STUDIES ON THE ASYNCHRONOUS SYNAPTIC RESPONSES AND ENDOGENOUS POTENTIATING SUBSTANCES OF NEUROTRANSMISSION IN THE HIPPOCAMPUS

By

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ABSTRACT

In the hippocampus, transient tetanic stimulations of inputs, or brief simultaneous pairings of conditioning intracellular postsynaptic depolarizations with activated presynaptic afferents at low stimulation frequencies, result in input specific long-term potentiation (LTP) of synaptic transmission. LTP lasts for hours *in vitro*, or weeks *in vivo*, and it is thought to be involved in memory and learning. Experimental evidence in the literature suggests that postsynaptic mechanisms mediate LTP induction, whereas presynaptic mechanisms are involved in its maintenance. Since LTP is thought to be generated by postsynaptic mechanisms and to be subsequently maintained by presynaptic processes, this suggests the presence of feedback interactions during LTP development. However, the experimental evidence for such interactions is presently not available. Consequently, the present studies were conducted to examine possible feedback interactions between postsynaptic and presynaptic elements in the hippocampus. Furthermore, the experiments tested the hypothesis that substances released during tetanic stimulations caused the release of endogenous substances that interacted with activated afferents resulting in alterations in presynaptic functions and LTP production.

Experiments were conducted using transversely sectioned guinea pig hippocampal slices. Briefly, physiological medium containing 3.5 mM Ba^{++} and 0.5 mM Ca^{++} (denoted as Ba^{++} medium) was used to induce the asynchronous release of transmitters, observed as evoked miniature EPSPs (minEPSPs) in CA_{1b} neurons after stimulation of the stratum radiatum. During transient Ba^{++} applications, short bursts of evoked minEPSPs were

observed following stimulations of the stratum radiatum or conditioning depolarizing current injections into CA_{1b} neurons. Moreover, the frequencies of minEPSPs were significantly increased immediately after simultaneous stimulations of the stratum radiatum and conditioning depolarizing current injections into CA_{1b} neurons. Significant increases in the frequencies of evoked minEPSPs were also observed during LTP induced by tetanic stimulations. The above increases in the frequencies of evoked minEPSPs were attributed, in part, to presynaptic changes resulting in increases in transmitters released. However, a thorough quantal analysis is required to substantiate this conclusion.

In order to determine whether any substances released during tetanic stimulations were involved in the modulation of presynaptic functions and induction of LTP, samples were collected from guinea pig hippocampus and rabbit neocortex. It was found that samples that were collected during tetanic stimulations of the guinea pig hippocampus in vivo or rabbit neocortex in vivo produced LTP in the guinea pig hippocampal slice in vitro. Applications of these samples after heating and cooling failed to induce LTP. Subsequent studies demonstrated that PC-12 cells incubated in growth medium treated with samples collected during tetanic stimulations of the rabbit neocortex developed extensive neurite growths. In contrast, PC-12 cell cultures incubated in (1) heated and cooled samples, (2) samples collected in the absence of tetanic stimulations of the rabbit neocortex, or (3) plain growth medium, failed to develop neurite growths. In addition, PC-12 cell cultures that were incubated in growth medium containing samples collected during tetanic stimulations plus saccharin (10 mM), a substance known to inhibit NGF-dependent neurite growth, failed to develop neurites.

In separate experiments it was found that saccharin could block (1) the synaptic potentiating effects of the above collected and applied endogenous substances, and (2) LTP induced with tetanic stimulations, in the guinea pig hippocampus *in vitro*. The concentrations of saccharin used in these studies had insignificant effects on resting membrane potentials, input resistances, spontaneous or evoked responses of CA_{1b} neurons. Furthermore, CA_{1b} neuronal depolarizations induced by N-methyl-DL-aspartate (NMDA) or with tetanic stimulations of the stratum radiatum, were not altered by saccharin applications. In addition, saccharin had insignificant effects on paired-pulse facilitation, post-tetanic potentiations, minEPSP frequencies in CA_{1b} neurons, and Schaffer collaterals terminal excitability. These results suggest that saccharin blocked LTP through mechanisms different from either non-specific alterations in CA_{1b} cell properties or NMDA receptor activation. Perhaps the agent antagonized LTP at a step beyond NMDA receptor activation. That saccharin blocked LTP caused by the applied neocortical sample as well as by tetanic stimulation of the stratum radiatum, and that saccharin also blocked neurite growth in PC-12 cells induced by the neocortical samples, raises the prospect that growth related substances are involved in LTP generation. In other control experiments, it was found that the potentiating effects of the collected endogenous substances were not antagonised by atropine or dihydro-β-erythroidine. Heated and then cooled solutions of glutamate (a putative transmitter at the Schaffer collaterals-CA_1 synapses) still maintained their actions on the CA_{1b} population spike. While brief applications of 2.5 μg/ml exogenous NGF (from *Vipera lebetina*) during low frequency stimulations of the stratum

radiatum did not consistently induce LTP, this peptide significantly facilitated the development of LTP when applied in association with tetanic stimulations of weak inputs in the CA_1 area. These weak inputs could not support LTP if tetanized in the absence of the exogenous NGF.

The results of the studies in this thesis suggested that postsynaptic depolarizations modulated presynaptic functions in the hippocampus. Tetanic stimulations in hippocampus and neocortex caused the release of diffusible substances, which were probably growth related macromolecules, that interacted with activated presynaptic afferents and/or subsynaptic dendritic elements resulting in LTP development. The precise locus of actions of these agents awaits further investigations.

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ABBREVIATIONS

AP5	D-2-amino-phosphonovalerate
AP7	D-2-amino-phosphonoheptanoate
APV	D-2-amino-phosphonovalerate
ACh	Acetylcholine
AHP	Afterhyperpolarization
ATP	Adenosine triphosphate
CA	Cornu ammonis.
cAMP	Adenosine 3':5'-cyclic phosphate
cGMP	Guanosine 3':5'-cyclic phosphate
DG	Dentate gyrus
DRG	Dorsal root ganglion
EMP	Embden-Meyerhof-Parnas pathway
EPP	End-plate potential
EPSP	Excitatory postsynaptic potential
FANFT	N-[-4-(5-nitro-2-furyl)-2-thiazoly]-formamide
GABA	γ-aminobutyric acid
GAD	Glutamic acid decarboxylase
HTHS	Heated-tetanised hippocampal sample
HTNS	Heated-tetanised neocortical sample
IPSP	Inhibitory postsynaptic potential
KA	Potassium acetate electrode
KCl	Potassium chloride electrode
LTP	Long-term potentiation
MEPP	miniature end-plate potential
MCD	Mast cell degranulating peptide
minEPSP	miniature excitatory postsynaptic potential
minIPSP	miniature inhibitory postsynaptic potential
NAD	Nicotinamide-adenine dinucleotide
NADP	Nicotinamide-adenine dinucleotide phosphate
NMDA	N-methyl-D-aspartate
NMDLA	N-methyl-DL-aspartate
NGF	nerve growth factor
NMJ	Neuro-muscular junction
PC-12	Rat adrenal pheochromocytoma cells
PKC	Protein Kinase C
PP	Perforant pathway
PS	Population spike
PTP	Post-tetanic potentiation
PW	Positive wave
RMP	Resting membrane potential
R_n	Input resistance
Sch	Schaffer collaterals
SKF10047	n-allynormetazocine.
STP	Short-term potentiation
THS	Tetanised hippocampal sample
TNS	Tetanised neocortical sample
UHS	Untetanised hippocampal sample
UNS	Untetanised neocortical sample

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It would mean nothing

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1. INTRODUCTION

An intriguing aspect of brain function is how learning and memory operations are accomplished, at the cellular level. Hebb (1949) postulated changes in synaptic weightings, as a consequence of use-dependent activities in neural networks. It was hypothesized that simultaneous activities in pre- and postsynaptic neurons led to synaptic modifications for learning and memory (Hebb, 1949). In recent years, the phenomenon of long-term potentiation (LTP) that has been extensively studied in the mammalian hippocampus is taken to be consistent with the concept of the "Hebbian synapse" and, therefore, provides a potential model for learning and memory (Gustafsson and Wigström, 1988; Kelso, Ganong and Brown, 1986). LTP in the hippocampus is described as an input specific increase in synaptic efficacy following brief tetanic stimulations of the input (in vivo: Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973; and in vitro: Alger and Teyler, 1976; Schwartzkroin and Wester, 1975). The tetanic stimulation frequencies used to induce LTP include those frequencies that occur in normal physiology (Larson and Lynch, 1986; Rose and Dunwiddie, 1986). The increase in synaptic efficacy lasts for several minutes to hours in vitro, or days to weeks in vivo (Barnes, 1979; Swanson, Teyler and Thompson, 1983). LTP is observed as decreases in onset latencies and/or increases in amplitudes of the evoked field responses. With intracellular recordings, LTP is seen as increased probability in cell discharges or enhancements of subthreshold EPSPs (Andersen, et al., 1980c; Schwartzkroin and Wester, 1975).

Using transversely sectioned hippocampal slices, different experimental methods can be used to elicit LTP. For example, brief pairings of adequate conditioning intracellular postsynaptic depolarizations with low frequency

activation of afferents induces LTP (Sastry, Goh and Auyeung, 1986). Long-lasting synaptic potentiations also occur following transient exposures to the following: raised extracellular Ca^{++} (Turner, Baimbridge and Miller, 1982), mast cell degranulating peptides from bee venom (Cherubini et al., 1987), phorbol analogs that activate protein kinase C (Madison, Malenka and Nicoll, 1986), and raised extracellular K^{+} in the absence of extracellular Ca^{++} (May, Goh and Sastry, 1987). During LTP several changes are observed in addition to increases in synaptic efficacy. These changes include release of newly synthesized proteins during tetanic stimulations (Duffy, Teyler and Shashoua, 1981), synaptic re-modeling (Chang and Greenough, 1984; Greenough, 1984; Desmond and Levy, 1981; Fifkova and Van Harreveld, 1977), increased release of glutamate and aspartate, the putative transmitters (Bliss, et al., 1986; Skrede and Malthe-Sørenssen, 1981), and decreased presynaptic terminal excitability (Sastry, 1982). It is not known whether the above changes reflect mechanisms mediating the expression of LTP, or if they represent secondary changes associated with the phenomenon. Furthermore, the identities and physiological functions of the released proteins have not been established. In addition, it is not clear what factor(s) control morphological alterations of synaptic structures during LTP. In fact, the exact mechanisms underlying the change(s) leading to this synaptic potentiation still remain to be determined (Gustafsson and Wigström, 1988).

A popular hypothesis that has received much attention in recent years advocates the involvement of transmitter and voltage regulated NMDA receptor channels. Briefly, both NMDA and non-NMDA receptors are thought to be present, for example, at the Schaffer collateral-commissural/CA_1 pyramidal cell synapses in the hippocampus (Collingridge, Kehl and McLennan, 1983;

Harris, Ganong and Cotman, 1984; Wigström and Gustafsson, 1984 and 1986). The putative transmitter, glutamate, is an agonist for both NMDA and non-NMDA receptors (Mayer and Westbrook, 1987; Watkins and Evans, 1981). Presumably, non-NMDA receptor channels mediate the fast EPSPs, whereas NMDA receptor channels are blocked by Mg^{++} and do not contribute significantly to low frequency synaptic transmission (Collingridge, 1985; Nowak, et al., 1984; Wigström and Gustafsson, 1985a). However, it is thought that tetanic stimulations (or adequate postsynaptic depolarizations) remove the Mg^{++} blockade of NMDA receptor channels, resulting in Ca^{++} influx through them. It has been postulated that these postsynaptic Ca^{++} influxes subsequently mediate secondary changes leading to long-lasting potentiation of the synaptic responses (Collingridge, 1985; Gustafsson and Wigström, 1988). The NMDA hypothesis is based on experimental data showing that amino-phosphonovalerate (APV) a presumed competitive antagonist at the NMDA receptors, blocks the induction but not the maintenance of LTP (Collingridge, Kehl and McLennan, 1983; Harris, Ganong and Cotman, 1984; Wigström and Gustafsson, 1984). Interestingly, APV also blocks LTP induced by raised extracellular Ca^{++} (Errington, Lynch and Bliss, 1987), mast cell degranulating peptides from bee venom (Cherubini, et al., 1987), and paired presynaptic and conditioning postsynaptic depolarizations (Kauer, Malenka and Nicoll, 1988). One intriguing feature concerning LTP expression is that presynaptic mechanisms seem to be involved in the maintenance of this phenomenon (Sastry, 1982; Teyler and DiScenna, 1987). Consistent with this notion are studies that have shown that LTP is associated with sustained increases in the release of glutamate, the putative transmitter (Bliss, et al., 1986; Skrede and Malthe-Sørenssen, 1981). How a process that is generated via postsynaptic mechanisms is subsequently sustained by presynap-

tic mechanisms has not been investigated.

The studies reported in this thesis arose from the following predictions. If LTP induction is postsynaptic but its maintenaince is presynaptic (Bliss and Lynch, 1988), this suggests that during LTP production feedback interactions occur between postsynaptic and presynaptic regions (cf. Eccles, 1983; Sastry, Goh and Auyeung, 1986). Consequently, studies presented in this thesis have examined these interactions through the analysis of changes in frequencies of evoked miniature excitatory postsynaptic potentials (minEPSP) in the guinea pig hippocampus in vitro. In these studies, Ba^{++} medium containing low Ca^{++} was used to induce the asynchronous release of transmitters (cf. Silinsky, 1978), and this was observed as increases in the frequencies of evoked minEPSPs in CA_1 neurons following stimulation of the stratum radiatum. The assessment of evoked minEPSPs provided a potential method for examining presynaptic functions. In this regard, changes in evoked minEPSP frequencies were used to assess directly increases in transmitter release during LTP. This was done for the following basic reason. The data in the literature showing sustained increases in release of transmitters during LTP are based on biochemical assays of glutamate, the excitatory transmitter candidates in the hippocampus. It is conceivable that increased glutamate release could be due to enhanced glutamate turnover associated with metabolic processes that are unrelated to transmitter release per se.

The major thrust of the studies, however, have been centered on determining some of the physiological effects of the substances that are released during tetanic stimulations. These studies stemmed from the prediction that proteins that are released during tetanic stimulations (Duffy, Teyler and Shashoua, 1981), may be involved in LTP development. Consistent with this

notion, for example, are reports in the literature showing that substances that inhibit protein synthesis also block LTP production (Stanton and Sarvey, 1984). The studies reported in this thesis involved the collection of samples during tetanic stimulations of the guinea pig hippocampus in vivo and rabbit neocortex in vivo, and subsequently applying them onto guinea pig hippocampus in vitro. Collections from the rabbit neocortex in vivo were used for the following reasons. The bigger surfaces of the rabbit neocortex made it possible to use relatively larger collecting cups. More importantly, LTP has been shown to occur in the mammalian neocortex (Lee, 1982). Related studies involved testing the effects of the samples collected during tetanic stimulations on neurite growth in cell cultures. The rationale for performing these experiments arose from the following observations. Proteins that are known to be released during tetanus-induced LTP present with molecular weights ranging between 14-86 kD (Duffy, Teyler and Shashoua, 1981; Hesse, Hofstein and Shashoua, 1984), and this is strikingly similar to the molecular weights of nerve growth factors (NGF) and other growth related substances (Berg, 1984; Wagner, 1986). Interestingly, the hippocampus and neocortex have the highest levels of NGF messenger RNA in the brain (Korsching et al., 1985; Whittemore, et al., 1986). Furthermore, NGF presumably is essential for nerve cell survival and axon sprouting that occurs following injury in the hippocampus (Crutcher and Collins, 1986; Hendry et al., 1974; Nietro-Sampedro and Cotman, 1985; Springer and Loy, 1985). Since LTP is associated with structural alterations in synapses, it is conceivable that these structural changes could be mediated by close relatives of NGF. If this is the case, then it raises the prospects that the released proteins during tetanic stimulations could be growth related macromolecules. Hence experiments were conducted to test the above ideas as

follows. Briefly, primary tissue cultures of rat pheochromocytoma cells (denoted as PC-12 cells) develop extensive neurite networks when incubated in growth medium containing NGF or related substances (Greene and Tischler, 1976). Hence, PC-12 cells cultures were incubated in growth medium containing samples collected during tetanic stimulations. In some experiments, saccharin was added to the growth medium containing the above samples. Saccharin is known to inhibit NGF "receptor" binding in a dose-dependent fashion, and the drug decreases NGF-dependent neurite growth in embryonic chick dorsal root ganglia cell cultures (Ishii, 1982). Several other experiments examined, for example, the physiological effects of saccharin in the guinea pig hippocampus in vitro. A few studies examined the effects of applied NGF on synaptic transmission in the CA_1 area.

Taken together, the results of the studies in this thesis were found to be consistent with the following predictions: (1) Postsynaptic depolarizations modulate presynaptic activities; (2) LTP was associated with an apparent increase in transmitter release; (3) Substances released during tetanic stimulations could elicit LTP, thereby indicating that they could be involved in the production of this phenomenon; and (4) The above samples contained neurite inducing factors, thereby indicating that these endogenous substances could be growth related macromolecules. Furthermore, it seemed that saccharin blocked both the induction of LTP in the hippocampus and neurite growth in PC-12 cell cultures induced by samples collected during tetanic stimulation of the rabbit neocortex via common mechanisms shared by these two processes. The concentration of saccharin (10 mM) used in these studies did not exert significant effects on CA_{1b} cell electrical properties, synaptic transmission or NMDA receptor mediated depolarizations. These results suggested that saccharin blocked the induction of LTP at a

step beyond NMDA receptor activation. In summary, the results presented in this thesis raise the prospect that growth related substances may be involved in the production of LTP. The above ideas, and their inherent implications, are further developed in the discussion sections.

2. BASIC MORPHOLOGY OF THE HIPPOCAMPAL FORMATION

2.1 General

A detailed knowledge of the neural networks involved in the generation of responses is useful for any meaningful interpretation of electrophysiological signals. For these reasons, comprehensive accounts of the hippocampal morphology and physiology will be discussed in this thesis. The present chapter will endeavour to illustrate the basic morphological features of the hippocampus that will have a bearing on the discussions presented in subsequent chapters. The descriptive morphological terms used here are consistent with the nomenclatures employed by Blackstad (1956), Cajal (1911) and Lorente De Nó (1934). Wherever appropriate, sections have been updated based on recent detailed anatomic studies (e.g. Schwerdtfeger, 1984; Swanson, Wyss and Cowan, 1978; White, 1959).

2.2 The hippocampal region

During ontogenic development, the cortical mantle is subdivided into the allocortex and the isocortex. The isocortex is commonly known as the neocortex, and it is a homogenous unit that separates completely from the cortical mantle. The allocortex consists of the archicortex and the palaecortex, and these are heterogenous units that remain attached to the cortical mantle (Filimonoff, 1947). A separate region denoted as the periallocortex is situated between the allocortex and the isocortex. The periallocortex gives rise to structures such as the peripalaeocortical claustral, entorhinal, presubicular, retrosplenal and periarchicortical cingulate cortices (Blackstad, 1956; Brodmann, 1909; Chronister and White, 1975; Lorente De Nó, 1934; Sanides, 1972; White, 1959; Vaz Ferreira, 1951). The palaecortex gives rise to the olfactory bulb, accessory bulb, retrobul-

bal region, periamygdalar region, the olfactory tubercle, the septum, the diagonal region and prepiriform region (Pribram and MacLean, 1953; Sanides, 1972; Schwerdtfeger, 1984). The archicortex is comprised of the subiculum, Ammon's horn, fascia dentata precommissural hippocampus and supracommissural hippocampus (Blackstad, 1956; Lorente De No, 1934; Swanson, Wyss and Cowan, 1978; Teyler and Discenna, 1984). Hence, the term "archicortex" is synonymous with "hippocampus proper" or simply "hippocampus" (Schwerdtfeger, 1984; Teyler and DiScenna, 1984).

2.3 The hippocampus

The hippocampus is seen as a curved elongated ridge that is situated along the floor of the descending horn of each lateral ventricle. A transverse section of the hippocampus reveals two distinct interdigitating fields termed cornu ammonis and dentate gyrus (Figure 2-1). Each of these fields contain densely packed sheets of cells. The predominant cell types of the hippocampus are the pyramidal cells in the cornu ammonis field and the granular cells in the dentate gyrus field. However, several other types of cells are distributed in both fields (Amaral, 1978; Cajal, 1893; Lorente De Nó, 1934).

2.4 The dentate gyrus

The dentate gyrus field is curved into a "V" shape. The blade (or side) of the curvature that is adjacent to the subiculum (Figure 2-1) is termed as the suprapyramidal blade. The infrapyramidal blade, therefore, is the intraventricular part of the curvature (Chronister and White, 1975; Swanson, Wyss and Cowan, 1978). The granular cells of the dentate gyrus are localised in a single layer termed the stratum granulosum. These cells possess apical dendrites that project into the stratum moleculare layer that is situated between the stratum granulosum and the pial surface within the

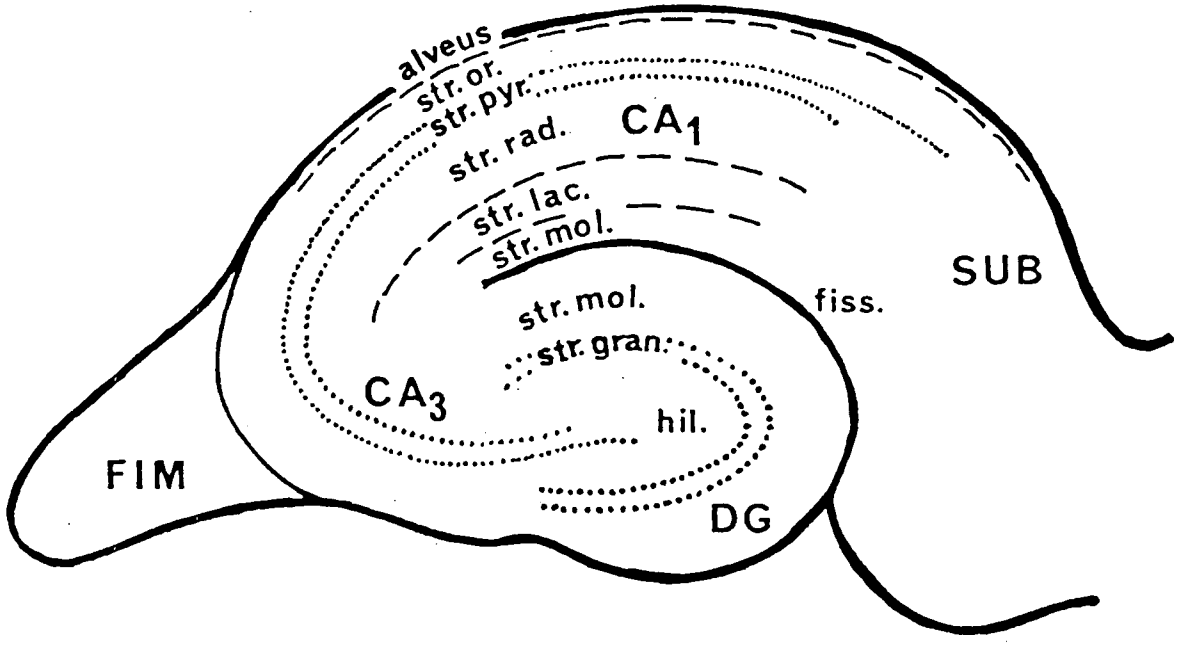

Figure 2-1. General morphology of the hippocampal formation. The stratum pyramidales (str. pyr.) layer of the cornu ammonis (CA) field and the stratum granulosun (str. gran.) layer of the dentate gyrus (DG) field contain densely packed sheets of cells which are mostly neuronal bodies of pyramidal cells and granule cells, respectively. Several other types of neurons are found in these layers as well as the subiculum (SUB) and the hilus regions. Typically, the axonal and dendritic processes are distributed in the following layers: stratum oriens (str. or.), stratum radiatum (str. rad.), stratum lacunosum (str. lac.), and stratum moleculares (str. mol.). The axons of CA_1 pyramidal cells course through the alveus and exit out via the subiculum or the fimbria (FIM). [fiss. denotes fissure].

hippocampal fissure (Figure 2-1). Inside the dentate gyrus curvatures are found several layers of polymorphic cells that make up the hilar region (Amaral, 1978; Lorente De Nó, 1934).

2.5 The hilus and CA_4 region

Within the dentate gyrus concavity and close to its apex is the hilus. The hilus is extremely variable in appearance across species. In the mammalian brain, it is least developed in rodents but increases in complexity in the rabbit, monkey and man (Lorente De Nó, 1934). Opinions are split on whether the hilus constitutes the third layer of the dentate gyrus (Blackstad, 1956; Cajal, 1911; Lorente De Nó, 1934). In rodents, the caudal end of the cornu ammonis extends into the hilus. Yet the boundaries between the cornu ammonis and the hilus are not readily discernible (Amaral, 1978; Swanson, Wyss and Cowan, 1978). Several cell types (e.g. basket cells, modified pyramidal cells) have been identified in the hilus, (Amaral, 1978; Cajal, 1893; Lorente De Nó, 1934). Some of the cell types in the hilus are similar to those found in the stratum oriens layer of the cornu ammonis field. In view of the above, the hilar region is taken to be a structural transition zone between the dentate gyrus and the cornu ammonis (Lorente De Nó, 1934). As proposed by Lorente De Nó (1934), the modified pyramidal cells that stream out of the hilar region make up the CA_4 region.

2.6 The cornu ammonis field

The rostral end of the cornu ammonis (CA) merges with the subiculum. The caudal part forms a continuum with the outer section of the CA_4-hilar region described above (Lorente De Nó, 1934; Schwerdtfeger, 1984). The cornu ammonis field has a curved cell layer termed the stratum pyramidale (Figure 2-1). In addition, the cornu ammonis field has the following layers. The alveus, lies next to the epithelium of the lateral ventricle,

and this layer marks the outer boundary of this field. The stratum oriens is situated between the alveus and the stratum pyramidale. 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 (Figure 2-1).

In vertebrates, the extent of development of different regions of the hippocampus is most varied in the cornu ammonis field. In rodents, for example, the division between stratum radiatum and stratum lacunosum is somewhat artificial. Consequently, in these animals, the stratum radiatum and stratum lacunosum are often described as a single layer, i.e. stratum radiatum (Lorente De Nó, 1934). Similarly, the stratum lucidum is considered to be part of the stratum pyramidale layer.

3. CELLULAR PROPERTIES AND INTRINSIC CIRCUITRY

3.1 Dentate gyrus granule cells

Granule cells are the predominant neurons in the stratum granulosum layer. These neurons are highly polar, and they possess characteristic ovoid cell bodies which are about 20 by 13 μm in size (Williams and Matthysse, 1983). A short stem dendrite emerges from the apical pole of each granule cell and extends into the stratum moleculare where it bifurcates repeatedly (Cajal, 1911; Lindsay and Scheibel, 1976; Lorente De Nó, 1934; Williams and Matthysse, 1983). The dendritic branches are covered with various types of spines. In general, spines are confined to segments beyond the first branch of the stem dendrites.

The granule cells possess thick axons, termed mossy fibers, which originate from the basal pole and extend transversely across subfield CA_3 (Blackstad, et al., 1970). Each mossy fiber has a distinct axon hillock situated at the basal tip of the soma (Williams and Matthysse, 1983). Mossy fibers are highly laminated and they form synaptic contacts with spines of proximal dendrites of CA_3 pyramidal cells. The mossy fibers of granule cells in the infrapyramidal dentate gyrus blade innervate CA_{3c} pyramidal cells. Granule cells in the suprapyramidal dentate gyrus blade send out mossy fibers across the entire CA_3 field (Lorente De Nó, 1934; Haug, 1974; Swanson, Wyss and Cowan, 1978). A small fraction of mossy fibers form synapses with some of the polymorphic cells in the hilar/CA_4 region (Blackstad and Kjaerheim, 1961) and interneurons in field CA_3 (Frotscher, 1985; Tombol, et al., 1979).

3.2 Cornu ammonis pyramidal neurons

Pyramidal cells are the predominant neurons in the stratum pyramidale.

