the pyramidal neurons described in intact animal preparations (Kandel and Spencer, 1961; Spencer and Brinley, 1961; Spencer and Kandel, 1961). The average spike amplitudes obtained were 64.9 ± 10 mV *in vitro*. The cell resistances, calculated from the slope of the current-voltage (I-V) curves yielded values of 16.3 ± 5.3 MΩ. The cell time constant, i.e., the latency from onset of the pulse to 1-(1/e) of the peak voltage deflection, was 9.8 ± 3.4 msec (Schwartzkroin, 1977). Schwartzkroin (1977) attributed the considerable variability of resistance and time constant measurements as being due to real differences among the sampled neurons. In any case, recent studies that have used advanced techniques (Turner and Schwartzkroin, 1980; Brown, Frickle and Perkel, 1981; Turner, 1982) have generated data on CA1 neuronal input resistance, time constant, time constant ratios and electrotonic length which are consistently similar among different investigators and are in agreement with values reported by Schwartzkroin in 1977.

5.2 CA1 pyramid and interneuron discharges

CA1 pyramidal neurons often generate a burst of 2-10 action potentials (Ranck, 1973) of decreasing amplitude and increasing duration, i.e., complex

spike. Intracellular records (Schwartzkroin, 1975) have shown that the complex-spike is comprised of long duration action potentials and depolarizing after-potentials. However, orthodromic and antidromic activation tend to elicit a single action potential due to the activation of recurrent inhibition (Andersen, Eccles and Løyning, 1964).

Unlike CA1 pyramids, the interneurons mediating synaptic inhibition give characteristic short-duration action potentials. Activated interneurons exhibit repetitive and high frequency firing (Andersen, Eccles and Løyning, 1964).

5.3 Basic features of evoked potentials

Andersen, Eccles and Loyning (1964) showed that large intracellular hyperpolarizations in CA1 neurons could be evoked by stimulation of the commissural and the Schaffer collaterals. Concurrent with these large intracellular hyperpolarizations, termed inhibitory postsynaptic potentials (IPSP), were the laminated extracellular positive potentials termed positive waves (PW). Similar times were demonstrated between the onset of the IPSP and of the PW. When there was an excitatory postsynaptic potential (EPSP) or action potential superimposed on the IPSP response, the latency of the IPSP was always longer by 0.8 to 2.3 msec. If such an EPSP and/or action potential preceded the IPSP, the PW exhibited an earlier onset than the intracellularly recorded IPSP.

It was concluded that the PW was the net field potential produced by hyperpolarizing current sources at or near the somata of CA1 neurons and by sinks in the dendrites. To a lesser extent and accounting for the earlier onset of the PW, relative to intracellular IPSP, was the addition of positive potentials produced by the action of depolarizing synapses on the

apical/basal dendrites. The soma region was the passive source to active sinks (Andersen, Eccles and Loyning, 1964).

It is now well established that with increasing stimulation strength the population PW can be interrupted by a negative-going potential which is the population spike (PS). Andersen, Bliss and Skrede (1971) demonstrated that the PS is the potential produced at the site of the individual action potentials of many neighbouring, synchronously discharging neurons. These investigators also showed a temporal correlation between PS and unit spikes in the same region. The PS amplitude introduced two peaks in the PW; and the second positive peak corresponded to the maximal intracellular IPSP. These features of the PS/PW are shown in figure 3.

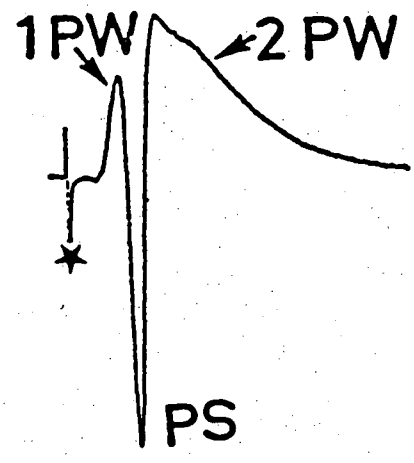


Fig. 3. Evoked population spike. The stimulus artifact is identified by the star. Abbreviations denote: 1PW, first positive wave component; PS, population spike; 2PW, second positive wave component. Negativity is downwards.

It should be noted that in CA1 neurons potential shifts can be caused by transient shifts in the ionic gradients. These ionic shifts and associated potential changes alter the magnitude of evoked EPSP, PW and PS. For instance, calcium activated K^+ conductances following neuronal activity (Hotson and Prince, 1980) probably enhances CA1 cell hyperpolarizations. Indeed CA1 pyramidal neurons possess a distribution of nonsynaptic ionic conductances (see section 6) that can modify the net signal transfer from input sites to the recording site.

6. INTRINSIC NONSYNAPTIC IONIC CONDUCTANCES

6.1 Basic features

A variety of ionic conductances are known to occur in the hippocampal and other central nervous system (CNS) neurons. These conductances have a localised distribution on the soma and/or dendritic regions. They exhibit ionic specificity, are voltage-dependent and can be modulated by neurotransmitters and neuropeptides (Llinás, 1984). Intrinsic ionic conductances participate in the neuronal integration of signals. In fact much of the electrophysiology observed in mammalian neurons derives from the intrinsic electrical properties of the cells, i.e., interplay of ionic conductances that co-occur with classic synaptic interactions.

6.2 Sodium conductances

At least two types of sodium (Na^+) conductances are known to operate in the CA1 pyramidal neurons. There is the classic inactivating Na^+ conductance (Terzuolo and Araki, 1961; Llinás and Sugimori, 1980) which is implicated in the generation of fast action potentials. A noninactivating Na^+ conductance has also been identified and is thought to mediate graded

plateau potentials lasting up to 15 seconds (Llinás and Sugimori, 1980; Strafstrom, Schwindt and Crill, 1982).

6.3 Calcium conductances

A high threshold inactivating calcium (Ca^{++}) conductance located in the soma and possibly in the dendrites (Sugimori and Llinás, 1982) is present in the hippocampus (Schwartzkroin and Slawsky, 1977). This inactivating Ca^{++} conductance is involved in the generation of Ca^{++}-dependent action potentials termed Ca^{++} spikes. There exists a second type of Ca^{++} conductance which does not inactivate but is able to induce Ca^{++} spikes. This somatic Ca^{++} conductance has a low threshold of activation since it is activated by hyperpolarizations from rest level (Llinás and Jahnsen, 1982).

6.4 Potassium conductances

Hitherto, the largest number of ionic conductances known are those of potassium (K^+). At least seven types of K^+ conductances are known and probably all of them occur in the hippocampus (Segal and Barker, 1984). First, there is the classic Hodgkin-Huxley type (Hodgkin and Huxley, 1952) delayed rectifier K^+ current which generates the falling phase of the fast action potential. Another K^+ current, denoted as the M-current, has been observed in the hippocampus. The M-current does not inactivate, is a low threshold K^+ current and is elicited by depolarizations and modulated by synaptic (cholinergic) neurotransmitter substances (Adams, Brown and Halliwell, 1981). The M-current increases input resistance and this effect could conceivably facilitate dendritic to soma communications.

Another type of inactivating delayed rectifying K^+ current differs from the M-current in that the former can be blocked by caesium and shows a dependency on Ca^{++} (Llinás and Yaro, 1980). Two and possibly three types

of anomalous inward rectifying K^+ conductances operate in the hippocampus (Holston, Prince and Schwartzkroin, 1979). Type one is the instantaneous rectifier whose characteristics were initially given by Katz in 1949 and Adrian in 1969. The second type is denoted as delayed or time-dependent rectifier K^+ current (Wilson and Goldner, 1975). Anomalous rectifications seem to modulate input resistance and invariably improve coupling between dendrite and soma (Llinás, 1984).

Lastly, a fast transient K^+ conductance has been noted in the hippocampus (Gustaffsson, Galvan, Graffe and Wigström, 1982) which presumably serves to prevent the rapid return of membrane potential to baseline, following hyperpolarizations. A possible function of this K^+ conductance would be in the prevention of rebound excitation as the cell membrane potential returned to baseline.

6.5 Functional role of ionic conductances

As stated previously, the hippocampal ionic conductances contribute significantly toward neuronal signal integration. For instance, the pyramidal cells' dendritic electrophysiology is in part generated by changes in Na^+, K^+ and Ca^{++} conductances (cf. Schwartzkroin and Slawsky, 1977). Spencer and Kandel (1961) postulated the existence of a fast and early prepotential, which is now thought to be mediated by Na^+ conductance, i.e., Na^+ spike. Wong, Prince and Basbaum (1979) have since confirmed the intradendritic Na^+ spike and proposed that these Na^+ spikes served as electrotonic couplers between the dendrites and the soma. However, the exact function of the potentials is not known.

All or some of the bursting behavior seen in pyramidal neurons seem to be caused by changes in intrinsic ionic conductance changes. Dentate gyrus

granule cells do not fire bursts but CA3 cells can burst spontaneously. Both somata and dendrites of CA3 cells burst readily (Wong, Prince and Basbaum, 1979). In contrast, CA1 pyramidal cells can burst but do not do so ordinarily (Masukawa, Bernado and Prince, 1982; Alger, 1984). CA1 pyramidal cell dendrites can be induced to burst (i.e. discharge) directly but CA1 somata cannot. The differences in bursting behaviour between soma and dendrite, or individual cornu ammonis fields and the dentate gyrus, may be due to differences in distribution and/or activity of recurrent or feed-forward inhibition (Alger, 1984). Anyhow, intradendritic and intrasomatic recordings from a pyramidal neuron seem to implicate both the soma and the dendrites as the most likely sites for generation of bursting activity (Wong and Prince, 1978; Wong, 1982). Wong (1982) postulated that sodium spikes initiated by membrane potential fluctuations in the hippocampal pyramidal neurons activate a Ca^{++} conductance. Upon membrane repolarization, the Ca^{++} conductance decays slowly and may induce secondary depolarizations. Whatever the mechanisms, tetrodotoxin resistant spikes that are thought to be mediated by Ca^{++} have been demonstrated in the mammalian CNS (Kandel and Spencer, 1961; Barrett and Barrett, 1976; Schwartzkroin and Slawsky, 1977; Wong and Prince, 1978).

In summary, the preceding discussion points out a variety of intrinsic ionic conductances that are present in the hippocampal pyramidal neurons. Among other functions, these ionic conductances participate in the generation of action potentials, soma-dendritic electrical coupling, modifications/control of synaptic signals and termination of neuronal activities. Some of the other known ionic conductances in the hippocampus have not been discussed since they have still to be properly characterized. Subsequent studies are likely to identify 'novel' ionic conductances. Clearly post-

synaptic neurons do not simply act as a slave to presynaptic commands. At least in the hippocampal pyramidal neurons, there exists a battery of ionic conductances that dictate the ultimate global response emanating from these postsynaptic neurons, following synaptic activation.

7. ANTIDROMIC, ORTHODROMIC AND OTHER EVOKED POTENTIALS

7.1 Antidromic field potentials

Synaptic potentials in CA1 pyramids cannot be evoked by stimulation of fibers in the alveus. Instead, a short latency spike (see figure 4A) showing little latency variation and capable of following high frequency (e.g., 100 Hz) is evoked. Thus alvear stimulation (where CA1 pyramidal axons traverse) elicits spikes which fulfil the criteria for antidromic invasion of the pyramidal neuron. These spikes are not abolished by high magnesium or manganese containing media (i.e., Ringer's solutions without Ca^{++}).

7.2 Orthodromic responses

Typically stimulation of the afferents in the stratum oriens or the stratum radiatum cause a presynaptic potential in a strip-like region at the stimulated level (Andersen, Silfvenius, Sundberg, Sveen and Wigström, 1978) This extracellularly recorded negative deflection, as shown by the arrow in figure 4D, is termed the presynaptic volley (PV) and its magnitude is an index of the number of activated fibers.

Evoked commissural or Schaffer collaterals elicit postsynaptic potentials in the CA1 pyramids. The negative field synaptic potentials have their maximum in the region where the activated fibers terminate and show reversal (i.e., to positivity) when recorded from distant positions along the dendritic axis (Andersen, Silfvenius, Sundberg and Sveen, 1980). Depending on the magnitude of the orthodromic excitatory potentials, i.e.,

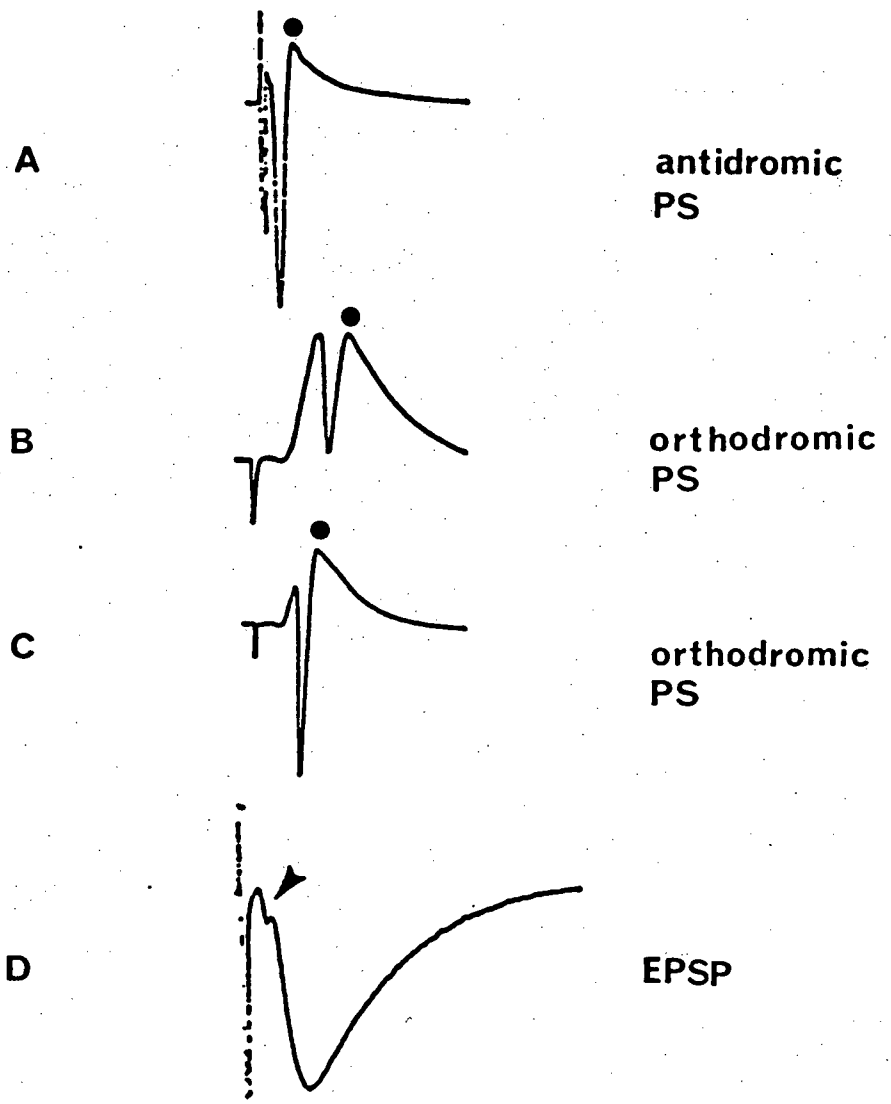


Fig. 4. Antidromic and orthodromic potentials recorded in CA1 field. Dot corresponds to 2PW and arrow points at the presynaptic volley. Note the early onset of PS in (A) relative to orthodromic PS in (B) and (C). Negativity is downwards.

reaching threshold, numerous action potentials (AP) are discharged in the pyramidal neurons. The magnitude of the summated APs generated is maximum, when recorded in the stratum pyramidale layer and show polarity reversal on both sides of the pyramidal layer.

Andersen, Silfvenius, Sundberg and Sveen (1980) caused selective activation of a small group of afferent fibers to elicit field potentials in the CA1 neurons. These investigators demonstrated that the proximal and distal synapses in CA1 were largely equipotent in evoking field potentials. The input across a similar number of proximal and distal synapses gave the same high probability of discharging single neurons.

7.3. Inhibitory postsynaptic potentials

Orthodromic, but not antidromic activation of CA1 pyramidal neurons is more effective in producing IPSPs (NB: same size field potentials and associated IPSP measured concurrently; see Alger and Nicoll, 1982). Pharmacological evidence supports the distinction between ortho- and antidromically evoked IPSPs (Alger and Nicoll, 1979). The morphology of IPSPs caused by antidromic stimulation differ from those evoked during orthodromic stimulation. Besides, pyramidal cell IPSPs are inducible following stratum radiatum stimulation even in the absence of recordable population spikes. Clearly recurrent inhibition (which operates only after CA1 discharges or after antidromic excitation of CA1 axons) alone cannot account for the discrepancies in antidromic versus orthodromic mediated IPSPs. The above evidence (cf. Alger, 1984) renders significant electrophysiological support for the existence of feed-forward inhibition.

7.4 Electrotonic coupling and ephaptic interactions

Though available evidence is incomplete, two types of nonsynaptic interactions have been implicated in the hippocampus. MacVicar and Dudek (1981) have proposed the operation of electrotonic coupling, where activity in one neuron is presumed to be transmitted directly to other neurons via anatomically identifiable junctions. The dye Lucifer Yellow, which is very sparsely taken up from the extracellular space and does not cross chemical synaptic junctions, has been used to reveal the gap junctions. In these studies, however, the possibility of mechanical coupling being introduced by the electrode itself partially impaling both cells (cf. Alger, McCarren and Fisher, 1983) has not been ruled out entirely. It should be noted that pyramidal neurons are tightly packed together (Lorente De Nó, 1934).

Ephaptic interactions are thought to be the influence on a neuron caused by extracellular current flow via extracellular resistances (Jefferys and Haas, 1982; Taylor and Dudek, 1982). The demonstration of ephaptic transmission requires that *in vitro* hippocampal slices be bathed for prolonged periods in a low Ca^{++} medium. Apparently there is complete chemical synaptic blockade. Thereafter rhythmic bursts lasting for many seconds and which can be blocked by high (6 mM) but not low (less than 4 mM) Mg^{++} are recorded (Taylor and Dudek, 1982; Alger, 1984). These bursts can be spontaneous or they can be evoked by electrical stimulation.

Taylor and Dudek (1982) analysed differential recordings of extracellular and intracellular potentials simultaneously and reported that the extracellular field potentials had effects on the membrane potentials. It is not clear why these ephaptic interactions take long to develop; and neither are the mechanisms for the synchronous bursting understood. If it is assumed that electrotonic and ephaptic interactions have a functional role in hippocampal physiology, these could be some of the means by which synchronization of cell firing occurs.

8. SYNAPTIC INTERACTIONS AND POSSIBLE NEUROTRANSMITTER CANDIDATES.

8.1 Recurrent and feed-forward inhibition

Inhibitory influences, both recurrent and feed-forward, probably use gamma-aminobutyric acid (GABA) as their principle neurotransmitter (Storm-Mathisen, 1977; Frotscher, Leranth, Lubbers and Oertel, 1984). GABA synthetic enzyme glutamic acid decarboxylase (GAD) as well as GABA-ergic receptor subtypes, are distributed in all layers of field CA1 (Storm-Mathisen, 1977; Andersen, Dingledine, Gjerstad, Langmoen and Mosfeldt-Laursen, 1980).

Evidence indicates that synaptically released GABA on cell bodies and around the initial parts of axons and/or dendrites, causes a conductance change that induces a net influx of chloride (Cl^-) ions. This influx of Cl^- causes hyperpolarizations in cells and thereby prevents them from discharging when synaptically driven.

In addition, inhibitory interneurons terminate on the primary and secondary parts of CA1 dendrites (Lorente De Nó, 1934) and release GABA. Here GABA presumably produces a conductance increase, involving sodium and chloride ions, and resulting in a depolarization action (Andersen, 1983). Andersen, Dingledine, Gjerstad, Lanmoen and Mosfeldt-Laursen (1980) proposed that this GABA-mediated depolarization in the dendritic regions created intense conductance changes which subsequently shunted the effects of excitatory synapses in the vicinity (cf. Alger and Nicoll, 1979; 1982).

8.2 Extrinsic modulatory pathways

A variety of extrinsic modulatory pathways in the hippocampus have been identified. In most cases, the exact target cells for these extrinsic inputs and/or their source of origin are not fully known. Some of these inputs that seem to innervate parts of CA1 include; the medial septum and

diagonal cholinergic input (Storm-Mathisen, 1977: Lynch, Rose and Gall, 1978), noradrenergic outflow from the locus coeruleus (Lindvall and Bjorkland, 1974) and the serotonergic projection from the medial and dorsal raphe nuclei (Azmitia and Segal, 1979).