The somata of a pyramidal cell is pear-shaped, and it is oriented such that the long axis is vertical to the alvear surface. On average the cell body size is about 40 by 20 μm. However, pyramidal cells exhibit a range of cell body sizes. The rostral cornu ammonis has the smallest pyramidal cells, whereas the caudal cornu ammonis is endowed with the largest cells. All pyramidal cells have apical and basal dendrites. There are important structural differences among pyramidal cells such as dendritic profiles and/or axonal ramifications. Lorente De Nó (1934) used these morphological differences among pyramidal cells to delineate the cornu ammonis into various subfields, namely CA_{1a-c}, CA_2, CA_{3a-c} and CA_4 (see also Cajal, 1911; Golgi, 1886; Schaffer, 1892; Blackstad, 1956).

3.2.1 Subfield CA_1. The stratum pyramidales in the rostral cornu ammonis starts as a diffuse region of mixed cells before it becomes a densely packed single layer consisting primarily of pyramidal cells. This initial part of the stratum pyramidales is denoted as CA_{1a}, and it is comprised of a mixed primary cell population (i.e contains cells belonging to the subiculum: Lorente De Nó, 1934). According to Lorente De Nó (1934), the Schaffer collaterals of CA_3 and CA_4 pyramidal cells cease at the border between CA_{1b} and CA_{1a}. The CA_{1b} contains much smaller but similar pyramidal cells, representative of the CA_1 subfield. The onset of CA_{1c} area is marked by the presence of small pyramidal cells similar to those of the CA_{1b} area, except that the dendrites of CA_{1c} pyramidal cells are smooth, with numerous side branches.

In general, basal dendrites of CA_1 pyramidal cells start from the soma in a bush-like fashion, with irregular branches that divide repeatedly in the stratum oriens. The apical dendrites extend out into the stratum radiatum for some distance before they begin to branch extensively. Both

apical and basal dendritic branches are covered with various types of spines (Andersen, et al., 1980a; Scheibel and Scheibel, 1968). The CA_1 pyramidal cells have axons that arise from the basal side and branch out in the stratum oriens. Some axonal branches cross the stratum pyramidale and ramify in the stratum radiatum or distribute back to CA_1 and CA_2 pyramidal layers, where they presumably make synaptic contacts with interneurons (Lorente De Nó, 1934). Most of the axonal collaterals descend down to the alveus, where they form pathways in both directions and leave the hippocampus via the fimbria or the subiculum. The CA_1 axons project out of the hippocampus to other brain regions such as the lateral septal nuclei and prefrontal cortex (Swanson, 1981; Swanson and Cowan, 1977). None of the axonal branches of CA_1 pyramids make contact with the pyramidal cells in CA_3 and CA_4, or the dentate gyrus granule cells (Lorente De Nó, 1934).

3.2.2 Subfield CA_3. The CA_3 pyramidal cells possess large apical dendrites that cross the stratum radiatum and only begin to branch when they reach the stratum moleculare (Lorente De Nó, 1934). In contrast, the basal dendrites of CA_3 pyramidal cells begin to branch early, as they traverse the stratum oriens. Both the basal dendrites and initial parts of the apical dendrites are endowed with numerous thick spines.

The pyramidal cells in CA_3 subfields have thick axons that originate from their basal poles. These axons cross the stratum oriens and travel towards the fimbria and leave the hippocampus (Figure 3-1). In addition, these axons give off several collaterals which terminate within the stratum oriens or in the CA_3 pyramidal cell layer (presumably to innervate interneurons; Lorente De Nó, 1934). Some collaterals cross the CA_3 cell layer and travel within the stratum radiatum/lacunosum layers (Lorente De Nó, 1934). These collaterals are myelinated, and they constitute a horizontal

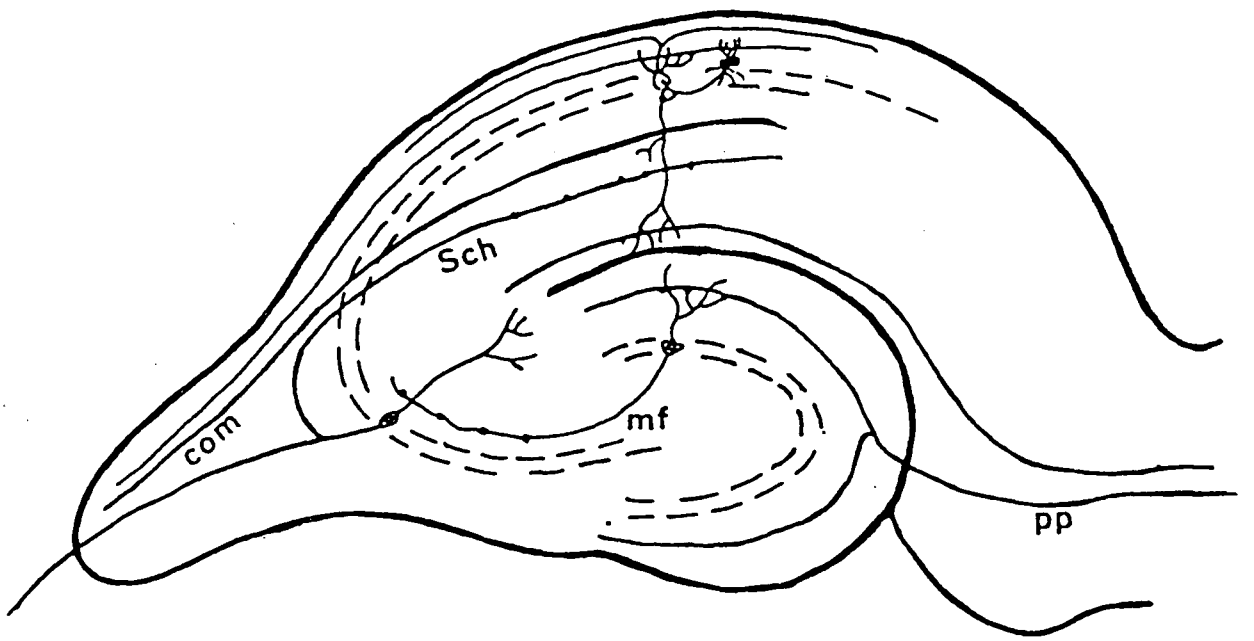

Figure 3-1. The major afferent systems in the hippocampus. The granule cells in the dentate gyrus give out mossy fibers (mf) that innervate CA_3 pyramidal cells. The axons of CA_3 pyramidal cells have thick axons, some of which branch and give rise to the Schaffer collaterals (Sch) that innervate CA_1 pyramidal cells. The commissural input (com) represents afferents from the contralateral hippocampus. Note the perforant path (pp) which is a major extrinsic input into the hippocampus originating from the entorhinal cortex and innervating the granule cells of the dentate gyrus.

fiber system termed Schaffer collaterals which innervate the apical dendrites of CA_{1b} and CA_{1c} cells.

Some CA_3 pyramidal cells do not give off Schaffer collaterals and this feature is one criterion that has been used to subdivide the CA_3 subfield. CA_{3c} pyramidal cells have axons that give out Schaffer collaterals. Furthermore, CA_{3c} pyramidal cells are innervated by the mossy fibers of both the infrapyramidal and the suprapyramidal granule cells (Lorente De Nó, 1934). In contrast, CA_{3b} and CA_{3a} pyramidal cells are only innervated by the mossy fibers of the suprapyramidal granule cells. The CA_{3b} area consists of mixed pyramidal cells, and presumably 50% of these cells have Schaffer collaterals (Lorente De Nó, 1934). The pyramidal cells of CA_{3a} do not have Schaffer collaterals (Lorente De Nó, 1934). Instead, most of the CA_{3a} pyramidal cells have thick axons that give off one or two myelinated collaterals. These collaterals ascend to the stratum radiatum where they form an associational pathway running within the stratum radiatum of the CA_3 and CA_{1b} subfields.

3.2.3 Subfield CA_2. The CA_2 subfield is rather small, and it is considered to be a transitional area, between CA_{1c} and CA_{3a} (Lorente De Nó, 1934; Schwerdtfeger, 1984). The CA_2 pyramidal cells are large (i.e., similar to those of CA_3), but their dendrites do not possess thick spines (Lorente De Nó, 1934; Haug, 1974; Swanson, Wyss and Cowan, 1978). Furthermore, the apical dendrites of CA_2 pyramidal cells start to divide into branches within the stratum radiatum, shortly after leaving the cell body layer. The axons of CA_2 pyramidal cells do not give Schaffer collaterals, but they possess thick collaterals that cross the cell body layer and enter the stratum radiatum where they constitute a strong axial pathway reaching up to the CA_{1b} region. In addition, the CA_2 pyramidal cell

axons form horizontal collaterals which travel within the stratum oriens, towards the subiculum and entorhinal cortex.

3.3 CA_4 and Hilus neurons

The CA_4 starts as a densely packed cell layer which begins to scatter towards the region bordering the dentate gyrus suprapyramidal blade. The cells in this latter section appear "unaligned", as if the caudal cornu ammonis layer had folded back on itself (Lorente De Nó, 1934). The basal dendrites of CA_4 pyramidal cells appose the infrapyramidal granule cells. Their apical dendrites, however, tend to branch within the region, or give branches that travel along the suprapyramidal blade to reach the molecular layer. The CA_4 pyramidal cell dendrites make synaptic contacts with mossy fibers. However, it is unclear what other afferents make synaptic contacts with the dendritic branches that extend into the molecular layer. The axons of CA_4 pyramidal cells innervate the ipsilateral and contralateral CA_3 fields (Gottlieb and Cowan, 1973). These fibers establish synaptic contacts with CA_3 cell dendrites in the stratum oriens and the stratum radiatum layers. In addition, the axons of CA_4 pyramidal cells give out Schaffer collaterals. These axons also have collaterals that constitute a commissural projection to the contralateral CA_1 subfield where they synapse with CA_1 cell dendrites, mostly in the stratum oriens (Laurberg and Sorensen, 1981; Schaffer, 1892).

The apex zone of the hilus is essentially devoid of neurons and consists mostly of mossy fiber bundles. This small zone is taken to be the polymorphic layer of the dentate gyrus (Amaral, 1978; Blackstad, 1956). The rest of the hilus is comprised of diverse cells that are dispersed throughout the region (Amaral, 1978). These cells exhibit complex dendritic profiles, much like those seen in CA_4. The axons from some of the hilar

cells approach the granule cells and ramify within the stratum granulosum. Some axonal collaterals travel along the suprapyramidal blade and terminate within the inner third of the dentate gyrus molecular layer. Yet other axonal collaterals form a commissural pathway that travels to the molecular layer of the contralateral dentate gyrus field. It is not known with certainty whether hilar projections terminate only on granule cells, or if these inputs make synaptic contacts with interneurons in the stratum granulosum (Swanson, Sawchenko and Cowan, 1981).

3.4 Interneurons

The pyramidal cells in the cornu ammonis and the granule cells in the dentate gyrus make up 96-98% of the neuropil in the hippocampus (Buzsaki, 1984). However, a variety of interneurons are distributed within these regions (Cajal, 1911; Lorente De Nó, 1934). These interneurons have been classified according to their morphological characteristics, e.g. pyramidal, horizontal, fusiform, inverted fusiform, multipolar and basket cells (Ribak and Seress, 1983; Buzsaki, 1984). Interneurons provide the pathways for inhibition (or modulation) of the principal cells in each hippocampal field (Andersen, Eccles and Løyning, 1963 and 1964; Kandel, Spencer and Brinley, 1961; Storm-Mathisen, 1977; Ribak, Vaugh and Saito, 1978; Seress and Ribak, 1984). Though it is likely that some interneurons are excitatory, the overwhelming evidence presently implicates interneurons as mediating inhibition (Andersen, 1975; Andersen, Eccles and Løyning, 1964; Fox and Ranck, 1981; Finch, Nowlin and Babb, 1983; Turner and Schwartzkroin, 1980).

The basket cells are the best studied inhibitory interneurons in the hippocampus. These interneurons have spherical to triangular cell bodies, measuring 50 by 50 μm on average (Andersen et al., 1969). Each basket cell gives out several dendrites that typically extend from the somata without

giving branches, but exhibit "frequent swellings like a string of pearls" (Andersen, et al., 1969). The dendrites of basket cells have few or no spines (Amaral, 1978; Buzsaki, 1984; Ribak and Seress, 1983; Ribak, Vaugh and Saito, 1978). In the cornu ammonis field, the somata of basket cells are situated close to the pyramidal cell bodies. The dendrites of these interneurons distribute within the stratum oriens, or ascend towards the stratum radiatum (Lorente De Nó, 1934). The axons of basket cells are very thin, but divide extensively, giving axonal terminals that form basket-like structures around the somata of pyramidal cells. Each basket cell innervates as many as 500 pyramidal cells (Andersen, et al., 1969). However, the extent of basket cell axons distribution in the hippocampus is not known.

4. EXTRINSIC AFFERENTS TO THE HIPPOCAMPUS

4.1 Entorhinal-hippocampal inputs

Afferents from the entorhinal cortices, termed perforant paths, constitute the major cortical inputs to the hippocampus. The perforant path originates from layers I-III of the entorhinal cortex (Segal and Landis, 1974; Steward and Scoville, 1976). Cells from each of these regions send out axons through the perforant path that distribute largely in the dentate gyrus molecular layers. The projections of the perforant path run perpendicular to the main axis of the hippocampus. Hence, the lateral entorhinal cortex perforant path terminates in the outer one-third of the dentate gyrus molecular layer whereas the medial entorhinal cortical perforant paths terminate in the middle one-third of this layer (Wyss, 1981).

Axons in the perforant path form synapses with the spines of the dentate gyrus granule cell dendrites (Andersen, Holmquist and Voorhoeve, 1966; Hjorth-Simonsen, 1973; Matthews, Cotman and Lynch, 1976; Steward, 1976). Since dentate gyrus interneurons, as well as cells in CA_4 and hilus, have dendritic branches that extend into the molecular layer (Ribak and Seress, 1983), it is likely that these dendritic projections are innervated by perforant paths. However, detailed morphometric studies have not yet confirmed such interactions. In addition, the entorhinal cortex innervates cells in CA_1, CA_3 and subiculum fields (Gottlieb and Cowan, 1972; Steward, 1976; Witter, et al., 1988).

4.2 Septo-hippocampal inputs

The septo-hippocampal pathways mainly originate from the nucleus of the medial septum (Swanson, 1978). However, some septal-hippocampal pathways start from the nuclei of the lateral septum. Both pathways are topographi-

cally organized along the septo-temporal axis of the hippocampus (Meibach and Sieger, 1977). The medial septal pathway is thought to be non-cholinergic (Amaral and Kurz, 1985; Wainer, et al., 1985), and it projects via the medial aspect of the fornix body before reaching the hippocampus (Lewis and Shute, 1967). The lateral septal area is considered to be cholinergic (Amaral and Kurz, 1985; Lewis and Shute, 1967; Nyakas, et al., 1987; Wainer, et al., 1985), and it projects through the lateral portion of the fornix column via the fimbria and then enters the ventral hippocampus (Lewis and Shute, 1967). Studies using intra-axonal markers or histochemical methods have demonstrated that the septal-hippocampal pathways terminate in the cornu ammonis and the dentate gyrus fields (Nyakas, et al., 1987). But the exact target cells in these hippocampal fields still remain to be identified.

4.3 Miscellaneous inputs

Other inputs that project to the hippocampal formation include the following. The locus ceruleus projects noradrenergic afferents (Madison and Nicoll, 1982; Swanson and Hartman, 1975) to the CA_1 and subiculum (Loy, et al., 1980; Pasquier and Reinoso-Suarez, 1978). The hippocampus also receives serotonergic afferents from the raphe nuclei, and dopaminergic afferents from the substantia nigra and the ventral tegmental area (Iversen, 1977; Scatton, et al., 1980; Segal, 1980). Morphometric studies have also revealed projections to the hippocampus that originate from the thalamus and the hypothalamus. The inputs from the thalamus terminate in the CA_1 and subiculum, whereas inputs from the hypothalamus terminate in the dentate gyrus and subiculum (Schwerdtfeger, 1984).

5. ELECTROPHYSIOLOGY OF THE HIPPOCAMPUS

5.1 Electrical properties of neurons

Many of the experimental data have come from studies using the hippocampal slice preparation. But these results obtained *in vitro* have been found to be strikingly similar to values obtained *in vivo* (Kandel and Spencer, 1961; Kandel, Spencer and Brinley, 1961; Spencer and Kandel, 1961). Hippocampal pyramidal and granule cells have resting membrane potentials of minus 50-70 mV, on average. Their input resistances, calculated from the slopes of the current-voltage relationships, typically are as follows (reported as ranges, in MΩ): 26-45, for CA_1; 34-42, for CA_3 and 35-70, for dentate gyrus (Brown, Fricke and Perkel, 1981; Durand, *et al.*, 1983; Turner, 1982; Turner and Schwartzkroin, 1980). The membrane time constants, which are the latencies from onset of the pulse to 1-(1/e) of the peak voltage deflection, exhibit the following values (reported as ranges, in msec); 10-20 for the CA_1 and dentate gyrus neurons, and 17-26 for CA_3 neurons (Brown, Fricke and Perkel, 1981; Durand, *et al.*, 1983; Turner, 1982; Turner and Schwartzkroin, 1980). The large variability in resistances and time constants measurements probably reflect real differences in the sampled neurons within and among the different hippocampal fields. In addition, hippocampal neurons have been modelled in order to assess other electrical features such as electrotonic lengths, dendrite-to-soma conductance ratios, etc (Brown, Fricke and Perkel, 1981; Durand, *et al.*, 1983; Johnston, 1981; Kawato and Tsukahara, 1984; Turner, 1982; Turner, 1984; Turner and Schwartzkroin, 1984). The calculated estimates for the electrotonic length and the dendrite-to-soma conductance ratios in the hippocampus are 0.8-1.0 and 1.5-3, respectively (Brown, Fricke and

Perkel, 1981; Durand, et al., 1983; Johnston, 1981; Turner, 1982; Turner and Schwartzkroin, 1984). These values indicate that the hippocampal neurons are electrically compact.

5.2 Intrinsic ionic conductances

Hippocampal neurons are endowed with a multitude of both chemical and/or voltage gated membrane ionic channels (Colino and Halliwell, 1987; Lancaster and Nicoll, 1987; Llinás, 1984; Moore, et al., 1988; Schwartzkroin and Slawsky, 1977). The specific ionic conductances that occur when these channels open contribute towards the genesis and outcome of local and propagated potentials. Some of the ionic conductances that have been described in the hippocampus include the following. Sodium spikes (i.e. classic action potentials) are generated via the Hodgkin-Huxley type inactivating Na^+ conductances (Llinás, 1984; Schwartzkroin and Slawsky, 1977). High threshold inactivating Ca^{++} conductances located in the soma and dendrites, generate Ca^{++}-dependent action potentials termed Ca^{++} spikes (Schwartzkroin and Slawsky, 1977; Wong and Prince, 1978). A second type of Ca^{++} conductance does not inactivate, and this conductance is able to induce Ca^{++} spikes. This presumably somatic Ca^{++} conductance has a low threshold of activation and is involved in the induction of an outward K^+ current (Hotson and Prince, 1980; Schwartzkroin and Slawsky, 1977). Presently, K^+ conductances constitute the largest number of ionic conductances that have been described (Colino and Halliwell, 1987; 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, is a low threshold non-inactivating K^+ conductance which is elicited by depolarizations and modulated by transmitters (Adams, Brown and

Halliwell, 1981; Moore, et al., 1988). Furthermore, hippocampal neurons exhibit inactivating delayed rectifying K^+ conductances which are activated by Ca^{++}. During neuronal activations, Ca^{++} influxes into CA_1 neurons subsequently induce outward K^+ conductances. These Ca^{++}-dependent K^+ effluxes cause intracellular membrane shifts termed afterhyperpolarizations, which diminish cell discharges (Hotson and Prince, 1980; Schwartzkroin and Stafstrom, 1980). In addition, fast transient K^+ conductances have been noted in the hippocampus (Gustafsson, et al., 1982) which presumably serve to prevent the rapid return of membrane potentials to baselines, following hyperpolarizations. This K^+ conductance probably contributes towards the prevention of rebound excitations, as the cell membrane potential returns to baseline following membrane potential perturbations.

5.3 Bursting activity

Hippocampal neurons can generate spontaneous bursts of 2-10 action potentials of decreasing amplitudes and increasing durations, i.e., complex spikes (Masukawa, Bernado and Prince, 1982; Schwartzkroin, 1975; Wong, Prince and Basbaum, 1979). Typically, dentate gyrus granule cells do not fire bursts but CA_3 cells readily support bursting activities (Wong, Prince and Basbaum, 1979). In contrast, CA_1 pyramidal cells can give bursts of spikes but do not do so ordinarily (Alger, 1984b; Masukawa, Bernado and Prince, 1982). The differences in bursting behaviour in different cornu ammonis subfields or dentate gyrus, may be due to differences in distributions and activities of recurrent/feed-forward inhibitory interneurons (Alger, 1984a).

Intracellular records have shown that complex-spikes are comprised of long duration action potentials and depolarizing after-potentials

(Schwartzkroin, 1975). Wong (1982) postulated that sodium spikes initiated by membrane potential fluctuations in the hippocampal pyramidal neurons activated Ca^{++} conductances. Upon membrane repolarizations, the Ca^{++} conductances decayed slowly, resulting in secondary depolarizations and Ca^{++} spikes (Kandel and Spencer, 1961; Schwartzkroin and Slawsky, 1977; Wong and Prince, 1978).

5.4 Miniature postsynaptic potentials

Hippocampal neurons are relatively electrically compact. This, and the long membrane time constants, can make possible the intrasomatic detections of small and discrete, potentials that are generated at dendritic subsynaptic membranes. These spontaneous small potentials are thought to be due to the quantal release of transmitters (Alger and Nicoll, 1982a; Brown, Wong and Prince, 1979; Johnston and Brown, 1984; Voronin, 1983; Yamamoto, 1982) and to be similar to the well characterized miniature end-plate potentials at the neuromuscular junction (del Castillo and Katz, 1952; Fatt and Katz, 1952; Katz, 1962). On the basis of their pharmacological profiles, the small potentials in the hippocampus have been termed miniature excitatory and inhibitory synaptic responses (i.e. minEPSP and minIPSP). The miniature IPSPs are effectively blocked by picrotoxin or bicuculline (Johnston and Brown, 1984), and these pharmacological agents selectively abolish Cl^--dependent inhibitory responses mediated by γ-aminobutyric acid (Johnston, 1978).

5.5 Evoked field responses

Stimulation of the CA_1 pyramidal cells axons in the alveus evokes antidromic responses in the CA_1 subfield. These responses are seen as population spikes with short onset latencies (Figure 5-1). These antidromic responses are capable of following high tetanic stimulation frequencies

(Chirwa, 1985). The evoked population spikes are not abolished by high magnesium and/or manganese containing media (i.e., physiological medium with little or no Ca^{++}). Stimulation of the afferents in the stratum oriens or the stratum radiatum (e.g. commissural or Schaffer collaterals, respectively) cause a presynaptic potential in a strip-like region at the stimulated level (Andersen, 1983; Andersen, et al., 1978). This extracellularly recorded negative deflection (arrow in Figure 5-1) is termed the presynaptic volley and its amplitude is usually taken as an index of the number of fibers activated. The presynaptic volley is followed by postsynaptic responses as shown in Figure 5-1. The negative-going synaptic field responses generated by stimulation of commissural or Schaffer collaterals, have their maximum in the region where the activated fibers terminate and show reversal when recorded from distant positions along the dendritic axis (Andersen, et al., 1980c). Depending on the magnitude of the orthodromic excitatory potentials, action potentials are discharged in several pyramidal neurons. The magnitude of the summated action potentials generated is maximum when recorded in the stratum pyramidale layer and shows polarity reversal on both sides of the pyramidal layer. Andersen, et al. (1980a) caused selective activation of a small group of afferent fibers to elicit field potentials in the CA_1 neurons. These investigators demonstrated that the proximal and distal synapses in CA_1 were largely equipotent in evoking field potentials. [NB: The density of the excitatory synapses was the same (see also Andersen, Storm and Wheal, 1987)].

5.6 Inhibitory postsynaptic potentials

The collaterals of CA_1 axons feedback onto inhibitory interneurons. These inhibitory interneurons, in turn, innervate the CA_1 neurons (i.e., recurrent inhibition). Hence, when a CA_1 pyramidal neuron

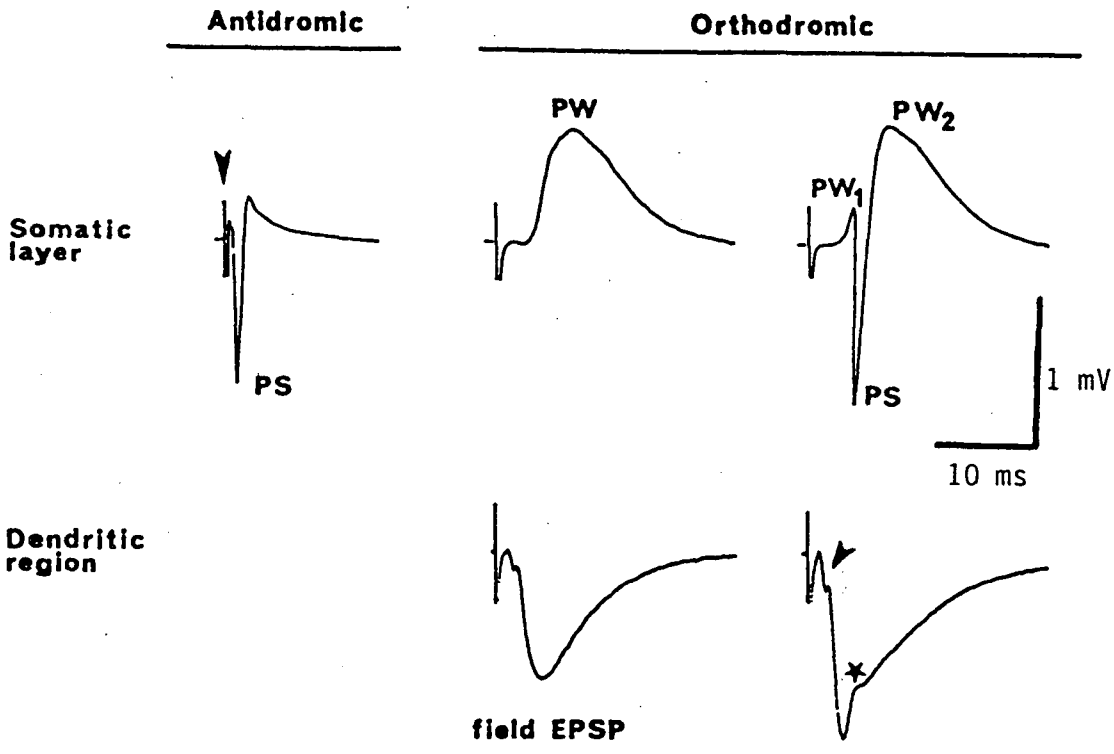

Figure 5-1. Representative evoked field responses in the hippocampus. The traces are from extracellular recordings in the somatic layer and the apical dendritic region of the CA_1 area. Stimulation of the CA_1 axons in the alveus evokes an antidromic population spike (PS) with short onset latencies (i.e. the time from start of stimulation artifact (arrow) to peak amplitude of population spike). Orthodromic stimulation of the stratum radiatum evokes the negative-going field EPSP in the dendritic region as well as the positive wave (PW) in the somatic region. The positive wave reflects mostly excitatory and inhibitory activities in CA_1 neurons. Increasing the orthodromic stimulation strength evokes a population spike in the somatic region (i.e. reflects the summation of synchronously discharging CA_1 neurons, see Andersen, Bliss and Skrede, 1971) that intoduces two peaks in the somatic positive wave (denoted as PW_1 and PW_2 in the illustration). In general, PW_1 mostly corresponds with excitatory influences whereas PW_2 mostly corresponds with inhibitory influences in CA_1 cells (Andersen, Eccles and Loyning, 1964). In addition, the field EPSP recorded in the dendrites can be interrupted by a positive peak (*) and this is a reflection of the fields generated by the population spike in the somatic region. Note the presence of the presynaptic volley (PV) that is usually discernable in these dendritic recordings.

is activated, it subsequently drives an interneuron, which then induces hyperpolarizing responses in the same CA_1 pyramidal cell. During this hyperpolarizing response in the CA_1 pyramidal neuron, incoming excitatory responses are shunted (Andersen, Eccles and Loyning, 1964). Some inhibitory interneurons are innervated directly by other afferents in the alveus, stratum oriens and stratum radiatum (e.g. Frotscher, 1985). These inhibitory interneurons then feed onto pyramidal neurons where they induce conductances that shunt excitatory responses (i.e. feed-forward inhibition). The presence of feed-forward inhibitions is supported by studies that have shown that stimulations of the afferents in the stratum oriens or the stratum radiatum induce prominent IPSPs in quiescent CA_1 pyramidal neurons (Buszaki, 1984). It appears that these hyperpolarizing responses could not have been caused by recurrent inhibitions, since these latter responses are dependent on prior discharges in CA_1 neurons.

5.7 Electrotonic couplings

Nonsynaptic interactions have been demonstrated in the hippocampus. MacVicar and Dudek (1981) reported the occurrence of electrotonic couplings, where activities in one neuron are transmitted directly to other neurons presumably 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, was used to reveal gap junctions (Dudek, et al., 1983). In these studies, however, the possibility of mechanical coupling being introduced by the electrode itself partially impaling both cells has not been entirely ruled out (cf. Alger, McCarren and Fisher, 1983). This is particularly important since pyramidal neurons are tightly packed together (Lorente De Nó, 1934).

5.8 Ephaptic interactions

Ephaptic interactions are thought to be the influences on a neuron caused by current flows via extracellular resistances (Jefferys and Haas, 1982; Taylor and Dudek, 1982). It was found that when hippocampal slices in vitro were perfused for prolonged periods with a low Ca^{++} medium (i.e. to block synaptic transmission), this resulted in the development of rhythmic bursts lasting for several seconds (Alger, 1984b; Taylor and Dudek, 1982). The bursts occurred spontaneously, or they could be evoked with direct or antidromic stimulations. Taylor and Dudek (1984) analysed differential recordings of intracellular and adjacent extracellular potentials. These investigators found that during population spikes, the associated extracellular electrical fields caused currents to flow passively across inactive pyramidal cell membranes. It has been suggested that electrotonic or ephaptic interactions could be involved in synchronization of cell discharges in the hippocampus (Richardson, Turner and Miller, 1984; Yim, Krnjević and Dalkara, 1988).

6. SYNAPTIC PHARMACOLOGY OF THE HIPPOCAMPUS

6.1 GABA

Inhibitory synaptic influences, both recurrent and feed-forward, use γ-aminobutyric acid (GABA) as their principle neurotransmitter (Storm-Mathisen, 1977; Frotscher, et al., 1984). Glutamic acid decarboxylase (GAD) as well as GABA-ergic receptors are distributed in all layers of field CA_1 (Storm-Mathisen, 1977; Andersen, et al., 1980b). Evidence indicates that inhibitory interneurons (at least the basket cell type; Lorente De Nó, 1934) release GABA onto CA_1 pyramidal cell bodies, axon hillock and/or dendrites, and activate conductances that shunt excitatory influences (Andersen, Bie and Ganes, 1982; Bowery, Hudson and Price, 1987).

Interestingly, the shape of IPSPs caused by antidromic stimulation differs from those evoked during orthodromic stimulation. Orthodromic, but not antidromic activation of CA_1 pyramidal neurons elicits larger IPSPs [NB: ame size field potentials and associated IPSP measured concurrently; Alger and Nicoll, 1982b]. Furthermore, recurrent IPSPs are completely abolished by GABA-ergic antagonists. In contrast, feed-forward IPSPs exhibit two time-dependent components, i.e., early and late components (Alger and Nicoll, 1979; Hablitz and Langmoen, 1987). The early component is abolished by picotroxin and bicuculline (GABA-ergic antagonists), whereas the late component is insensitive to these agents. These differing pharmacological profiles for IPSPs have formed the basis for the conclusion that GABA actions are mediated by at least two types of receptors, termed $GABA_A$ and $GABA_B$ (Alger and Nicoll, 1979).

6.2 $GABA_A$ receptors

$GABA_A$ receptors are largely distributed on the soma, axon hillock and

proximal parts of stem dendrites of pyramidal cells. Activation of $GABA_A$ receptors induce Cl^- currents that cause hyperpolarizations. The inhibitory conductance induced by GABA appears to be due to Cl^- ions since these fluxes are diminished in media containing low Cl^- concentrations (Thalmann, Peck and Ayala, 1981). Furthermore, the reversal potential for these conductances range between minus 60-65 mV (Andersen, et al., 1980b). These values are thought to be consistent with those calculated using the Nernst equation, which take into account the presumed chemical gradients between intracellular and extracellular Cl^-. While picrotoxin appears to block the $GABA_A$ receptor coupled Cl^- channel, bicuculline is thought to prevent the interaction of GABA with the $GABA_A$ receptors (Johnston, 1978; Olsen, 1982; Peck, Schaffer and Clark, 1973).

6.3 $GABA_B$ receptors

Exogenous GABA applications to the CA_1 pyramidal layer elicits hyperpolarizing responses that are blocked by picrotoxin or bicuculline. In contrast, applications of GABA to the dendritic regions of CA_1 pyramidal cells elicits a depolarizing response, as recorded in the soma (Andersen, Bie and Ganes, 1982). This depolarizing response to GABA is presumably inhibitory since it effectively shunts excitatory synaptic responses. The depolarizing responses induced by GABA, however, are not sensitive to changes in Cl^- gradients. Moreover, the ionic conductances associated with the depolarizing actions of GABA exhibit reversal potentials that are more negative than would be expected for Cl^- conductances. Instead these ionic conductances show reversal potentials similar to those of K^+. The above findings led to the implication of a second GABA-ergic receptor subtype termed $GABA_B$. Hence GABA released in the dendritic sites interacts with $GABA_B$ receptors to initiate inhibitory conductances that are

probably mediated by K^+ fluxes (Alger, 1984a; Alger and Nicoll, 1979; 1982b; Andersen, et al., 1980b). Ca^{++} conductances have also been implicated in the responses mediated by $GABA_B$ activation (Gahwiler and Brown, 1985; Inoue, Matsuo and Ogata, 1985). Recent pharmacological experiments have shown that baclofen is a selective agonist at $GABA_B$ receptors (e.g. Bowery, Hudson and Price, 1987), and phaclofen is purported to be a specific antagonist at these receptors (Dudar and Nicoll, 1988).

6.4 Putative excitatory transmitters

The evidence for an excitatory transmitter role of glutamate and/or aspartate in the hippocampal commissural and Schaffer collateral axons is based on biochemical and autoradiographic localizations of high affinity uptake sites (Storm-Mathisen and Iversen, 1979; Fonnum, et al., 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, et al., 1978; Wieraszko and Lynch, 1979; Malthe-Sørenssen, Skrede and Fonnum, 1979). While the abundant biochemical evidence implicates glutamate (or aspartate) as putative excitatory transmitters (e.g., Koerner and Cotman, 1982; Nadler, et al., 1976; White, Nadler and Cotman, 1979), it is far from clear whether these substances constitute the actual endogenous neurotransmitters in the hippocampus. 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 with glutamate or aspartate (Foster and Fagg, 1984). Even though Ca^{++} and voltage dependencies have been demonstrated in the release studies, it is not known for certain whether the released tritiated transmitters come from the same intracellular compartments as the endogenous

transmitters themselves (Laduron, 1984). In other systems, it is known that tritiated ligands can be trapped in different intracellular compartments of intact cells (Maloteaux, et al., 1983).

6.5 Exogenous glutamate actions in hippocampus

Glutamate application by iontophoresis near single hippocampal neurons, causes a fast onset excitation followed by a rapid termination of action. This excitant action is thought to be mediated by a direct glutamate-induced depolarization of the hippocampal neurons (Mayer and Westbrook, 1987). The electrophysiological studies indicate that glutamate induces an inward movement of cations, mostly Na^{+} and/or perhaps Ca^{++} (Mayer and Westbrook, 1987). There are "hot" spots along the dendritic trees of CA_1 pyramidal cells, at which the depolarizing actions of glutamate are most prominent (Dudar, 1974; Schwartzkroin and Andersen, 1975). These hot spots presumably reflect receptor sites for glutamate. Hablitz and Langmoen (1982) reported that the reversal potentials for the glutamate-mediated depolarization in the hippocampus were comparable to those of the EPSPs. Both shifted in a negative direction in low Na^{+} medium.

6.6 NMDA and Quisqualate/Kainate receptors

Though many antagonists of excitatory acidic amino acids have been discovered, their blocking actions have been against exogenous acidic amino acids. Many of the acidic amino acids, notably aspartate, quisqualate and kainic acid, exhibit actions similar to glutamate (Mayer and Wesbrook, 1987; Puil, 1981). Their differences in potencies, 'antagonistic' profiles and/or ionic conductances activated have led to the implication of different types of acidic amino acid receptors. At least two types of acidic amino acid receptors have been implicated; the NMDA receptor and the non-NMDA receptor(s) (Dingledine, 1984; Foster and Fagg, 1984; Mayer and Westbrook,

1987; McDonald and Wojtowicz, 1982; Watkins, 1984).

N-Methyl-D-aspartate (NMDA) is selective for NMDA receptors which, when activated, presumably increase a voltage-dependent cationic conductance (Cotman and Iversen, 1987; Mayer and Westbrook, 1987; Watkins and Olverman, 1987). NMDA receptor activation is highly voltage-dependent, due to a Mg^{++} blockade near the resting membrane potential (Cotman and Iversen, 1987; Mayer and Wesbrook, 1987; Watkins and Olverman, 1987). Experimental evidence indicates that adequate depolarizations, however, remove the block by Mg^{++} ions, leading presumably to regenerative Ca^{+} currents (Cotman and Iversen, 1987; Watkins and Olverman, 1987). Both quisqualate and kainate exhibit preferences at non-NMDA receptors, whose activations elicit Na^{+} and possibly K^{+} conductances. Glutamate is active at both NMDA and non-NMDA receptors.

A variety of substances have been shown to antagonise responses mediated by applied NMDA, quisqualate or kainate. Substances such as γ-D-glutamylaminomethylsulphate or 1-(p-chlorobenzoyl)-piperazine-2,3-dicarboxylate are non-specific antagonists of both quisqualate and kainate responses. However, these substances also diminish NMDA responses, but with lower potencies. Selective antagonists have only been discovered for responses mediated by NMDA receptors. Competitive NMDA receptor antagonists include D-2-amino-5-phosphonovalerate (AP5; APV), D-2-amino-phosphonoheptanoate (AP7) and 3-3(2-carboxypiperazine-4-yl)propyl-1-phosphonate (Cotman and Iversen, 1987; Watkins and Olverman, 1987). Non-competitive NMDA receptors antagonists include n-allylnormetazocine (SKF10047) and MK-801 (Cotman and Iversen, 1987; Mayer and Wesbrook, 1987; Watkins and Olverman, 1987).

6.7 Subsynaptic receptors

In terms of excitatory synaptic transmission in the hippocampus, the

antagonist profiles are somewhat incomplete. Both NMDA and non-NMDA receptor subtypes are thought to be distributed in the same subsynaptic regions of the hippocampus. In addition, there is evidence indicating that NMDA (and possibly non-NMDA) receptors are distributed in presynaptic regions (Dingledine, 1983a). The fast EPSPs are thought to be mediated by the non-NMDA receptors. At the present time, specific (and indeed selective) antagonists of the fast synaptic transmission have not been found. Recently, it has been proposed that the slow depolarizing wave that develops during tetanic stimulations of afferents (particularly in the presence of GABA-ergic inhibitors; see Wigström and Gufstaffson, 1985b) was due to NMDA receptor activation. APV in low doses has been found to abolish these responses. However, in higher doses APV will also diminish the fast synaptic responses. More studies are needed to clarify further the synaptic pharmacology of excitatory transmission in the hippocampus.

6.8 Other putative transmitters

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 origin are not fully known. Some of these inputs that seem to innervate parts of CA_1 include; the medial septum and diagonal band cholinergic input (Storm-Mathisen, 1977: Lynch, Rose and Gall, 1978), the noradrenergic outflow from the locus ceruleus (Lindvall and Björkland, 1974) and the serotonergic projection from the medial and dorsal raphe nuclei (Azmitia and Segal, 1979). The probable modulatory role for some of these extrinsic pathways is illustrated by the actions of acetylcholine. Acetylcholine is known to cause a reduction in the M-current which is active over the -70 mV to -40 mV membrane potential range (Dodd, Dingledine and Kelly, 1981; Bernado and Prince, 1982). This antagonism of the M-current

causes slow depolarizations in the cells and raises input resistances. These actions probably improve electrical compactness of target cells. Such an action would facilitate the invasion of small synaptic signals to the soma (Dingledine, 1984). In addition, acetylcholine has been shown to inhibit the release of inhibitory and excitatory neurotransmitters in the CA_1 hippocampal field (Yamamoto and Kawai, 1967; Ben-Ari, et al., 1981). It is not known, however, whether the cholinergic septal inputs also form axo-axonic contacts with inhibitory or excitatory afferents.

6.9 Neuromodulators

Recent findings have revealed a diverse distribution of neuroactive substances in the hippocampus (Dingledine, 1984). It remains to be established whether these neuroactive substances form separate pathways and/or co-exist with other neurotransmitters. It can be speculated that neuroactive substances might even reside and/or be released from dendritic spine structures and thereby influence synaptic interactions. But the experimental evidence for these possibilities is presently lacking.

The main neuroactive substances that have been characterized so far in the CA_1 field include enkephalin-like substances (Gall, et al., 1981), cholecystokinin and somatostatin (Greenwood, et al., 1981), vasoactive intestinal polypeptides (Loren, et al., 1979), substance-P (Vincent, Kimura and McGeer, 1981) and angiotensin-II (Haas, et al., 1980).

7. LONG-TERM POTENTIATION IN THE HIPPOCAMPUS

7.1 Introduction

Long-term synaptic potentiation is generally viewed as a potential model for cellular mechanisms involved in learning and memory. The use-dependent increase in synaptic efficacy invariably alters the intricate balances in synaptic weighting within and among neural networks. Clearly, signals that traverse potentiated synapses exert biased influences on their target cell(s). The attractiveness of long-term potentiation (LTP) as a model for learning and memory is discernable in the following observations. LTP is readily inducible in the hippocampus, a structure that has long been considered to subserve learning and memory functions (Swanson, Teyler and Thompson, 1983; Teyler and DiScenna, 1984). The basic requirements for LTP induction are within physiological ranges (Byrne, 1987; Larson and Lynch, 1986; Rose and Dunwiddie, 1986). Even if memory functions are ultimately encoded as biochemical changes within selected neuronal groups, preferential mechanisms for the transfer or retrieval of stored information are probably present. Enduring changes in synaptic efficacy in selected neural circuits could be one such method. Moreover, that synapses remain potentiated after priming is itself an example of learning (cf. Eccles, 1977). Whatever is the role of LTP in physiology, the understanding of this phenomena is bound to yield significant insights into the diversity of nervous system plasticity. It is for such reasons that the phenomenon of long-term potentiation continues to attract great interests among neurobiologists.

7.2 Basic features of long-term potentiation

7.2.1 Long-term potentiation. In the early seventies, Bliss and his co-workers gave the first detailed account of long-term potentiation (LTP)

in the hippocampus in vivo (Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973). These investigators discovered that conditioning stimuli of 10-20 Hz (for one or more seconds) given to a selected perforant path bundle, induced post-tetanic synaptic potentiations of the population spikes in the stratum granulosum and field EPSPs in the molecular layer of the dentate gyrus evoked by the same inputs (pre- and post-tetanic test pulses were evoked at 0.5 Hz, same stimulation parameters; Bliss and Lømo, 1973). LTP lasted for hours to several days, and it was manifested as decreases in population spike latency, and increases in amplitudes of population spikes and/or field EPSPs. Often, depressions of evoked responses lasting from seconds to several minutes followed a low frequency tetanus (10-20 Hz) before LTP was observed. In contrast, the higher frequency conditioning trains (i.e., 100 Hz) induced LTP without prior post-tetanic depressions of evoked responses. But LTP elicited by high and low frequency tetanic trains exhibited the same characteristics (Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973).

LTP expression was confined to the tetanized perforant paths bundle only. However, repeated tetanic stimulations of the same input caused an augmentation of the established LTP until an asymptote was reached. Bliss and co-workers found that LTP was not due to changes in stimulating electrode properties after tetanus. Furthermore, LTP was not a result of a simple upward shift along the stimulus-response curves (Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973). The post-tetanus field EPSPs elicited bigger population spike relative to matched pre-tetanus field EPSPs, over a wide range of stimulation intensities. These studies also revealed that the potentiation of the population spike was not always accompanied by the potentiation of the field EPSP. Hence there were two basic expressions of LTP; that which presented with the potentiation of the population spike

alone, and that which presented with simultaneous increases to population spikes and field EPSPs. These two forms of LTP expression were subsequently classified as "E-S potentiation" and "synaptic potentiation", respectively (Andersen, et al., 1980c).

7.2.2 Distribution of LTP. Recent studies have demonstrated that many other afferent systems can be potentiated in the mammalian brain (see Byrne, 1987, for review). Within the hippocampus, LTP has been produced at the following synapses: Schaffer collaterals-CA_1 (Schwartzkroin and Wester, 1975), and mossy fibers-CA_3 (Alger and Teyler, 1976). In addition, LTP occurs in the synapses of CA_3 commissural projections and the contralateral CA_1 and CA_3 neurons (Bliss, Lancaster and Wheal, 1983; Buzsaki, 1980). LTP has also been induced in the excitatory projections to feed-forward inhibitory interneurons (Buzsaki and Eidelberg, 1982; Kairiss, et al., 1987). Outside the hippocampus, LTP has been found in the following structures in vertebrates; limbic system (Racine, Milgram and Hafner, 1983), cerebral cortex (Lee, 1982), pyriform cortex (Stripling and Patneau, 1985), and the medial geniculate nucleus (Gerren and Weinberger, 1983). Long lasting synaptic potentiations have been studied in the invertebrate nervous systems as well (see Byrne, 1987, for review), but these will not be considered here.

7.2.3 Homosynaptic and heterosynaptic LTP. In the CA_1 subfield, like the dentate gyrus, only input-specific LTP has been demonstrated. Hence, when the Schaffer collaterals are tetanized, only the Schaffer collateral-CA_1 synapses become potentiated (Andersen, et al., 1977; Andersen, et al., 1980c). McNaughton (1983) described this input-specific LTP as "homosynaptic LTP". In the CA_3 subfield, input specificity of LTP is not always preserved (Yamamoto and Chujo, 1978) since tetanic stimulations of

inputs can result in LTP across the synapses of both the tetanized and non-tetanized inputs (i.e. "heterosynaptic LTP"; Misgeld, Sarvey and Klee, 1979; Yamamoto and Chujo, 1978). Significant differences between homosynaptic LTP and heterosynaptic LTP have been reported. Heterosynaptic LTP development is apparently restricted to the polysynaptic components of the evoked response, and the expression of homosynaptic LTP is limited to the responses evoked by monosynaptic inputs in the CA_3 subfield (Higashima and Yamamoto, 1985). [NB: Orthodromic stimulations of inputs to the CA_3 often elicit dual population spikes (or field EPSPs) with different onset latencies. The early responses are due to the activation of monosynaptic inputs, whereas the late responses are caused by polysynaptic activations (Higashima and Yamamoto, 1985)].

7.2.4 LTP in single neurons. In single neurons, LTP is expressed as increases in probabilities for neuronal discharges and/or augmented amplitudes of subthreshold intracellular EPSPs (Schwartzkroin and Wester, 1975). However, LTP is not accompanied by changes in input resistances or resting membrane potentials measured in the soma (Andersen, et al., 1980c). Using single-electrode voltage clamp methods, Barrionuevo, et al., (1986) found that the currents associated with the excitatory postsynaptic potentials are greatly increased after LTP development in the mossy fibers-CA_3 synapses. In addition, the ability of intracellular EPSPs to propagate to the soma did not change during LTP. Presumably, the changes that mediate increases in intracellular EPSPs in CA_3 neurons during LTP are localised to subsynaptic and/or presynaptic elements (Barrionuevo, et al., 1986).

7.3 Production of LTP

7.3.1 Induction. LTP can not be induced by antidromic tetanizations alone (Chirwa, 1985; Schwartzkroin and Wester, 1975). The classic LTP is

seen only with orthodromic tetanizations. Though a whole range of tetanic frequencies are capable of inducing LTP, higher tetanic frequencies most reliably induce synaptic potentiations (Chirwa, 1985; Dunwiddie and Lynch, 1978). Tetanic stimulations delivered during perfusions with physiological medium without Ca^{++} or medium with raised concentrations of Ca^{++} entry blockers, (i.e. Mg^{++} and/or Mn^{++}) do not elicit LTP (Dunwiddie and Lynch, 1979; Wigström, Swann and Andersen, 1979). Prior induction of LTP, however, is not reversed by subsequent transient exposures to Ca^{++}-free medium (Dunwiddie and Lynch, 1979). Hence, only the induction of LTP with tetanic stimulations, but not its maintenance, is dependent on extracellular Ca^{++} levels.

Conflicting reports have been presented on whether induction of LTP is dependent on postsynaptic discharges. Scharfmann and Sarvey (1985) reported that LTP induction was blocked when bath applications of GABA were used to inhibit postsynaptic spiking during tetanic stimulations of inputs. These results were interpreted as reflecting the need for postsynaptic spiking during LTP production. In contrast, Kelso, Ganong and Brown (1986) used intracellular injections of QX-222, a quartenary anaesthetic agent, to block intracellular action potentials in CA_1 neurons. Yet tetanic stimulations in the stratum radiatum induced LTP across the Schaffer collaterals-CA_1 synapses. Interestingly, postsynaptic discharges are much more pronounced during lower frequency tetanic stimulation compared with higher frequency tetanic stimulation (Chirwa, 1985; Chirwa, et al., 1983; Dunwiddie and Lynch, 1978). But the higher tetanic stimulation frequencies (i.e. associated with minimal postsynaptic spiking) most reliably induce LTP. Taken together, the available experimental evidence favors the notion that postsynaptic spiking per se is not a necessary pre-requisite for LTP develop-

ment. Rather, postsynaptic membrane depolarization seem to be essential for LTP induction in the hippocampus (Malinow and Miller, 1986).

7.3.2 Co-operative LTP. In the studies of Bliss and Gardner-Medwin (1973), it was evident that LTP induction was dependent on the stimulus strengths used during tetanus. McNaughton, Douglas and Goddard, (1978) further examined the relationships of stimulus strength with LTP induction in the dentate gyrus and found that low stimulus strengths mostly elicited brief potentiations that lasted for 3-5 minutes (i.e., LTP failed to develop). LTP production, however, was consistently evoked with high stimulus strength tetanus, suggesting the presence of stimulus intensity "threshold" for LTP induction. It was inferred that LTP production required co-activation of a minimum number of afferents during tetanic stimulations. This effect was termed "co-operative" interactions or co-operativity (McNaughton, Douglas and Goddard, 1978). Co-operative interactions have also been demonstrated in the CA_3 (Yamamoto and Sawada, 1981) and the CA_1 subfields (Lee, 1983).

7.3.3 Associative LTP. McNaughton, Douglas and Goddard, (1978) used two separate weak inputs to the same target neurons, and none of these inputs supported LTP when tetanized independently. However, simultaneous tetanizations of both inputs produced LTP. In comparable experiments, Levy and Steward (1979) found that conjoint tetanic stimulations of a strong input with a weak input, produced LTP in the weak input. [NB: The strong input could support LTP, when tetanized independently.]. These co-operative interactions between two separate inputs have now been classified as "associative interactions" or "associative LTP" (Johnston and Brown, 1984). It was not determined how associative interactions occurred between separate inputs to the same dendritic tree or those that impinged on the apical and

basal dendrites. McNaughton, Douglas and Goddard (1978) postulated that postsynaptic neurons were the conduit for these interactions. However, Goh and Sastry (1985) could induce transient increases in the threshold for antidromic activation of Schaffer collaterals, following tetanic stimulations of nearby but separate inputs to the same target cells in the CA_1 subfield. These studies illustrated that interactions among presynaptic terminals were possible and could account for associative LTP.

7.3.4 Coupled LTP. Sastry, Goh and Auyeung (1986) found that repeated pairings of test inputs that were activated at low frequencies (0.2 Hz), with the simultaneous applications of adequate conditioning intracellular depolarizations of CA_1 neurons, resulted in LTP that was localised to the test inputs. LTP induction was dependent on (1) the intensity of the depolarizing current injections, (2) concomitant activations of test inputs, and (3) the total number of pairings used. Fewer pairing episodes elicited transient short-term potentiations, lasting for 3-5 minutes. Increasing the number of pairings (i.e. more than 10) produced LTP (Sastry, Goh and Auyeung, 1986). It was found that LTP production, using the pre- and postsynaptic pairings, was facilitated if picrotoxin ($GABA_A$ receptor antagonist) was present in the physiological medium. That LTP could be induced by pairing postsynaptic depolarizations with presynaptic activation was also independently reported by other investigators (Kelso, Ganong and Brown, 1986; Wigström, et al., 1986).

7.4 LTP production with pharmacological methods

7.4.1 Raised extracellular K^+. May, Goh and Sastry (1987) could induce LTP in the CA_1 subfield with the application of raised extracellular K^+ (10-80 mM), in the absence of extracellular Ca^{++}. This report presented an important finding showing that the induction of LTP was not

absolutely dependent on extracellular Ca^{++}. Rather, adequate depolarizations of pre- and postsynaptic regions were necessary. In view of this, the requirement for extracellular Ca^{++} during the induction of LTP with tetanic stimulations previously reported by other investigators (cf. Dunwiddie and Lynch, 1979; Wigström, Swann and Andersen, 1979) probably reflected the need for Ca^{++} for evoked transmitter release. Hence the evoked release of transmitters during tetanic stimulations subsequently depolarized the postsynaptic regions, that were necesssary for LTP production. Interestingly, Wigström and Swann (1980) could elicit LTP with tetanic stimulations in medium containing Sr^{++} (a Ca^{++} agonist that supports transmitter release) instead of extracellular Ca^{++}. It seemed that Sr^{++} substituted for Ca^{++} in mediating depolarization-coupled transmitter release (cf. Zengel and Magleby, 1980). It is presently not known whether Sr^{++} can also substitute for Ca^{++} in other biological processes. Even though extracellular Ca^{++} is not always necessary to induce synaptic potentiations (May, Goh and Sastry, 1987), this does not rule out the possible involvement of intracellular Ca^{++} in LTP. Lynch, et al., (1983) reported that the induction of LTP in single neurons could be blocked with prior intracellular ejections of EGTA, (a Ca^{++} chelating substance). This intriguing study awaits replication by other investigators.

7.4.2 Raised extracellular Ca^{++}. Turner, Baimbridge and Miller (1982) caused long term increases in synaptic efficacy in the CA_1 subfield following transient exposures to elevated extracellular Ca^{++} (4 mM Ca^{++} perfused for 10 min; control medium: 2 mM Ca^{++}). Both the PS and the field EPSPs evoked with stimulations of the Schaffer collaterals were potentiated for periods beyond 3 hrs. There were no changes to the amplitudes of the antidromic PS in the CA_1 subfield evoked by stimulation of CA_1 axons

in the alveus (Turner, Baimbridge and Miller, 1982). The Ca^{++}-induced LTP was associated with the accumulation of presumably intracellular Ca^{++}. However, it could not be determined whether the increased Ca^{++} was limited to presynaptic and/or postsynaptic regions. The induction of LTP with brief exposures to elevated extracellular Ca^{++} has also been reported by other investigators (e.g. Bliss, Dolphin and Feasey, 1984; Higashima and Yamamoto, 1985; Sastry, et al., 1983; Reymann, et al., 1986). However, the significance of these effects of Ca^{++} is at present unclear.