The probable modulatory role of these extrinsic pathways is illustrated by acetylcholine actions (ACh). Acetylcholine is known to cause a reduction in the M-current which is active over the -70 mV to -40 mV range (Dodd, Dingledine and Kelly, 1981; Bernado and Prince, 1982). The actions of ACh are accompanied by slow depolarizations in the cells and a raised input resistance. These actions can conceivably predispose the CA1 pyramids to bursting (Dingledine, 1984) especially since ACh also inhibits the release of inhibitory and/or excitatory afferents to CA1 pyramidal neurons (Yamamoto and Kawai, 1967; Ben-Ari, Krnjević, Reiffenstein and Ropert, 1981).

8.3 Neuroactive substances

Recent findings have revealed a diverse distribution of neuroactive substances in the hippocampus (reviewed by Dingledine, 1984). It remains to be established whether these neuroactive substances form separate pathways and/or co-exist with other neurotranmitters. It can be speculated that neuroactive substances might even reside and/or be released from the CA1 neurons dendritic spines and influence synaptic interactions. But the experimental evidence for these possibilties are presently lacking.

The main neuroactive substances that have been characterized so far in the CA1 field include enkephalin-like substances (Gall, Brecha, Karten and Chang, 1981), cholecystokinin and somatostatin (Greenwood, Godar, Reaves and Hayward, 1981), vasoactive intestinal polypeptides (Loren, Emson, Fahrenkrug, Alumets, Hakanson and Sundler, 1979), substance-P (Vincent, Kimura and

McGeer, 1981) and angiotensin-II (Haas, Felix, Celio and Inagami, 1980). The list of neuroactive substances presented here is by no means exhaustive. A more detailed discussion of neuroactive substances and their mechanism of actions has been presented by Dingledine (1984).

8.4 Excitatory amino acids in commissural and Schaffer afferents

Several of the hippocampal excitatory pathways seem to utilise acidic amino acids as neurotransmitters. The evidence for a neurotransmitter role of glutamate and/or aspartate in the hippocampal commissural and Schaffer afferents are based on the biochemical and autoradiographic localization of high affinity uptake sites (Storm-Mathisen and Iversen, 1979; Fonnum, Lund-Karlsen, Malthe-Sorensen, Skrede and Walaas, 1979), induction of changes in the endogenous levels of amino acids after selective lesions (Fonnum and Walaas, 1978) and the demonstration of Ca^{++} mediated release following K^{+} or electrical stimulation (Nadler, White, Vaca, Perry and Cotman, 1978; Wieraszko and Lynch, 1979; Malthe-Sorensesen, Skrede and Fonnum, 1979).

It needs emphasizing that the abundant data in the literature (e.g., Nadler, Vaca, White, Lynch and Cotman, 1976; White, Nadler and Cotman, 1979; Koerner and Cotman, 1982) only implicates L-glutamate and/or L-aspartate or their analogues, as likely neurotransmitter candidates. In fact the methods used to implicate L-glutamate (Glu) or L-aspartate (Asp) as neurotransmitters are not without criticism. For example, many antagonists of acidic amino acid mediated excitation have been characterized, but their antagonism was to exogenously applied acidic amino acids. Firstly, these studies employ iontophoretic and/or other means of acidic amino acid applications, which is not necessarily the same as directly stimulating the fibers thought to contain acidic amino acids as neurotransmitters. There is lack of evidence for the selective/specicic antagonism of endogenous acidic amino acid

transmission (reviewed by Puil, 1981). Secondly, binding studies and electrophysiological manipulations have been used to characterize acidic amino acid subtypes. The validity of any ligand binding technique is dependent on the demonstration that the radioactive ligand selectively labels the physiological or pharmacological receptors under study. This requirement is often not fulfilled (Foster and Fagg, 1984). Thirdly, even though a Ca^{++} and voltage dependency are demonstrated in the release studies, it is not known for certain whether the released tritiated transmitter comes from the same intracellular compartment as the endogenous transmitter itself (Laduron, 1984). In other systems, it has been shown that tritiated ligands can be trapped in different intracellular compartments of intact cells (Maloteaux, Gossuin, Waterkeyn and Laduron, 1983). These ligands can be displaced by unlabelled compounds.

Still, Glu or Asp are probably the neurotransmitter substances in the commissural and Schaffer afferents and are treated as such here. In spinal neurons, microiontophoresed Glu causes a fast onset excitation followed by a rapid termination of action. This excitant action is mediated by a direct Glu- induced depolarization of the spinal neurons (reviewed by Puil, 1981). The electrophysiological studies indicate that Glu induces an inward movement of cations, mostly Na^{+} and/or perhaps Ca^{++}. Puil (1981) summarised that the initiating ionic event in Glu-induced depolarizations could be an increase in Ca^{++} conductance or even release of membrane-bound Ca^{++}. The subsequent inward movement of Ca^{++} could cause significant depolarizations, since Ca^{++} has a steep driving force and carries twice the amount of charge of Na^{+}. Whatever the initial mechanisms of activation, Glu- dependent depolarizations subsequently cause an intracellular accumu-

lation of Na^+ (which probably activates electrogenic Na^+ extrusion) and also an outward K^+ flux. Both these processes probably contribute towards repolarization.

Most of the actions/effects of Glu on spinal neurons have now been demonstrated in the hippocampal pyramidal neurones (Dingledine, 1983a; Collingridge, Kehl and McLennan, 1983). According to Hablitz and Langmoen (1982) the reversal potential for the Glu-mediated depolarization in the hippocampus was comparable to that of the EPSP. Both shifted in a negative direction in low Na^+ medium.

In CA1 pyramids, Dingledine (1983b) found that N-methyl-DL-aspartate (NMA) evoked Ca^{++} spikes accompanied by an apparent increase in input resistances. But Glu caused depolarizations with an associated decrease in resistance. Another observed difference was the selective blockade of NMA-induced responses by Ca^{++} antagonists whereas Glu actions remained (Dingledine, 1983b). It was concluded that NMA activated Ca^{++} conductances but Glu activated Na^+ and possibly K^+ conductances (Dingledine, 1983b; 1984; cf. Westbrook and Mayer, 1984).

Many of the acidic amino acids, notably aspartate, quisqualate and kainic acid, exhibit similar actions to Glu. Their differences in potencies, 'antagonistic' profiles and/or ionic conductances activated have led to the implication of acidic amino acid receptor subtypes. At least two types of acidic amino acid receptors have been implicated; the NMA receptor and the non-NMA receptor(s). (McDonald and Wojtowicz, 1982; Westbrook and Mayer,1984; Watkins, 1984; Foster and Fagg, 1984; Dingledine,1984). N-methyl-D-aspartate (NMDA) is selective for NMA receptors which, when activated, increase a voltage-dependent Ca^{++} conductance. Both quisqualate and kainate are

preferred at non-NMA receptors, whose activation elicits Na^+ and possibly K^+ conductances. Glu is active at both NMA and non-NMA receptors. Conceivably both receptor subtypes may be distributed in the same subsynaptic regions of the hippocampus.

9. CENTRAL NERVOUS SYSTEM SYNAPTIC PLASTICITY

9.1 Simple model of brain function

Once primitive neuroblasts differentiate into nerve cells, they loose their mitotic competency and never divide again (Gazen 1970; Jacobson, 1970). Subsequent nerve cell development is in completing genetically determined structural and functional specializations whose phenotypic expression is governed by their regional location, i.e., environmental influences. While microgrowths still occur that replace components such as synapses, none of the nerve cells divide to give rise to daughter nerve cells. The nervous system therefore is fully constructed in its cell numbers and connections before it is used (Eccles, 1977).

In very simple terms, the nervous system uses its own language to perform its integrative functions. Hence any external signals presented to it must first be converted into this unique nervous system language before processing. Eccles (1977) has stated that signal intensity is translated into increased frequency of action potential propagation in some peripheral afferents. In this scheme, then, transient changes in action potential amplitude and duration or frequency of action potential propagation or post-signal potentiation then represent some of the elements comprising the nervous system alphabet. It then becomes conceivable how any signal can be modified and encoded to constitute a specific message to the nervous system

for integration. If changes in the performance of the brain during life are not due to the addition of new daughter nerve cells then there must exist other mechanisms that could mediate, for instance, learning and memory functions. It could be argued that these complex processes involve subtle changes in the chemistry or microstructure and microfunction of the existing neuronal populations.

Let us suppose that ultimately memory functions are encoded as biochemical changes within selected neuron populations in specific regions of the brain then conceivably preferential mechanisms exist that mediate signal transfer or retrieval from neuronal memory banks.

At any one time arrays of memory and non-memory signals criss-cross in the same neuronal networks. If the fore-going summation is accurate then how can the preferential transfer of information be achieved? Most investigators in neurobiology implicate the phenomena of long-lasting potentiation as the likely mechanism for the selective and efficient transfer of learning and memory signals (review Swanson,Teyler and Thompson, 1982). During long-lasting potentiation, synaptic efficacies of specific inputs are greatly augmented for very prolonged periods (cf. Hebb, 1949).

9.2 Features of long-lasting potentiation and depression

Bliss and his co-workers (Bliss and Lømo, 1973; Bliss and GardnerMedwin, 1973) gave the first detailed account of post-tetanic long-lasting potentiation (also called long-term potentiation) of evoked field potentials in the dentate gyrus. In anaesthetized rabbits, conditioning stimuli of 10-20 Hz for one or more seconds given to the perforant path caused longlasting potentiation (LLP) of DG field responses evoked by post-tetanic test pulses (0.5 Hz frequency) to that input (Bliss and Lomo, 1973). LLP was of

several hours duration and was manifested as a decrease in PS latency and/or an increase in amplitude of the PS or field EPSP. LLP was found to be associated with a decrease in PS variability to the same test pulses. During the low frequency (10-20 Hz) train a rapid build up of PS was observed, a phenomena termed as frequency potentiation (Andersen, Holmqvist and Voorhoeve,1966). On cessation of this conditioning stimuli, a decaying PS potentiation to test pulses first occurred which was quickly followed by a phase of PS depression lasting from seconds to minutes. Thereafter, LLP became apparent. In contrast, high frequency (i.e., 100 Hz) conditioning stimuli caused a rapid diminution of the synchronouusly discharging PS during the trains but this led to a post-tetanic development of LLP without the initial phase of depression. The LLP elicited by high and low frequency tetanic trains exhibited similar characteristics. Once LLP was established it only began to diminish when the quality of recordings in the experimental and the control inputs were poor. Whenever there were changes reflective of LLP, these were confined to the tetanized and not the non-tetanized perforant path sections. In the same tetanized perforant path, subsequent conditioning stimuli caused an augmentation of the established LLP until an asymptote was reached.

LLP could not be accounted by changes in stimulation electrode after tetanic stimulations since a second electrode (along the same tetanized PP) that was not used to deliver the conditioning trains to this PP, could still evoke LLP in the DG. Furthermore, conditioning stimuli caused the stimulus versus response (i.e., EPSP or PS) curves to shift to the left. However, potentiation of the PS and EPSP did not always co-occur (Bliss and Lømo, 1973). It was argued that LLP was not a simple shift up the stimulus versus

response curves since a post-conditionong dendritic EPSP, though matched in amplitude with a pre-conditioning dendritic EPSP, elicited a bigger PS.

The above studies were subsequently reproduced in unanaesthetized rabbits (Bliss and Gardner-Medwin, 1973) where it also became clear that LLP could be maintained for up to three days. Presently it is known that LLP can be maintained for weeks in intact animals (review, Swanson, Teyler and Thompson, 1982). A more significant development, however, was the demonstration of LLP in the CA1 region of the guinea-pig transverse hippocampal slice in vitro (Schwartzkroin and Wester, 1975). Alger and Teyler (1976) were later to show that LLP could be elicited in the CA1, CA3 and DG subregions of the rat hippocampus in vitro. Schwartzkroin and Wester (1975) noted that a stimulating electrode placed in the stratum radiatum could evoke an orthodromic potential in CA1 and an antidromic potential in CA3. Yet tetanization with this radiatum placed stimulation electrode only potentiated the orthodromic potentials. In addition, the antidromic tetanization of CA1 axons in the alveus could not elicit LLP in the CA1 region.

Two separate inputs, one in the stratum oriens and the second in the stratum radiatum , presumably to the same CA1 output neurons were used to test for the input specificity of LLP (Andersen, Sundberg, Sveen and Wigstrom, 1977; Andersen, Sundberg, Sveen, Swann and Wigstrom, 1980). Immediately after the conditioning stimulation to the stratum radiatum afferents there was a generalised post-tetanic brief depression to both inputs. After this depressive phase, only the tetanised afferents showed LLP. Schwartzkroin and Wester (1975) had previously found that conditioning stimuli caused an increase in the probability of discharge in single CA1 units (cf.review Voronin, 1983). Subsequent studies in individual cells indicated that membrane resistance and resting membrane potential were

apparently not changed during LLP (Andersen, Sundberg,Sveen, Swann and Wigström, 1980).

Dunwiddie and Lynch (1979) reported that frequency potentiation, paired-pulse facilitation and short-term post-tetanic potentiation could readily be elicited in low Ca^{++} Ringer's solution whereas LLP was only occassionally induced in these conditions. Hence LLP seemed to differ from these other forms of neuronal plasticity since LLP induction was most sensitive to extracellular Ca^{++} levels. The dependence of LLP initiation on extracellular Ca^{++} was further investigated as follows. Ca^{++}-free Ringer's and Ringer's solutions containing increased manganese (Wigström, Swann and Andersen, 1979) or increased magnesium (Dunwiddie and Lynch, 1979) were used to block synaptic transmission. The presynaptic volley amplitude in normal Ringer's and during the synaptic blockade was essentially the same (Wigström, Swann and Andersen, 1979).

It was found that tetanic stimulations given during the synaptic blockade did not elicit LLP when post-tetanic responses were monitored in normal Ringer's. Yet after this return to normal Ringer's, if the same input was similarly tetanised, this caused the development of LLP (Dunwiddie and Lynch, 1979; Wigström, Swann and Andersen, 1979). Interestingly, the institution of a brief synaptic blockade with high magnesium Ringer's, <u>after</u> LLP was initiated, did not abolish LLP (Dunwiddie, Madison and Lynch, 1978). This indicated that only the initiation of LLP was dependent on the extracellular Ca^{++} but its maintenance seemed to be independent of this Ca^{++}.

Most studies that have used low frequency tetany (i.e., 10-30 Hz) to induce LLP have usually reported an initial phase of PS depression prior to LLP formation. In fact under these conditions, LLP has been reported as

'developing gradually' (Alger and Teyler, 1976). In contrast, high frequency tetanic stimulations i.e., greater than 100 Hz) cause the 'rapid' development of LLP without an associated depressive phase (Schwartzkroin and Wester, 1975; Swanson, Teyler and Thompson, 1982). Dunwiddie and Lynch (1978) examined the effects of stimulation trains of 100 pulses at rates of 5-100 Hz, on LLP development. The magnitude of the elicited LLP was shown to be greatest with the highest frequencies. It was confirmed that the lower frequencies evoked a post-tetanic depression prior to any other post-tetanic changes (Barrionuevo, Schottler and Lynch, 1980; Chirwa, Maretic and Sastry, 1983). The duration of this depression varied from minutes to hours.

Prior to tetanus, a constant depolarizing current of 0.56 nA was used to elicit action potentials in a CA1 pyramid (Andersen, Sundberg, Sveen, Swann and Wigström, 1980). The latency for action potential discharge, which was the time from the onset of the depolarizing pulse until the firing of an action potential was then determined. This latency was termed as the depolarizing current pulse (abbreviated as DPP). Immediately after the tetanic stimulation, and only during the time when the transient depression was present, the DPP was found to be increased. It was suggested that when the post-tetanus depression was present, this was associated with a generalized but transient decrease in CA1 neuronal excitability.

9.3 Summary

In conclusion it can be stated that LLP presents itself as an increase in synaptic efficacy, following appropriate conditioning of specific inputs. During LLP, previously subthreshold neurons are recruited and even neurons that were previously reaching threshold have an increased probability to discharge. With electrical manipulations, only Ca^{++}-dependent orthodromic

conditioning caused the development of LLP. Under these conditions, LLP will still develop even if the postsynaptic neurons are kept 'silent' with intracellularly injected hyperpolarizing currents. In fact there is no evidence in the literature that has shown the induction of LLP by injecting depolarizing currents into the CA1 neurons. It is presumed that antidromic influences or hyperpolarizing (and even depolarizing currents) in the soma do not 'reach' the dendritic/subsynaptic regions or the terminal boutons, where the changes mediating LLP seem to occur.

Recently, a variant to LLP in DG and CA3, termed associative LLP,has been described. Associative LLP is an enhanced synaptic efficacy that occurs in one (weak) synaptic input only if it is tetanically stimulated in conjunction with nearly concurrent stimulation of a second (strong) synaptic input (summary of Johnston and Brown, 1984). Furthermore, potentiation phenomena in other brain regions have been described (e.g., Racine, Milgram and Hafner, 1983) though their characteristics are not exactly like those of hippocampal LLP.

Lastly, the wide range of frequencies that induce LLP also cover the frequencies operative in normal physiology. LLP should therefore be inducible in a behaving animal. As stated previously, LLP is an attractive candidate for mediating some memory and learning functions. The role of the depressions that can be induced following repetitive stimulation is unclear. More importantly the actual mechanisms mediating LLP (and depression) or the loci for the change remain to be conclusively elucidated. The next section examines some of the mechanisms thought to account for LLP development. The possible loci for these changes are discussed.

10 CONSIDERATION OF POSSIBLE POSTSYNAPTIC MECHANISMS MEDIATING LLP

10.1 Post-tetanic potentiation

Post-tetanic potentiation (PTP) is a short duration (2-5 min) increase in synaptic efficacy which occurs after repetitive stimulation of an input and has been shown to be mediated by an increase in neurotransmitter released. The basic change in PTP is an afferent terminal hyperpolarization which leads to augmented action potential amplitudes (Eccles and Krnjević, 1959). It is unlikely that LLP is an extension of PTP per se, since the latter can be evoked in low Ca^{++} (Dunwiddie and Lynch, 1979) and is independent of tetanic stimulation intensities (McNaughton, Douglas and Goddard, 1978; McNaughton, 1982). PTP can be evoked in a single fiber (Eccles and Krnjević, 1959) whereas LLP has yet to be demonstrated in one hippocampal afferent. McNaughton, Douglas and Goddard (1978) contended that there existed a threshold stimulus intensity below which LLP could not be elicited. The available evidence in the literature rules out a common process between PTP and LLP.

10.2 Increases in afferent volley

Bliss and Lømo (1973) proposed that LLP could be explained in terms of increases in presynaptic volley (PV), i.e., increase in number of recruited fibers. Andersen, Sundberg, Sveen and Wigström (1977) failed to detect any changes in the afferent fibers field potentials after tetanus, indicating that the number of afferents being activated was unaltered. The use of constant current stimulators or biphasic stimuli has shown that stimulation electrode resistances are not changed by tetanus. Lastly, it has been reported that even when an ipsilateral input to a subfield in the hippocampus is potentiated, its collaterals to the contralateral hippocampus do not ex-

hibit LLP (Voronin, 1983 review). Such an effect would be unattainable, if indeed there was an increase in the presynaptic fibers recruited.

Still none of the above objections conclusively rule out an increase in PV in LLP. The early component which is often taken to be the PV merges into the dendritic EPSP without giving a clear demarcation (see fig. 4). The early onset of a potentiated dendritic EPSP tends to distort this PV and complicates analysis. The absence of LLP in a contralateral component of a tetanised ipsilateral input could be due to the inexact positioning of recording electrodes in the target areas of the contralateral hippocampal subfields. Besides, it is known that each single afferent fiber gives out a series of en passage boutons along its trajectory. It is not known for certain whether all the boutons belonging to the same afferent fiber are activated, each time an action potential is generated in that fiber. While there may not be any discernable changes in PV amplitude, it is likely that in LLP more inactive boutons are recruited. Such a possibility is functionally equivalent to an increase in PV and has yet to be tested.

10.3 Increased transmitter release

Voronin (1983) summarised that LLP was mediated by an increase in the number of transmitter quanta released per presynaptic spike. LLP presents itself as an increase in amplitudes and/or a decrease in rise time of evoked potentials. More importantly, various investigators have reported an increase in the intracellularly recorded EPSP during LLP (Yamamoto and Chujo, 1978; Andersen, Sundberg, Sveen, Swann and Wigström, 1980). These findings are consistent with an increase in neurotransmitter released (for alternative explanations, see later sections). A presynaptic change was indicated when it was found that terminal excitability of tetanised inputs decreased

after LLP initiation (Sastry, 1982). Lastly, an increase in glutamate release after the initiation of LLP has been demonstrated (Skrede and Malthe-Sorenssen, 1981; Dolphin, Errington and Bliss, 1982). However, these last studies quoted here have not constituted the definitive proof that LLP is mediated by an increase in transmitter released. Some objections will be cited here. It is not known for certain whether labelled ligands actually occupy the same sites as the endogenous neurotransmitter (Laduron, 1984). Besides, L-glutamate has been implicated but not conclusively shown to be the neurotransmitter in Schaffer collaterals (see section 8.4 in this thesis).

10.4 Spine morphology

Van Harreveld and Fifkova (1975) observed swellings of spines following tetanic stimulations and linked this to LLP. It was assumed that the evoked tetanus caused LLP even though this potentiation was not actually checked. Mixed results on spine changes and LLP have since been reported. Desmond and Levy (1981) reported increases in spine size and synaptic densities and associated these changes with LLP. Yet it was found by Lee, Schottler, Oliver and Lynch (1980) that LLP was not associated with significant changes in spine area, spine number, spine neck diameter or length of the postsynaptic density. Similar findings have been reported by Chang and Greenough (1984) who further noted that the only consistent morphological change seen during LLP was an increase in the number of sessile spines and direct contact synapses.

It should be noted that the functional significance of dendritic spines has yet to be elucidated. It has been proposed that spines might serve to attenuate synaptic signals (Chang, 1952) or permit the 'weighting' of sig-

nals from different afferents impinging on the same dendrite (Rall, 1970). Spines could be a structural mechanism for the separation of synaptic apparatus and thereby delimit synaptic cross-talk. Few studies have elaborated on the above possibilities. Computer modelling studies that have used dimensions obtained from hippocampal histological studies indicate that spine signal transients are only attenuated by less than 2% in conduction across the spine neck (Turner, 1984; Kawato and Tsukahara, 1984). Therefore, the dendrites essentially 'see' the total signal transduced at the subsynaptic membrane. Clearly no advantage would be offered by increased spine size, even if there is a concomittant reduction in axial resistance (if spines increase in volume), making such a change of less importance in LLP. It is apparent from the above discussion that if the intracellular dendritic EPSP increases after LLP (Yamamoto and Chujo, 1978) only two processes could account for the increase namely (a) increase in neurotransmitter depolarizing the subsynaptic membrane or (b) an increase in the conducting ability of the subsynaptic membrane. Point (a) can be incorporated in mechanisms discussed in section 10.3. Point (b) will be considered in section 10.5.

10.5 Increased synaptic receptors

Baudry and Lynch (1980) proposed the following scheme for LLP initiation. Tetanic stimulations caused Ca^{++} influx into postsynaptic dendrites. The increased intra-dendritic Ca^{++} triggered a biochemical change which involved phosphorylation of γ-pyruvate dehydrogenase. Then a membrane bound proteinase was activated which in turn caused proteolysis of some membrane associated component(s) thereby effecting re-organization of the dendritic membranes. Ultimately these changes caused the uncovering of glutamate receptors previously not accessible in synaptic transmission (Baudry

and Lynch, 1980; Eccles,1983 review) Since glutamate receptors are assumed to be coupled to ionic channels, clearly the above changes would lead to an increased conducting capability (i.e., more channels now available) of the subsynaptic membrane. Such an effect would satisfy point (b) in section 10.4 above.

Much of the evidence in support of the receptor increase hypothesis was obtained from binding studies that showed an increase in specific glutamate binding sites following the induction of LLP (Baudry and Lynch, 1979; Baudry, Oliver, Creager and Wieraszko, 1980). However, these studies can be faulted for the following reasons, Sastry and Goh (1984) carefully reproduced these binding studies but found that the increase in glutamate binding correlated better with depression rather than LLP. Results from the studies conducted in this thesis will further demonstrate some inconsistencies of the receptor hypothesis as a mechanism for LLP.

10.6 Miscellaneous changes.

Intracellular recordings have so far failed to reveal long-lasting changes in resting membrane potential, spike threshold or input resistances (Andersen, Sundberg, Sveen, Swann and Wigström, 1980). In any case, the specificity of CA1b LLP seem to rule out the possibility of a generalised postsynaptic increase in excitability (Andersen, Sundberg, Sveen and Wigström, 1977). In fact immediately after tetanic stimulations, post-tetanic hyperpolarizations occur which invariably decrease cell excitability (Yamamoto and Chujo, 1978). Other studies have shown a decrease in L-glutamate sensitivity after tetanus (Lynch, Gribkoff and Deadwyler, 1976). These changes do not support the notion that generalised postsynaptic changes can account for LLP. In addition, disinhibtion of interneurons and/or net in-

creases in synaptic excitatory to inhibitory influences (Schwartzkroin and Wester, 1975, Haas and Rose, 1982) have been examined and found to be unlikely candidates for LLP changes (Swanson, Teyler and Thompson, 1983 review).

However, one postsynaptic change offers a possible contributing mechanism to LLP. It was shown in section 6 that CA1b neurons are endowed with a multitude of ionic conductances, which participate in neuronal integration of synaptic signals. These conductances have been shown to contribute towards dendritic spike generation (Wong,1982) and may be involved in the soma-dendritic electrotonic coupling (Spencer and Kandel, 1961; Wong, Prince and Basbaum, 1979). It is feasible that dendritic changes in efficacy of some ionic conductances, which may not be readily detected with intracellular recordings, improve electrotonic coupling during LLP. Lastly, another possible mechanism for LLP that is presently incompletely characterised is the involvement of neuromodulators and related substances. For instance, it has been reported that depletion of noradrenaline reduces LLP (Bliss, Goddard, Robertson and Sutherland, 1981). Future studies will elaborate on these mechanisms.

10.7 Homo- and heterosynaptic depression

Homo- and heterosynaptic depression have yet to be fully characterised. It is known that lengthy low frequency tetanus induce homo- and heterosynaptic depressions (Dunwiddie and Lynch, 1978). Unlike LLP, depressions can be induced by nonsynaptic activation. Lynch, Gribkoff and Deadwyler (1976) reported a cell-wide reduction in responsiveness to glutamate iontophoresis. Andersen, Sunberg, Swann and Wigstrom (1980) used intracellular methods and detected a decrease in cell excitability. However, this testing was only

done for intervals immediately after tetanus and not when a fully developed homo- and heterosynaptic depression was present. It still has to be determined what intracellular changes are associated with the depressions. Recently it became evident that both depression and potentiation could co-exist. A balance is established between potentiation and depression (Sastry, Chirwa, Goh, Maretic and Pandanaboina, 1984). As best as can be determined, the locus for homo- and heterosynaptic depression is postsynaptic. The exact mechanisms mediating these changes are still under investigation.

11 SOME INHIBITORS OF POTASSIUM AND CALCIUM FLUXES

11.1 Barium

Barium (Ba^{++}) competitively hinders Ca^{++} entry into cells, and when inside the cell, Ba^{++} diminishes K^{+} efflux (Werman and Grundfest, 1961). Sastry (1979) demonstrated a Ba^{++}-mediated increase in presynaptic terminal action potential (AP) refractory period, indicative of a widened AP caused by the blockade of the delayed K^{+} rectifier current. In the hippocampus, Hotson and Prince (1981) found that bath applications of Ba^{++} augmented the Ca^{++} potentials but attenuated the K^{+}-dependent hyperpolarizations. It was concluded that Ba^{++} effects were caused by the influx of Ba^{++} through Ca^{++} channels followed by the ions' reduction of K^{+} conductance.

Ba^{++} is closely related to Ca^{++} in atomic number, valence and chemical properties. The hydrated radius of Ba^{++} is smaller than that of Ca^{++} (Stokes, 1964) and may account for the favoured entry of Ba^{++} than Ca^{++} through physiological Ca^{++} channels (Potreau and Raymond, 1980). Extensive studies are lacking that have explored all the Ca^{++} functions

that Ba^{++} can mimic. Ba^{++} will bind to calmodulin though with a lower affinity than Ca^{++} (Wolff, Hubner and Siegel, 1972). It was of interest to find out whether Ba^{++} can substitute for Ca^{++} during the initiation of LLP.

11.2 4-Aminopyridine

There are relatively few studies that have been concerned with the possible correlation between pharmacologically induced enhancement of field responses (Haas and Ryall, 1980; Linsemann and Corrigall, 1981; Turner, Baimbridge and Miller, 1982; Goh and Sastry, 1983) and the electrically evoked LLP (Bliss and Lømo, 1973). Pharmacological agents such as 4-Aminopyridine (4-AP) enhance inhibitory and excitatory influences in the hippocampus (Buckle and Haas, 1982). 4-AP blocks voltage sensitive, fast K^{+} channels in a variety of excitable tissues (Lechat, Thesleff and Bowman, 1981). Consequently 4-AP will enhance most processes that are dependent on action potentials whose declining phase is partly due to fast K^{+} channel conductances. In parallel fibers of the rat cerebellar cortex, 4-AP caused the widening of the presynaptic volley (Kocsis, Malenka and Waxman, 1982). In presynaptic terminals, K^{+} blockade invariably leads to prolongation of the action potential and this causes voltage-sensitive Ca^{++} channels to open for a longer period (Yeh, Oxford, Klu and Narahashi, 1976; Tokunaga, Sandri and Akeset, 1979; Sastry, 1979; Thesleff, 1980; Letchat, 1981). Hence Ca^{++} influx is enhanced and this facilitates coupling between terminal depolarizations and transmitter release at chemical synapses. The actions of 4-AP therefore lead to an increase in impulse-evoked transmitter release (Thesleff, 1980). An interesting feature of 4-AP interactions is in its enhancement of Ca^{++} entry into presynaptic terminals (Buckle and Haas, 1982), which is a possible loci for LLP (Sastry, 1982). This property of

4-AP was utilized in an attempt to initiate LLP via pharmacological methods.

11.3 Verapamil

Verapamil is a synthetic derivative of papaverine, an alkaloid found in opium poppy. In cardiac pharmacology, verapamil blocks activated and inactivated Ca^{++}-channels. Its effects are more marked in tissues that discharge frequently or are less polarized at rest and where activation is dependent on the Ca^{++} current (Henry, 1980; Katzung and Chatterjee, 1982). The sinoatrial and atrioventricular nodes exhibit both these characteristics. Verapamil activity is stereospecific (i.e., possible receptor interaction) with the S(-) isomer presenting a much greater potency than the R(+) isomer. Presumably verapamil acts from the inner side of the membrane and binds more effectively to channels in depolarized membranes. This block is partially reversible by elevating extracellular Ca^{++} or by drugs that increase the transmembrane flux of Ca^{++}, e.g., adrenergic stimulants (Katzung and Chatterjee, 1982). The actions of verapamil stated above have made this substance useful in clinical therapeutics Katzung and Chatterjee, 1982) where it is indicated for the following conditions; (a) atrial fibrillation or atrial flutter of recent onset, with rapid ventricular response (especially if not controllable with digitalis preparations) (b) paroxysmal supraventricular tachycardia (c) chronic stable angina of effort (d) angina resulting from coronary artery spasm and (e) non-surgical obstructive hypertrophic cardiomyopathy (Canadian Pharmaceutical Association, 1984). The therapeutic effects correspond to verapamil plasma levels of 60-70 ng/mL., i.e., 0.13-0.15 μM, typically less than 1 μM. At these concentrations, verapamil exerts its inhibition of Ca^{++} entry into cardiac tissue.

Data from cardiac and other excitable tissues indicate that low verapamil concentrations do not prevent the Ca^{++}-dependent release of transmitter

substances. Annunziato, DiRenzo, Amoroso and Quattrone (1984) found that the K^+-evoked release of endogenous dopamine from tuberoinfundibular neurons in vitro was enhanced by 1μM verapamil but depressed by verapamil concentrations greater than 10 μM. Similarly low verapamil (less than 10 μM) interfered with the Ca^{++}-dependent contractile responses of isolated canine saphenous veins caused by transmural nerve stimulation and yet this same verapamil concentration did not reduce release of tritiated norepinephrine from the adrenergic nerve endings in this system (Takata and Kato, 1984). Only verapamil concentrations greater than 30 μM could reduce the release of tritiated norepinephrine following transmural adrenergic nerve stimulation. In rat cerebrocortical synaptosomes, Norris, Dhaliwall, Druce and Bradford (1983) demonstrated that while low verapamil concentrations (equal to or less than 1 μM) could not prevent transmitter release, it was effective in blocking $^{45}Ca^{++}$ entry. In excised rabbit nodose ganglia C somata, Ca^{++} spikes could be elicited which were maintained by further stimulation of Ca^{++} influx (Ito, Sakanashi, Kawamura and Nishi, 1984). In this tissue, it was reported that in Na^+ free solutions intracellularly recorded Ca^{++} spikes were reduced in amplitude by 10 μM verapamil. In normal physiological solutions, 1 μM verapamil caused reductions in maximal rate of rise and maximal rate of fall. Ito, Sakanashi, Kawamura and Nishi (1984) concluded that these low concentrations of verapamil depressed Ca^{++} influx and possibly could interfere with Na^+ and K^+ fluxes.

The preceding studies demonstrate that low verapamil concentrations interfere with Ca^{++} entry into neurons and/or other excitable cells but do not prevent the Ca^{++}-dependent neurotransmitter release. It is well docu-

mented that the initiation of hippocampal plasticity are Ca^{++}-dependent. However, it remains to be resolved whether this Ca^{++} requirement is needed to the pre- and/or postsynaptic elements. The effects of low verapamil concentration was thought to be a potential method that could be used to determine which Ca^{++} currents (i.e., presynaptic versus post-synaptic) were important for the development of LLP. For that reason, low verapamil concentrations were selected and used in some of the studies reported in this thesis.

12. METHODS

12.1 Animals

Male Sprague-Dawley rats obtained from Charles River in Ontario (Canada) were used for some of the early experiments reported in the present thesis, i.e., the demonstration and characterization of LLP and depression and the assessment of 4-AP effects in the hippocampus. But to minimise costs as well as facilitate rapid delivery of animals, male Wistar rats obtained from the university of British Columbia (Canada) animal unit were subsequently used.

The animal unit uses standard animal care procedures for the maintenance of laboratory animals. Their Wistar rats are weaned after 21 days. Once a week, typically on Mondays, 8-12 male Wistar rats were received from the animal unit and used for studies in that same week. Upon arrival and prior to experimentation, Wistar rats (about 30 days old; 75-100g) were kept in wire cages in the modern departmental animal room. These rats had free access to food (rat chow) and water. The animal room had controlled temperature (22-23° C) and humidity (50-55%) with set 12-hourly day and night periods.

First of all, studies on LLP and depression as well as effects of 4-AP in Sprague-Dawley rats could be duplicated in Wistar rats in vitro. In fact no significant differences in pharmacological responsiveness and/or electrophysiological behavior could be detected between slices from Sprague-Dawley rats and Wistar rats (cf. Chirwa, Goh, Maretic and Satry, 1983 to Sastry, Chirwa, Goh, Maretic and Pandanaboina, 1984). Hence whenever applicable the overall experimental data from Sprague-Dawley rats and Wistar rats have been assessed together without critical considerations of the rat subtypes used.

12.2 Slice preparations

Hippocampal slices were prepared from male Wistar rats as follows. Animals were initially cooled (30-40 min) on an ice pack in a dessicator to a rectal temperature of 30-31° C and maintained on a mixture of halothane and oxygen. The dessicator was pre-equilibrated with this halothane/oxygen mixture (in concentrations sufficient for general anaesthesia) before introducing the animal. To obtain slices, the skin was cut and an insertion made under the base of the skull. A pair of small scissors was used to cut through the skull along the sagittal suture line and the sides pulled apart to expose the brain. The brain was carefully removed and hippocampus dissected free on a cooled glass surface. The dorsal surface of the hippocampus was then sectioned transversely to the septotemporal axis at a thickness of 500 µ on a McIlwain tissue chopper. Serial sections were separated with a fine brush and a stainless steel spatula in a plate containing previously cooled (5° C) artificial cerebrospinal fluid (termed 'standard medium' or 'standard solution' in this thesis) and equilibrated with 5% carbon dioxide and 95% oxygen (carbogen). The procedure from surgery to slice preparetion was completed within 3 minutes. Initial

cooling of the animal significantly increased the proportion of viable slices obtained from each hippocampus. Finally slices were transferred to the slice chamber. Slices were positioned between two nylon nets to minimise movement as well as permit submersion. The chambers were perfused at a rate of 2-3 mL/min with the standard medium containing in mM: NaCl, 120; KCl, 3.1; $NaHCO_3$3, 26; NaH_2PO_4, 1.3; $CaCl_2$, 2.0; $MgCl_2$, 2.0 and glucose, 10.0. The standard medium was pre-gassed with carbogen (pH of medium, ca. 7.4) and maintained at 32 ± 0.5° C. In addition, the carbogen flowed over the top of the slices in the slice chamber. Slices were allowed to equilibrate with the standard medium in the slice chamber for a minimum of 60 minutes prior to recording.

12.3 Slice selection and viability

Significant damage occurs along the cut surfaces of the slice (Schurr, Reid, Tseng and Edmonds, 1984). So electrophysiological recordings were usually obtained from a depth of about 100-300 μ from the surface of a transversely cut hippocampus section. It is known that immediately after slicing, the metabolic status of brain slices is significantly altered relative to *in vivo* activity. For instance, the levels of total adenylates and creatines or peak cAMP and cGMP levels are decreased (Whittingham, Lust, Christakis and Passonneau, 1984). In fact redistribution of cations occurs, i.e., lowered intracellular K^+/Na^+ ratio. Evidence indicates that some 1-2 hours are needed for the slices to return back to a steady metabolic state (Whittingham, Lust, Christakis and Passonneau, 1984). This steady metabolic state in slices is presumably maintained for 6-8 hours of incubation. Perez-saad, Valouskova and Bures (1984) found that immediately after slice cutting and submerging, the extracellular K^+ profiles were very

high. After 1-2 hours these investigators found that the extracellular K^+ profiles decreased and approached the K^+ concentrations of the artificial cerebrospinal fluid used.

It is for reasons as presented above that in the present studies, slices were incubated for at least one hour to permit recovery from the mechanical and metabolic trauma. To begin with, selected slices (4-6 out of a possible 8-10 obtained in slicing) had the following characteristics. They were intact with well defined and unmashed borders, i.e., slice edges. Only slices with a clear, translucent CA and DG cellular layers and in addition, a V- and/or U-shaped DG were chosen. Furthermore, the selected slices had clean and even (smooth) surfaces, i.e., not 'mashy' or 'flaky'.

Evoked responses could be obtained after one-hour incubations and maintained for up to 12 hours (Sastry, Chirwa, Goh, Maretic and Pandanaboina, 1984). Each experiment was typically of 2-4 hours duration. Hence 2-3 of the selected slices could have been used concurrently after the incubation period. But to minimise variability between slices from the same animal (and those of other animals) caused by different exposure times to the standard medium, only one slice per animal was ultimately used. Consequently, the total times of exposures to bathing medium and experimentation for different slices were comparable.

12.4 Slice chamber and perfusion method

Detailed descriptions of the slice chamber used were reported in a publication from this laboratory (Pandanaboina and Sastry, 1984). Figure 5 is a simple sketch of the slice chamber. The main components of the chamber were (a) a raised stage constructed of plexiglass and (b) with a circular chamber of diameter 7.5 cm and depth 0.7 cm, bored into the top surface and (c) a special temperature-regulating aluminum bar that was stuck beneath the circular chamber.

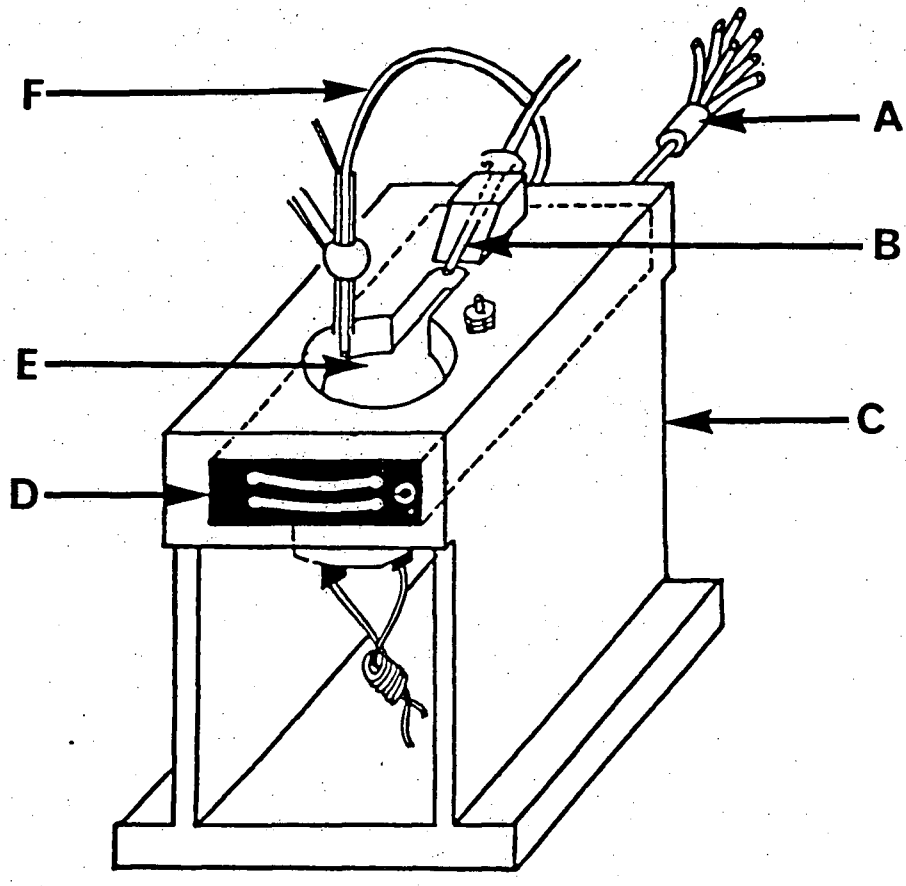


Fig. 5. Slice chamber.