7.4.3 Phorbol esters. Malenka, Madison and Nicoll (1986) induced synaptic potentiations in the CA_1 subfield with transient applications of phorbol analogs known to activate protein kinase C (PKC). Both the population spike and field EPSPs were potentiated for periods beyond 2 hrs post-application. The probabilities for single cell discharges or the amplitudes of subthreshold intracellular EPSPs were increased (Malenka, Madison and Nicoll, 1986). The characteristics of the potentiated responses induced with phorbol esters were similar to those seen with tetanus-induced LTP (cf. Bliss and Lømo, 1973). In a separate report, Nicoll and co-workers showed that the synaptic potentiations induced with phorbol esters were associated with augmented transmitter release, as evidenced by the increased frequencies of minEPSPs and minIPSPs (Malenka, Ayoub and Nicoll, 1987). In addition, there was an increase in K^+ stimulated release of endogenous glutamate (Malenka, Ayoub and Nicoll, 1987). Taken together, the above studies were consistent with the involvement of presynaptic mechanisms in LTP induced by phorbol esters. However, postsynaptic mechanisms also contributed towards the expression of LTP since the phorbol esters blocked specific chloride conductances that were active at resting membrane potentials (Madison, Malenka and Nicoll, 1986). Interestingly, a report appeared in

the literature that showed that intracellular injections of the active enzyme protein kinase C (PKC) into CA_1 neurons (i.e. PKC mixed with electrolytes of recording electrodes) resulted in synaptic potentiations that were similar to LTP (Hu, et al., 1987) However, PKC was continuously present (i.e., in the recording micropipette) throughout the duration of the experiments (Hu, et al., 1987).

7.4.4 Mast cell degranulating peptides. A report has also appeared in the literature showing that brief exposures to mast cell degranulating peptides (MCD) isolated from bee venom caused LTP development in the CA_1 subfield (Cherubini, et al., 1987). LTP was expressed as post-application increases (lasting beyond 6 hr) in evoked EPSPs, following stimulation of the Schaffer collaterals, without concomitant alterations in membrane resistance, cellular excitability or the magnitude of afferent volleys. MCD applications were associated with reversible depolarizations that could be blocked if TTX or Co^{++} (a Ca^{++} entry blocker) were present in the perfusion medium, indicating that these depolarizations were synaptic in origin. The potentiating effects of MCD required synaptic activations since TTX or Co^{++} blocked the induction of LTP (Cherubini, et al., 1987). However, neither TTX nor Co^{++} could reverse developed LTP induced by MCD. Interestingly, prior treatment of the MCD with trypsin destroyed its potentiating effects. These authors suggested that MCD may be mimicking an endogenous peptide (Cherubini et al., 1987).

7.4.5 Glutamate. Recently, it was reported that input-specific synaptic potentiation could be induced with simultaneous pairings of test stimuli to Schaffer collaterals and brief iontophoretic applications of glutamate to sensitive spots along the CA_1 apical dendrites (Hvalby, et al., 1987). LTP was expressed as long lasting (beyond 1 hr) increases in

cell discharge probabilities and shorter spike onset latencies following stimulation of the Schaffer collaterals.

7.4.6 Miscellaneous. GABA-ergic blockade with picrotoxin facilitates the induction of LTP (Douglas, 1978; Wigström and Gustafsson, 1983). It is generally presumed that picrotoxin diminishes GABA mediated shunting of excitatory responses. Reports have also appeared showing that septal input stimulation (which is partly cholinergic) enhances the E-S type of LTP (Robinson, 1986). It is known that exogenous acetylcholine exerts disinhibitory influences on hippocampal neurons through the suppressions of (1) M-currents, and/or (2) release of GABA (i.e. disinhibition; Krnjević and Ropert, 1982). Some of the above effects of acetylcholine are probably mimicked by septal stimulations. Depletion of cortical noradrenaline or serotonin diminishes the magnitude of LTP that can be obtained (Bliss, Goddard and Riives, 1983; Hopkins and Johnston, 1984). Substances such as noradrenaline are known to reduce neuronal accommodations in the hippocampus (Madison and Nicoll, 1986), and these effects probably prolong membrane depolarizations. Interestingly, substances that block certain K^+ conductances (e.g 4-aminopyridine) facilitate the development of LTP induced by tetanic stimulations (Chirwa, 1985; Lee, Anwyl and Rowan, 1986). Clearly, pharmacological manipulations that enhance neuronal depolarizations generally facilitate LTP production, and vice-versa.

7.5 Blockade of LTP induction

Several reports have shown that tetanic stimulations of the Schaffer collaterals in the presence of APV (in doses that did not antagonise non-NMDA receptors) blocked the induction of LTP across the Schaffer collaterals and CA_1 synapses (Collingridge, Kehl and McLennan, 1983; Harris, Ganong and Cotman, 1984; Wigström and Gustafsson, 1984). Similarly, APV

has been found to block tetanus-induced LTP in the dentate gyrus (Morris, et al., 1986). These results have led to the implication of NMDA receptors in the induction of LTP (Collingridge, 1985; Wigström and Gustafsson, 1985a). It has been suggested that the intense synaptic activation during tetanic stimulations of afferents caused postsynaptic depolarizations that were sufficient to remove the voltage-dependent blockade by Mg^{++} of the NMDA receptor-gated ionic channels. This led to the influx of Ca^{++} through these channels, and the intracellular accumulations of Ca^{++} subsequently mediated the changes underlying LTP production (Collingridge, 1985; Wigström and Gustafsson, 1985a). That NMDA could be a common denominator in the induction of LTP was reflected in recent findings showing that APV also blocked Ca^{++}-induced LTP (Errington, Lynch and Bliss, 1987). However, the anticipated universality of the NMDA hypothesis in LTP production was found to be limited. Harris and Cotman (1986) found that APV did not block tetanus-induced LTP at the mossy fiber-CA_3 synapses.

7.6 Maintenance of LTP

7.6.1 Biochemical and structural changes. Though LTP has been shown to last for several weeks in vivo (Bliss and Gardner-Medwin, 1973), experimental evidence indicates that LTP gradually decays over time (Barnes, 1979; Swanson, Teyler and Thompson, 1983). However, the exact duration of LTP is not clear. A variety of changes have been described that probably contribute towards the maintenance of LTP, and some of the best studied mechanisms are reviewed here. Distinct ultrastructural changes in dendritic spines occur in preparations exhibiting LTP (Fifkova and Van Harreveld, 1977; Van Harreveld and Fifkova, 1975). The observed changes included (1) enlargement of spine head (Desmond and Levy, 1981; Fifkova and Van Harreveld, 1977), (2) widening and shortening of spine stalks (Fifkova and

Anderson, 1981, (3) increased length of synaptic appositions (Fifkova, 1985), (4) increased number of synapses (Greenough, 1984; Greenough, Hwang and Gorman, 1985; Routtenberg, 1985), and (5) increased number of sessile spines and direct contact synapses (Chang and Greenough, 1984).

In view of the above structural changes, it has been speculated that contractile proteins could be involved in LTP development (Fifkova, 1985; Markham and Fifkova, 1986). In contrast, Lynch and co-workers have argued for different types of dendritic morphological correlates for LTP. These investigators reported an increase in the number of specific glutamate binding sites, presumed to be excitatory synaptic receptors, following LTP induction (Baudry and Lynch, 1979; Baudry, et al., 1980). In separate experiments, Lynch and co-workers could not detect significant changes in spine area, spine number, spine neck diameter or length of the postsynaptic density during LTP (Lee, et al., 1980; see also Chang and Greenough, 1984). Baudry and Lynch (1980) subsequently proposed the involvement of increased subsynaptic receptors during LTP. It was hypothesized that 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 (calpain I) was activated which in turn caused proteolysis of some membrane associated component(s) (fodrin) that subsequently mediated the uncovering of subsynaptic glutamate receptors previously not accessible in synaptic transmission (Baudry and Lynch, 1980; Eccles, 1983). The initial promise that was inherent in this hypothesis has subsided, however, in light of contradictory reports that have appeared in the literature. Sastry and Goh (1984) replicated the glutamate binding studies used by Lynch and co-workers, but found that the increase in glutamate binding correlated

better with synaptic depressions rather than LTP. Lynch, Feasey and Bliss (1985) confirmed Sastry and Goh's finding that LTP is not associated with increases in glutamate binding sites. In recent years, Lynch and co-workers have de-emphasized their postulate correlating increased subsynaptic receptors and LTP. Rather, it is now speculated that calpain degrades brain spectrin and other cytoskeletal proteins, leading to alterations in the morphology of synaptic contacts (Lynch, 1986; cf. Markham and Fifkova, 1986).

7.6.2 Protein kinase C. In recent years, a number of protein kinases have been implicated, for example, in transmitter release or membrane ionic channel functions. At least three types of protein kinases have been identified, namely (1) cAMP or cGMP dependent kinases, (2) Ca^{++}-calmodulin dependent kinases, and (3) Ca^{++}-phospholipid dependent kinases (also termed "PKC") (Nairn, Hemmings and Greengard, 1985). The activity of PKC is dependent upon intracellular levels of diacylglycerol, phosphatidylserine (or related phospholipids) and intracellular Ca^{++} (Nishizuka, 1984). In addition, the actions of diacylglycerol are mimicked by certain phorbol esters (Castagna, et al., 1982). PKC fractions exist in soluble forms in the cytosol, or they are bound to membranes. Akers, et al., (1986) have recently reported that LTP is associated with the translocation of cytosolic PKC to membranes. The translocation of PKC was not associated with any changes in the total PKC (i.e. soluble + bound form). It was concluded that the membrane bound PKC subsequently phosphorylated "F1" proteins, which are thought to be involved in the formation of new synapses (Routtenberg, 1985).

7.6.3 Increased transmitter release. Several studies have reported that LTP is associated with increases in the release of the putative trans-

mitters glutamate and aspartate. Using hippocampal slices pre-loaded with radiolabelled aspartate, Skrede and Malthe-Sørenssen (1981) observed an increase in the resting release of labelled aspartate following LTP induction. Dolphin, Errington and Bliss (1982) subsequently reported long-lasting increases in the release of labelled glutamate that was newly synthesized from infused radiolabelled glutamine, during LTP in the dentate gyrus in vivo (see also Bliss, et al., 1986). At the present time, interpretations of these release studies have been confounded by the fact that it is not known for certain if glutamate is indeed the endogenous transmitter, and whether labelled ligands actually occupied the same sites as the endogenous neurotransmitter (cf. Laduron, 1984).

Recently, different experimental approaches were utilized in examining probable increases in the release of endogenous transmitters during LTP. In a combined electrophysiological and neurochemical study, Agoston and Kuhnt (1986) observed a significant increase in K^+-induced $^{45}Ca^{++}$ uptake in relatively pure synaptosomes prepared from minislices of area CA_1 following LTP induction across the Schaffer collaterals-CA_1 synapses. Applegate, Kerr and Landfield (1987) observed that micrographs prepared from ultrathin sections of the CA_1 subfield obtained from potentiated animals (i.e. LTP was initially established in CA_1 of rat hippocampus in vivo) showed significant reductions in both local vesicle densities and distant vesicle densities. However, these investigators observed significant increases in the number of vesicles attached to the presynaptic membranes of active zones (Applegate, Kerr and Landfield, 1987). In addition, it was found that the average area and perimeter per spine significantly increased in the LTP tissue. Taken together, the above studies were consistent with the notion that LTP was, in part, due to increases in Ca^{++} dependent

transmitter release.

7.7 Summary

Much of the available evidence in the literature indicates that LTP production is dependent on the generation of sufficient depolarizations to presynaptic and postsynaptic regions. Consequently, maneuvers that enhance both presynaptic and postsynaptic depolarizations facilitate the induction of LTP (Wigström and Gustafsson, 1983 and 1985a). Conversely, manipulations that diminish and/or interfere with the occurrence of these depolarizations antagonise LTP development (Malinow and Miller, 1986). To some extent these predictions have been borne out with experimental data (Gustafsson and Wigström, 1988). However, there are many outstanding features (and questions) about LTP production that remain to be resolved. It is not known for certain whether the various types of potentiations that have been described in the hippocampus (e.g. Ca^{++} induced LTP, homosynaptic LTP, heterosynaptic LTP, co-operative LTP, etc) represent the same phenomenon. Furthermore, it has not been directly tested whether inhibitory synapses can support LTP. This may very well be likely, in view of the finding showing that transient exposures to phorbol esters increased the frequencies of spontaneous minIPSPs and minEPSPs in CA_1 neurons (Malenka, Madison and Nicoll, 1986). Presuming that LTP across inhibitory synapses is possible, then the requirements for pre- and postsynaptic depolarizations in the induction of LTP would be unattainable at these inhibitory synapses. Clearly, there are insufficient data on the exact role of depolarizations during LTP production.

Studies that have utilized agents such as APV or picrotoxin, have tended to disregard potential presynaptic interactions of these drugs. The explanations given for the facilitatory effects of picrotoxin during the

induction of LTP tend to ignore the powerful inhibitory influences exerted by activated "dendritic" $GABA_B$ receptors. Dendritic $GABA_B$ receptors are rather insensitive to picrotoxin or bicucculine. Then there is the more basic question of the identity of the endogenous excitatory transmitters in the hippocampus. The failure of many exogenous antagonists of glutamate (and its analogs) to selectively block synaptic transmissions in the hippocampus raises the real prospect that the endogenous transmitters may not be glutamate or aspartate. Moreover, the failure of APV to block the induction of LTP in the mossy fiber-CA_3 system is puzzling since glutamate is the putative transmitter in mossy fibers. It is not known if the mossy fiber-CA_3 synapses are endowed with a class of receptors that function like NMDA receptors, though insensitive to APV.

The physiological significance of the observed synaptic morphological changes during LTP will only be established once the functional role of dendritic spines is elucidated. It has been proposed that spines might serve to attenuate synaptic signals (Chang, 1952) or permit the 'weighting' of signals 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. Yet simulation studies that have used structural dimensions obtained from hippocampal histological studies indicated that subsynaptic signal transients were only attenuated by less than 2 across the spine neck (Kawato and Tsukahara, 1984; Turner, 1984). Presuming that these simulations accurately reflected synaptic transmission in the hippocampus, then the apparent advantages inherent in increased spine sizes during LTP, for example, become somewhat less important. It is evident from the above discussions that more studies will be needed to clarify the mechanisms underlying LTP.

8. BARIUM AND SACCHARIN AS EXPERIMENTAL TOOLS

8.1 General

Ba^{++} and saccharin have certain characteristics that make these substances useful experimental tools. Ba^{++} supports depolarization-coupled asynchronous release of transmitters (Quastel and Saint, 1988; Silinsky, 1978). Silinsky (1978) found that, in the presence of Ba^{++}, stimulation of the motor axons caused a burst of miniature end-plate potentials (MEPP) at the neuromuscular junction (NMJ). These MEPPs were caused by the asynchronous release of transmitter. Changes in MEPP frequencies at the NMJ reflect presynaptic mechanisms (del Castillo and Katz, 1954). In view of the above, the effects of Ba^{++} on depolarization-coupled transmitter release provide a method for examining presynaptic functions in neural circuitries (Quastel, et al., 1988). In the case of saccharin, this agent interferes with the binding of nerve growth factor (NGF) to its "receptors", and it also decreases NGF-dependent neurite growth in embryonic dorsal root ganglia cell (DRG) cultures (Ishii, 1982). Therefore, the effects of saccharin on NGF-dependent cell differentiation can be used to screen substances that have neurite-inducing activities. The above features of Ba^{++} and saccharin were used in the experiments in this thesis. The characteristics of these substances are briefly reviewed in this chapter.

8.2 Barium

8.2.1 Chemistry. Ba^{++} is closely related to Ca^{++} in atomic number, valence and chemical properties (Rosseinsky, 1965). The hydrated radius of Ba^{++} is smaller than that of the other alkali earth metals Ca^{++}, Mg^{++} and Sr^{++} (Stokes, 1964). Ba^{++} readily permeates physiological Ca^{++} channels, and Ba^{++} currents are usually larger than cur-

rents carried by Ca^{++} (Augustine and Eckert, 1984; Nachshen and Blaustein, 1982; Potreau and Raymond, 1980). In this regard, most of the physiological effects of Ba^{++} that have been examined pertain to its actions on membrane ionic currents and transmitter release.

8.2.2 Transmitter release. Transmitter release during neurotransmission is thought to be critically dependent on Ca^{++} entry into presynaptic terminals via specific voltage sensitive channels which open in response to membrane depolarizations induced by presynaptic action potentials (Augustine, Charlton and Smith, 1987; Baker, 1972; Baker, Hodgkin and Ridgway, 1971; Dodge and Rahamimoff, 1967; Katz, 1969; Krnjević, 1974; Miledi, 1973; Quastel et al., 1988; Rubin, 1970). Once inside, Ca^{++} mediates the synchronous quantal release of transmitter. At the NMJ, the vesicles in nerve terminals are taken to be the morphological correlates of acetylcholine (ACh) quanta (Ceccarelli and Hurlbut, 1980, Whittaker, 1959). Each MEPP recorded at the postsynaptic junction reflects the all-or-none discharge of the ACh content of one synaptic vesicle (del Castillo and Katz, 1954; Fatt and Katz, 1950; Fatt and Katz, 1952). The end-plate potential (EPP), therefore, consists of the "synchronous" release of two or more ACh quanta (del Castillo and Katz, 1954). According to stochastic models of transmitter release, the mean number of quanta released by an action potential (i.e. quantal content, m) is equal to the product of the number (n) of quanta capable of responding and the average probability (p) that they respond (del Castillo and Katz, 1954; Katz, 1969; McLachlan, 1978); thus

$$m = np$$

Under conditions where "p" is very small (e.g. in the absence of extracellu-

lar Ca^{++}), the distribution of "m" in time is adequately described by Poisson's statistics. When conditions increase "p", and presumably "n" (e.g. in the presence of extracellular Ca^{++}), "m" is best described with binomial statistics. In general, the above models have adequately described quantal events at various chemical synapses wherever such recordings have been feasible (Augustine, 1987; Aurbach, 1971; Hackett, Cochran and Greenfield Jr, 1986; Johnston and Brown, 1984; Katz, 1969; Kuno, 1971). The entry of Ca^{++} into presynaptic terminals, in response to an impulse, causes increases in "p" and "n" and results in multiquantal release of transmitters, which is necessary to support the EPP or EPSP. Ba^{++} readily permeates these Ca^{++} channels in response to a single nerve impulse during depolarizations but only initiates the "asynchronous" quantal discharge of transmitter in response to a single nerve impulse (Quastel and Saint, 1988; Quastel et al., 1988; Silinsky, 1978). Therefore, Ba^{++} cannot support the multiquantal EPP or EPSP in response to a single nerve impulse. However, Silinsky (1978) found that repetitive nerve stimulation in the presence of Ba^{++} produced "...a slowly developing avalanche of MEPPs", which was associated with an underlying slow depolarization of the postsynaptic membrane (Silinsky, 1985). Interestingly, the slow depolarization produced by repetitive high frequency nerve stimulation in the presense of Ba^{++} was linearly related to frequencies of MEPPs. Silinsky (1978) concluded that increases in MEPP frequencies at the NMJ represented the electrophysiological correlate of ACh release by stimulated preparations bathed in Ba^{++} solutions (cf. Douglas, Lywood and Straub, 1961).

8.2.3 K^+ currents. The effects of Ba^{++} on K^+ conductances have been demonstrated in various preparations such as spinal neurons (Ribera and Spitzer, 1987), mammalian hippocampus (Hotson and Prince, 1981),

dorsal root ganglion cells (Yoshida and Matsuda, 1980), and pancreatic acinar cells (Iwatsuki and Petersen, 1985). In pancreatic acinar cells, apparently all the K^+ conductances are accounted for by the same Ca^{++} and voltage-activated K^+ channels (Iwatsuki and Petersen, 1985). In this preparation, Ba^{++} can substitute for Ca^{++} in inducing the Ca^{++} activated K^+ conductance (Iwatsuki and Petersen, 1985). Presumably Ba^{++} and Ca^{++} possess similar efficacies in subsequently inhibiting K^+ channels, from both sides of the membrane. However, Ba^{++} has a much greater potency for inhibition of the Ca^{++} activated K^+ channels, whereas Ca^{++} is much more potent at activating these K^+ channels (Iwatsuki and Petersen, 1985). In the hippocampus, Hotson and Prince (1981) found that bath applications of Ba^{++} augmented Ca^{++} potentials but attenuated K^+-dependent hyperpolarizations. These effects of Ba^{++} were presumed to be caused by the influx of Ba^{++} through Ca^{++} channels followed by the Ba^{++}-mediated reduction of K^+ conductances. In presynaptic terminals, Sastry (1979) demonstrated a Ba^{++}-mediated increase in the presynaptic terminal action potential refractory period, presumed to be a reflection of a widened action potential caused by the blockade of the delayed K^+ rectifier current. Hence the effects of Ba^{++} on K^+ currents seem to be present in all cell membranes that are endowed with Ca^{++} and K^+ conductances. Whether Ba^{++} interferes with all types of K^+ currents in cells is unclear.

8.3 Saccharin

8.3.1 Chemistry. Saccharin (chemical name: 2,3-dihydro-3-oxobenzisosulfonazole), discovered by Fahlberg in 1879, is a potent non-caloric, synthetic, non-sucrose sweetening agent (Arnold, Krewski and Munro, 1983 for review). Saccharin has a low solubility (1 g dissolves in 290 ml water), but its sodium salt is very soluble (1 g dissolves in 1.5 ml water).

Furthermore, saccharin is very stable, particularly at pH ranges between 3.3-8.0, and only decomposes at temperatures in excess of 230° C (Arnold, Krewski and Munro, 1983; Swinyard and Lowenthal, 1980).

8.3.2 Disposition. The use of saccharin as an artificial sweetener was considered to be safe, since it was thought to be an inert substance in humans. Saccharin is not metabolized in the body (Byard and Golberg, 1973; Lethco and Wallace, 1975; Sweatman and Renwick, 1979), and it is largely excreted via the kidneys (mostly through tubular secretions without reabsorption) (Colburn, Bekersky and Blumenthal, 1981; Renwick and Sweatman, 1979). The substance crosses the placenta in pregnancy (Ball, Renwick and Williams, 1977; Matthews, Fields and Fishbein, 1973; Pitkin, et al., 1971; West, 1979), and it is excreted into milk during lactation (Arnold, Krewski and Munro, 1983). It is unclear whether saccharin crosses the blood-brain barrier in any significant amounts (Pitkin, et al., 1971). An extensive search of the literature only produced a few studies demonstrating direct physiological effects of saccharin, with the exception of controversial reports related to its presumed involvement in certain tumors of the urinary bladder (Arnold, Krewski and Munro, 1983 for review). This, more than any other single factor, has led to the almost complete disuse of saccharin as an artificial sweetener.

8.3.3 Tumor promoter. On reviewing the literature it was interesting to note that the causal relationship between saccharin and carcinogenesis remains unresolved, due to the equivocal nature of the toxicological studies (Arnold, Krewski and Munro, 1983 for review). Some of the early studies on saccharin failed to control for the mutagenic and/or carcinogenic properties of impurities that were present in the preparations. Moreover, in most animal studies, excessively large doses of saccharin (relative to

the maximum saccharin concentrations used in food and beverages) were used to demonstrate purported saccharin-induced tumors (Arnold, Krewski and Munro, 1983 for review). In recent years, saccharin has been suggested to be a tumor promoter in carcinogenesis (Bryan, Erturk and Yoshida, 1970).

The process of carcinogenesis is thought to involve two distinct biochemical phases, namely (1) initiation, and (2) promotion (Scaga, Sivak and Boutwell, 1978). Initiation is irreversible, and it is thought to be due to a mutagenic event. In contrast, promotion is the result of epigenetic changes. Substances that induce cancer presumably act through one or both of these phases. Initiation alone does not result in cancer, rather initiation requires the action of a promoting agent to produce cancer. Batzinger, Ou and Bueding (1977) examined directly the effects of several saccharin preparations on base pair substitution and frameshift mutations in his$^-$ Salmonella typhimurium tester strains TA100 and TA98. These investigators could not detect any mutagenic activities with purified saccharin preparations (Batzinger, Ou and Bueding, 1977). Similar results have been reported by several other investigators (Bryan, Erturk and Yoshida, 1970; Trosko, et al., 1980; Mondal, Brankow and Heidelberger, 1978), using different direct assay methods (e.g. Ames salmonella assay with or without rat liver microsomal enzymes, host-mediated assay, dominant lethal test). In contrast, the tumor-promoting actions of saccharin is suggested in studies such as the following. Cohen, et al., (1979) fed Fischer rats with "subthreshold" doses of a tumor initiating substance, N-[-4-(5-nitro-2-furyl)-2-thiazoly]-formamide (FANFT), for six consecutive weeks at which time the animals were separated into several groups. The various groups were subsequently fed as follows: (1) control rat chow, (2) rat chow mixed with 2% or 5% saccharin, (3) a period of six weeks post-FANFT was allowed

before instituting rat chow mixed with 2% or 5% saccharin. In separate controls, some rats were maintained on (a) control rat chow, (b) rat chow with FANFT, or (c) rat chow with 2% or 5% saccharin. After two years, animals were sacrificed for histological examinations. It was found that only rats fed with FANFT followed by saccharin (groups 2 and 3 above) presented with urinary bladder tumors (Cohen, et al., 1979). The above results were consistent with the notion that saccharin might be a tumor promoter.

8.3.4 Neurite growth. Tumor promoters are thought to act by altering the process of cellular differentiation through as yet unknown mechanisms (Cohen et al., 1977; Diamond, O'Brien and Rovera, 1977; Ishii, 1978; Ishii et al., 1978; Rovera, O'Brien and Diamond, 1977; Yamasaki et al., 1977). Ishii (1982) tested the effects of saccharin on the nerve growth factor-dependent neurite development in embryonic chick dorsal root ganglion (DRG) cell cultures. The DRG cell cultures were incubated in growth media containing the β-subunit of the mouse submaxillary gland nerve growth factor (βNGF). In addition, possible interactions between saccharin and the binding of radiolabelled βNGF to intact cells obtained from dissociated GDR were examined (Ishii, 1982). It was found that 48.8 mM saccharin reversibly inhibited neurite outgrowth in DRG cell cultures (Ishii, 1982). In contrast, sucrose, glucose or sodium chloride (50 mM used in each case) did not antagonise βNGF-dependent neurite development in DRG cell cultures. These findings illustrated the specificity of the saccharin mediated inhibition of βNGF-dependent neurite development, and that it was not due to changes in osmolality. Ishii (1982) noted that developing neurites did not retract when subsequently exposed to saccharin, rather their rates of growth were greatly diminished by saccharin. In the binding studies, 10-100 mM

saccharin significantly diminished the amount of labelled βNGF that was bound to cells (48.8 mM saccharin reduced βNGF binding by 60-65%; Ishii, 1982). The relationship between the saccharin-mediated inhibitions of (1) βNGF binding, and (2) βNGF-dependent neurite growth, was not established.

8.3.5 Inhibition of enzymes. At about the same time that saccharin was being reported to be a potential tumor promotor, other studies began to show that this agent inhibited the activities of specific enzymes. In the mid-seventies, Lygre (1974, 1976) discovered that saccharin caused a reduction of close to 50% in the enzymatic activities of beef and rat glucose-6-phosphatases. Later, Vesely and Levey (1978) showed that saccharin significantly inhibited guanylate cyclase in different tissues, including that from the urinary bladder. In recent years, the effects of enzyme inhibition by saccharin have clearly been established in micro-organisms. For example, Streptococcus mutans is considered to be the major etiological agent responsible for dental caries (Hamada and Slade, 1980). Many reports in the literature demonstrated that saccharin inhibited the carbohydrate-dependent growth and acid production of S. mutans (Linke, 1977; Linke, 1980; Linke and Chang, 1976; Tanzer and Slee, 1983). Linke and Kohn (1984) assayed the specific activities of glycolytic enzymes in cell-free extracts of S. mutans NCTC 10449 and found that saccharin caused significant reductions (maximum reductions in the order of 37-58%; for saccharin concentrations of 0.02-20 mg/ml, respectively) in the activities of hexokinase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate mutase and pyruvate kinase. The inhibition of specific activities of glycolytic enzymes was proposed to be the mechanism by which saccharin inhibited the growth of micro-organisms. Micro-organisms such as S. mutans generate their ATP via the Embden-Meyerhof-Parnas (EMP) pathway (Brown and Wittenberger, 1971). In view of this, it

was suggested that the diminution of the specific activities of enzymes in the EMP pathway resulted in the reduction of both vegetative growth and fermentative acid production in these micro-organisms (Linke and Kohn, 1984).

A recent study has extended the number of enzymes that are inhibited by saccharin. Brown and Best (1986) reported that saccharin also diminished the activities of the following enzymes; lactate dehydrogenase, sorbitol-6-phosphate dehydrogenase, mannitol-1-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, glutamate dehydrogenase, glucose6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. More importantly, Brown and Best (1986) found that saccharin competitively antagonised the binding of reduced coenzyme, NAD, to lactate dehydrogenase (saccharin increased K_m values for NAD from 0.033 to 0.250 mM; K_i for saccharin was 6.2 mM). Interestingly, all the enzymes inhibited by saccharin were those that bound to substrates or coenzymes which contained adenine and/or pyridine (i.e. ATP, NAD(H), NADP(H)) (Brown and Best, 1986, cf. Linke and Kohn, 1984). It was noted that saccharin shared spatial and structural similarities with adenine and pyridine, the functional groups of NAD, and it was surmised that these similarities accounted for the observed competitive antagonism (Brown and Best, 1986).

It is intriguing to note that saccharin antagonises NGF-dependent activities as well as inhibits enzyme activities. Whether these two processes are related is not clear. Brown and Best (1986) suggested that the negative interactions of saccharin with the EMP pathways, for example, could result in the accumulation of intracellular carbon. This carbon could then be channelled into other metabolic pathways in the cell. How this channelling of carbon subsequently modulates NGF-dependent growth or cellular differentiation in general is not clear.

9. METHODS AND MATERIALS

9.1 Animals

9.1.1 Source. Male Duncan Hartley guinea-pigs and New Zealand White rabbits (either sex) were obtained from the Animal Care Centre of The University of British Columbia, Vancouver (Canada). The Animal Care Centre used standard animal care procedures for the maintenance of laboratory animals. Their guinea-pigs were weaned after 14 days, and were fed on guinea pig chow that was supplemented with vitamin C. The rabbits were kept in a communal pen comprising of indoor and outdoor areas. These animals were fed rabbit chow supplemented with cabbage. Both guinea pigs and rabbits had access to water ad libidum.

9.1.2 Animal feed and housing. Once a week, typically on Mondays, 8-12 male guinea-pigs (200-250 g, approximately 28 day old) were received from the animal unit and used for studies in that same week. Rabbits of either sex (2-3 kg, approximately 42-56 days old) were usually obtained in sets of two, 2-3 times in a week. Animals were housed in the animal rooms of the Department of Pharmacology and Therapeutics at the University of British Columbia. About 4-6 guinea pigs were placed in each wire cage (58 x 35 x 53 cm, in size) in the animal room. These guinea pigs had free access to food (guinea pig chow) and water. Each rabbit was kept in a separate cage (58 x 35 x 53 cm, in size; different room from that used for guinea pigs). The animal rooms had controlled temperatures (22-23° C) and humidity (50-55) with set 12-hourly day and night periods.

9.2 Slice preparation

Hippocampal slices were prepared from male guinea pigs as follows. Animals were initially cooled (30-40 min) on an ice pack in a dessicator to

a rectal temperature of 28-30° C and maintained on a mixture of 1.5-2% halothane in carbogen (95% O_2 and 5% CO_2). The dessicator was pre-equilibrated with this halothane-carbogen mixture (in concentrations sufficient for general anaesthesia) before introducing the animal. To obtain slices, the skin on the head 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 placed on dissecting paper. Each hippocampus was dissected free and quickly transferred to cooled physiological medium that was continuously being oxygenated. Subsequently, each hippocampus was then sectioned transversely to the septotemporal axis at a thickness of 450 μm on a McIlwain tissue chopper. Serial sections were separated with fine stainless steel spatulae in a plate containing previously cooled (5° C) physiological medium and equilibrated with carbogen. The procedure from surgery to slice preparation 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 1.5-2 mL/min with the standard medium containing in mM: NaCl, 120; KCl, 3.1; $NaHCO_3$, 26; NaH_2PO_4, 1.3; $CaCl_2$, 2.0; $MgCl_2$, 2.0 and glucose, 10.0 (see Table 9-1, for list of physiological media used). 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.

9.3 Slice selection

About 8-10 slices were selected from the middle portion of each hippocampus in vitro. The selection of slices to be used was based on the following criteria. Each slice had to be intact with well defined and unmashed borders, i.e., slice edges. Only slices with a clearly discernable cornu ammonis and dentate gyrus cellular layers were chosen. Furthermore, the selected slices had to have clean and even (smooth) surfaces, i.e., not 'mashy' or 'flaky'. Such slices could support physiological responses for periods up to 12 hours. Each experiment was typically of 2-4 hours duration. Hence 2-3 of the selected slices from a given animal could have been used sequentially. 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 usually used. Consequently, the total times of exposures to bathing medium and experimentation for different slices were essentially comparable during each series of experiments.

9.3.1 Slice chamber and perfusion method. The slice chamber used in the present experiments (Figure 9-1) was manufactured by Mr. C. Caritey, Department of Pharmacology and Therapeutics, The University of British Columbia. The full descriptions of the slice chamber used were reported in a publication from this laboratory (Pandanaboina and Sastry, 1984). The basic components of the slice chamber were as follows; (a) a raised stage constructed of plexiglass with (b) 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 plate that was attached beneath the circular chamber (Figure 9-1).

9.3.2 Standard and test media. The standard and test media were contained in separate 50-mL polyethylene barrels. Carbogen lines for media

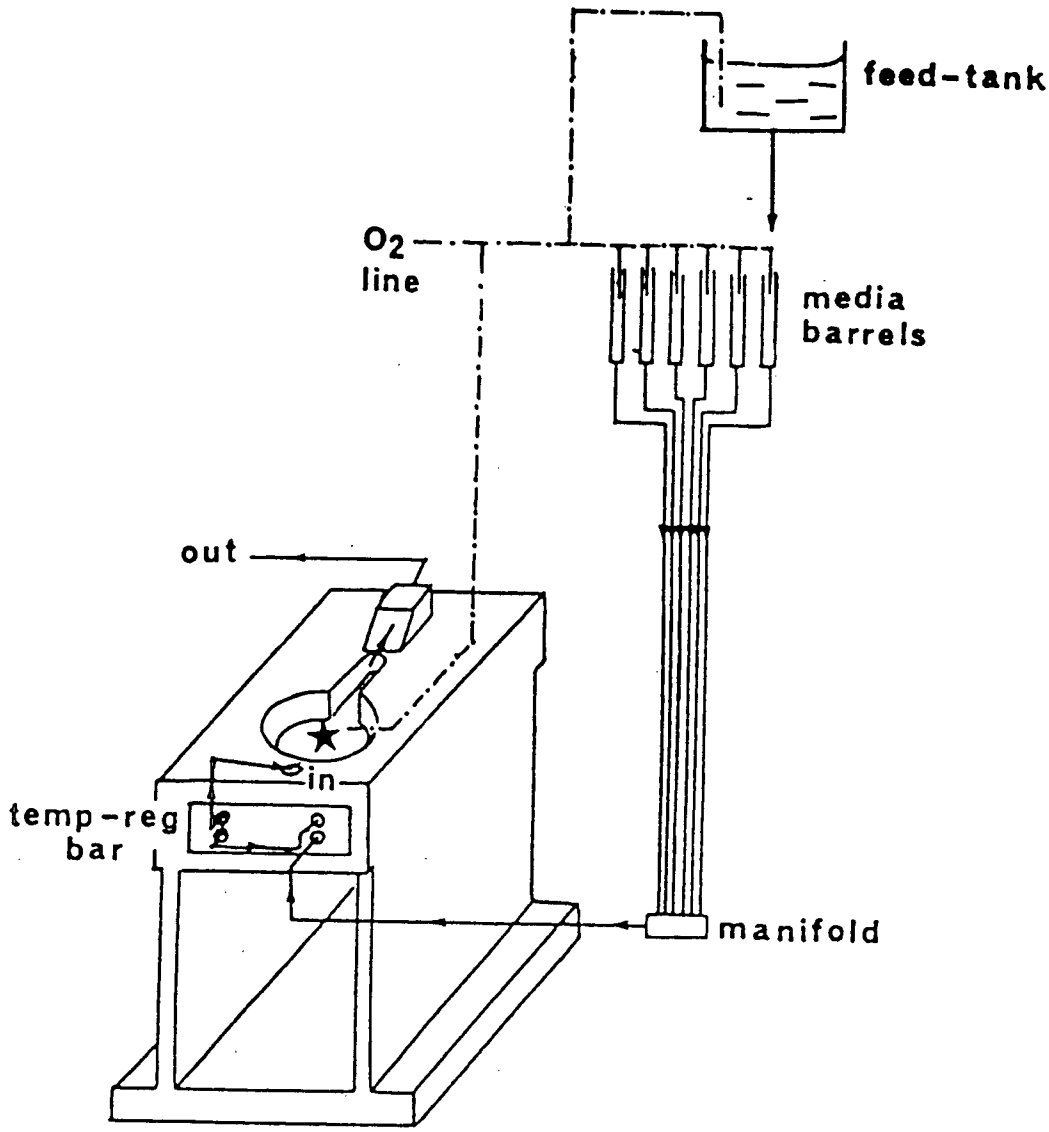

Figure 9-1. Recording chamber and perfusion method for the maintainance of transversely sectioned guinea pig hippocampal slices. The slice chamber consisted of a raised stage made from plexiglass that had a circular incubating chamber (*) bored into the top surface and, a temperature-regulating bar was fixed within the stage. The standard physiological medium was added into the reservoir (feed-tank) with a flow-line leading into one of the media barrels. The test physiological media were added into the remaining media barrels. Any one of these madia barrels could be used to perfuse the incubation chamber through flow-lines (continuous lines with arrows) that travelled via the common manifold then made turns within the temperature-regulating aluminum bar before entering the incubation chamber. The oxygenation flow-lines are indicated by the dotted lines.

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 (Figure 9-1). The feed-tank was continuously oxygenated as well. A tube from each 50-mL barrel was connected to a common manifold. A single outlet from the opposite end of the manifold led to the slice chamber via a connecting polyethylene tube. Control and/or test solutions were introduced into the slice chamber via an inlet at the bottom of the slice chamber. Continuous drainage was via a suction outlet created for that purpose (Figure 9-1). A balanced inflow and outflow of solutions ensured the maintenance of constant solution levels in the slice chamber. The slices were submerged in the medium at all times during the experiments. Among other properties, the whole perfusion set-up permitted; (a) the rapid exchange of standard and test solutions; (b) adequate oxygenation of solutions; (c) minimum dead spaces within the system; and (d) the regulation of solution temperature with a maximum of 0.5° C fluctuation.

The standard and test media used in the experiments are summarized in Table 9-1. Most of these media were freshly prepared to their final constituent concentrations on the day of the experiment. However, stock solutions of substances such as N-methyl-DL-aspartate or sodium saccharin, were prepared once a week. These stock solutions were refrigerated when not being used.

9.4 Endogenous sample collections

9.4.1 Guinea pig hippocampus. Guinea pigs were initially anaesthetized with 1.5 g/kg urethane, given intraperitoneally. When each guinea pig was adequately anaesthetized, it was transferred and then positioned into a

Table 9-1. Composition of media (in mM) used for hippocampal slices

Medium	[1]NaCl	[2]KCl	[1]$NaHCO_3$	[1]NaH_2PO_4	[2]d-Glucose	[1]$CaCl_2$	[1]$MgCl_2$	[3]$BaCl_2$	[4]$MnCl_2$
Control	120	3.1	26	1.8	10	2.0	2.0	-	-
Raised Ca^{++}	120	5.0	26	-	10	4.0	4.0	-	-
Ca^{++} free	120	3.1	26	1.8	10	-	3.5	-	0.5
Low Ba^{++}	120	5.0	26	-	10	3.5	4.0	0.5	-
Moderate Ba^{++}	120	5.0	26	-	10	2.0	4.0	2.0	-
High Ba^{++}	120	5.0	26	-	10	0.5	4.0	3.5	-

Source: (1) Fisher Scientific Co.; (2) BDH Chemicals Ltd.; (3) Sigma Chemicals Co.; (4) J.T. Baker Chemicals Co.

stereotaxic holder. For continued anaesthesia, each ginuea pig received maintenance doses of urethane whenever necessary (typically every 1-3 hr) during the collection experiments. Once in the stereotaxic holder, the following surgery was done. The skin above the skull was cut and retracted. Two spherical holes (4 mm in diameter; one on each side of the sagittal suture line) were carefully drilled into the exposed skull. The centre of these holes was around the following co-ordinates (with the bregma as the reference point); posterior, 8 mm, and lateral 5 mm. It was important to prevent bleeding by ensuring that the dura was not punctured when the holes were made in the skull. Small spherical cups (10 mm long, outside diameter approximately 4 mm) constructed from drinking straws, were positioned into each hole as follows. The dura was removed carefully with fine scissors and forceps. Then one end of the straw-cup was pushed deeper into the brain tissue down to a depth of ~ 7 mm from the surface of the skull. Dental wax was applied between the straw and the skull, and around much of the exposed skull.

Suction was used to clear the cortical tissue within the cup, but never beyond the depth of the inserted cups. Once the unwanted tissue was cleared, the cups were rinsed with oxygenated physiological medium until the fluid was clear. At this time, the animals were ready for the collection experiments. Using micromanipulators, a bipolar stimulating electrode was introduced into each cup. The stimulating electrode was inserted down to a distance of about 7.25 mm from the surface of the skull (i.e. to penetrate the CA_1 area of the hippocampus). Modified suction lines were used to collect fluids from each cup at the appropriate intervals (see section 10.5.1). When all the collections were done, the animals were sacrificed, and their brains removed. The hippocampi were dissected out and examined.

It was clear from the markings on the hippocampal surfaces that they had been penetrated by the stimulating electrode down to the stratum radiatum regions. In some cases, the bottom end of the cup had cut through into the alveus; samples from these animals were discarded.

9.4.2 Rabbit neocortex. Rabbits (2-3 kg) were anaesthetized and maintained on halothane (1.5-2%) and carbogen (95% O_2 and 5% CO_2) mixtures and were restrained in a stereotaxic apparatus. Through bore-holes made in the skull, two cups (diameter: 8 mm) were positioned on the surface of the neocortex that was exposed by removal of the dura (Figure 9-2). A ring-shaped stimulation electrode was fixed to the inner end of each cup that was in contact with the neocortex. In addition, a single on-off suction line was lowered with micromanipulators into each cup. These suction lines were used to collect fluid in each cup, whenever necessary. The suction lines led to a common container that was kept on dry ice.

9.5 PC-12 Rat Pheochromocytoma cell line

Frozen cultures of rat pheochromocytoma PC-12 cell lines (Batch No. F-5876) were obtained from American Type Cultures, USA. These frozen cells were maintained in sealed ampules. Each ampule contained approximately $4x10^6$ PC-12 cells. These ampules were packaged on dry ice during shipment and delivery was effected within three days (i.e. between Rockville (U.S.A.) and Vancouver (Canada)). Upon receipt, each ampule with PC-12 cells was attached to a string and suspended in a jar filled with liquid nitrogen. The jar with its contents was placed in a walk-in refrigerator. Every alternate day, liquid nitrogen was added to the jar, to replace amounts lost through evaporation.

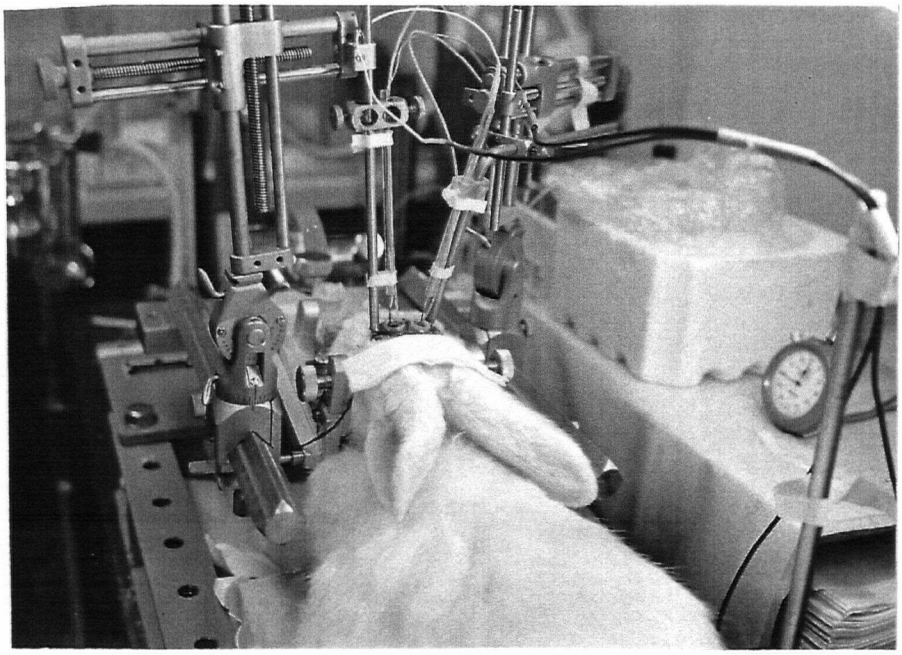

Figure 9-2. Positioning of small cups onto rabbit neocortical surface for collection of samples in vivo. Through bore-holes made in the skull, two cups (diameter: 8 mm) were positioned on top of the neocortex that was exposed by removal of the dura.

9.6 Electrical instruments

9.6.1 Amplifiers. Extracellular responses were amplified with the Western Precision Instruments (WPI) differential pre-amplifier model DAM-5A. This pre-amplifier had a maximum gain of 1000X. During recordings, the low frequency (10 Hz) and high frequency (10 KHz) filters were set at 0.1 Hz and Wide-Band, respectively. The amplified physiological potentials were then led to the Data Precision 6000 waveform analyser (see section 9.6.3). Intracellular responses were fed into the Dagan single electrode system, model 8100-1. This unit had three operational modes namely: (a) pre-amplifier only (bridge-current clamp), (b) pre-amplifier and switched current stimulator (switched current clamp), and (c) switched voltage clamp. The current clamp or the voltage clamp mode, had sample and hold times of 10 microseconds, and an adjustable switching frequency of 500 Hz-25 KHz. The probe had an input resistance of 10^{10} M with an input bias current of 1 pA. Some other operational features of this unit included an adjustable DC offset (range ± 1000 mV) and adjustable capacitance compensations (0-15 pF). Most of the intracellular recordings were done with the unit set in the switched current clamp mode (filters at wide-band), and 10X gain.

9.6.2 Stimulators. 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 the photoelectric constant current units.

9.6.3 Oscilloscopes. The amplified field potentials were fed into the Data Precision 6000 Universal Waveform Analyser model 611. This programmable unit had capabilities for digitizing and storing analog signals (sampling speeds; 100 KHz; digitizing resolution up to 14 bits; memory:

48K bytes). The processed signals were displayed on the cathode ray tube. An outlet from the Data Precesion 6000 unit fed into a plotter. Outputs from the Dagan amplifying system were fed into the Tektronix 5113 Dual Beam Storage Oscilloscope. This unit had the following plug-in modules; (a) Tektronix 5A14N four-channel amplifier, (b) Tektronix 5A22N differential amplifier, and (c) Tektronix 5B12N dual time base. Intracellular recording outputs from the Dagan amplifier were fed into the differential amplifier module (DC-offset, and high frequency filters at 0.1-1 KHz). Current signals were fed into one or more of the four-channels amplifier. Any of these signals could be viewed on the oscilloscope.

9.6.4 Magnetic tape recorder. Most intracellular experiments were taped in their entirety on magnetic tape using the Hewlett Packard (HP) 3968A instrument recorder. Outputs from the Tektronix 5A22N differential amplifier were fed into one of the channels of the HP tape recorder (on-line). Typically, the recording (and play-back) speed of the HP recorder was set at 9.52 cm/sec (FM Data, band-width 1250 Hz). Selected segments of these tapings could be charted out on the HP-7404A plotter or analysed on the oscilloscopes, as necessary.

9.6.5 Paper plotter and chart recorder. 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 Precesion 6000 programmable acquisition unit. In addition, signals displayed on the oscilloscope type 5113 could be photographed directly.

9.6.6 Miscellaneous. During intracellular recordings, it was desirable to pick up characteristic sounds associated with specific neuronal activities, as the recording electrode approached cells. From these sounds, it was possible to determine the "position" of the electrode tip within the

tissue. For this purpose, an output from the Dagan amplifier was fed into the Grass AM-8 Audio Monitor (low filter, 100 Hz; and high filter, 0.3 KHz). To facilitate the penetration of cells, the intracellular micro-electrode assembly was attached to a David Kopf Instrument Microdrive unit, model 607W. With this unit, the microelectrode could be lowered into the recording region in μm steps (100-200 steps/sec).

9.7 Stimulating and recording electrodes

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

9.7.2 Recording electrodes. Standard fiber filled borosilicate glass micropipettes (internal diameter, 1.02 mm; outside diameter, 1.5 mm: WPI) were used to prepare extracellular recording electrodes. These micropipettes were pulled to fine tips (tip diameter, 1-3 μm) on Narishige Scientific Instruments' vertical electrode puller type PA-2, and filled with 4 M NaCl (resistances; 0.5-1.5 MΩ. Intracellular electrodes were made from fiber filled micropipettes (internal diameter, 0.76 mm; outside diameter, 1.0 mm: WPI) pulled to fine tips (submicrons tip diameters, could not be resolved under microscope set at 400X magnification) on the Narishige Scientific Instruments' vertical electrode puller type PA-81. These pipettes were filled with either 3 M potassium chloride (resistances; 50-90 MΩ), 2 M potassium acetate (50-90 MΩ) or 3 M cesium chloride (resistances; 50-90 MΩ).

10. EXPERIMENTAL SCHEMES

10.1 Intracellular recordings

Standard intracellular techniques were used in the present studies. Briefly, microelectrode impedances were determined with the "Z-test" and "null-bridge" method. The Z-test was done by using the Dagan 8100-1 to pass 1 nA (10 ms, at 100 Hz) through the microelectrodes, and the resulting voltage outputs were proportional in amplitude to microelectrode impedances. The null-bridge method used external trigger sources to generate currents (0.2-1 nA, for 200 msec) that were passed (via the Dagan 8100-1) through the microelectrodes. The amplifier bridge was subsequently adjusted to provide a zero stimulus voltage at the output. The magnitude of the adjustment needed to balance the bridge, corresponded linearly to the resistance of each microelectrode (in MΩ). Microelectrode impedances were determined at three different times (null-bridge method): (1) before cell penetrations; (2) upon impalements; and (3) after retrieval from the cells, when the experiments were completed. The values obtained for (1) and (3) were similar in each experiment. The values for (2) were higher due to the contributions of membrane resistances in these measurements. Hence membrane resistances could be estimated by subtracting values in (1) or (3) from those of (2). In some experiments, the membrane input resistance (R_n) was continuously monitored with constant hyperpolarizing intracellular currents (0.5-1 nA, 200-300 msec at 1 Hz). Whenever appropriate, R_n was also determined with graded hyperpolarizing intracellular current pulses (0.5-1 nA, 100-200 msec, at 1 Hz). The amplitudes of the resulting membrane potential shifts at plateau were plotted as a function of the currents used (Figure 10-1). The calculated slopes of these curves corresponded to the input resistances in MΩ.

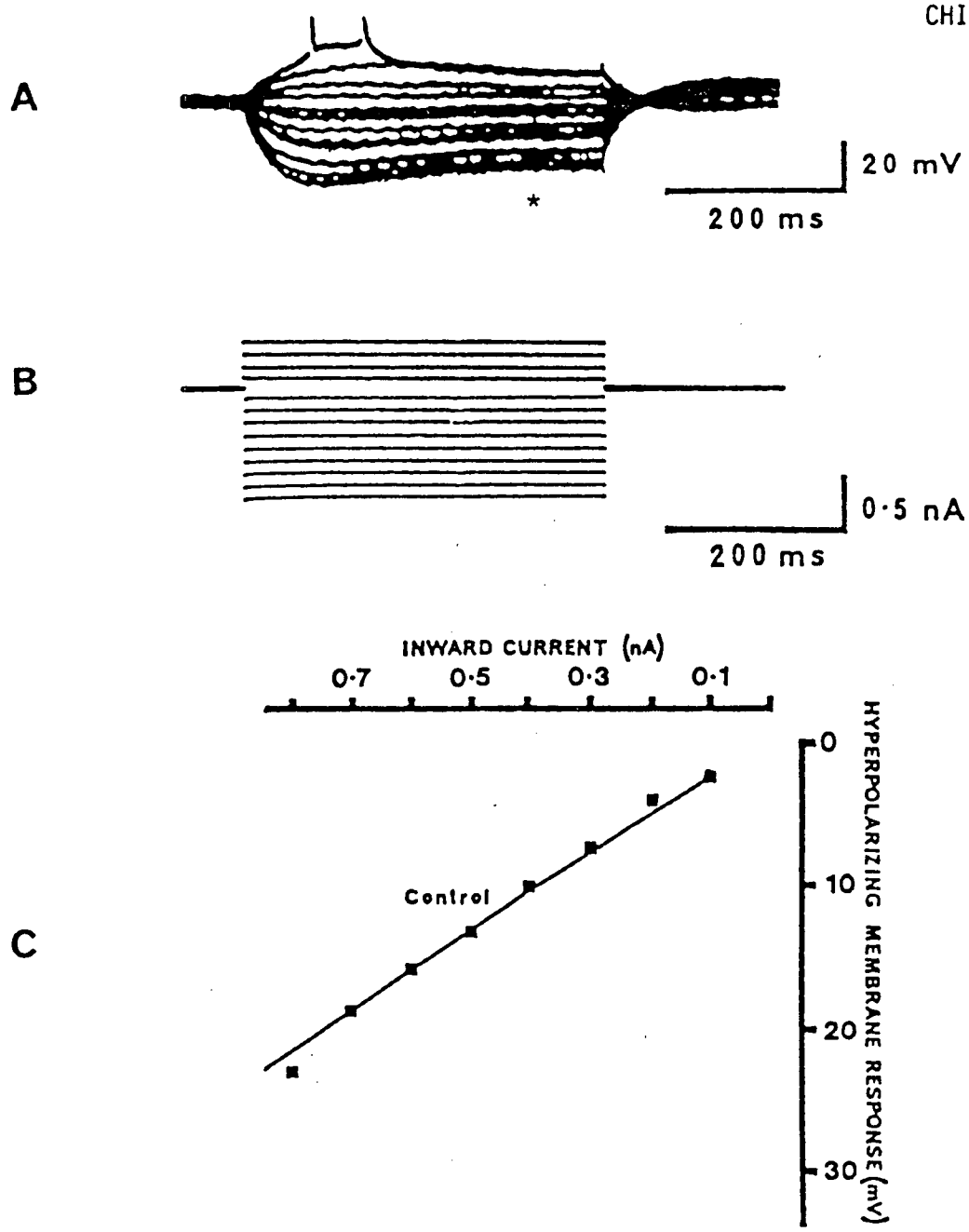

Figure 10-1. Determination of cell input resistances with intracellular injections of graded hyperpolarizing current pulses into CA_{1b} neurons. In (A) are shown the membrane potential shifts from rest in a CA_{1b} neuron induced by graded intracellular current pulses (B) applied in random order. Note that action potentials could be induced with a depolarizing current pulse thereby confirming that this cell was a neuron. [NB: Low resolution and truncation of action potentials is due to photographic reproduction of the traces from the oscilloscope.] In determining the cell input resistances, the amplitudes of the membrane shifts at steady-state (*) were plotted as a function of the hyperpolarizing current pulses as illustrated in (C). The slopes obtained from these I-V curves yielded the cell input resistances in MΩ. [CA_{1b} neuron; RMP, -60 mV; R_n, ~27 M . KCl electrode.]

The values of the resting membrane potentials (RMP) were obtained directly from the oscilloscope. Typically, the cell membrane potentials were continuously monitored during the experiments. Whenever appropriate, the cell membrane potential could be clamped to desired voltage levels, with passage of appropriate steady DC currents generated by the Dagan 8100-1 but this was not necessary for most cells studied. Only cells that presented with stable (i.e. non-fluctuating) RMP of -60 or more negative and R_n greater than 25 MΩ, were used for data collection. Intracellular responses in CA_{1b} or CA_{3b-c} neurons were evoked with direct current injections (0.1-7 nA, 50-400 msec; 0.2-0.02 Hz) and stimulations of the stratum radiatum (10-150 μA, 0.01-0.3 msec, at 0.01-0.2 Hz). In some experiments, antidromic action potentials in CA_{3b-c} neurons were evoked with stimulating electrodes positioned in the CA_{1b} apical dendritic region (Figure 10-2), where Schaffer collaterals make synaptic contacts with CA_{1b} neurons. These antidromic action potentials presented with distinct thresholds of activation and could be evoked in Ca^{++}-free medium.