A. Manifold.
B. Suction line.
C. Raised plexiglass stage.
D. Temperature regulating aluminum bar.
E. The slice chamber.
F. Medium inlet.

The standard and test media were contained in separate 50-mL polyethylene barrels. Carbogen lines for medium oxygenation terminated in each of these barrels. The barrel containing standard medium was in turn connected to an elevated feeding tank (volume, ca. 2 L) which was the source of standard medium.

From each 50-mL barrel came out a connecting tube (that could be opened or closed with a clip) that led to a common 'manifold' on one end and which in turn, gave out a single outlet on the opposite side. This single outlet, i.e, via connecting polyethylene tubes, eventually fed the selected medium to the slice chamber. Briefly, both standard and/or test solutions were introduced into the slice chamber from above. Continuous drainage out of the slice chamber was via a side suction outlet created for that purpose. A balanced inflow and outflow of solutions ensured the maintenance of constant solution levels in the slice chamber. At all times, the slices were submerged in the medium.

Among other properties, the whole perfusion set-up permitted (a) the rapid exchange of standard and test solutions (b) complete oxygenation of solutions (c) removal of dead spaces within the system and (d) the regulation of solution temperature.

12.5 Preparation of standard and test media

The standard medium was freshly prepared on each experimental day, to the final constituent concentrations described in section 12.2. Test media of Ba^{++}, Mn^{++} and Mg^{++} were prepared by omitting 2 mM Ca^{++} in the standard medium and replacing it with 2 mM of Ba^{++} or Mn^{++} or Mg^{++} respectively. Stock solutions of L-glutamate (the monosodium salt) and 4-Aminopyridine, were prepared by dissolving appropriate amounts of each of

these substances in de-ionised water to make final concentrations of 100 mM. The L-glutamate solution was used to fill the iontophoretic microelectrode. The 4-AP solutions were diluted to the appropriate final concentrations by adding measured amounts to the standard medium or the other test media. In all cases, the pH of the final 4-AP containing media was found to be ca. 7.4 while being bubbled with carbogen.

The stock solutions were refrigerated when not being used. The remaining standard media at the end of the (experiment) day was discarded. The laboratory chemicals used in the preparation of solutions were obtained from the following sources; (a) $BaCl_2$, 4-Aminopyridine and L-glucose came from Sigma, (b) $NaHCO_3$, NaCl, NaH_2PO4, $MgCl_2$ and $CaCl_2$, came from Fisher and (c) KCl and $MnCl_2$ came from Baker.

12.6 Stimulating and recording electrodes

Concentric bipolar stimulating electrodes, model SNEX-100 with shaft lengths of 50 mm (Rhodes Medical Instruments) were used. These electrodes had resistances of 1.0 MΩ. Subsequently each stimulating electrode was replaced, whenever its resistance significantly increased, i.e., greater than 3 MΩ (occurred after 5-7 weeks of continuous use) and if this resistance could not be lowered by basic techniques of cleaning the electrode.

Standard fiber filled glass micropipettes (internal diameter, 1.02 ± 0.15; outside diameter, 1.5 ± 0.1 mm: WPI) were used to prepare recording electrodes. Most of these micropipettes were pulled to fine tips (tip diameter, 1-3 micrometers) on the Narishige vertical puller type PE-2. Single barrel recording micropipettes were filled with 4M NaCl and had resistances of 0.5-1.5 MΩ. These micropipettes were used to monitor or record spontaneous and evoked potentials in the hippocampus in vitro. For

iontophoretic applications and recordings, seven-barrel micropipettes were assembled, then pulled (tip diameter, 2-3 micrometers) and filled as follows. The central barrel, which was usually used to record potentials was filled with 4M NaCl. Three side barrels also contained 4M NaCl, and were used to check for current effects and to initiate current balancing when necessary. The remaining three side barrels were filled with 0.1M L-glutamate (resistance typically 30-50 MΩ).

A third type of recording micropipette was assembled and used to monitor Ca^{++} and K^{+} levels. A double-barrel ion selective micropipette was pulled to fine tip sizes (diameter 0.5-1 micrometers) on a model P-77, Brown-Flaming micropipette puller. Then a very fine hypodermic needle was inserted down to the taper of one barrel. Using a double-switch system, this hypodermic needle could be used to pass either nitrogen (N_2) or vapors of dichloromethylsilane (silane) caused by first passing N_2 over silane.

Initially, the taper of the micropipette was heated to 200°C in a coil. During this heating, one barrel was dried by passing N_2 for one minute. This was followed by passing silane vapors down the taper for 5 minutes. This process led to the baking of silane onto the inner surfaces of the treated micropipette barrel thereby making it hydrophobic. Each silaned tip was syringe-filled with either ion-exchange resin IE-190 for K^{+} or ion-exchange resin IE-202 for Ca^{++} (both resins obtained from Narco). Then the barrels were back-filled with 0.1M KCl or 0.1M $CaCl_2$ respectively. The non-silaned barrel in the double-barrel assembly was used as a reference electrode and filled with 0.1M NaCl (for K^{+}-electrode) and 0.1M KCl (for the Ca^{++}-electrode).

The K^+ electrode was calibrated by measuring the mV deflections on a vibronmeter, after exposure to graded $[K^+]$ solutions. In the range 10-150 mM K^+, a linear relationship was observed. Typically a 40-45 mV deflection was seen with every 10 mM increase in $[K^+]$. Similarly, the Ca^{++}-electrode was calibrated using graded $[Ca^{++}]$ solutions. In the linear range, (within physiological Ca^{++} ranges), increases of 10 mM $[Ca^{++}]$ caused approximately 25 mV deflections. These calibrated values for K^+ and Ca^{++} were used as references for comparison when recording K^+ and Ca^{++} changes in the hippocampus, in vitro.

12.7 Positioning of electrodes

Placement of electrodes was done with the aid of a dissecting microscope. The stimulating electrodes were positioned in the stratum radiatum or in the CA3 region for stimulating Schaffer collaterals. In some cases, stimulating electrodes were placed in the alveus or the stratum oriens for the stimulation of CA1 pyramids axons or commissural afferents respectively. The terminal region excitability was examined with stimulation electrodes placed in the Schaffer-CA1 dendrites synaptic regions to evoke discharges in CA3 pyramids. These stimulation and recording electrode positions are shown in fig. 6A and 6B.

The recording micropipettes, i.e., single barrel, double-barrel ion selective electrode or seven-barrel iontophoretic electrode, were positioned in the stratum oriens, stratum pyramidale or stratum radiatum in the CA1b subfield (Masukawa, Bernado and Prince, 1982). This arrangement permitted the recording from basal dendrites, CA1 cell bodies and apical dendrites. Once positioned, the depth of the tip of the micropipette from the slice surface was adjusted to maximise the observed responses evoked by stimulation of

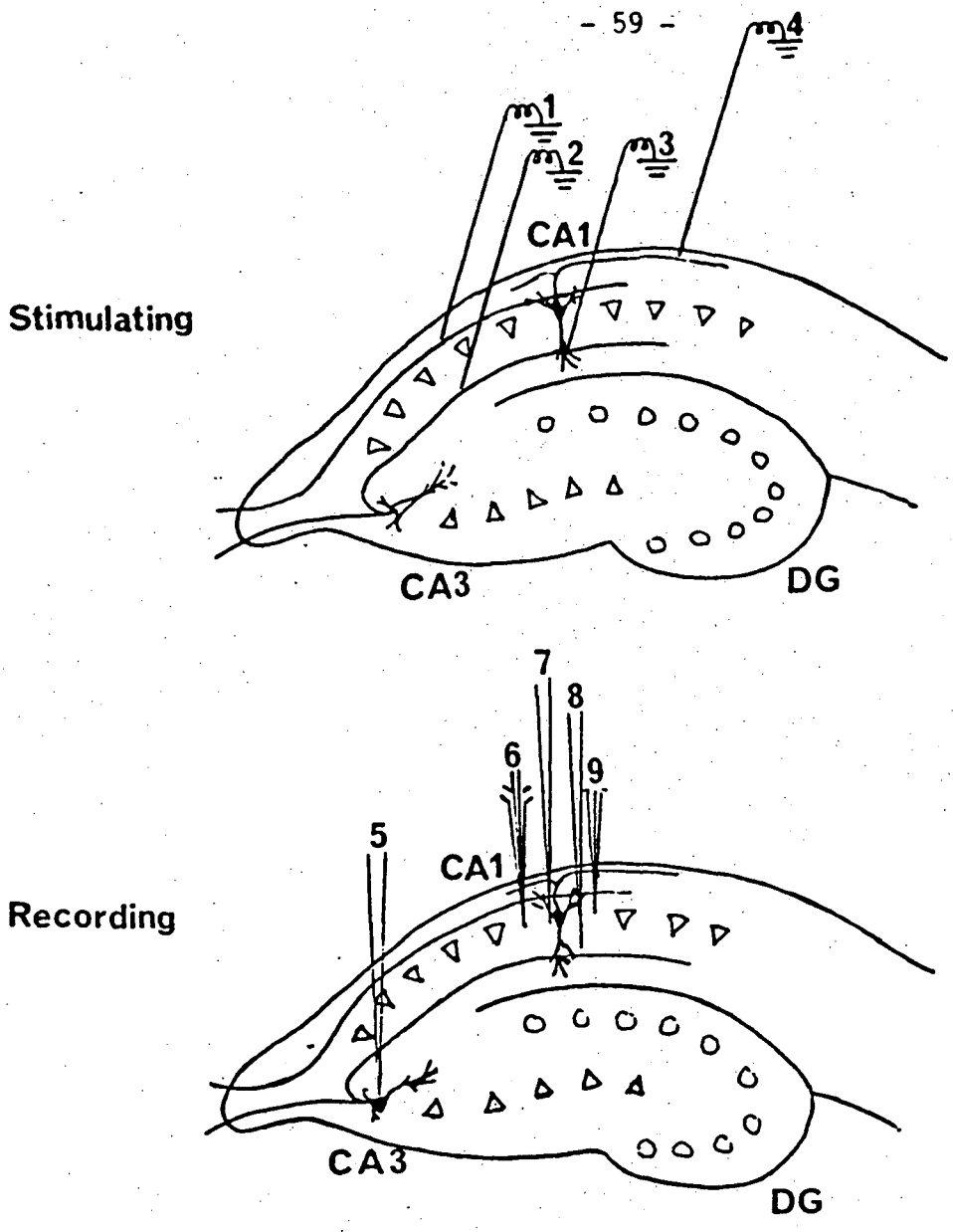


Fig. 6. Positioning of stimulating and recording electrodes.

A. Stimulating:
1. Commissural afferents.
2. Schaffer collaterals.
3. Sch/CA1b synaptic regions.
4. CA1b axons.

B. Recording:
5. Antidromic single spikes in CA3.
6. PS with central barrel; ejecting. L-glutamate with side barrels.
7. CA1b PS.
8. EPSP in dendritic regions.
9. Monitoring K^+ or Ca^{++} in cell body layer.

CA1b axons, commissural and/or Schaffer collaterals. During Schaffer collateral boutons excitability testing, the single-barrel recording micropipette was positioned in the CA3 subfield, for the recording of antidromic single cell discharges.

12.8 Laboratory electrical instruments

The main electrical apparatus/instruments used are briefly summarised below. Square wave pulses were delivered through an isolation unit type DS2 regulated by the digitimer programmer D4030. Alternatively, the Grass S88 stimulator was used to drive either the stimulus isolation unit S1115 or the photoelectric constant current unit, which was used in terminal region excitability testing. Recording electrode signals were amplified on the Neurolog systems or the Dam-5A differential pre-amplifier. The amplified signals could be viewed on any of the three types of oscilloscopes namely; the Data 6000 programmable acquisition unit, the Tektronix dual beam storage type 565 or type 5113.

Ion selective signals were led into the Vibronmeter which, in turn, fed signals to the Grass polygraph to be amplified and plotted out. Typically, permanent records of all observed signals could be plotted on the HP 7404A recorder and the HP 7470A plotter. This last plotter was driven and controlled by the Data 6000 programmable acquisition unit. Signals could also be plotted out on the Grass polygraph. In addition, displayed signals on the oscilloscope type 5113 could be photographed directly. In some cases complete experiments, (especially those examining individual pulses during tetanic stimulations) were taped on Sony PR-150 magnetic tape using the HP-3968A instrument recorder. Then selected segments of these tapings could be charted out on the HP-7404A plotter for analysis or played back on one of

the oscilloscopes. Lastly, a six channel Neurophore BH-2 (Medical Systems) was used for iontophoretic applications of selected ionic species. Among other features, this Neurophore gave controlled current ejections of selected ionic species as well as induction of set backing and/or balancing currents. The availability of these electrical instruments made it possible to combine two or more units and independently stimulate and/or record from more than one hippocampal site.

13. EXPERIMENT SCHEMES

13.1 Induction of evoked responses

Depolarizing square wave pulses (2-15 V; 0.02-0.8 msec) delivered at 0.2 Hz test frequency were used to stimulate CA1b cell axons or commissural (Com) or Schaffer collaterals (Sch).

Components of the somatic and dendritic recorded potentials were examined in standard medium and in Mg^{++}-medium. This latter procedure permitted the visualization of the presynaptic volley as well as antidromic components or non-Ca^{++} dependent evoked potentials.

Furthermore, depolarizing square wave pulses of 4-15 uA amplitudes and 0.2-0.8 msec durations (test frequency), 0.2 Hz) were applied in the Sch-CA1b apical dendritic synaptic regions to evoke antidromic single cell discharges in CA3 pyramids. These all-or-none discharges could be evoked in standard medium and in Mg^{++} or Mn^{++} media. Subsequent use of these antidromic single cell discharges was dependent on the demonstration of (a) constant onset latency, which was the time from onset of stimulation artifact to peak single discharge negativity (b) constant shape and amplitude in

standard medium, comparable to spontaneous discharges in these CA3 pyramids and (c) distinct thresholds of activation.

The selected sets of electrical parameters in each experiment were those that elicited PS amplitudes of 1.0-1.5 mV or dendritic EPSP amplitudes of approximately 1.0 mV. Once these initial population responses were obtained, particularly those of orthodromic stimulation, their amplitudes were constantly monitored (at fixed pulse amplitude and duration; 0.2 Hz) for a minimum 30 minutes. These were the baseline responses. Subsequent experimentation was conducted only if these baseline responses remained unchanged for at least 30 minutes. Then changes to evoked potentials caused by electrical conditioning and/or pharmacological manipulations were compared to baseline responses and where applicable these changes were expressed as fractions of the baseline responses respectively.

13.2 Tetanic frequencies

Studies on potentiation and depression of evoked responses were conducted by using one or more of the following frequencies: 20 Hz, 200 or 600 pulses; 100 Hz, 100 or 500 pulses and 400 Hz, 200 pulses. These tetanic trains were evoked using the same stimulation strengths and durations as was used to obtain baseline (control) responses respectively.

Tetanic stimulations were delivered to any of the following inputs (a) commissural (b) Schaffer collaterals (c) Sch-CA1b synaptic regions and (d) CA1b cell axons. To facilitate analysis individual pulses in the tetanic trains were taped and charted out at the end of the experiment.

13.3 4-AP dose-response curves

In a given slice, dose response curves were constructed by the method of stepwise cumulative addition of the drug. After a control period the ini-

tial concentration of 4-AP used was 25 μM. The amount of 4-AP was then doubled after each 5 minute interval to a maximum of 400 μM. On completion of the dose-response curve, 4-AP was washed out from the slices by continuous perfusion with standard medium. All responses to 4-AP and the subsequent recovery were expressed as a percentage of the control responses obtained in standard medium prior to the determination of the dose response curves.

13.4 Effects of 4-AP

A concentration of 100 μM 4-AP was selected from the dose-response curve above and used for subsequent studies. In one series of experiments, each slice was perfused with 4-AP for 5 minutes only and then the drug was washed out with standard medium. A second 5-minute 4-AP perfusion was started after an interval of 40-60 minutes. Responses in 4-AP and during washing out were monitored.

Similar experiments were conducted (different slices) with the following modifications. After obtaining control responses, stimulation was stopped for 15 minutes and then resumed again. This was done to check for the effects, if any, of temporarily stopping stimulation. Spontaneous activity was also monitored. After a minimum of 30 minutes stimulation was stopped again in the same slice, but this time 4-AP was perfused for 5 minutes. Effects of 4-AP in calcium-free medium was examined as follows: Mg^{++}-medium was prefused to block synaptic transmission for 15 minutes. 100 μM 4-AP (previously added to a second Mg^{++} medium) was perfused for 5 minutes during synaptic blockade i.e., 5 min. after synaptic blockade was established).

13.5 Verapamil studies

Dose-response curves to 0.083-2.64 μM verapamil were obtained using the single application method (i.e., with washing between doses). After

exposure to the initial 0.083 μM for 3-4 min, the verapamil dose was doubled. This process was repeated until a maximum 2.64 μM verapamil was applied. From the completed dose-response curves, an appropriate verapamil concentration (0.33 μM) was selected and used in subsequent experiments. The effects of a single concentration (0.33 μM) verapamil were tested as follows. After establishing baseline responses to Sch stimulation, 0.33 μM verapamil in standard medium was perfused for 3 minutes and then washed out with just standard medium perfusions. A similar application was repeated some 40-60 minutes after the first application. In both cases, evoked responses during verapamil were monitored.

13.6 Iontophoretic L-glutamate

Using ejection currents of 50-200 nA, L-glutamate was locally applied for 1 minute to the CA1b pyramidal layer. In these experiments the Sch were continuously stimulated at 0.2 Hz. In another set of experiments, modified standard medium containing 0.33 μM verapamil preceded the ejection of L-glutamate by 1 minute This modified standard medium with verapamil was left on for a total 3 minutes. Lastly, tetanic stimulations (100 Hz, 1 sec) to Sch were evoked (a) while L-glu was ejected alone or (b) when L-Glu was ejected concurrently with the verapamil medium.

13.7 Effects of Ba^{++}

At pre-determined intervals, Ba^{++} medium was perfused for 3, 10 or 15 minutes. Each selected perfusion time constituted a complete experiment in a given slice. The Ba^{++} medium perfusion was instituted while the Sch were being stimulated at 0.2 Hz.

13.8 Monitoring extracellular Ca^{++} and K^{+} changes

Changes in soma extracellular Ca^{++} or extracellular K^{+} during tetanic stimulations (20 Hz or 100 Hz) were recorded using the ion-sensitive

electrodes (ISE). These ISE were positioned in the pyramidal layer as previously described (see section 12.7).

13.9 Sch boutons excitability testing

Control thresholds in standard medium for the initiation of antidromic CA3 single cell discharges were first determined in standard medium. The threshold was defined as the stimulation strength used to evoke 4-5 antidromic discharges out of 10 possible attempts. The thresholds were then determined during as well as after 4-AP or Ba^{++} perfusions.

13.10 Homo- and heterosynaptic plasticity

To investigate homo- and heterosynaptic plasticity the commissural and the Schaffer collaterals were stimulated as follows. Initially baseline responses and their appropriate stimulation parameters were determined for each input independently. Thereafter, 10-15 depolarizing pulses at 0.2 Hz were given to the Sch while the Com was not stimulated. After these pulses, stimulation was stopped and then another 10-15 depolarizing pulses at 0.2 Hz were given to the commissural input. This alternate manner of stimulating these two inputs was repeated during the whole experiment. However, at appropriate intervals, selected tetanic trains were evoked in the Sch or the Com. In some experiments tetanic stimulations were evoked while 0.33 µM verapamil was perfused for 3 minutes. In these experiments, tetanic stimulations were evoked during the last minute of verapamil perfusions.