10.2 Extracellular recordings

Square wave pulses (10-150 μA, 0.02-0.8 msec, at 0.2 Hz) were used to stimulate the stratum radiatum (Figure 10-2). Components of the somatic and dendritic recorded potentials were examined in standard medium and in Ca^{++}-free medium. This latter procedure permitted visualization of presynaptic volleys and antidromic responses. The selected stimulation parameters in each experiment were those that elicited population spike amplitudes of 1.0-1.5 mV or dendritic EPSP amplitudes of 0.5-1 mV. Once these initial population responses were obtained, their amplitudes were constantly monitored for stability (at 0.2 Hz) for a minimum of 30 min.

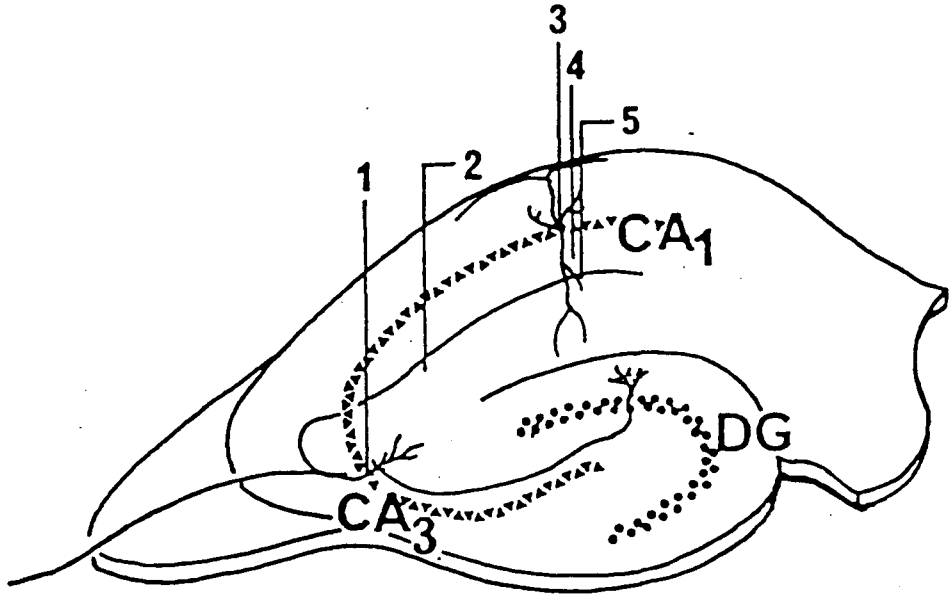

Figure 10-2. A schematic illustration of the extracellular positioning of stimulating and recording electrodes in the guinea pig hippocampus in vitro. Orthodromic extracellular and intracellular responses in the CA_1 area were evoked with stimulating electrodes positioned in the stratum radiatum (2). Antidromic action potentials in individual CA_3 neurons were evoked with stimulating electrodes positioned in the Schaffer collaterals-CA_1 pyramidal cell synaptic regions (5). Orthodromic field EPSPs were recorded with recording electrodes positioned in the CA_1 dendritic region (4) and, population spikes were recorded with recording electrodes positioned in the CA_1 pyramidal layer (3). Intracellular potentials in CA_1 neurons were recorded with intracellular electrodes positioned in the CA_1 pyramidal layer (3) and, these electrodes could also be used for intracellular stimulations. Similarly, antidromic intracellular action potentials in CA_3 neurons were recorded with stimulating-recording intracellular electrodes positioned in the CA_3 pyramidal layer (1).

These responses constituted the control responses. Subsequent experiments were conducted only if these control responses remained stable.

10.3 Induction of long term potentiation

10.3.1 Tetanic stimulations. Postsynaptic responses in CA_{1b} area were evoked with stimulations of the stratum radiatum (test frequencies, 0.02 or 0.2 Hz). The following train frequencies were used to induce synaptic potentiations of the population spike, field EPSP or intracellular EPSP; (1) 50 Hz, 5-250 pulses, (2) 100 Hz, 100, and (3) 400 Hz, 200 pulses. In each case, the same stimulus intensity was used throughout the experiment. In some experiments, intracellular responses were recorded using micropipettes filled with 3 M CsCl. This was done to test whether LTP could be induced even if most postsynaptic K^+ currents were blocked by Cs^+ applied internally.

10.3.2 Paired depolarizations. The following experiments were done with picrotoxin (50 μM) added to the control medium to facilitate LTP induction by this method (cf. Sastry, Goh and Auyeung, 1986). In each case, an impaled CA_{1b} neuron was directly depolarised with current injections (3-7 nA, 300-400 msec) while the inputs in the stratum radiatum were being activated at the onset of the intracellular depolarization. These conjoint stimulations, termed pairings, were evoked at 0.2 Hz. Typically, 5 to 15 consecutive pairings were given at any one time.

10.4 Effects of Ba^{++} in hippocampus

10.4.1 Ba^{++} and evoked responses. A Ba^{++} medium with low Ca^{++} was used in these experiments to facilitate the occurrence of evoked minEPSPs in Ca_{1b} neurons following stimulation of the stratum radiatum. It was anticipated that changes in the frequency of evoked minEPSPs could be used in assessing presynaptic functions in the hippocampus (cf, Silinsky,

1978). In order to select an ideal combination of Ba^{++} and Ca^{++}, the following media were tested (in mM): (1) 0.5 Ba^{++} and 3.5 Ca^{++}; termed low Ba^{++} medium, (2) 2 Ba^{++} and 2 Ca^{++}; termed moderate Ba^{++} medium, and (3) 3.5 Ba^{++} and 0.5 Ca^{++}; termed high Ba^{++} medium (see Table 9-1 of chapter 9). Each impaled cell was exposed to one type of Ba^{++} medium perfused for 2-10 min. Whenever appropriate, picrotoxin (50 μM) or tetrodotoxin (1 μM) was added to these solutions to block GABA-ergic inhibition or inhibit Na^{+} dependent action potentials, respectively. Changes in membrane potential levels and R_n were determined for each impaled neuron, both in control medium and during Ba^{++} applications. In addition, spontaneous and evoked intracellular responses were recorded. In some experiments, Ba^{++} applications were repeated in the same cells, at intervals of 30 min. The results obtained in the above experiments were used to select an appropriate Ba^{++} medium to be utilized in subsequent studies.

10.4.2 Asynchronous release of transmitter and LTP. Both the control and the selected Ba^{++} media contained 50 μM picrotoxin. The CA_{1b} neurons examined were impaled with recording electrodes filled with either 3 M KCl (KCl electrode) or 2 M CH_3COOK (KA electrode). A bipolar concentric stimulating electrode was positioned in the stratum radiatum within 50 μm distance from the CA_{1b} pyramidal layer, to stimulate mostly proximal synapses (Andersen, et al., 1980a). During 2-5 min Ba^{++} applications, the frequencies of evoked minEPSPs were determined immediately following; (1) single subthreshold or suprathreshold stimulations of the stratum radiatum, (2) direct intracellular depolarizing current injections (3-7 nA, 200-300 msec), and (3) after pairings of subthreshold stimulations of the stratum radiatum with direct current injections into a CA_{1b} neuron.

In studies with tetanic stimulations, slices had their CA_2-CA_4

pyramidal cell layers removed. This was done to minimise the occurrence of minEPSPs due to action potentials generated in these fields, particularly during picrotoxin and Ba^{++} applications. The present experiments aimed at determining whether evoked minEPSPs in the CA_{1b} neurons in the presence of Ba^{++} were increased during LTP. This provided one method for directly assessing increases in released transmitter during LTP (cf. Silinsky, 1978). Each slice was exposed to medium containing Ba^{++} (3.5 mM) and Ca^{++} (0.5 mM) for 2 min whenever necessary. During Ba^{++} perfusion, the number of minEPSPs following the EPSP evoked with stimulation of the stratum radiatum was determined. Slices were then reexposed to normal medium for 15 min and the stratum radiatum was tetanized (400 Hz, 200 pulses; stimulation strength adjusted to evoke subthreshold EPSP only) to induce LTP. Long-term potentiation was detected as long-lasting post-tetanus increases of previously subthreshold EPSPs, sometimes reaching threshold. Slices were then reexposed to Ba^{++} containing medium 15 min after the induction of LTP. During this second Ba^{++} application, the number of evoked minEPSPs following the EPSP evoked with stimulation of the stratum radiatum was determined. In addition, the presynaptic volleys during these Ba^{++} applications were monitored. In separate experiments, slices were exposed to the same Ba^{++} medium twice with a 30 min interval without the LTP-inducing tetanus. During these Ba^{++} applications, the frequencies of minEPSPs after stimulation of the stratum radiatum were determined.

10.5 Effects of released endogenous substances in the hippocampus

10.5.1 Collection of endogenous substances. It has been reported in the literature that proteins are released during the induction of LTP with tetanic stimulations (Duffy, Teyler and Shashoua, 1981). Whether these proteins and other substances that are released during tetanic stimulations

exert any effects on synaptic transmission, for example, has not been tested. The present experiments, therefore, were designed to collect substances released during tetanic stimulations with the view of examining their effects on synaptic transmission. The methods used to collect samples from guinea pig hippocampus in vivo were as follows. Every 5 min, 0.05 ml of oxygenated medium (i.e control medium used for incubating guinea pig hippocampal slices) was added into each cup. An extra oxygen line was positioned in each cup, on top of the fluid, to ensure that the added medium remained adequately oxygenated. A stimulating electrode was placed 250 μm inside the hippocampal tissue through the cup. At the end of each 5 min incubation period, each hippocampus was tetanized (bipolar pulses of 0.5 msec duration at 100 Hz, 100 pulses, 15 V, given every 5 sec, 6 trains), and the suction lines were opened during the fifth second to collect the fluids in the cups. These samples were denoted as the "tetanized hippocampal samples" (THS), and they were collected in a common container that was kept on dry ice. The above procedures were repeated until the desired volume of THS (4 ml) was collected. Control samples (2 ml of "untetanized hippocampal samples", i.e. UHS) were collected into a separate container using the same techniques as described but without tetanization of the hippocampus. [NB: From each guinea pig 2 ml of UHS were collected before collecting 4 ml of THS.]

Similar methods as described above were used to collect substances from the rabbit neocortex in vivo. Briefly, every 5 min, 0.1 ml of oxygenated medium was added into each cup (the procedures for placing the cups was described in chapter 9). At the end of each 5 min incubation period, the neocortical surface was tetanized (bipolar pulses of 0.5 msec duration at 50 Hz, 100 pulses, 30 V), and the samples were collected (i.e. tetanized

neocortical samples; TNS; 4 ml). Two ml samples of "untetanized neocortical samples" (UNS) were collected prior to any TNS collections.

The samples (i.e. UNS, UHS, TNS, or THS) from a particular animal were stored in separate collection tubes that were adequately identified to indicate the following; (1) animal type, (2) date of sample collection, and (3) type of sample, i.e. UNS. At the end of each collection experiment, the samples were stored at around -60° C until used in subsequent experiments.

10.5.2 Guinea pig hippocampal samples and LTP production. To examine the effects of the collected samples on synaptic transmission in the hippocampus in vitro, the following procedures were followed. The samples (UHS and THS) were quickly thawed by placing the sample tubes in luke-warm water, and the THS samples were split into two portions of 2 ml each. One portion of the THS was then heated by suspending the capped tube containing the sample in boiling water for 30 min and then cooled. This sample was termed the heated-tetanized hippocampal sample (HTHS). This heating procedure was done to inactivate heat sensitive macromolecules that could be present in the THS (cf. Duffy, Teyler and Shashoua, 1981).

Typically, samples collected from one animal in vivo were used in a single experimental series in vitro. Prior to application, the appropriate sample was transferred into one of the perfusion barrels where it was oxygenated for at least 5 minutes. To allow for longer contact times between the applied samples and the hippocampal slices, the flow rates were adjusted to 1 ml/min. The samples were applied blind to the guinea pig hippocampal slices, as each slice was exposed to one sample only (i.e. THS, HTHS or UNS). After obtaining stable responses (i.e. population spikes recorded in CA_{1b} pyramidal layer evoked by stimulation of the stratum radiatum), the sample was perfused (about 2 min) onto the hippocampal

slice. The population spikes were evoked at 0.2 Hz and monitored during these experiments.

10.5.3 Rabbit neocortical samples and LTP production. Effects of the samples collected from rabbit cerebral cortex in vivo on synaptic transmission in the hippocampus in vitro, were examined in the same way as described in section 10.5.2, with the following modifications. Briefly, the rabbit neocortical samples were identified as follows; (1) UNS, for untetanised neocortical samples, (2) THS, for tetanised neocortical samples, and (3) HTNS, for heated-tetanised neocortical samples. In these experiments, the order of application was UNS, HTNS and TNS and these applications were given during low frequency stimulations (0.2 Hz) of the stratum radiatum. Each application was followed by a 60 minute wash with standard medium. In separate slices, TNS was applied in the absence of stimulations of the stratum radiatum, and at least 5 min after stopping any such stimulation. Stimulations of the stratum radiatum were reinstituted 5 min post-TNS applications. In another series of experiments, TNS was applied to the hippocampus in the presence of 10 mM saccharin. Saccharin is known to inhibit binding of nerve growth factor to its receptors (Ishii, 1982). It was anticipated that saccharin would antagonise the effects of NGF-like substances if they were present in TNS. The saccharin was applied for 10 minutes and during the 7-8th minute of this saccharin perfusion, TNS was applied. This was followed by washing with standard medium for 60 minutes. At this time a second application of TNS was repeated in the absence of saccharin. In other experiments, the above procedures were used to apply TNS with (1) atropine (100 μM), or (2) dihydro-β-erythroidine (100 μM). These experiments were done to check for possible effects of any acetylcholine that could be present in the TNS. In addition, the effects of 2 min

applications of exogenous glutamate (100 μM), and, pre-heated and cooled glutamate on the stratum radiatum-induced CA_{1b} population spikes were examined.

10.6 Effects of rabbit neocortical samples on cultured PC-12 cells

10.6.1 PC-12 cell growth. PC-12 cells are clonal lines of rat adrenal pheochromocytoma cells which develop neurites when incubated with nerve-growth factor or related compounds (Greene and Tischler, 1976). Therefore, PC-12 cell cultures provide a method for screening substances with NGF-like activities. In the present experiments it was hypothesized that samples collected during tetanic stimulations contained NGF-like substances. It is known that LTP is associated with morphological changes to synaptic structures (Teyler and DiScenna, 1987, for review). It is conceivable that substances released during tetanic stimulations could be growth related macromolecules that mediate the above structural changes. In view of this, the present experiments were done to determine whether samples collected during tetanic stimulations could induce neurite growth in PC-12 cell cultures. The growth medium used for cell cultures contained Dulbecco's modified Eagles medium, 5% fetal calf serum and 10% heat-inactivated horse serum (ingredients obtained from Gibco Laboratories). Sufficient Gentamicin was added to make a final antibiotic concentration of 0.005%. The frozen PC-12 cells were activated as follows. The ampule containing PC-12 cells was quickly submerged in warm water (temp: 38-40° C) in a beaker. With agitation, the frozen contents in the ampule melted within a minute, at which time the ampule was quickly immersed in 70% ethanol at room temperature (25-26° C). The neck of the ampule was carefully broken and its contents (i.e. PC-12 cell suspensions) emptied into a centrifuge tube

containing 6 ml of growth medium. Using a pipette, 0.2 ml of PC-12 cell suspensions were transferred into each culture dish (35 x 10 mm size), to which 1.3 ml of the growth medium was added (final volume of 1.5 ml in each dish). A total of 30 culture dishes were plated in this way. These culture dishes were placed in one large tray that was transferred into the incubator. After twenty-four hours of incubation, each culture dish was examined for cell growth using a phase-contrast microscope. PC-12 cells were found to be growing in all 30 culture dishes. At this time, the culture dishes were randomly assigned to five groups. Each group, therefore, had six culture dishes. Colored dots were used to identify the five groups.

10.6.2 Preparation of PC-12 cells feeding media. The various rabbit neocortical samples were thawed and mixed with freshly prepared growth media as illustrated in Figure 10-3. Briefly, 5 ml of double-concentrated growth medium was added to each one of four separate centrifuge tubes. Then the following neocortical samples were added blind to the above tubes (5 ml in each case): (1) TNS, (2) HTNS, (3) UNS, and (4) TNS with 10 mM saccharin (Figure 10-3). The contents of each tube were filter-sterilized, and stored in color coded tubes to maintain blinding. The above media were used to feed the PC-12 cell cultures. [NB: To obtain 5 ml of each type of neocortical sample, similar collections (i.e. TNS) from two rabbits were pooled together.]

10.6.3 Neurite induction. On day two of incubation (i.e 24 hours after plating), the growth medium was carefully decanted from the culture dishes. Culture dishes from the same color-coded group were replenished

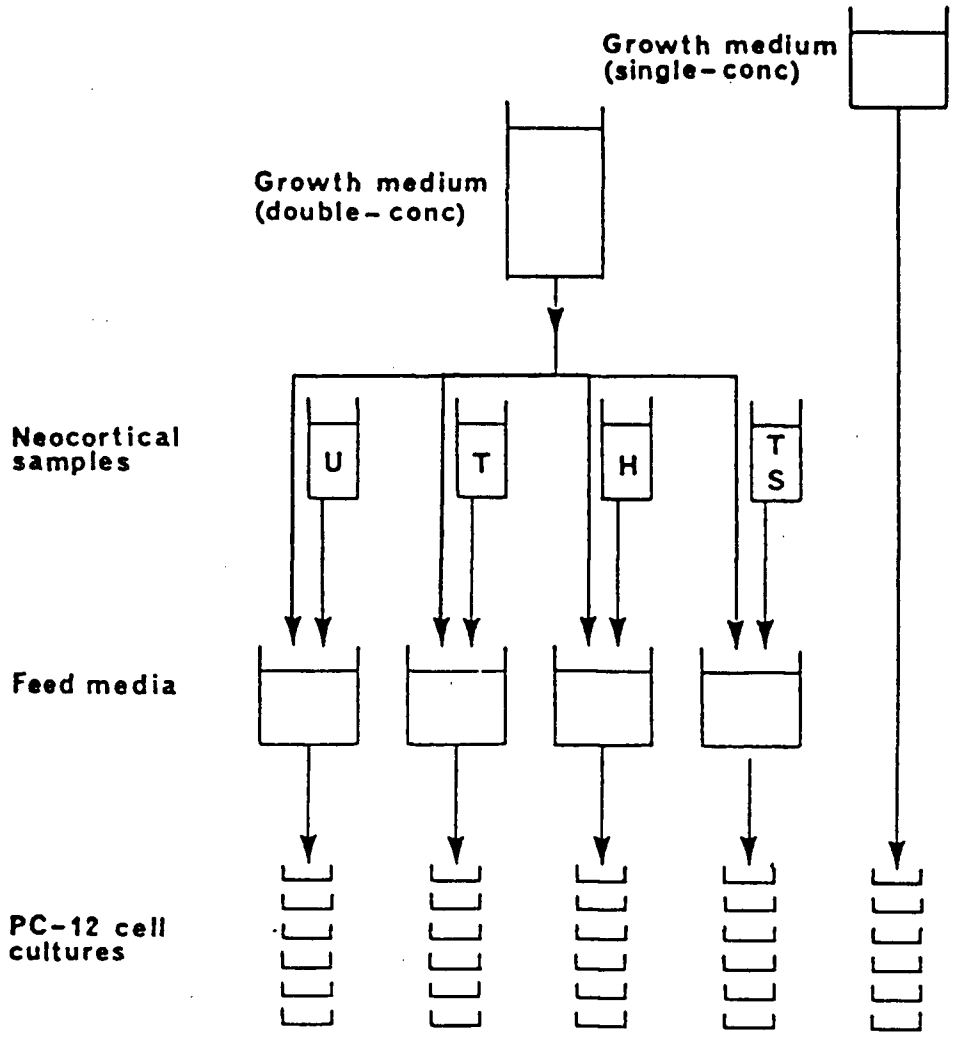

Figure 10-3. Preparation and composition of the different types of feeding media used for incubation of rat adrenal pheochromocytoma (PC-12) cell cultures. The control growth medium (single-conc.) contained Dulbeccos' modified Eagle's medium, 5% fetal calf serum, 10% heat-inactivated horse serum and 0.005% gentamicin. The growth medium (double-conc.) contained twice the concentration of the control growth medium (single-conc.). To make feed media, aliquots of growth medium (double-conc.) were mixed with equal volumes of (1) untetanised neocortical samples, (U), (2) tetanised neocortical samples, (T), (3) heated-tetanised neocortical samples, (H), and (4) tetanised neocortical samples containing 10 mM saccharin, (TS). The feed media, therefore, contained normal concentrations of Dulbeccos' modifield Eagles' medium and 0.005% gentamicin. The above feed-media were used to maintain the various PC-12 cell cultures as illustrated. One group of PC-12 cell cultures was continued on control growth media (single-conc.).

with 1.5 ml of the feed media described in section 10.6.2 above. The different types of feed media comprised of equal mixtures of growth medium and: (1) TNS, (2) UNS, (3) HTNS, (4) TNS and saccharin (Figure 10-3). Plain growth medium was maintained in one group of PC-12 cell cultures (Figure 10-3). Saccharin, a substance that inhibits NGF-dependent neurite growth (Ishii, 1982), was added to the feeding medium of another group. In the present studies, it was wondered whether NGF-related compounds were present in TNS. It was anticipated that if such factors were present in TNS, their effects on PC-12 cell growth could be antagonised by saccharin. After adding the feed media, the culture dishes were returned to the incubator. Thereafter, the cultured PC-12 cells were examined under a phase-contrast microscope each day, starting with the second day and ending on the eighth day of incubation.

10.7 Studies on the possible mechanisms of action of saccharin

10.7.1 General. Experiments were done to determine if saccharin could prevent the effects of samples collected during tetanic stimulations when applied to the hippocampus, or block tetanus-induced LTP. To facilitate analysis of results obtained from experiments in which saccharin was used, it was necessary to determine the electrophysiological effects of saccharin in the hippocampus.

10.7.2 Dose-response curves. Briefly, the population spike in CA_{1b} area was evoked with stimulation of the stratum radiatum at 0.2 Hz. In a given slice, dose-response curves to saccharin were obtained using the single application, randomised design. The saccharin concentrations examined were (in mM); 2.5. 5, 10, 20, 40 and 80. Each drug concentration was perfused for 10 minutes. This was generally followed by a wash period of 15 minutes, which was found to be a sufficient interval for the popula-

tion spike to return to pre-drug levels. The only time the wash time was extended was after applications of 80 mM saccharin, when 20-30 min of wash were required to bring the population spikes back to pre-drug levels.

10.7.3 Saccharin and electrical properties of neurons. Possible effects of saccharin on electrical properties of neurons in the hippocampus were examined as follows. In these experiments, CA_{1b} neurons were impaled with microelectrodes filled with either 3 M KCl or 2 M potassium acetate. In addition, a stimulating electrode was positioned in the stratum radiatum within 50-100 microns distance to the CA_{1b} pyramidal layer. This was done in order to stimulate mostly proximal synapses on CA_{1b} apical dendrites. Under these conditions, the effects of saccharin on the following responses were examined: (1) RMP and R_n; (2) spontaneous minEPSPs and minIPSPs; (3) evoked EPSPs, IPSPs, action potentials and AHPs. In these studies, 10 mM saccharin was perfused for 2-10 minutes. In some experiments, these saccharin applications were repeated at 30 min intervals.

10.7.4 Saccharin and LTP. The present experiments were aimed at determining the minimum dose of saccharin that could interfere with the development of LTP following tetanic stimulations. The population spike in the CA_{1b} area was evoked by stimulation of the stratum radiatum at 0.2 Hz. After obtaining control population spikes, 2.5 mM saccharin was applied for 10 minutes, and the responses were monitored. During the last minute of saccharin applications, the stratum radiatum was tetanized (400 Hz, 200 pulses). This was followed by re-institution of the standard medium to wash out the saccharin. After a washing period of 30 minutes, the stratum radiatum was tetanized (400 Hz, 200 pulses, same stimulus strength and durations as used in the first tetanus). The responses were monitored for another 30 minutes. In different slices, the above procedures were repeated with the

following saccharin conncentrations (in mM); 5, 7.5 and 10 saccharin. In some control experiments, tetanic stimulations were applied twice, separated by 30 min intervals, in standard medium only (i.e. the slices were not exposed to saccharin).

10.7.5 Saccharin and post-tetanic potentiation. Post-tetanic potentiation (PTP) is thought to be mediated by presynaptic mechanisms (e.g Eccles and Krnjević, 1959; McNaughton, Douglas and Goddard, 1978). Therefore, changes in the magnitudes of PTP during different treatments provide one method for assessing presynaptic functions. In view of this, the present experiments examined the effects of saccharin on PTP size. In these experiments, the control medium used contained reduced Ca^{++} relative to Mg^{++} concentrations (low Ca^{++} medium: 1 mM Ca^{++} and 3 mM Mg^{++}). Secondly, the stimulation parameters were adjusted to evoke small population spike of about 0.3-0.5 mV amplitudes. Under these conditions, tetanic stimulations of the stratum radiatum (400 Hz, 200 pulses) only induced post-tetanic potentiation of the population spike in the CA_{1b} pyramidal cell layer (cf. Dunwiddie, Madison and Lynch, 1978). In each slice, PTPs were evoked (1) in control medium, (2) during 10 mM saccharin applied for 10 min, and (3) after drug applications.

10.7.6 Saccharin and presynaptic excitability. Another method for assessing presynaptic functions involves determination of the threshold for antidromic activations of presynaptic terminal regions. Changes in values of antidromic thresholds during treatments are useful indicators of the conditions in the presynaptic regions. For example, hyperpolarizations in presynaptic regions are associated with increases in antidromic thresholds (e.g. Wall, 1958; Saint, Quastel and Chirwa, 1986; Sastry, 1982). Hence the methods of presynaptic excitability testing were used to assess further the

effects of saccharin on presynaptic regions. In these experiments, the physiological medium used did not contain Ca^{++} (i.e. 2 mM Ca^{++} was substituted with 1.5 mM Mg^{++} and 0.5 mM Mn^{++}; see Table 9-1 in chapter 9), in order to abolish synaptic transmissions. Intracellular recordings were obtained from CA_{3b-c} neurons. The selection of cells for inclusion in the experiments was the same as that described for CA_{1b} neuron impalements. In these experiments, the stimulating electrode was positioned in the Schaffer collaterals-CA_{1b} apical dendritic region and used to evoke antidromic action potentials in Schaffer collaterals that invaded CA_{3b-c} neurons. Stimulation test pulses at each fixed duration (given at 0.2 Hz) were adjusted to threshold for antidromic invasion on 50% of 6-8 consecutive trials. The threshold values were determined for a range of stimulus durations (i.e. 0.1-5 msec stimulus pulse durations). The rheobase was estimated from the strength-duration curves of each recording, and these stimulus durations were used to determine threshold values in (1) control medium, (2) during 10 mM saccharin applied for 10 min, and (3) 15 min after drug applications. In saccharin, the threshold values were obtained in the last min of drug application.

10.7.7 Saccharin and paired-pulse facilitation. Paired-pulse facilitation is well characterised in the hippocampus and it is thought to be due to an increase in transmitter released with the second pulse in the stimulation pair (e.g. McNaughton, 1980). Presumably, "residual" presynaptic effects (e.g. Ca^{++} influx into presynaptic terminals) associated with depolarizations induced by the first pulse add up with those of the second pulse and augment the effects of the latter pulse on transmitter release. In principle, alterations in the ratio of the second response relative to the first response during paired-pulse stimulations reflect changes to

presynaptic mechanisms. In view of the above, the effects of saccharin on paired-pulse facilitations were examined. Briefly, stimulus pairs that were separated by an interval of 30 msec were applied to the stratum radiatum to evoke a pair of population spikes in the CA_{1b} field respectively. The pulse interval used was sufficient to cause an increase in the second response (hence facilitated) relative to the first response in each evoked pair. These responses were obtained during control medium perfusions. This was then followed by the application of 10 mM saccharin for 10 min. During saccharin applications, responses to paired pulse stimulation of the stratum radiatum were recorded during the 1, 3, 5 and 9 min intervals. Fifteen minutes after saccharin applications, the responses to paired pulse stimulations of the stratum radiatum were also determined.

10.7.8 Saccharin and NMDLA responses. The effects of saccharin on N-methyl-DL-aspartate (NMDLA) responses in CA_{1b} neurons were examined since NMDA receptors are thought to be involved in the induction of LTP (Collingridge, Kehl and McLennan, 1983). It was necessary to test if saccharin antagonised NMDLA responses since at this stage in the studies it was clear that saccharin blocked the induction of LTP with tetanic stimulations. Intracellular responses were obtained from CA_{1b} neurons as previously described elsewhere. In these experiments, the racemate NMDLA was used since it was available in the laboratory when these studies were planned. Moreover, it is known that the effects of NMDLA are similar to those of N-methyl-D-aspartate (NMDA), the active enantiomer (e.g. Dingledine, 1984). After successful impalements, NMDLA media were applied for 1 or 1.5 min and then washed out. One or two different concentrations of NMDLA (in μM; 25, 50, 75 and 100) were tested during each intracellular recording. Typically, NMDLA applications were repeated at different times in the same

cell, usually at intervals of 8-10 min. Subsequently, 10 mM saccharin was perfused for 10 min. During the 7-9th min of saccharin perfusion, the effects of NMDLA concentrations were tested. After returning to control medium (i.e. post-saccharin), NMDLA applications were repeated.

10.8 Effects of exogenous NGF in the hippocampus

Again these next series of experiments stemmed from the results obtained from experiments described in sections 10.5-10.6, which showed that substances released during tetanic stimulations induced LTP when applied in the hippocampus in vitro, and these same substances could initiate neurite growth in cultured PC-12 cells. This raised the possibility that growth related substances were present in the collected samples. Hence the effects of exogenous nerve growth factor (2.5 µg/ml NGF from Vipera lebetina) were examined in some experiments. This NGF was used in these studies since it was available in the laboratory when these experiments were planned. In these studies, NGF was applied for 5-10 min with or without stimulation of the stratum radiatum to evoke population spikes in the CA_{1b} pyramidal layer. In separate experiments, the effects of NGF were also tested as follows. Stimulation of the stratum radiatum was adjusted to evoke a weak dendritic EPSP in the CA_{1b} field. Repeated tetanic stimulations (50-100 Hz, 100 pulses) applied to the stratum radiatum only induced PTP (cf. McNaughton, Douglas and Goddard, 1978). Slices were then perfused with NGF for 5-10 min. Then a similar tetanus to that described above was applied to the stratum radiatum, during the last minute of NGF application. In other experiments, this tetanus was applied during perfusions of NGF and saccharin.

10.9 Data analysis

Standard control procedures, with blinding wherever applicable, formed the protocol of all experiments. The following statistical methods were

used to analyse the data. Briefly, α was set at 0.05, and two-tailed tests were employed unless the research hypothesis specified the direction in which a difference would occur. In this latter case, one-tailed tests were used. Furthermore, the paired Students' t-test was used for comparisons between two related samples. For this purpose, a variate (e.g. amplitude of population spike) before treatment was compared with its counterpart after treatment. However, for comparisons among a series of Means for which there was one criterion of classification (e.g. Mean amplitude of population spike), one-way ANOVA was used and, if statistical differences were indicated, Duncans' multiple comparisons method was the a posteriori test used to determine which pairs of Means were statistically different.

11. RESULTS

11.1 Recordings in CA_{1b} field of the hippocampus

11.1.1 Features of intracellular recordings. The quality of cell penetrations was initially examined in 20 CA_{1b} cells. In these and subsequent results, each cell represents a complete recording in one hippocampal slice prepared from a different guinea pig. Cells were impaled with micropipettes filled with either 3 M KCl (KCl electrode) or 2 M CH_3COOK (KA electrode). Successful impalements presented with stable RMP between -55 and -80 mV and R_n values between 7 and 57 MΩ (null-bridge method; n = 20 cells). Seventeen out of 20 cells could support action potentials when challenged with depolarizing current injections (0.2-3 nA, 200-400 msec, 0.2 Hz). These same cells presented with fast EPSPs and synaptically activated action potentials following stimulations of the stratum radiatum (10-150 μA, 0.1-0.3 msec, 0.2 Hz; 17 of 17 cells). Action potentials had amplitudes of 80 ± 20 mV and widths, at half-maximum height, of ~1 msec. The above 17 cells were taken to be neurons, and their characteristics were further examined.

Most of the above CA_{1b} neurons did not give spontaneous action potentials (11 of 17 neurons), but some neurons presented with occasional spontaneous single action potentials (< 6 per min; 6 of 17 neurons). However, sporadic small discrete potentials (< 5 mV) were commonly encountered (see section 11.1.2). Figure 11-1 illustrates the features of the evoked intracellular responses in CA_{1b} neurons. The EPSPs and/or action potentials evoked with direct depolarizing current steps or synaptic activations, were followed by transient membrane hyperpolarizations, which reflected IPSPs and/or afterhyperpolarizations (AHP; cf. Schwartzkroin, 1987). Typically, intracellular recordings could be maintained for periods

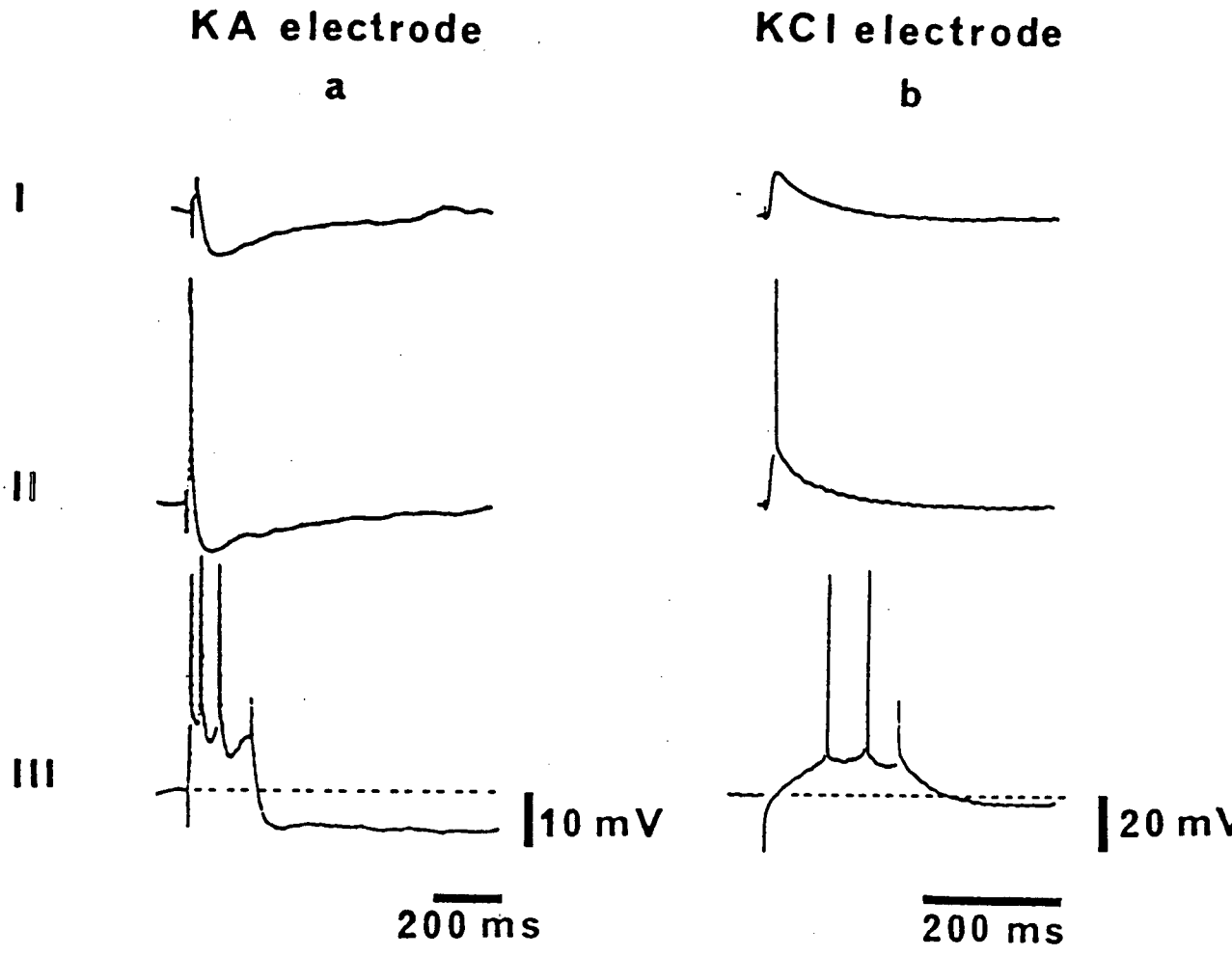

Figure 11-1. Characteristic features of evoked intracellular potentials in the CA_{1b} neurons of the guinea pig hippocampus in vitro. Intracellular potentials in CA_{1b} neurons were evoked by stimulation of the stratum radiatum or by direct depolarizing current injections into these neurons. The evoked intracellular potentials were recorded with micropipettes filled with either 2 M potassium acetate (KA electrode) or 3 M potassium chloride (KCl electrode). The undershoots following the evoked EPSP (Ia) or the synaptically activated action potential (IIa) probably reflected IPSPs and/or afterhyperpolarizations (AHP). Leakage of Cl^- from KCl electrodes caused reversal of IPSPs and, this probably accounts for the apparent increase in duration of the "EPSP" (Ib) or the "hump" following the synaptically activated action potential (IIb) when compared to similar responses in column (a). The occurrence of "reversed IPSPs" tended to mask the underlying AHPs in recordings using KCl electrodes. However, AHPs could clearly be observed after direct depolarizing current injections into CA_{1b} neurons in recordings using both KA electrodes and KCl electrodes (III). [NB: Traces in column (a) were recorded on a strip-chart recorder and, traces in column (b) were photographed directly from the oscilloscope. Some traces have been re-touched to compensate for loss of clarity during photographic reproduction.

of 60-150 min with no significant changes to membrane potentials or input resistances (RMP, start: -63 ± 2 mV, end: -62 ± 2 mV; R_n, start: 31 ± 1.5 MΩ, end: 32 ± 3.1 MΩ, values are Mean ± S.E.M.; n = 17 neurons; $p > 0.05$ by two-tailed paired Student's t-test in both cases). The above results formed the basis for establishing the criteria used to select neuronal impalements for data collections, namely: (1) stable RMP of -60 mV or more negative; (2) R_n greater than 25 MΩ; (3) clear fast EPSPs and synaptically activated action potentials; and (4) direct action potentials induced with depolarizing current injections.

11.1.2 Miniature postsynaptic potentials. Small discrete +ve and -ve membrane potentials were detected in CA_{1b} neurons impaled with KA electrodes (n = 7 neurons). Only small +ve potentials were detected in CA_{1b} neurons impaled with KCl electrodes (n = 10 neurons). These transient postsynaptic responses exhibited varied amplitudes (< 5 mV) and frequencies (1-20 per sec) among different CA_{1b} neurons (n = 17 neurons; Table 11-1). In recordings with KA, 10 μM picrotoxin reduced the frequencies of small -ve potentials by at least 50% and 50 μM picrotoxin abolished all -ve potentials. Recordings with KCl electrode revealed decreases of about 40-60 % in frequencies of +ve potentials in 10 μM picrotoxin, and these decreases were as much as 90% in 50 μM picrotoxin. The changes in the frequencies of small potentials in the presence of picrotoxin were significantly different from controls, as determined by one-way ANOVA with Duncan's multiple comparison tests (n = 17 neurons; quantitative data in Table 11-1). Typically, the effects of picrotoxin were reversible within 15-20 min after returning to control medium. The above small potentials were taken to be simultaneous recordings of "miniature" EPSPs and "miniature" IPSPs (minEPSPs

Table 11-1. Small discrete potentials in CA_{1b} neurons in the guinea pig hippocampus in vitro

	KA electrodes		KCl electrodes
	-ve	+ve	+ve
Control	9 ± 3	3 ± 1	12 ± 5
10 µM picrotoxin	4 ± 2*	3 ± 1	8 ± 2*
50 µM picrotoxin	---	3 ± 1	2 ± 1*
15 min post-drug	8 ± 2	3 ± 1	14 ± 2

Values are Mean ± S.E.M. per sec (numbers rounded up to nearest integer other than 0; n = 7 neurons for recordings with KA electrodes and, n = 10 neurons for recordings with KCl electrodes. In these experiments, each hippocampal slice was exposed to 10 µM picrotoxin (applied for 10 min) followed by 50 µM picrotoxin (applied for 10 min). Subsequently, control medium was reperfused. Astericks indicate significant differences as determined by one-way ANOVA with Duncans' multiple comparisons tests.

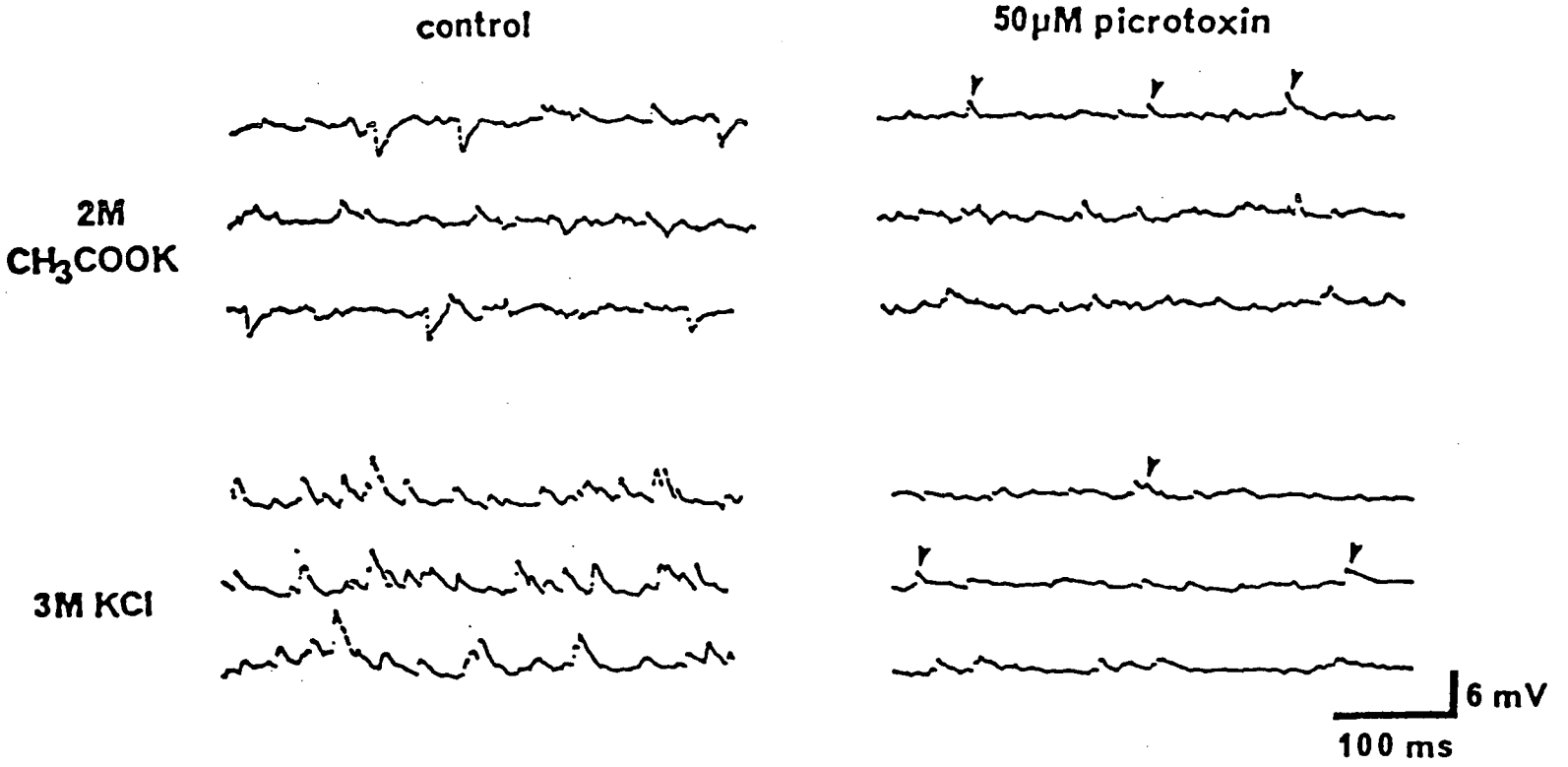

Figure 11-2. Intrasomatic recordings of spontaneous small discrete potentials in CA_{1b} neurons in the guinea pig hippocampus in vitro. Both -ve and +ve spontaneous small potentials were present in recordings with micropipettes filled with potassium acetate (2 M CH_3COOK). However, the -ve spontaneous small potentials were abolished in 50 μM picrotoxin. By comparison, only +ve spontaneous small potentials were present in recordings with micropipettes filled with potassium chloride (3 M KCl) but, as much as 90% of these +ve spontaneous small potentials were abolished in 50 μM picrotoxin. The spontaneous small potentials recorded in the presence of 50 μM picrotoxin (e.g. arrows), with both types of recording micropipettes, were taken to be "miniature" EPSPs in CA_{1b} neurons. The traces were recorded on a strip-chart recorder and, the three sweeps in each set were taken in immediate succession.

and minIPSPs; Figure 11-2). It was inferred that picrotoxin diminished or abolished minIPSPs that were probably due to the quantal release of GABA (cf. Alger and Nicoll, 1980; Brown, Wong and Prince, 1979; Turner, 1988). Recordings with KCl electrodes revealed only small +ve potentials because of the presence of minEPSPs and reversed minIPSPs, the latter being a result of equilibrium shifts in Cl^- conductances due to leakage of Cl^- from microelectrodes.

In separate experiments conducted in 50 μM picrotoxin (different neurons from above), minEPSPs were detected even though tetrodotoxin (1 μM) was present in the medium (n = 4 neurons; Figure 11-3). These results demonstrated that the observed minEPSPs were not all due to presynaptic action potentials. However, tetrodotoxin was not used in subsequent experiments since it was necessary to have activatable afferents (see below). The recorded minEPSPs had amplitudes of < 2 mV (Figure 11-2 and Figure 11-3). But the frequency of spontaneous minEPSPs was found to be low (< 10 per sec) and extremely variable among CA_{1b} neurons (1-20 per min). Occasionally, however, stimulation of the stratum radiatum evoked EPSPs or synaptically activated action potentials that were immediately followed by a burst of minEPSPs. These were denoted as evoked minEPSPs. This discovery motivated the search for experimental methods that could consistently produce evoked minEPSPs. For this reason, Ba^{++} was used to induce the asynchronous release of transmitters in some experiments (Chirwa, 1985; Quastel and Saint, 1988; Silinsky, 1978; [NB: The use of Ba^{++} in these studies was suggested by Dr. Quastel, who has been using Ba^{++} to examine presynaptic functions at the neuromuscular junction].

11.1.3 Recordings with Cs^+ electrodes. Figure 11-4 illustrates typical intracellular responses recorded with micropipettes filled with 3 M CsCl. After cell impalements, membrane potentials gradually shifted to

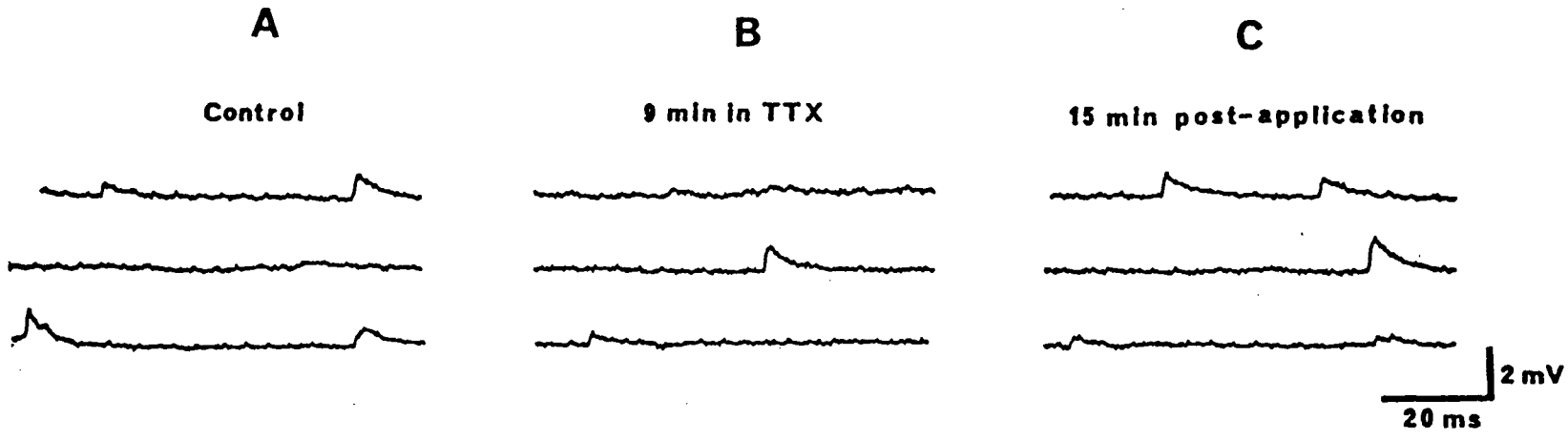

Figure 11-3. The occurence of miniature EPSPs potentials in CA_{1b} neurons in guinea pig hippocampal slices incubated in tetrodotoxin. Spontaneous miniature EPSPs were detected in CA_{1b} neurons both in control medium containing 50 μM picrotoxin (A) or test medium containing 1 μM tetrodotoxin (TTX) and 50 μM picrotoxin (B). Note the decrease in the frequency of spontaneous miniature EPSPs recorded at 9 min after starting TTX applications (i.e. TTX was applied for 10 min in this experiment). The frequency of spontaneous miniature EPSPs returned to pre-TTX levels after about 15 minute washing in control medium (C). The sweeps in each set are of continuous recordings taken from a strip-chart recorder, respectively.

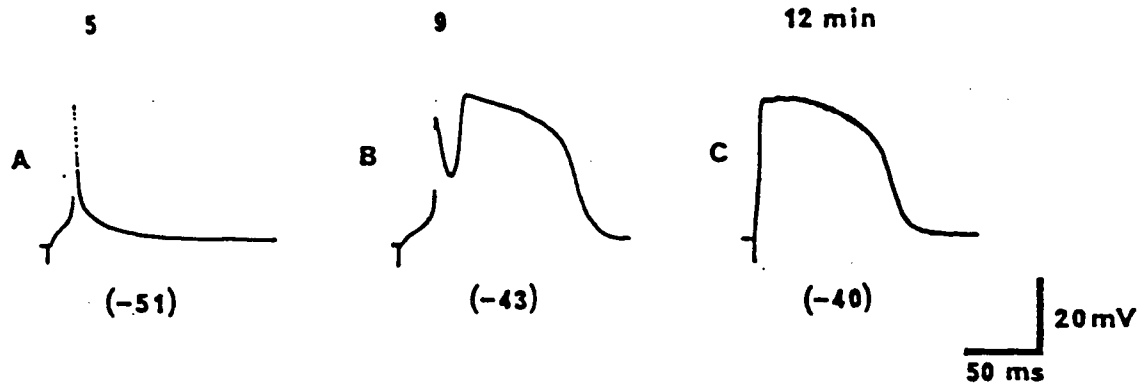

Figure 11-4. Characteristic features of intracellular potentials in a CA_{1b} neuron recorded with micropipettes filled with Cs^+. In (A) is shown a synaptically activated action potential in a CA_{1b} neuron following suprathreshold stimulation of the stratum radiatum, 5 min after cell impalement with a micropipette filled with 3 M CsCl . Four min later (i.e at 9 min) the same suprathreshold stimulation of the stratum radiatum evoked intracellular potentials with two peaks that are, denoted as "I" and "II" in the illustration. The first peak, I, was taken to be a "widened" sodium spike due to blockade of some K^+ currents by internal Cs^+. The second peak, II, was probably a Ca^{++} spike. Subsequently, suprathreshold stimulation of the stratum radiatum (e.g at 12 min after cell impalement) evoked a single, very long-lasting peak (C). [NB: The cell membrane potential in mV at time of each recording are given in brackets.]

depolarized levels (cell depolarizations of 10-50 mV, from initial resting values; n = 10 neurons). In the early stages, membrane depolarizations were associated with increased frequencies of spontaneous action potentials which subsequently stopped, probably due to Na^{+} channel inactivations caused by the prolonged membrane depolarizations. Prolonged subthreshold EPSPs could still be evoked, however, with stimulations of the stratum radiatum (Figure 11-4). In addition, Ca^{++} spikes could be evoked with suprathreshold EPSPs or adequate intracellular depolarizing current injections (0.5-2 nA, 100-200 msec; Figure 11-4).

11.1.4 Features of extracellular responses. Figure 11-5 illustrates features of evoked responses recorded in the CA_{1b} pyramidal cell layer and apical dendritic regions, following stimulation of the stratum radiatum. The evoked field responses in the CA_{1b} pyramidal cell layer exhibited stimulus-dependent biphasic positive waves that could be bisected with negative-going peaks, which were population spikes (Figure 11-5; n = 10 slices). Stimulus strengths between 50-150 μA (0.1-0.8 msec; 0.2 Hz) applied to the stratum radiatum elicited population spikes with amplitudes of 1.0-1.5 mV and onset latencies (i.e., time from onset of artifact to peak negativity of the population spike; Figure 11-5) ranging between 6-10 msec. Evoked field responses recorded in the CA_{1b} apical dendrites presented with negative-going waves which were caused by sinks associated with dendritic field EPSPs. Positive-going peaks could be superimposed on these dendritic field EPSPs (i.e., reflected sinks of population spike in the soma region), by increasing stimulus strengths applied to the stratum radiatum. Both the population spike and the field EPSP could be abolished by transient perfusions with Mg^{++}-Mn^{++} medium (Figure 11-5). During these recordings in Ca^{++}-free medium, however, the presynaptic volleys (especially with

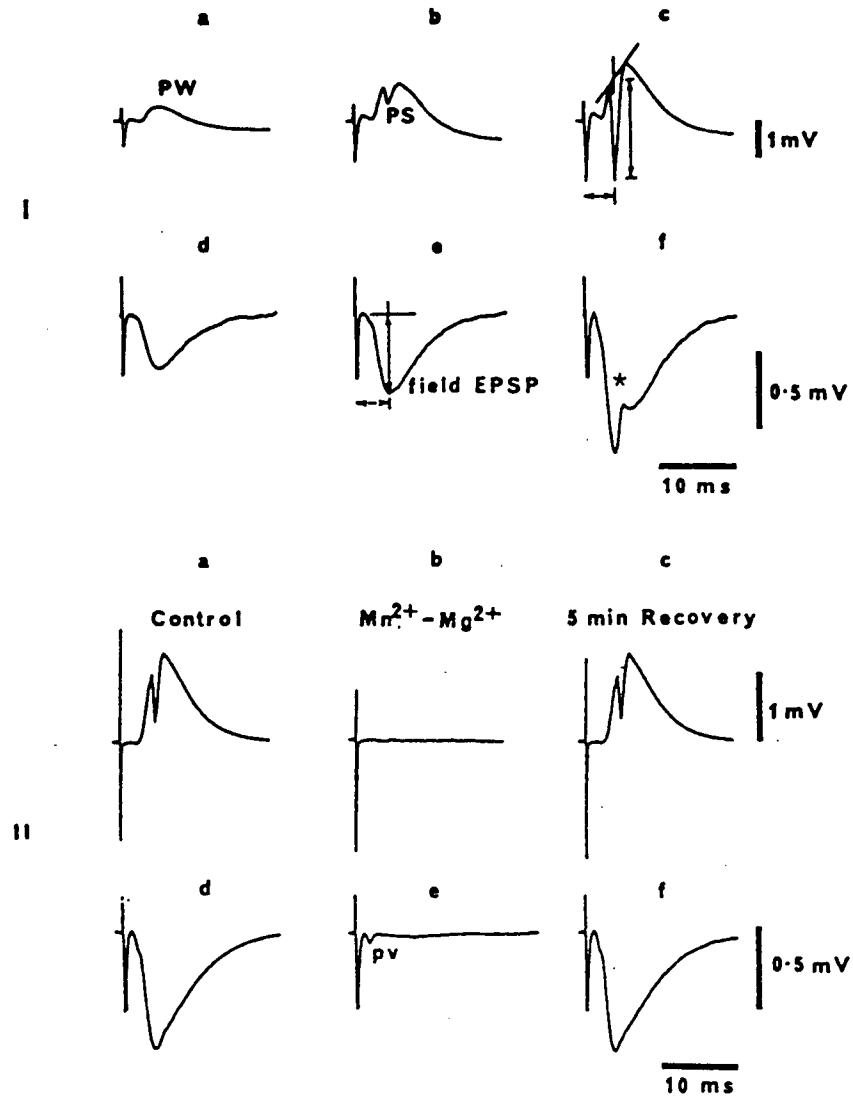

Figure 11-5. Characteristic features of evoked field potentials recorded in the CA_{1b} area following stimulation of the stratum radiatum in the guinea pig hippocampus in vitro. A "weak positive wave" (PW) recorded in the somatic layer (Ia) and a "weak EPSP" recorded in the dendritic region (Id) could be evoked with low stimulation strengths applied to the stratum radiatum (i.e. taken to be stimulation of "weak inputs"). However, higher stimulus strengths evoked population spikes in the pyramidal layer (Ib-c) and dendritic field EPSPs (Ie-f). The onset latencies (horizontal arrows) and amplitudes (vertical arrows) of evoked responses were measured as illustrated in (Ic) for the population spike and in (Ie) for the dendritic field EPSP. The electrical fields generated by the population spike in the somatic layer sometimes appeared as a small peak (*) that contaminated the dendritic field EPSP as shown in (If). In (II) are presented recordings taken from a different slice. Both the somatic population spike (IIa) and the dendritic field EPSP (IId), evoked by stimulation of the stratum radiatum in control medium, were abolished during a 10 min application of Ca^{++}-free medium (IIb and IIe; responses taken at 5 min after starting Mn^{++}-Mg^{++} medium) but, these evoked responses recovered within 5 min after returning to control medium (IIc and IIf). The presynaptic volley (PV) is readily discernable during blockade of synaptic transmission.

dendritic recordings) were clearly discernible (Figure 11-5).