14. RESULTS

14.1 Evoked potentials

The evoked potentials recorded in CA1b cell layer had a biphasic positive wave (PW) that could be interrupted with a negative going peak which

was the population spike (PS). The presence of the PS and its increasing magnitude, i.e., more neurons recruited was dependent on the stimulus strength used. Stimulus strengths between 2-15 volts (0.02-0.8 msec; 0.2 Hz) to Sch or Com elicited PS amplitudes of 1.0-1.5 mV. Figure 7 shows how the PS amplitude and its latency was measured. The observed latencies from onset of artifact to peak negativity of the orthodromic PS were 6-10 msec. The longer latencies were associated with stimulating electrodes positioned farthest from the recording electrodes in CA1b. Similarly, antidromic activation of CA1b axons in the alveus, used lower stimulus strengths but elicited comparable PS amplitudes (1.0-1.5 mV) that had shorter latencies (1-3 msec). Orthodromic potentials recorded at the CA1b dendrites in stratum oriens or stratum radiatum induced a slow, negative going potential which was the population EPSP. Depending on the stimulation strength, a positive going peak was sometimes superimposed on this dendritic EPSP wave, which was the soma generated PS. The relationship of stimulation strength and amplitude of PW, PS and EPSP obtained in a typical experiment are given in figure 8. In Mg^{++} or Mn^{++} media, orthodromic potentials were abolished but not the antidromic evoked potentials. During Mg^{++} or Mn^{++} perfusions, the presynaptic volley, especially in dendritic recordings, was clearly discernible. Lastly, visual inspection of the continuous oscilloscope signals revealed little or no spontaneous activity in standard medium. In Mg^{++} or Mn^{++} media, however, the spontaneous activity increased and sometimes led to bursting activity.

14.2 4-AP dose-response curves

Figure 9 shows the dose-response curves obtained from the cumulative addition of 4-AP. Maximal enhancements in evoked PS were obtained with con

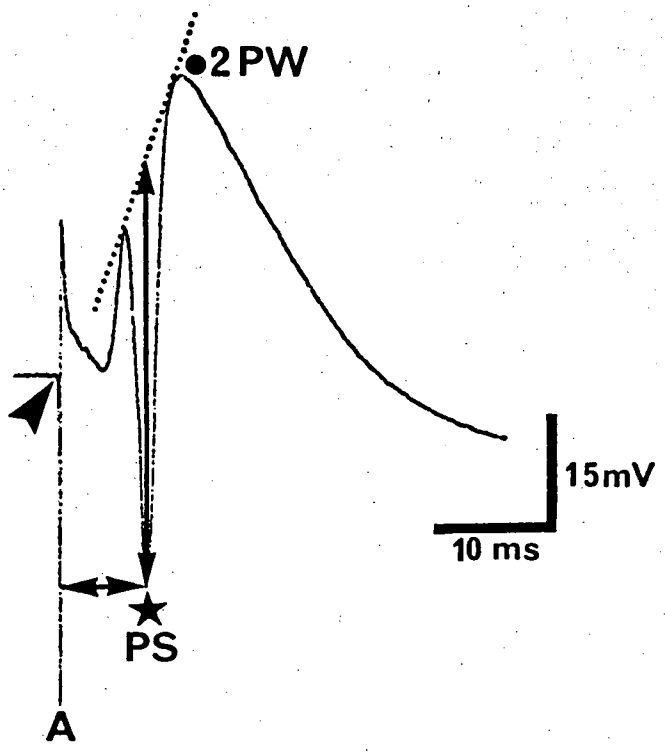


Fig. 7. PS amplitude and latency measurement. Vertical line corresponds to PS amplitude measurement. Horizontal line represents PS onset latency. Diagonal arrow shows onset of stimulus artifact. Negativity is downwards. Abbreviations: A, stimulus artifact; PS, population spike, 2PW, second component of positive wave.

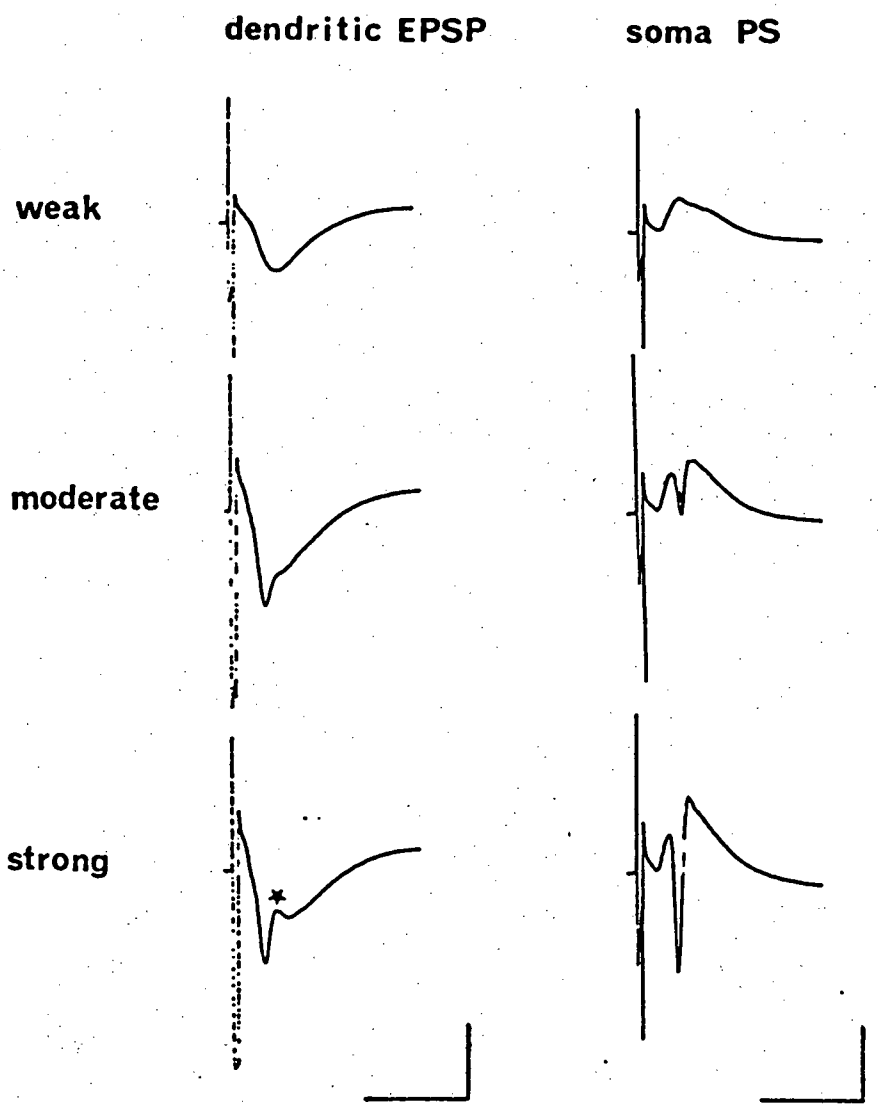


Fig. 8. Stimulus strength and evoked potentials. Terms on side refer to relative stimulus strength used to evoke the presented potentials. Note the presence of soma generated PS in the dendritic EPSP (*). Scale: 1.0 mV by 10 msec for EPSP; 1.5 mV by 10 msec for PS. Negativity is downwards.

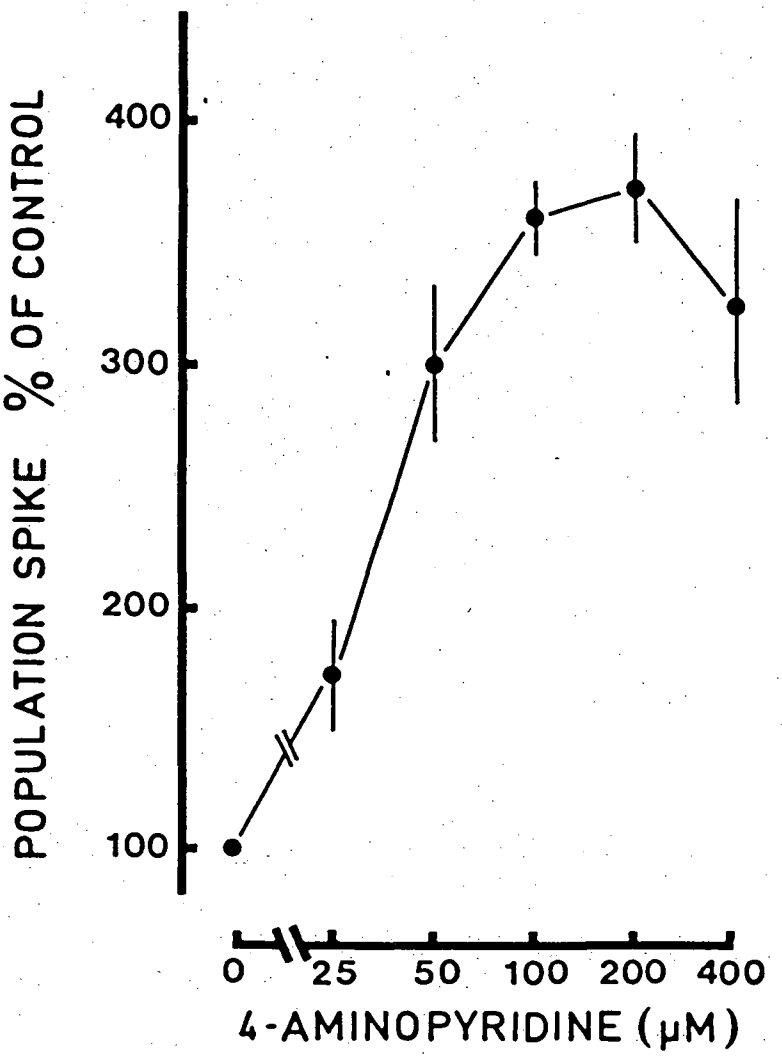


Fig. 9. Dose response curves to the cumulative addition of 4-AP showing increases in the amplitudes of evoked population spikes. Responses are expressed as percentages of the responses obtained in standard medium. Each value represents mean ± S.E.M. (n = 5).

centrations between 100-200 μM 4-AP. Cumulative drug concentrations above 200 μM often caused reductions in the evoked responses during perfusions. In addition, a second spike was often associated with cumulative doses above 100 μM 4-AP. The last two records in figure 10 show these spikes which rapidly diminished with washing. Further analysis of the effects of 4-AP were conducted using 100 μM concentrations.

14.3 Verapamil

From the verapamil studies (for methods see section 13.5) it was found that verapamil concentrations greater than 0.66μM tended to induce some depression (down to 85-95% of control PS) of the evoked PS. Yet verapamil concentrations less than 0.66μM did not cause a suppression of these potentials. Hence 0.33μM verapamil was selected and used in the remaining experiments. During 0.33μM verapamil applied for 3-4 min, there was little or no increase in the evoked PS. After verapamil perfusions, the evoked PS was slightly enhanced as illustrated in figure 11.

14.4 LLP

In the experiments described here, LLP induction trials were considered successful or not after one tetanic stimulation. As stated previously, antidromic tetanic stimulations at all tested frequencies, failed to elicit LLP. Only orthodromic tetani evoked in standard medium induced LLP. This LLP was seen as an increase in PS, PW and EPSP amplitudes with reduced latencies. Figure 12 depicts these potentiated potentials. The potentiated responses (PS increases of 200-500% or EPSP increases of 150-200% relative to controls) showed little or no decay even after 60 min. Interestingly, the best LLP (in terms of magnitude) was elicited by 400 Hz then 100 Hz and lastly 20 Hz tetanic stimulations in that order. In fact the higher tetanic

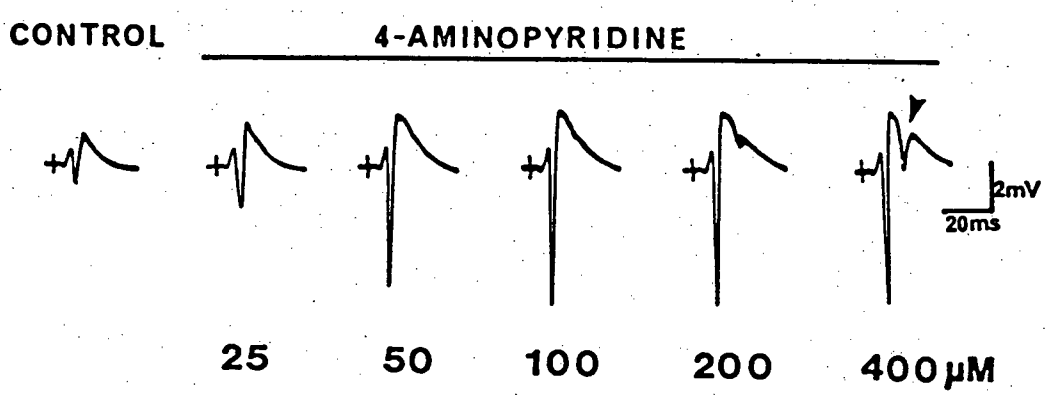


Fig. 10. Representative enhancements in evoked population spikes of CA1b pyramidal cells to cumulative addition of 4-AP during the determination of the dose response curves. Stimulation was in the stratum radiatum. The last trace clearly shows a second spike (arrow). Negativity is downwards; each trace is an average of eight consecutive sweeps.

frequencies were associated with an immediate post-tetanic expression of LLP (100 Hz, LLP in 7/9 slices; 400 Hz, LLP in 6/6 slices). Yet after 20Hz, LLP expression was gradual and tended to peak at 15-20 min. after tetanus and here the success rate was low (20Hz, LLP in 5/9 slices).

14.5 Homo- and heterosynaptic plasticity

In preliminary experiments it was found that 20Hz for 30 sec could produce a post-tetanic depression (PS, 20-80% Of controls; n = 14) that lasted for 10-110 min. In some cases, this depressive phase was followed by a return to pre-tetanus PS or LLP. Yet tetanus of 100-150 Hz for 1-5 sec consistently caused the development of LLP (PS, 100-720% of controls; in 20/22 slices) without a depressive phase. LLP produced by 100-150Hz could be interrupted temporarily by a subsequent 20Hz tetanus (five slices) for 10-20 min. Thereafter, LLP could be seen again. This phenomena (potentiation and depression) was further investigated on two separate inputs, Com and Sch afferents to the same output CA1b neurons, monitored simultaneously. In addition, the involvement of Ca^{++} in potentiation and depression was tested by using 0.33μM verapamil, which selectively blocked Ca^{++} entry into the postsynaptic neurons (see section 11.3).

High frequency tetanus (400Hz, 200 pulses) to Com or Sch elicited a rapid development of LLP of the CA1b PS that was limited to the tetanized input. This LLP after 400Hz was preceded by a post-tetanic potentiation (PTP, discussed in section 10.1) that quickly decayed down in 2-3 min, revealing the underlying LLP. The tetanus given in the presence of verapamil (0.33μM) also induced an input-specific LLP. Table 1 gives a summary of the results on LLP following 400Hz. The PTP preceding LLP elicited by 400Hz during verapamil perfusions was much greater than PTP obtained with

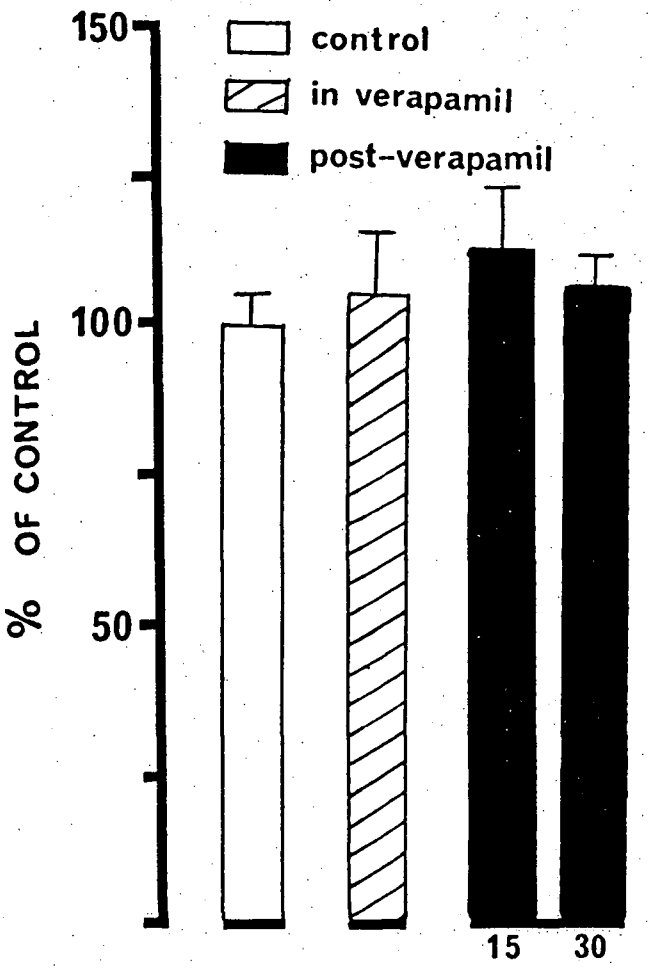


Fig. 11. Verapamil and evoked potentials. Bars represent mean amplitude of population spike Small vertical lines are +S.E.M. for n=7 slices. The post-verapamil recordings were taken 15 and 30 min after 0.33 micromolar verapamil was applied for 3 min.

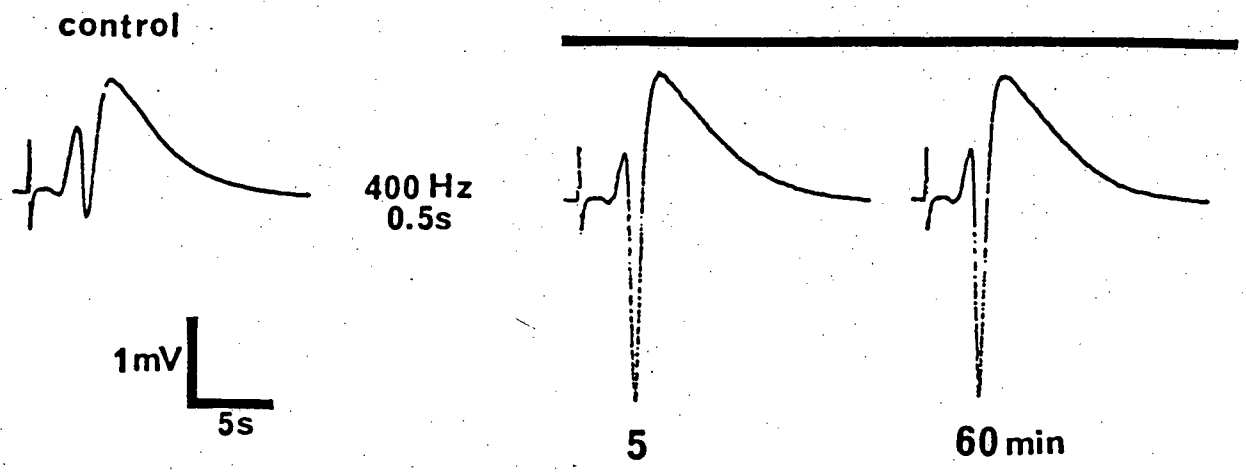


Fig. 12. Illustration of hippocampal long lasting potentiation. Note that even after 60 min there is no decrement in potentiated population spike. Negativity is downwards. Each record is an average of four sweeps.

400Hz alone. Furthermore, during the LLP of Sch PS, if Com was tetanized with 400Hz to induce LLP, there was no detectable homo- or heterosynaptic depression (cf. with 20Hz tetanus). Thereafter, the second LLP in Com co-existed with the LLP previously initiated in the Sch input.

The results obtained with low frequency tetanus (20Hz, 200 pulses) are compiled in table 2. It was found that 20Hz to Com or Sch initially induced a post-tetanic depression in both inputs (homo- and heterosynaptic depression) that was followed by return to pre-tetanus PS in the non-tetanized input (heterosynaptic) and sometimes in the tetanized (homosynaptic) input or LLP in the tetanized input only. Though not carefully characterized in the present studies, the 'PTP' associated with 20Hz seemed to be an extension of the frequency potentiation during the tetanic trains. This 'PTP' after 20Hz (cf. Bliss and Lømo, 1973) quickly decayed down to within 1-1.5 min to reveal a marked depression of evoked responses. When 20Hz tetanus was evoked while verapamil (0.33 μM) was perfused, this verapamil was found to counteract the homo- and heterosynaptic depression (see table 2). A remarkable finding was the observation that an established LLP could temporarily be masked by a subsequent 20Hz to the same input (homosynaptic) or to a non-potentiated (heterosynaptic) input.

The above results prompted the need to examine the characteristics of individual pulses associated with the tetanic trains used in these studies. The findings from such analyses are presented in the following section.

14.6 Characteristics of tetanic frequencies

Figure 13 depicts the individual PS evoked in a representative slice, during antidromic tetanization of CA1b axons. These PS associated with each pulse in the tetanus could be evoked in standard solution, Mg^{++} or Mn^{++}

	Sch-induced population spike (% of control)	Comm-induced population spike (% of control)
	30 min post-tetanus	30 min post-tetanus
400 Hz tet Sch	243 ± 56 (n = 7)	103 ± 4 (n = 7)
400 Hz tet Sch with verapamil	494 ± 95 (n = 5)	108 ± 4 (n = 5)

*All values are mean ± SEM.

Table 1. Effects of verapamil on long lasting potentiation. Note that only Schaffer (Sch) was tetanised and this input developed LLP. Verapamil did not block the initiation of LLP but was found to enhance LLP development (bottom row).

	n	Sch-induced population spike (% of control)			Comm-induced population spike (% of control)		
		5 min	10 min (post-tetanus)	20 min	5 min	10 min (post-tetanus)	20 min
20 Hz (Sch)	8	56 ± 6	77 ± 3	79 ± 3	47 ± 6	64 ± 5	70 ± 3
20 Hz tet (Sch) with verapamil	8	93 ± 7 ($p < 0.01$)	118 ± 16 ($p < 0.05$)	139 ± 21 ($p < 0.02$)	81 ± 10 ($p < 0.02$)	89 ± 7 ($p < 0.02$)	101 ± 5 ($p < 0.01$)
20 Hz tet (Sch) during 400 Hz induced LLP	10	53 ± 10	80 ± 7	94 ± 7	59 ± 18	68 ± 12	88 ± 13
20 Hz tet (Sch) with verapamil during LLP	8	98 ± 4 ($p < 0.01$)	101 ± 4 ($p < 0.05$)	102 ± 3	103 ± 5 ($p < 0.05$)	101 ± 4 ($p < 0.05$)	114 ± 7

[1]All values are mean ± SEM. p values are obtained using unpaired t-test between verapamil-treated and untreated responses.

Table 2. Effects of verapamil on homosynaptic and heterosynaptic depression. In first row, note the time-course of the depression of evoked PS in the tetanised (Sch) and non-tetanised (Com) inputs after 20Hz tetanus. This depression was counteracted if the 20Hz tetanus was evoked during verapamil perfusions (second and fourth rows). The 20Hz-induced depression masked developed LLP (third row; pre-20Hz data of LLP not given). See text for discussions.

media. The amplitudes and latencies of individual PS evoked during 20 Hz (200-600 pulses) or 100 Hz (100-500) pulses) were essentially unaltered. In a few experiments the amplitudes of some individual PS during tetanic stimulations, particularly with 100 Hz, were insignificantly depressed. This effect was apparent in the latter phases of a long antidromic tetanus and only in standard medium. A significant finding was that none of the antidromic tetani in more than twenty separate attempts was associated with frequency potentiation. Above all, antidromic tetanic stimulation at all tested frequencies, evoked during standard, Mg^{++} or Mn^{++} perfusions, failed to elicit LLP.

Orthodromic 400 Hz tetanus to Sch evoked 2-4 rapidly declining synchronously discharged PS only (fig. 14A). The remaining pulses in the 400 Hz train were not associated with a clear PS or PW. Without exception, the first pulse in the 400 Hz tetanic stimulation gave the maximum PS amplitude, i.e., no frequency potentiation. In contrast, 100 Hz trains (fig. 14B) typically gave a synchronous PS associated with the initial 1-50 pulses of the train. Thereafter, the PS were usually absent. Sometimes, PS to the 2-4 pulses in the 100 Hz train were slightly bigger than that evoked by the first pulse. Even after the PS were no longer evoked in the latter pulses of the 100 Hz train, a gradually decaying PW could still be observed (cf. with 20 Hz). Rarely did all the pulses in the 100 Hz train evoke a PS. 20 Hz tetanic stimulations typically induced greatly facilitated PS (frequency potentiation) in a manner shown in figure 14C. In the 20 Hz trains, pulses after 150-250 tended to evoke less frequency potentiation. During frequency potentiation, the PS was greatly enhanced (increases of 500-900% relative to the first pulse in the train), with associated two or more secondary

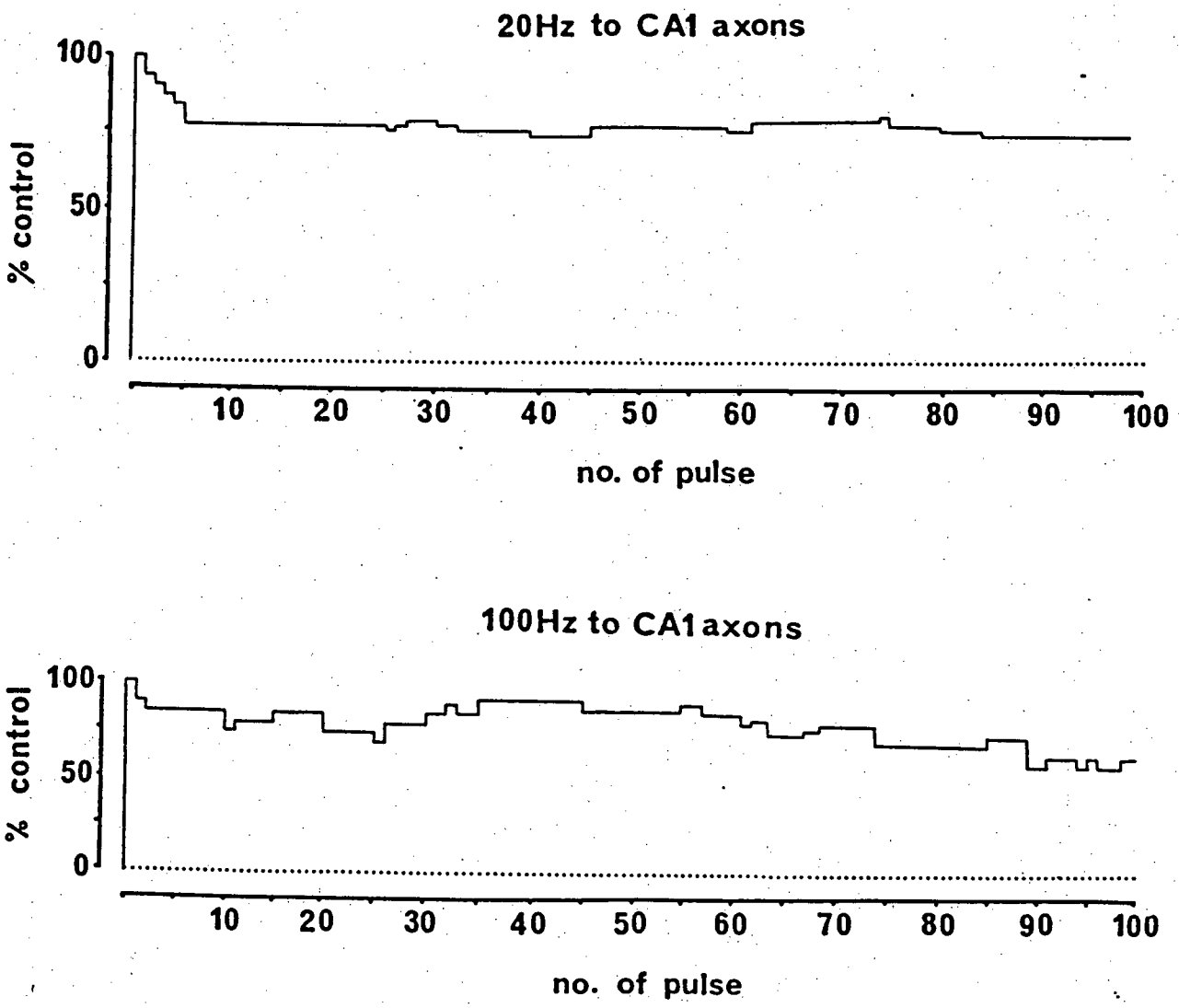


Fig. 13. Records of individual pulses during antidromic low and high frequency tetanic stimulations. In each case, only the first 100 pulses of each train are shown. Data was obtained from a single representative slice. See text for complete descriptions.

spikes. The PS during 20 Hz eventually declined below the amplitude of the PS evoke by the first pulse within the 300-350 pulse range. Thereafter, the PS was abolished. Though not shown in figure 14C the PS and PW components during the 20 Hz tetanus seemed to decay at different rates (cf. with 100 Hz above). Initially, both the PS and PW were greatly facilitated. Then even though the PS could still be seen with the late pulses prior to being blocked there was no discernable second PW component associated with these spikes.

Lastly, in some orthodromic tetanic experiments, the clarity of the presynaptic volley was enhanced by blocking synaptic potentials with Mg^{++} or Mn^{++} media. Under these conditions, it was found that the presynaptic volley (PV) followed both the 20 Hz or 100 Hz tetani. However, during the latter phases of the 100 Hz tetanic stimulation, the PV began to taper down. Figure 15 shows the results on PV during 20 Hz and 100 Hz trains obtained from two different slices.

14.7 Extracellular Ca^{++} and K^{+}

Among other things, the preceding results (particularly those on tetanic trains and potentiation/depression with verapamil) indicated that the synchronous discharge of PS consistently followed the low and not the high frequency tetanus. Yet LLP was better elicited with the high frequency tetanic stimulations i.e., there was little or no synchronous discharge of the postsynaptic CA1b neurons during tetanus. It was the depression which was associated with low tetanic stimulations (or the continuous discharges of CA1b neurons during the tetanus). It also became clear that interference with Ca^{++} entry to postsynaptic CA1b neurons during 20Hz tetanus counteracted the depression associated with this tetanus. These observations

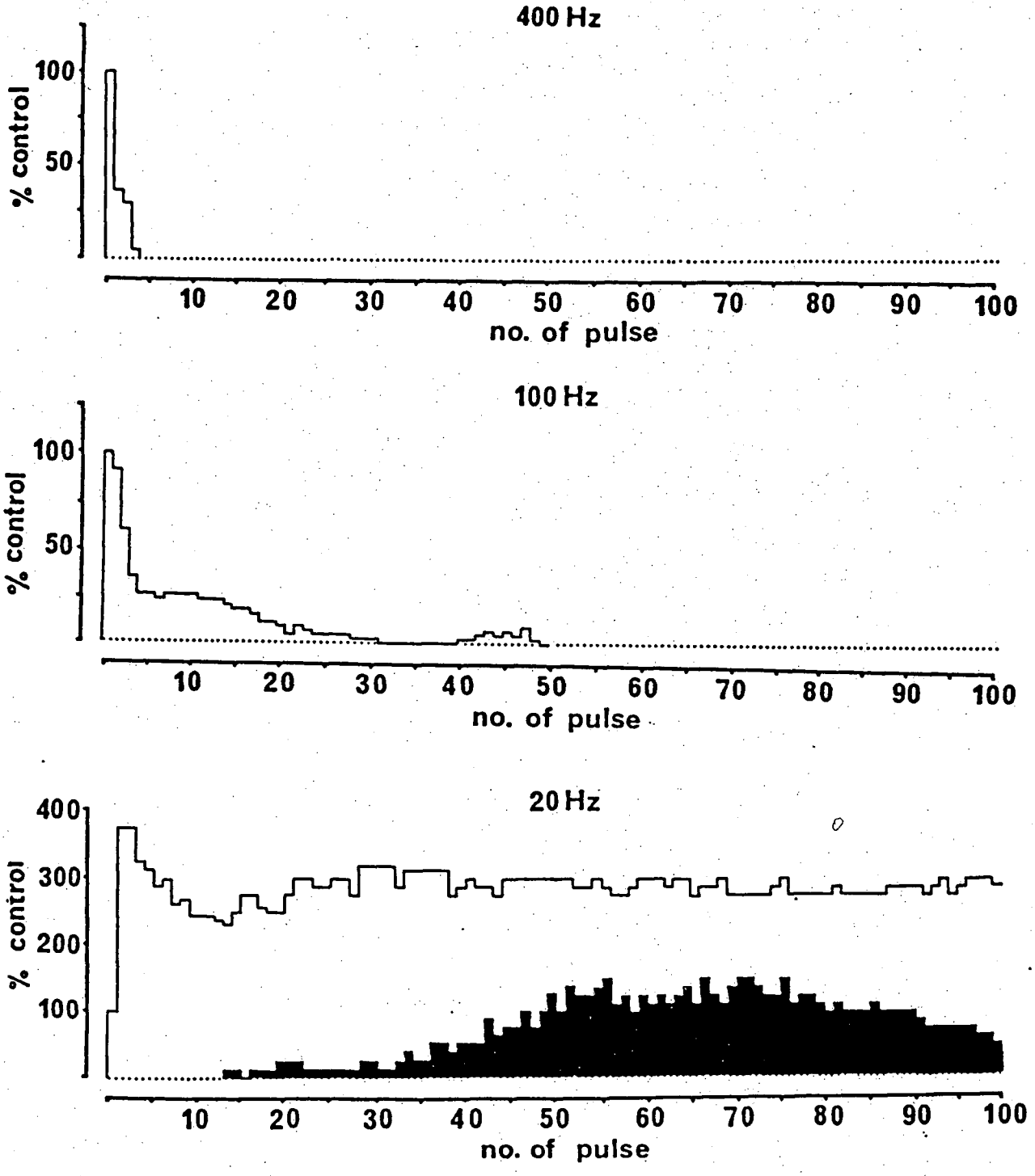


Fig. 14. Records of individual pulses during orthodromic low and high frequency tetanic stimulations. In each case, only the first 100 pulses of each train are shown. Data was obtained from a single representative slice. See text for complete descriptions. Shaded parts indicate presence of secondary spikes.

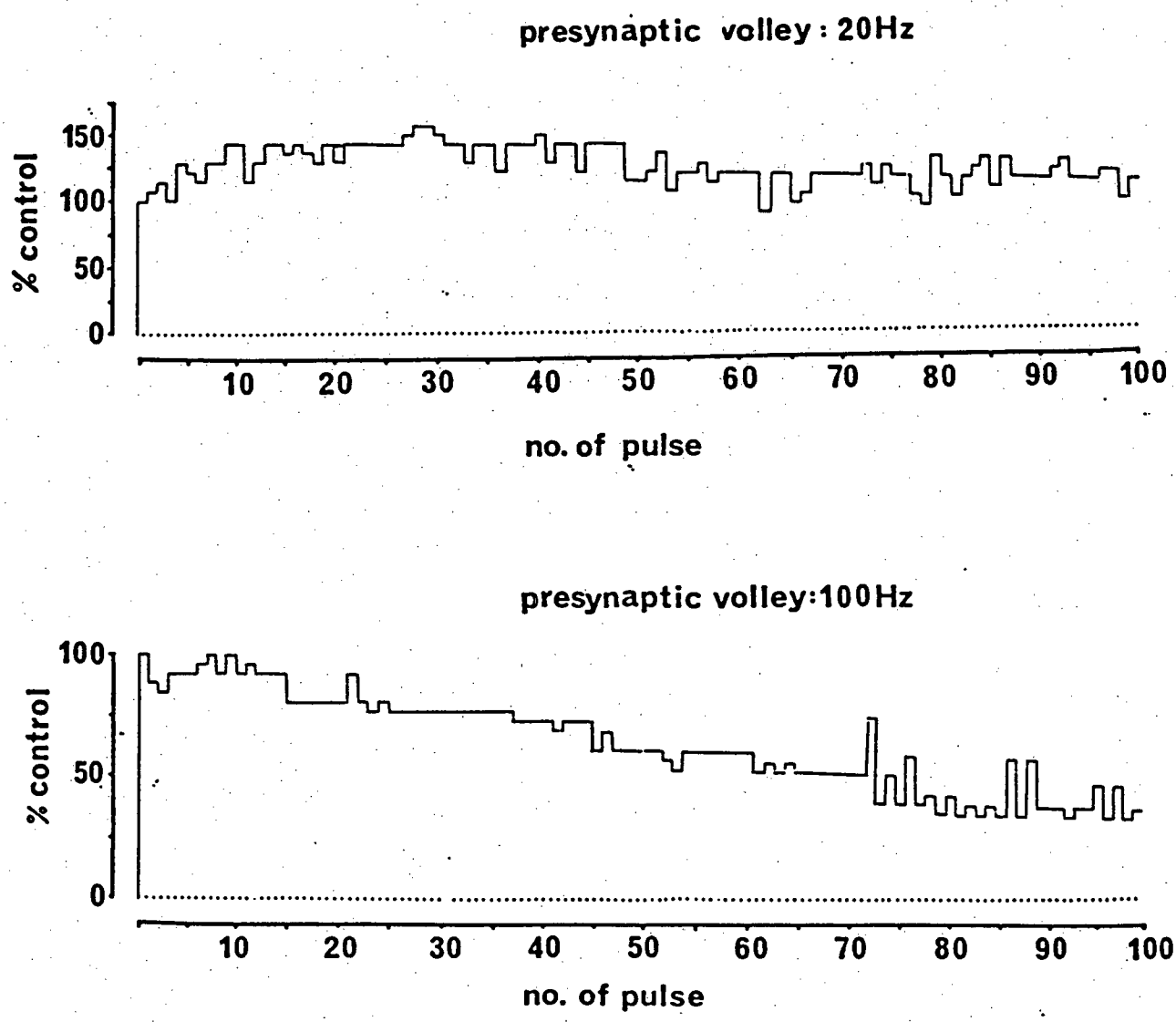


Fig. 15. Records of individual presynaptic volleys during low and high frequency tetanic stimulation of Schaffer collaterals. In each case, only the first 100 pulses of each train are shown. Data was obtained from a single representative slice. See text for complete description.

formed the basis of a formulated hypothesis that stated that low frequency tetanic stimulations caused an influx of Ca^{++} into CA1b neurons which mediated the development of a generalised postsynaptic depression. In order to substantiate this working hypothesis , studies were conducted that monitored changes in extracellular Ca^{++} during low and high frequency tetanic stimulations. The results obtained from these experiments are presented here.

Using calibrated Ca^{++} selective electrodes, it was determined that 20Hz given for 20-40 sec produced a reduction in the extracellular Ca^{++} (from 2 to 0.7-1.2 mM; n = 5: CA1b pyramidal layer recordings). In contrast, 100Hz given for 5-10 sec did not produce a significant change in Ca^{++} levels (n = 5). The PS was depressed after the 20Hz tetanus but not after 100Hz. Yet the 100Hz elicited LLP.

Extracellular K^{+} levels increase during tetanus (Benninger, Kadis and Prince, 1979). It was therefore likely that the observed depression following 20Hz (and even the LLP initiation for that matter!) could have been caused by elevated extracellular K^{+} during tetanus. This possibility was examined by first determining the extracellular K^{+} levels with K^{+} selective electrodes. Then in separate experiments, hippocampal slices were exposed to modified medium with substituted K^{+} (i.e., K^{+} medium) in concentrations comparable to those present during 20Hz tetanus. 20Hz tetanus for (20-40sec) produced an increase in extracellular K^{+} of 10-15mM (recorded in the CA1b cell body; n = 6). Consequently, the K^{+} medium prepared contained 15mM K^{+}. When this K^{+} medium was perfused for 10 min it caused a sustained increase in evoked PS (PS, 300-400% times controls; n = 4) during the application. Spontaneous activity increased and secondary spikes were present. Yet after K^{+} medium perfusions, on average, the evoked PS returned to the control levels. These results are presented in figure 16.

14.8 Ba^{++} and evoked potentials

Figure 17 illustrates the sequence of changes in evoked potentials associated with Ba^{++} perfusions. Ba^{++} applications caused an initial increase in the amplitudes of these potentials. The onset latency for the PS and PW were greatly enhanced. The delayed and widened PW was inundated with a multitude of single neuron spikes (see fig. 17). With continued Ba^{++} perfusions, the PS and PW were abolished. Where the presynaptic volleys were discernable these showed increases in amplitudes but their onset latencies seemed unaltered. These effects to the artifact and pre-synaptic volley were reversed to pre-Ba^{++} levels after Ba^{++} perfusions.

Upon returning to standard medium, there was an immediate recovery of evoked potentials and these potentials were still greatly potentiated. Gradually these potentials decayed resulting in one of the following outcomes. After 60 minutes, which was the selected post-Ba^{++} monitoring period, evoked potentials (a) remained augmented or (b) were depressed. The average changes, for different Ba^{++} application times are summarised in fig. 18 and 19. Furthermore, Sch terminal region excitability was tested in five fibers (different slices) before, during and after Ba^{++} perfusions. Interestingly it was found that the Sch antidromic thresholds were greatly increased only during the Ba^{++} perfusions. Yet the post-Ba^{++} Sch antidromic threshold were essentially the same ($n = 5$) as the pre-Ba^{++} levels.

14.9 Effects of 4-AP

Single 5 minute perfusions with 100 μM 4-AP caused larger enhancements (400-800%; $n = 4$) in evoked PS than those obtained with cumulative additions

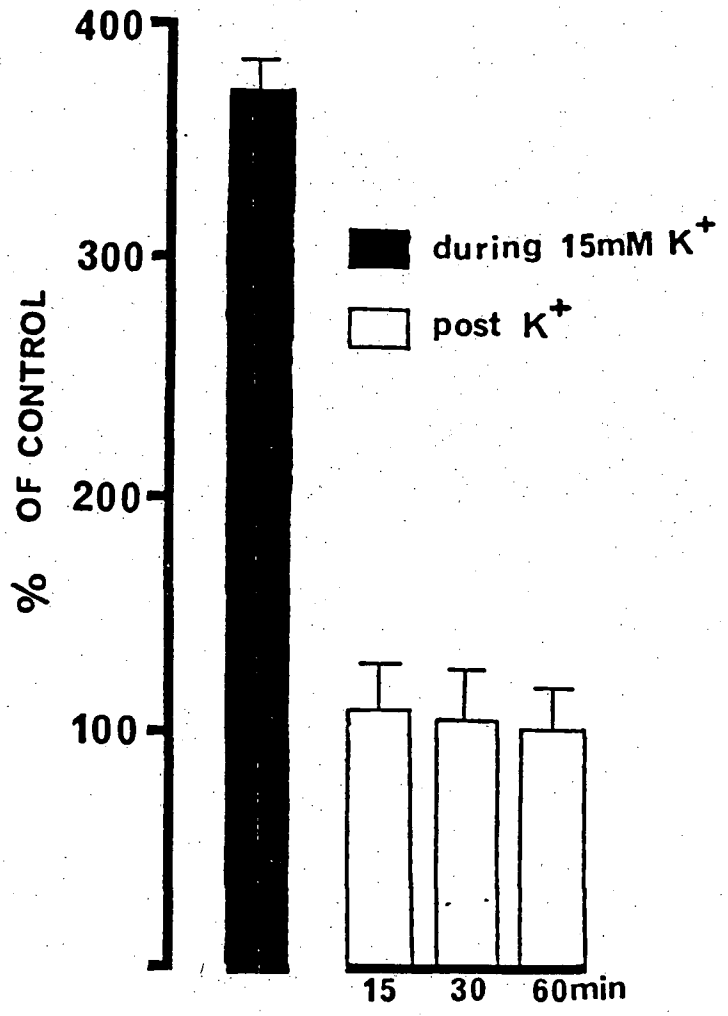


Fig. 16. Effects of raised potassium concentrations on evoked population spike. Bars represent mean amplitude of evoked population spike. Vertical lines are +S.E.M. (n = 4). K^+ medium was perfused for 10 min.

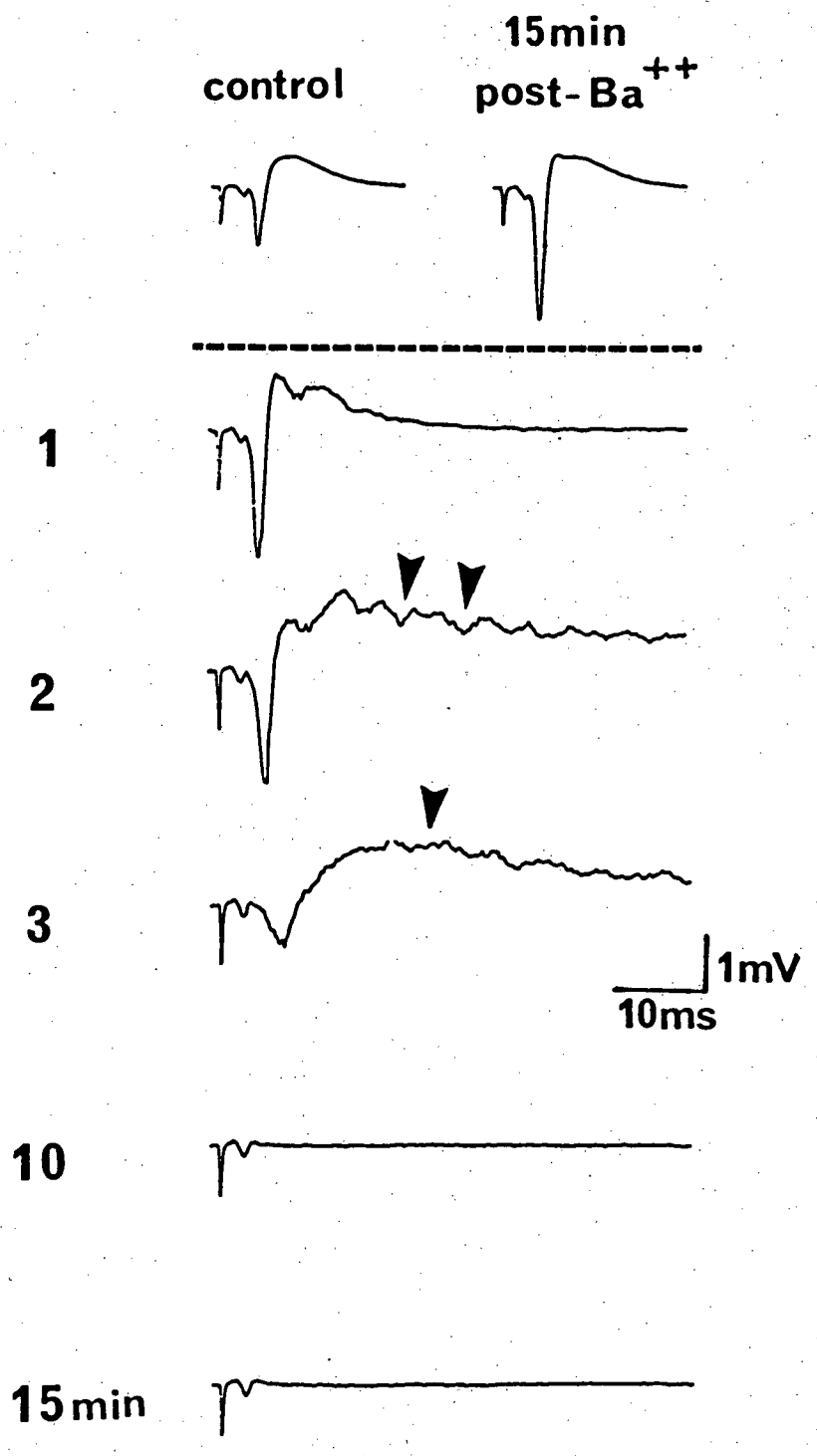


Fig. 17. Effects of barium on evoked population spike. Numbers on side indicate elapsed time during barium perfusion. Cell disharges are shown by arrows. See text for discussion.

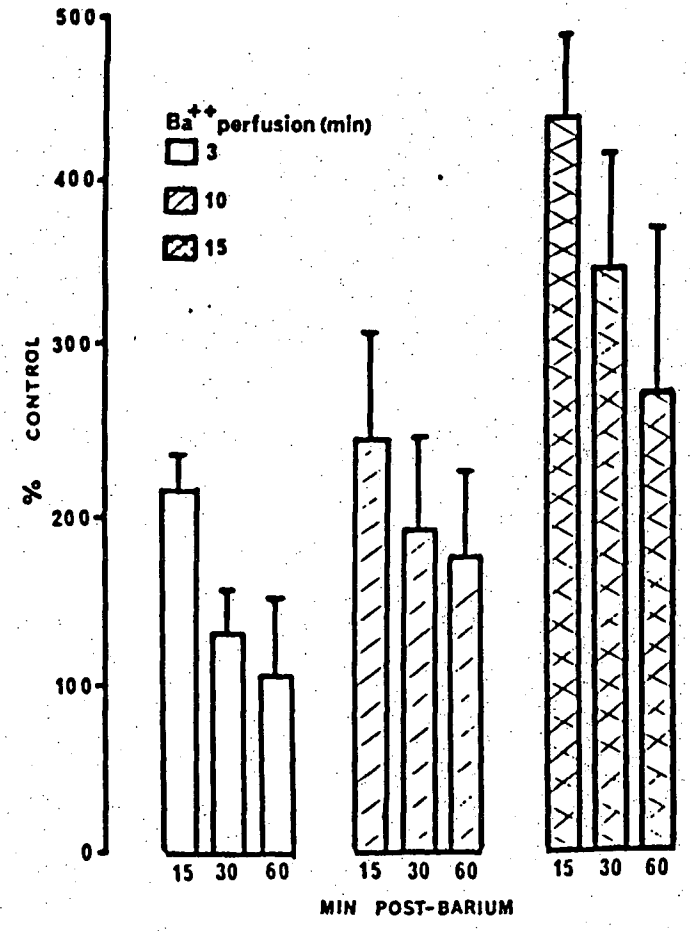


Fig. 18. Changes in evoked population spike following 3, 10 and 15 in. exposures to barium medium. Vertical lines represent S.E.M. (n=5).

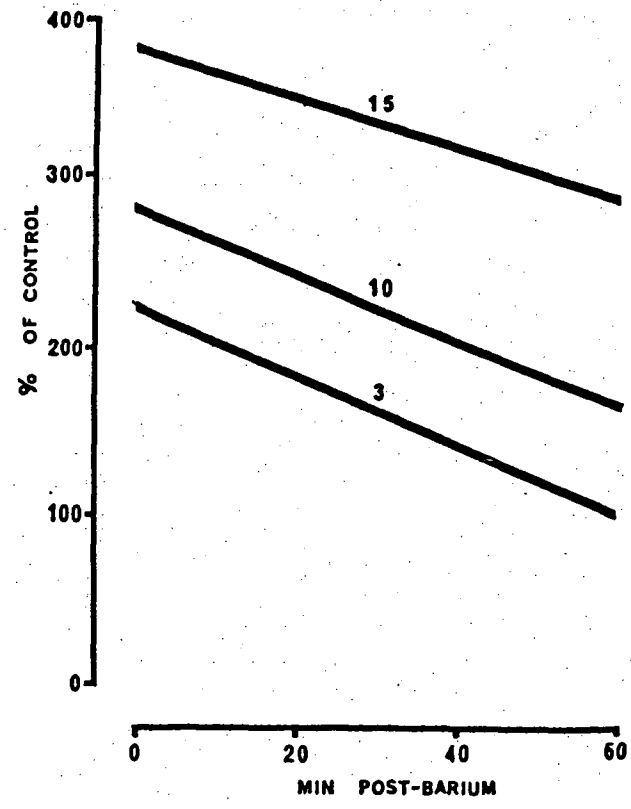


Fig. 19. Linear regression plots of decay times of evoked population spike following barium applications.

up to 400 μM 4-AP (300-400% ; n = 5). Furthermore, maximal enhancements with 100 μM 4-AP were obtained if the drug was perfused for 5 minute during stimulation. However, absence of synaptic transmission (i.e., Mg^{++} blockade or temporary cessation of stimulation) when the drug was applied did not prevent subsequent enhancement of the evoked PS (see table 3 and figure 20). Presumably the sites of action for 4-AP are located on the inner parts of the membranes (Mashall, 1981). The results in table 3 seem to indicate that 4-AP movement across the membrane may not be entirely dependent on voltage-sensitive channels. Alternatively, it may be that sufficient 4-AP was present (i.e., not completey washed) when synaptic transmission was re-instituted which then facilitated movement across the membranes. It is also possible that the incubation for 5 minutes with Mg^{++} was inadequate to completely remove extracellular Ca^{++} and, therefore, block all synaptic transmission.

In addition, repetitive bursting activity at 1-2 minutes intervals were observed during perfusion with 4-AP. Initiation of this bursting behavior is shown in figure 21. Bursting activity was not observed if 4-AP was added during synaptic transmission blockade with Mg^{++} medium. Effects of 4-AP including the bursting activity when present were reversible by washing for 30-60 minutes. However, in some slices, depressions of evoked responses occurred following reversal of 4-AP effects with washing. Repeated applications did not alter the recovery times.

As has been shown in earlier sections, effects of 4-AP were reversible by washing for 30-60 minutes. In contrast, when the stratum radiatum or the CA3 region afferents to CA1b were tetanized (100 Hz, 5 sec) during 4-AP perfusions, the subsequent enhancements in evoked PS were not reversed to con

	n	Post 4-AP time (min)[a]		Recovery time to control level (min)
		15[b]	30	
Stimulation	4	6.75 ± 0.75	1.65 ± 0.15	45 ± 12.72
No stimulation	3	5.8 ± 1.06	0.8 ± 0.95	40 ± 6.26
In Mg^{++}	4	2.89 ± 1.18	1.98 ± 0.83	45.75 ± 10.46

Table 3. Drug induced enhancements associated with 4-AP perfusions during stimulation, no stimulation and magnesium-mediated synaptic blockade.

Evoked responses reported as a ratio (a) of population spike in standard medium. Early time (b) chosen for making comparisons since reversal of synaptic transmission blockade with magnesium medium occurred within 10 min (Maretic, Chirwa and Sastry, unpublished observations).

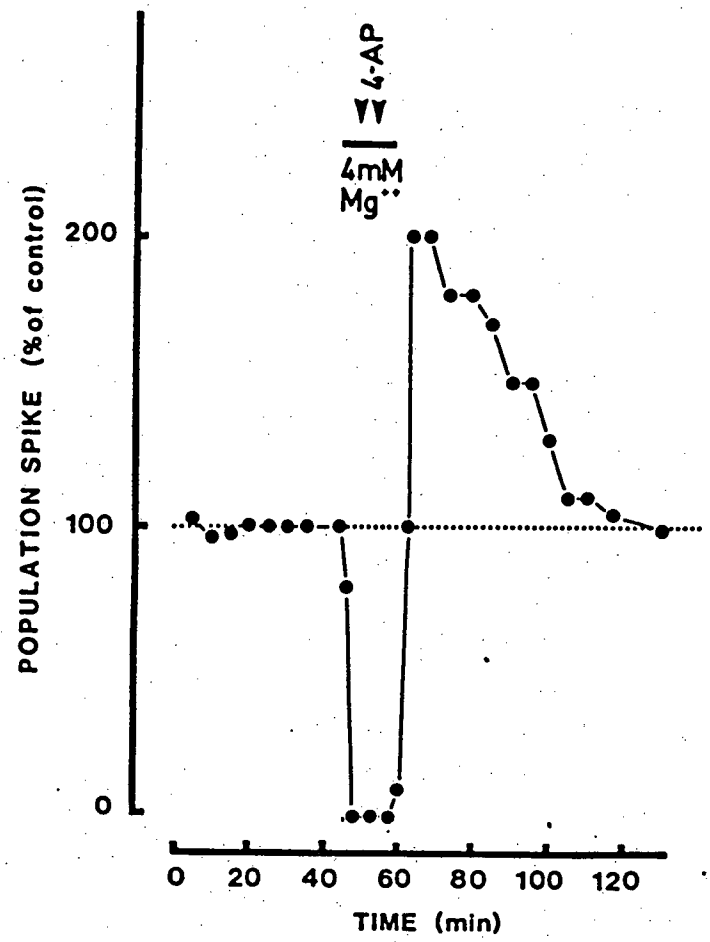


Fig. 20. Plot of 4-aminopyridine perfused during synaptic transmission blockade with raised magnesium medium.

trol levels. Figure 22 illustrates the methods used and results obtained from one slice. Large enhancements (400-500% ; n = 4) in evoked PS were obtained immediately after the 4-AP/100 Hz, 5 sec treatment and decayed to 150-200% limits within 30-40 minutes. The initial enhancements probably were due to 4-AP effects and decayed as the drug was washed out. The second phase presumably represented LLP induced by tetanus that could now be seen after washing out of 4-AP. These potentiated responses could be followed for two or more hours (see figure 22).

14.10 Iontophoretic glutamate

L-glutamate ejected with 200nA currents for 1 min on CA1b cell bodies initially facilitated then rapidly diminished the PS evoked by Sch stimulation (test pulse,0.2Hz). Spontaneous activity was greatly increased by L-glutamate. After L-glutamate iontophoresis, the depressed PS recovered slowly (PS, 11-90% of control at 5 min post L-glutamate; 6/8 slices) Surprisingly, if the Sch was tetanized with 100Hz (100 pulses) during the last seconds of L-glutamate ejections, the post-drug depression was much more pronounced (PS, 1-12% of control at 5 min post-drug; 4/4 slices) and this depression was still present even after 30 min.

In some experiments, verapamil perfusions preceded the L-glutamate ejections. If this treatment was instituted, verapamil counteracted the post-L-glutamate depression (PS, 84-130% of controls at 5 min post-L-glutamate; 4/5 slices). Even more striking was the finding that verapamil counteracted the depression following L-glutamate /100Hz treatments and actually 'unmasked' the tetanus induced LLP to Sch (PS, 130-325% of controls at 5 min postdrugs; 5/5 slices).

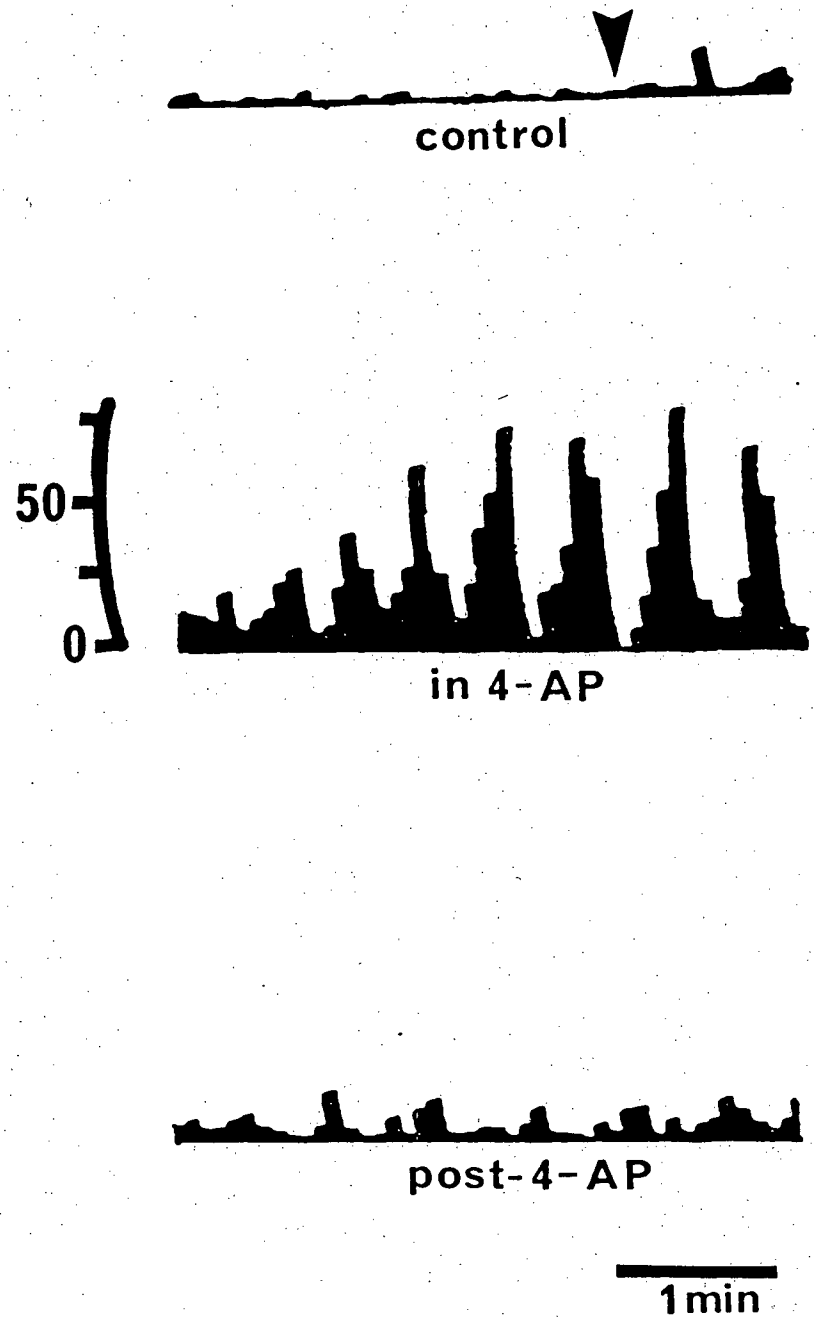


Fig. 21. 4-aminopyridine induced bursting activity in CA1b subfield. Vertical line is spikes per second. Arrow indicates time when 4-AP was started. The post-4-AP records were taken at the 10 min interval. Figures were re-touched to enhance clarity.

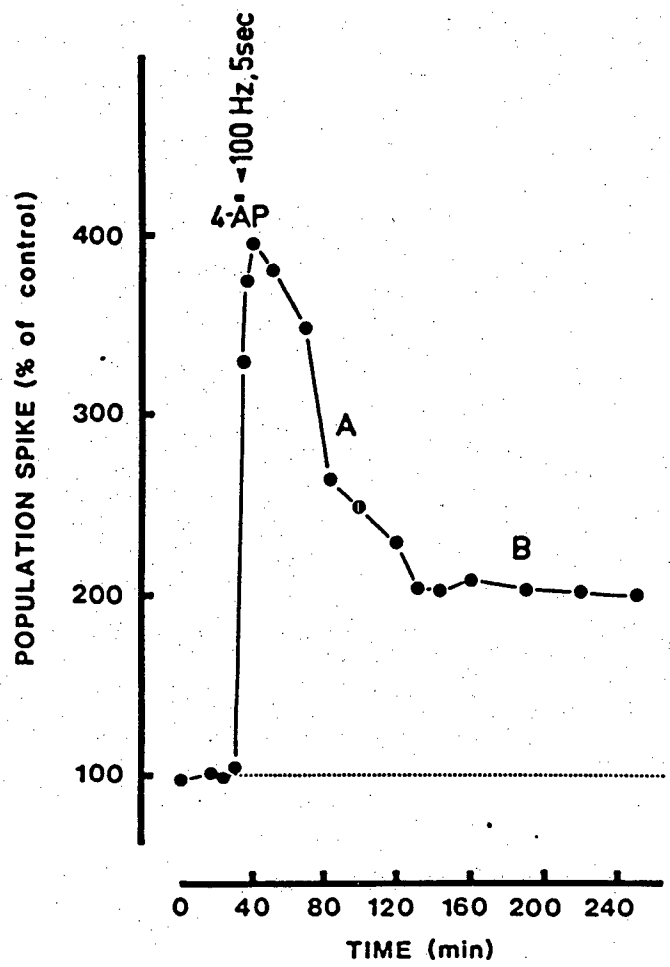


Fig. 22. Maintained enhancements of evoked population spike following 4-aminopyridine and 100Hz treatment. The two rates of decay are indicated by the letters A and B (see text for full discussion). Results shown were obtained from one slice.

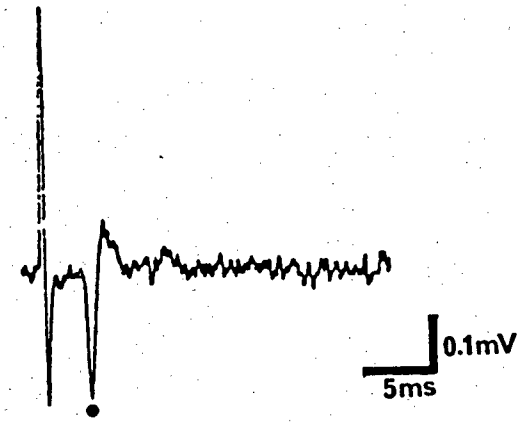


Fig. 23. Illustration of an antidromic single spike (●) evoked in field CA3. Stimulation was at Sch/CA1b synaptic region.

14.11 Excitability of Schaffer collateral terminals

Figure 23 is a record of the CA3 single spike evoked by the antidromic stimulation of Sch. The threshold for this antidromic activation was determined during Ba^{++}, and 4-AP. Ba^{++} caused more than a 3-fold increase in threshold during perfusions. Yet this increase in Sch excitability threshold was reversed to pre-Ba^{++} levels once standard medium was re-instituted. On the other hand, 4-AP perfusions slightly decreased (93-97% of control threshold, n = 4) the Sch excitability threshold during applications but this was reversed to pre-4-AP levels within 10-15 min after 4-AP perfusions.

15. DISCUSSION

15.1 LLP

All the evoked potentials reported in this thesis had the clarity and characteristics of similar potentials reported in the literature (Swanson, Teyler and Thompson, 1983). The electrically evoked LLP was seen as a reduction in PS latency and/or a net increase in the amplitude of PS, PW or EPSP. Many overlapping currents, both inward and outward to CA1b pyramids, interneurons and possibly glia cells, contributed to the global shape of the evoked potentials (see section 6). However, the specific contribution of each ionic current is indeterminate and therefore a detailed account of the exact ionic conductance changes associated with LLP cannot be given.

It is conclusively clear that antidromic tetanic stimulations failed to elicit LLP. Only orthodromic trains evoked in standard medium elicited LLP. It became clear during the analysis of individual pulses of the varied tetanic stimulations that LLP was best elicited by high frequency trains

that caused little or no discharges of the postsynaptic CA1b neurons. Yet the presynaptic volley under these conditions could follow the high frequency tetanus. Since Ca^{++} influx is a prerequisite for LLP development, clearly the Ca^{++} influx must have been largest in the presynaptic terminals as these were continuously activated by all orthodromic tetanus examined. It was possible that asynchronous release of neurotransmitters, though insufficient to discharge the postsynaptic neurons, still depolarised the local dendrites. This could have led to the influx of Ca^{++} into the dendritic regions which might have mediated LLP development. A few observations negate this possibility. Firstly, twin-pulse studies show a marked reduction in the second PS if the interval between the two pulses is 2.5 msec, i.e., 400Hz (Chirwa, unpublished; Turner, Richardson and Miller, 1983). This is due to the depletion of terminal neurotransmitters caused by the first pulse and incomplete neurotransmitter recovery (i.e., replenishment) after this pulse (cf. Elmqvist and Quastel, 1965). Secondly, it is known that eight pulses given at 400Hz can elicit LLP (Swanson, Teyler and Thompson, 1983 review). Obviously, the last 4-6 pulses in this train would cause little or no release of neurotransmitters since they are probably evoked when the terminals (a) have been emptied and (b) the interval is small for complete replenishment of neurotransmitter substances. If it is argued that sufficient Ca^{++} influx to postsynaptic dendrites still occur under these conditions (400Hz, 8 pulses) then it remains to be explained why strong single shocks to Sch do not elicit LLP. Lastly, verapamil in doses that selectively interfered with the Ca^{++} influx to postsynaptic neurons (see later sections) augmented PTP and LLP development. These results are consistent with a presynaptic locus for LLP development.

15.2 Tetanic stimulations

The major differences between antidromic and orthodromic tetanus were that (a) synchronous discharges accompanied all pulses in the antidromic tetanus, whereas (b) few, if any synchronous PS followed the high frequency orthodromic tetanus and (c) only the low frequency orthodromic tetanus gave well developed frequency potentiation and secondary PS. Many hypothetical schemes can be given that can account for these differences. However, when the known intrinsic circuitry of the CA1b region are taken into consideration (Knowles and Schwartzkroin, 1981) then the following series of events seem to occur.

The intense depolarizations on CA1b dendrites caused by repetitive orthodromic stimulation made neurons to fire repeatedly. These CA1b neurons in turn fed signals to inhibitory interneurons (via the recurrent inhibitory loop) intensely depolarizing them. Hence the interneurons then generated huge IPSPs in CA1b pyramids (Andersen and Lømo, 1967). Conceivably these huge IPSPs could have reversed and became depolarizing influences during the train and 'potentiated' on-going CA1b dendritic depolarizations. Alternatively, the intense depolarizations on interneurons during 20Hz may have caused depolarization blockade of interneurons, thereby effectively abolishing IPSPs, i.e., inhibitory interneurons inactivated (Andersen and Lømo, 1967). In any case, the net effect was a greatly enhanced CA1b neuron excitability resulting in the recruitment of inactive CA1b neurons. In fact the increased extracellular K^+ during tetanus possibly reduced CA1b neuron excitability and thereby facilitated CA1b discharges (Alger and Teyler, 1978). These processes probably accounted for the frequency potentiation and secondary spiking seen with 20Hz. Blockade of these processes set in

when (a) inactivation or depolarization blockade in CA1b dendrites/soma components occured or (b) neurotransmitters were depleted or (c) action potentials were blocked in Sch boutons. It seemed likely that high frequency orthodromic tetanus rapidly caused depolarization blockade and/or terminal neurotransmitter depletion and therefore not all of the above events, i.e., synchronous discharges, frequency potentiation or secondary spikes, were seen. From the above descriptions , it can also be seen why antidromic tetanus did not cause frequency facilitation or secondary spikes. These events were mainly generated and/or perpetrated by synaptic interactions.

Lastly, the reduction in PS sometimes seen with antidromic tetanus in standard medium could be explained as follows. While the CA1b neurons were being antidromically activated, collaterals of these axons also fed orthodromic signals to inhibitory interneurons, which then caused IPSPs that subsequently shunted some CA1b neuron discharges. Following the antidromic tetanus, it was likely that more inhibitory interneurons were recruited since the CA1b axon collaterals must have developed PTP immediately after the tetanus. This then would explain the transient depression to Sch evoked potentials after antidromic tetanus (Chirwa, Murali Mohan and Sastry, unpubl.) Alternatively, since the slight reduction in PS sometimes seen during antidromic tetanus was not present in Mg^{++} or Mn^{++} media, it could be that the reductions were caused by Ca^{++} influx into CA1b neurons, an effect that was extended to the post-tetanus period.

15.3 Homo- and heterosynaptic depression

It is evident that homo- and heterosynaptic depression is a generalised phenomena occuring in the postsynaptic CA1b neurons. Initiation of this depression is dependent on the CA1b neuronal accumulation of Ca^{++},

which is greater with low frequency tetanus. In support of this conclusion were the following results. Using Ca^{++} selective electrodes it was found that the largest drop in extracellular Ca^{++} in the pyramidal layer occurred with low frequency tetanus. Iontophoresed Ca^{++} on CA1b cell bodies is known to induce homo- and heterosynaptic depression (Sastry, Chirwa, Goh and Maretic, 1983). Iontophoresed glutamate on CA1b cell bodies initiated a post-drug depression. This depression was more pronounced after glutamate/100Hz treatments. The simplest and plausible explanation of the results was that glutamate depolarized CA1b cell bodies and let in Ca^{++}. Tetanus caused even more Ca^{++} to enter the dendrites. That indeed Ca^{++} mediated the above changes was confirmed by the discovery that verapamil could counteract the glutamate-induced depressions. In fact it was predicted and subsequently confirmed that reduction of the glutamate/100Hz postsynaptic depression by verapamil led to the unmasking of a possibly presynaptic mediated LLP. Both LLP and depressions co-existed together. In low tetanus, depressions were more pronounced and invariably masked the expression of LLP. At higher frequencies, LLP development was favoured which masked the less favoured depressions. These conclusions are consistent with the findings that homo- and heterosynaptic initiation could reversibly interrupt established LLP. Such an effect was not seen with high frequency tetanus to a potentiated input or a separate input to the same output neurons.

15.4 Barium

Turner, Baimbridge and Miller (1982) caused a long term increase in synaptic efficacy by the transient exposures to elevated extracellular Ca^{++}. In similar experiments, Sastry, Chirwa, Goh and Maretic (1983) reported that

a sustained potentiation followed a 10 min exposure of hippocampal slices to Ca^{++}/K^{+} combinations of 4 and 5 mM and not 4 and 3.1 mM respectively. It seemed that a voltage-dependent Ca^{++} channel was involved in the development of the potentiation during these exposures to Ca^{++}. A major outcome of the above studies included the demonstration of a technique, i.e., exposure to elevated Ca^{++}, that seemed to be eliciting a potentiation similar to that evoked with electrical stimulations.

In the present studies, the method of exposing hippocampal slices to Ba^{++} medium was used to test Ba^{++} capability to induce LLP. Other factors that governed the selection of these technique included the following. From the initial experiments, it was clear that Ba^{++} caused a massive but asynchronous release of neurotransmitters which led to the observed delay in the latency of the evoked PS and its PW. Further evidence for the asynchronous neurotransmitter release became apparent when it was noted that superimposed along the entire length of the delayed PW, were numerous single cell discharges. Besides, it is known that Ba^{++} causes an increase in the terminal action potential refractory period (Sastry, 1979). Since the duration of a similar Ba^{++} induced terminal action potential refractory period in the hippocampus was not known, it seemed inappropriate to evoke tetanic stimulations in Ba^{++} medium and thereby test for post-tetanic LLP. The possibility existed that an increase in terminal action potential refractory period could cause the failure of some axonal action potentials generated in Sch to invade the Sch synaptic boutons (which are a possible loci for LLP; Sastry, 1982) in a manner necessary to induce LLP.

On average, all exposures to Ba^{++} medium for 3, 10 or 15 min. led to the post-Ba^{++} potentiation of the evoked PS. A huge asynchronous release

in neurotransmitter was evident in the initial phases of Ba^{++} perfusions. Continuation of Ba^{++} applications led to the blockade of synaptic responses. The mechanisms mediating this blockade were not clear, since Ba^{++} is known to support neurotransmitter release. It can be speculated that Ba^{++} caused the depletion of releasable neurotransmitter pools. If this is correct, then it must be argued that unlike Ca^{++}, Ba^{++} possibly hinders the replacement of depleted releasable neurotransmitter pools. Secondly, a combination of Ca^{++}/Ba^{++}, unlike Ca^{++} alone, that must have existed in the initial post-Ba^{++} periods greatly enhanced the total neurotransmitter released. After all, Ba^{++} prolongs the terminal action potential duration (Sastry, 1979) and this could account for the better efficacy in neurotransmitter release caused by Ca^{++}/Ba^{++} combinations.

Given that neurotransmitter release was augmented, this could therefore account for the observed post-Ba^{++} potentiation. An alternative explanation such as an increase in postsynaptic receptors is unattainable since Ba^{++} exposures presumably do not increase glutamate receptors (Baudry and Lynch, 1979). Since there were no changes to amplitudes of the stimulation artifact or the presynaptic volley after Ba^{++} prefusions, it was unlikely that there were alterations to the stimulation electrodes that could account for the potentiation. What seemed likely was that the actions of a Ba^{++} increase in neurotransmitter release were further 'amplified' by the postsynaptic effects of this ion. Ba^{++} is known to augment the Ca^{++}-mediated depolarizations and also blocked the outward K^{+} currents (Hotson and Prince, 1981). These actions could conceivably facilitate the recruitment of CA1b neurons during synaptic transmission. However, the potentiation seen with Ba^{++} differed from the electrically induced LLP since the former

quickly decayed in time. There was a relationship between the level of potentiation and decay times to the Ba^{++} exposure times. It seemed that long exposures to Ba^{++} led to increased accumulation of Ba^{++} in slices, which subsequently took longer to passively wash out after Ba^{++} applications. Moreover, LLP is known to be associated with a decrease in the excitability of Sch terminals following an LLP-inducing tetanus to this input (Sastry, Murali Mohan and Goh, 1985). Yet Ba^{++} did not alter the post-Ba^{++} threshold for the antidromic activation of Sch.

The increase in Sch threshold seen <u>during</u> Ba^{++} perfusions further confirmed the presynaptic actions of this ion. However, under the conditions employed with Ba^{++} in the present study, it is concluded that Ba^{++} did not elicit LLP. Perhaps the most ideal exposure times or methods for Ba^{++} exposures that could elicit LLP were not attained in the present studies.

15.5 <u>4-Aminopyridine</u>

The bursting activity induced by 4-AP perfusions may have originated from intrinsic sites on the CA1b pyramidal cells. It is conceivable that substances such as 4-AP enhance Ca^{++} influx into the soma-dendritic sites and promote bursting activity (Wong and Prince, 1978; Simmons and Dun, 1984). However, since this bursting activity was not detected during 4-AP/Mg^{++} medium perfusions it seemed likely that they were caused by synaptic interactions. In any case, Ca^{++} binding sites presumably increase along the stimulated afferents and their synaptic regions with 4-AP treatment (Kuhnt, Mihaly and Joo, 1983). Hence these regions could be the source of ectopic action potentials in these afferents, leading to bursting activity in CA1b neurons. Besides, it was observed that 4-AP caused a slight decrease in Sch excitability threshold during application an effect that could have enhanced spontaneous transmitter release.

Single 100 μM 4-AP perfusions caused larger PS enhancements than cumulative doses of 400 μM 4-AP. It was possible that the long exposure times needed to reach a cumulative dose of 400 μM 4-AP made it possible for secondary interactions to occur that diminished synaptic responsiveness. Yet again it is known that 4-AP facilitated neurotransmitter release at lower concentrations and exerted a curare-like effect at higher concentrations (Van Der Sprong and Voskuyl, 1982; Simmons and Dun, 1984). In addition single 100 μM 4-AP perfusions elicited maximum enhancements greater than those initiated in 4-AP/100Hz treatments. Tetanic stimulations are known to partly reverse the 4-AP mediated blockade of fast K^+ channels and this effect could account for the differences in enhancements reported above (Yeh, Oxford, Klu and Naharashi, 1976). More importantly, the 4-AP mediated enhancements were reversible with washing for 30-60 min. In contrast, the 4-AP/100Hz treatment led to enhancements that exhibited two rates of decay. The first enhancement decayed within 40 min to reveal the second potentiated phase, which was maintained throughout the duration of the experiments. It is proposed here that the second potentiated phase was the LLP (caused by 100Hz in the 4-AP/100Hz combination) that could now be seen after 4-AP had washed out.

4-AP has mostly dose-dependent presynaptic and some postsynaptic actions (Ikemoto, Klee and Daunicht, 1982). In the 4-AP doses used in the present studies, it was expected that enhanced Ca^{++} influx into presynaptic terminals as a result of blockade of K^+ conductance by 4-AP could initiate LLP. However it was found that 100 μM 4-AP added to medium containing 0 to 2 mM Ca^{++} (test frequency, 0.2Hz) did not induce LLP. A possible postsynaptic location for LLP initiation could not explain the absence of 4-AP

mediated LLP since this drug also had reversible effects on CA1b neurons (Buckle and Haas, 1982; Van Harreveld, 1984). It was possible that the 4-AP mediated increase in Ca^{++} influx though sufficient to facilitate transmitter release, may not have attained critical intraterminal Ca^{++} levels that were maintained for a period necessary to elicit LLP. In fact 4-AP may have been enhancing transmitter release in both active and mostly inactive Sch boutons. 4-AP facilitates afferent transmission, as evidenced by increased presynaptic volleys (Kocsis, Malenka and Waxman, 1980; Haas, Wieser and Yasargil, 1983) and could result in the recruitment of inactive synaptic boutons. The net results would be an apparent increase in the neurotransmitter released. In contrast, electrically induced LLP might involve (a) an improved influx of Ca^{++} into terminals and (b) an actual increase in terminal neurotransmitter replacement and/or increase in releasable pools (Voronin, 1983 review). The results reported by Sastry (1982), though not attributed to such an effect, are suggestive of a possible increase in terminal boutons volume during LLP and hence the associated decrease in terminal excitability. This is mere speculation.

15.6 Miscellaneous

Raised extracellular K^+ in concetrations that mimicked increases in K^+ during tetanus did not induce significant changes. These results seem to suggest that increased extracellular potassium is not involved in the initiation of LLP. Alternatively, the raised K^+ which must have depolarized presynaptic neurons, may have induced potentiation and depression that then 'cancelled' each other. Sufficient experiments were not done to examine these possibilities.

16. CONCLUSION

The results from the studies presented in this thesis illustrate that continuous synchronous discharge of CA1b neurons during tetanus, in the presence of extracellular Ca^{++} i.e., in standard medium, correlated with the development of post-tetanic homo- and heterosynaptic depressions. L-glutamate applications to CA1b neurons, which is known to cause Ca^{++} entry into neurons, led to depressions. Verapamil in doses that selectively interfered with postsynaptic entry of Ca^{++} counteracted the late depression associated with Glu or low frequency induced depressions. While the exact mechanisms of these depressions are uncertain, the following statements can be made. Cell death cannot account for the depressions since it is known that subsequent development of LLP to the same input (and other inputs) can be induced with high frequency tetanus (Dunwiddie and Lynch, 1978; Chirwa, Goh, Maretic and Sastry, 1983). In any case, complete recovery of evoked potentials after the heterosynaptic depression usually occured. Depolarization blockade and/or increased post-activity hyperpolarizations contributed towards the early phase of the depressions and this could not be reversed by verapamil. It seemed therefore that CA1b repetitive cell discharges led to the entry of Ca^{++} into these neurons, which then effected a generalised postsynaptic depressions.

The dependency of LLP initiation on extracellular Ca^{++} is well documented. Yet verapamil did not interfere with the development of LLP. It could be that the subsynaptic Ca^{++} current responsible for LLP development was insensitive to verapamil. Alternatively, and this is the favoured interpretation, verapamil did not antagonise neurotransmitter release. This

was suggestive of the fact that verapamil did not interfere with Ca^{++} influx into presynaptic regions. A pertinent observation was that the presynaptic volley followed low and high frequency tetanic stimulations. The high frequencies must have preferentially caused Ca^{++} influx into continuously activated synaptic boutons. Yet high frequencies continuously elicited LLP. Clearly this is strong evidence for a presynaptic mediated LLP.

Barium and 4-aminopyridine induced enhancements of evoked potentials differed from the electrically induced LLP, i.e., drug induced enhancements were reversed with washing. Firstly, these results are suggestive of the fact that LLP is not mediated by blockade and/or alterations to K^{+} fluxes. This seemed likely since elevated K^{+} medium did not induce LLP. Still, it is not clear why the enhanced Ca^{++} influxes that was secondary to the actions of these drugs did not elicit LLP. It is tentatively suggested here, that at the level of the presynaptic terminals, while these drugs enhanced neurotransmitter released, more importantly they facilitated the recruitment of subthreshold Sch boutons (i.e., through K^{+} blockade and at this level the afferent sections are not myelinated). In addition, the depletion of neurotransmitter pools that may have accompanied Ba^{++} applications was not readily replenished as occurs with Ca^{++}. It is proposed here that the changes in LLP possibly involve (a) presynaptic alterations of the <u>active</u> boutons that facilitates improved invasion of terminal action potentials and (b) a more efficacious neurotransmitter mobilization, which then translates into an increase in quantal content. These are only speculations needing further experimentation to confirm them.

Subsequent studies should be aimed at the exploration of intracellular activities in these synaptic boutons following tetanic stimulations. In general, there is a need for intracellular studies to further characterise mechanisms of depressions as well as the pharmacological properties of verapamil in neurons. Lastly, it should be resolved whether in fact net inward synaptic currents increase and whether there are changes in the subsynaptics' active or passive properties.

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