11.2 Saccharin dose-response curves

Saccharin (10-100 mM) competitively antagonises the binding of NGF to its "receptors" and inhibits NGF-dependent neurite growth (Ishii, 1982). Saccharin, therefore, provided a method for screening NGF-dependent activities. This feature was utilized in certain experiments in this thesis (see later sections). In this regard, it was necessary to establish the dose-response characteristics of saccharin in the hippocampus and this is illustrated in Figure 11-6. In these experiments, the population spike in the CA_{1b} area was evoked by stimulation of the stratum radiatum. Saccharin exhibited steep dose-response relationships, suggestive of some specific mechanism of actions. During the initial 2 min of drug application (saccharin was applied for 10 min each time), there were increases in population spikes in the CA_{1b} area evoked by stimulations of the stratum radiatum, at all drug concentrations tested (amplitudes of population spikes as % of controls: 105-117; n = 6 slices) but these were insignificant changes as determined by one-way ANOVA (quantitative data in Figure 11-7). During the last 5-7 min of drug applications, saccharin concentrations > 5 mM but < 20 mM were associated with insignificant decreases of the population spikes in the CA_{1b} area evoked by stimulation of the stratum radiatum (amplitudes of population spikes as a % of controls: 87-92; n = 6 slices; $p > 0.05$, one-way ANOVA; quantitative data in Figure 11-7). However, saccharin concentrations > 20 mM induced significant depressions of the population spike (amplitudes of population spikes as a % of controls: 0-35; n = 6 slices; $p < 0.05$, one-way ANOVA with Duncan's multiple comparisons tests; quantitative data in Figure 11-7). In the case of 80 mM saccharin,

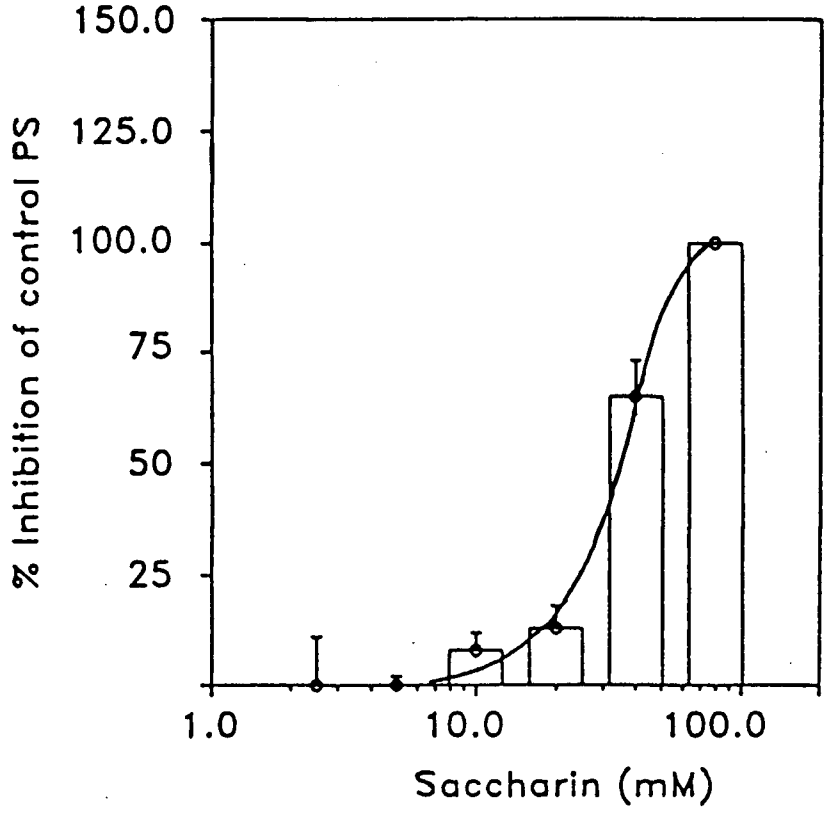

Figure 11-6. Dose-response curves of saccharin obtained by the method of single application, randomised design. Plotted are amplitudes of evoked population spikes in CA_{1b} area recorded during the 9th minute of a 10 min application of saccharin, expressed as a % inhibition of evoked population spikes obtained in control medium (values are Mean ± S.E.M., n = 6 slices). Saccharin presented with a steep dose-response relationship which was distributed within two logarithmic units as illustrated.

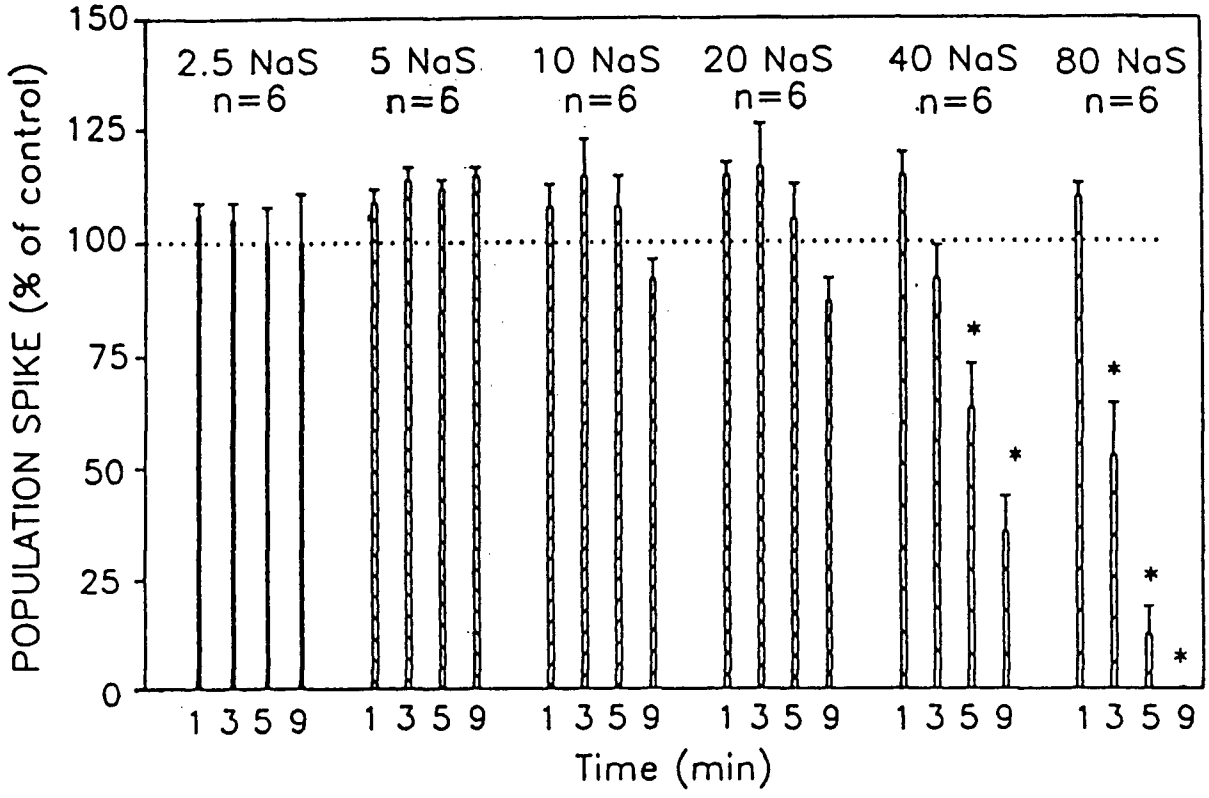

Figure 11-7. Changes in amplitudes of population spikes in CA_{1b} area evoked by stimulation of the stratum radiatum during applications of different doses of saccharin in guinea pig hippocampus in vitro. The graph shows the amplitudes of evoked population spikes recorded during a 10 min application of saccharin, expressed as a of evoked population spikes recorded in control medium. The time intervals along the X-axis illustrate the time when the evoked population spikes were recorded for each concentration of saccharin tested (values are Mean ± S.E.M.). [NB. These are the same experiments from which the dose-response relationships for saccharin were obtained (see Figure 11-6).] The asterisks indicate significant differences between population spikes evoked during saccharin application and population spikes evoked in control medium (n = 6 slices; $p < 0.05$; one-way ANOVA with Duncan's multiple comparisons test).

the evoked population spike was rapidly abolished (Figure 11-7). From these dose-response curves, 10 mM saccharin was selected and used in subsequent experiments since this drug concentration did not induce significant depressions of the population spike during applications.

11.3 Effects of barium in the hippocampus

The Ba^{++} media tested were classified as low (0.5 mM Ba^{++} and 3.5 Ca^{++}), moderate (2 mM Ba^{++} and 2 Ca^{++} mM), and high Ba^{++} media (3.5 mM Ba^{++} and 0.5 Ca^{++})(see Table 9-1 of Chapter 9, for media constituents). Within 1-3 minutes of Ba^{++} perfusions, CA_{1b} neurons became depolarized by 3-20 mV (n = 30 neurons). The onset of these depolarizations was quicker in high Ba^{++} medium than in low or moderate Ba^{++} media. In all applications, Ba^{++} increased R_n by at least 75% (checked in the initial 1-3 min of Ba^{++} applications). Typically, the evoked responses (e.g. EPSPs and action potentials) changed as follows. In low Ba^{++} medium, the amplitudes of evoked intracellular EPSP and associated IPSP were generally increased. However, in moderate and high Ba^{++} media, the initial increases in amplitudes of evoked intracellular EPSP and associated IPSP rapidly diminished. Subsequently, stimulations of the stratum radiatum evoked delayed and staggered synchronous synaptic responses that were followed by bursts of miniature postsynaptic potentials as illustrated in Figure 11-8. Injections of depolarizing current pulses (0.2-1 nA, 50-200 msec) into CA_{1b} neurons caused large depolarizing shifts with bursts of miniature postsynaptic potentials. Continued Ba^{++} perfusions (beyond 4 min) triggered spontaneous membrane depolarizing shifts with spikings (similar to epileptogenic discharges) as illustrated in Figure 11-8. Subsequently, synchronous synaptic responses were abolished, except

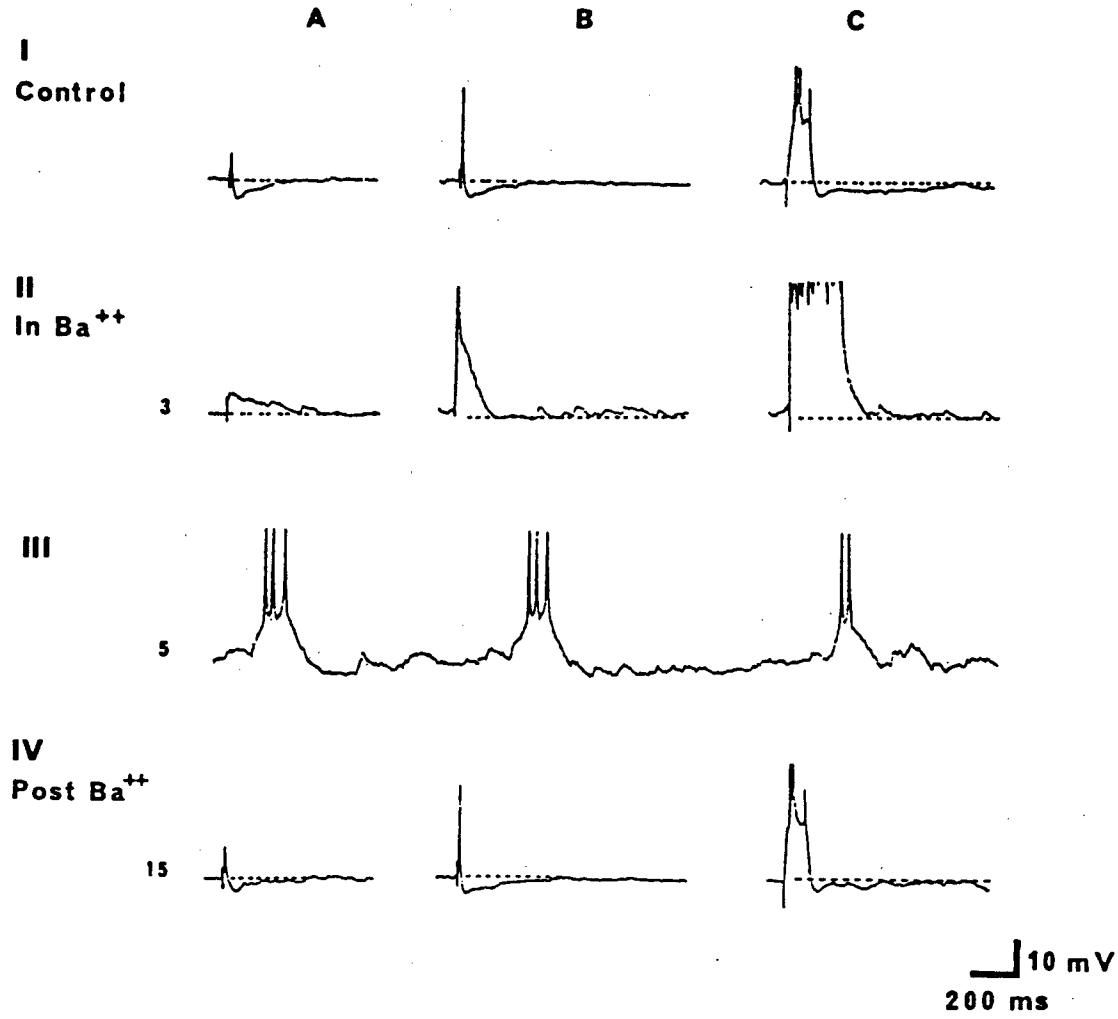

Figure 11-8. Intracellular potentials in CA_{1b} neurons in guinea pig hippocampal slices incubated in barium. The Ba^{++} media tested contained 3.5 mM Ba^{++} and 0.5 Ca^{++}. Typically, an intracellular EPSP (IA) or a synaptically activated action potential (IB), and their associated afterhyperpolarizations (i.e picrotoxin not added to media), were evoked in a CA_{1b} neuron by stimulation of the statum radiatum. In adition, action action potentials were evoked by injection of depolarizing current pulses (0.4 nA, 200 msec) into a CA_{1b} neuron. In Ba^{++} medium (applied for 10 min), stimulation of the stratum radiatum subsequently evoked delayed and staggered synchronous synaptic responses that were followed by bursts of miniature postsynaptic potentials (II, number next to each trace in column A indicates time in min when record taken). Injections of depolarizing current pulses (0.4 nA, 200 msec) into a CA_{1b} neuron evoked several action potentials, followed by a bursts of miniature postsynaptic potentials (IIC). Continued Ba^{++} perfusions triggered spontaneous membrane depolarizing shifts with spikings as shown by the continuous trace in (III). The effects of Ba^{++} applications were essentially reversible within 15 min of re-perfusing with control medium (IV, see also Figure 11-13). [NB: Action potentials truncated by strip-chart recorder. Some traces have been re-touched to compensate for loss of clarity during photographic reproduction.]

in low Ba^{++} medium. However, bursting discharges could still be triggered with stimulations of the stratum radiatum. These bursting discharges made it difficult to visualise miniature postsynaptic responses that might be present.

The effects of Ba^{++} applications were essentially reversible within 15 min of re-perfusing with control medium (Figure 11-8). From the above experiments, high Ba^{++} medium was selected and used in subsequent studies since this Ba^{++} medium exerted its effects relatively early during perfusions. More importantly, high Ba^{++} medium was found to be most efficacious in evoking the asynchronous release of transmitters following stimulations of the stratum radiatum.

11.4 Induction of long-term potentiation

11.4.1 Tetanic stimulations. Single high frequency tetanic stimulations of the stratum radiatum (400Hz, 200 pulses) induced synaptic LTP of the population spike and field EPSPs in the CA_{1b} area (% increases in amplitudes: population spikes, 250-600; field EPSPs, 150-200; 10 of 10 slices). Typically, LTP development was preceded by post-tetanic potentiations (PTP) that rapidly decayed in 3-5 min, revealing the underlying long-lasting synaptic potentiation as illustrated in Figure 11-9. The potentiated responses were associated with reductions in onset latencies, and LTP showed little or no decay even after 60 min as illustrated in Figure 11-10. With intracellular recordings, LTP in 9 out of 10 CA_{1b} neurons was observed as increases in the amplitudes of subthreshold intracellular EPSPs (intracellular EPSPs as a % of control 15 min after induction of LTP: 167 ± 7.9; values are Mean ± S.E.M.; $n = 10$ neurons; $p < 0.05$; one-tailed paired Student's t-test). In most experiments, previously subthreshold EPSPs reached threshold after LTP development as illustrated in Figure 11-10

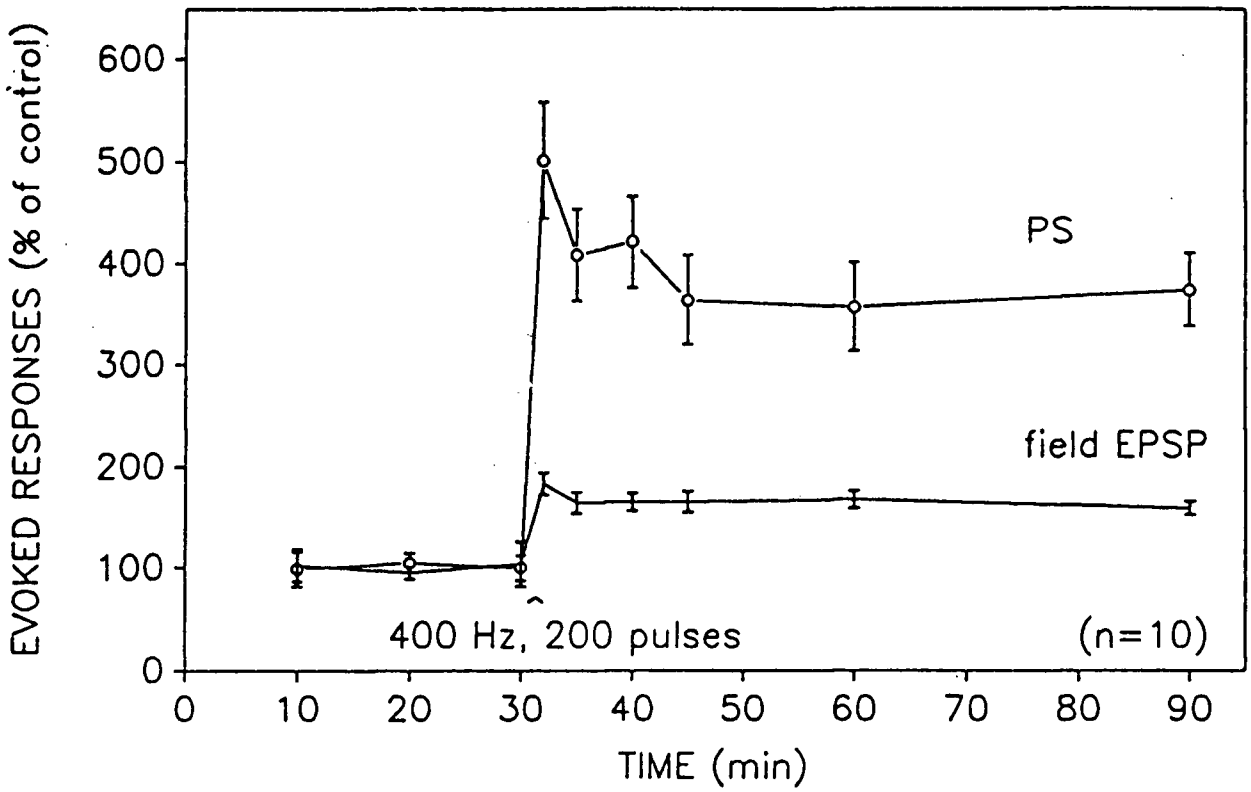

Figure 11-9. Illustration of long-term potentiation induced by high frequency tetanic stimulations of the stratum radiatum in guinea pig hippocampus in vitro. The graph shows both the population spike (PS) recorded in the CA_{1b} pyramidal layer and the field EPSP (field EPSP) recorded in the dendritic region evoked by stimulation of the stratum radiatum in these experiments. Tetanic stimulation of the stratum radiatum subsequently induced post-tetanic potentiations (*) that decayed within 5 min to reveal the underlying long-term potentiation of both the population spike and the field EPSP. Note that LTP was present throughout the one-hour 1 observation period (values are Mean ± S.E.M.).

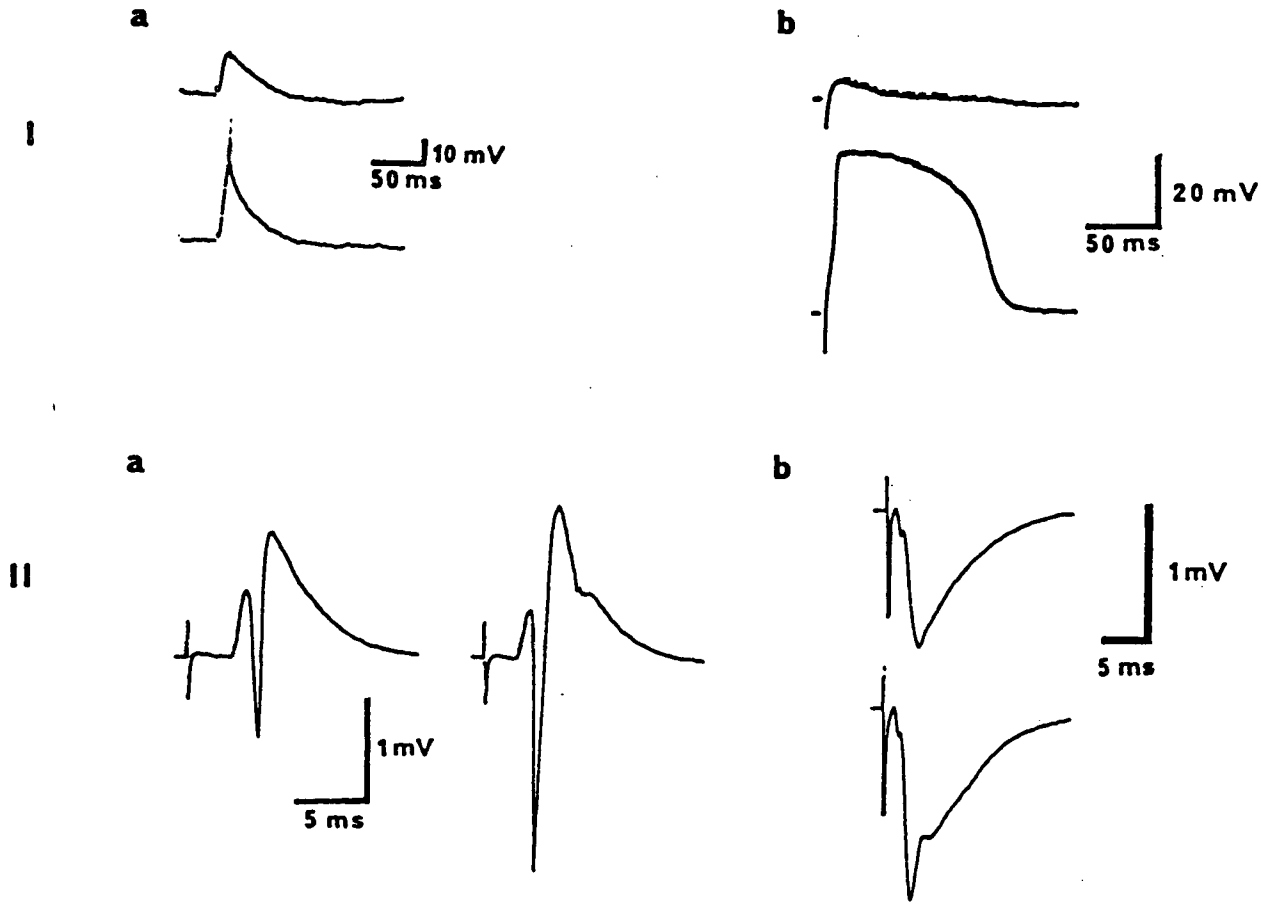

Figure 11-10. Representative recordings of intracellular and extracellular potentials in CA_{1b} area evoked by stimulation of the stratum radiatum before and after induction of long-term potentiation. In (I) are shown pre- and post-tetanus intracellular potentials in representative CA_{1b} neurons recorded with micropipettes filled with 3 M potassium chloride (Ia) or 3 M cesium chloride (Ib). Note that in both cases, LTP is seen as increases in previously subthreshold intracellular EPSPs sufficient to evoke a synaptically activated Na^+ spike (Ia) or a presumed Ca^{++} spike (Ib). In (II) are shown pre- and post-tetanus population spikes (IIa) recorded in the CA_{1b} pyramidal layer and field EPSPs (IIb) recorded in the CA_{1b} dendritic region. The reduction in onset latencies of the potentiated synaptic responses is discernable in the field responses (II).

(6 of 9 neurons). In these neurons, resting membrane potentials and input resistances recorded in the soma remained unchanged during LTP (RMP, before: -63.4 ± 1.3, after: -63 ± 2.2; R_n in MΩ: before: 32.5 ± 2.5, after: 32.2 ± 1.9; values are Mean ± S.E.M.; n = 9 neurons; $p > 0.05$ as determined by two-tailed paired Student's t-test). These results are in agreement with those reported by Andersen et al. (1980c). In separate CA_{1b} neurons recorded with micropipettes filled with Cs^+ (same neurons described in section 14.1.3), tetanus-induced LTP was expressed as increases in subthreshold EPSPs in 7 of 10 neurons tested (intracellular EPSPs as a% of control 15 min after induction of LTP: 150.8 ± 5.7; values are Mean ± S.E.M.; n = 10 neurons; $p < 0.05$; one-tailed paired Student's t-test), even reaching threshold for Ca^{++} spikes (4 of 7 neurons) as illustrated in Figure 11-10. The above results demonstrated that LTP could still be induced even if K^+ effluxes in a neuron were diminished by Cs^+ applied internally.

11.4.2 Paired depolarizations. In these experiments, slices were incubated in physiological medium containing 50 μM picrotoxin, to facilitate LTP development (Sastry, Goh and Auyeung, 1986; Wigstrom, et al., 1986). Repeated pairings (10-15 pairings) at 0.2 Hz of intracellular depolarizations of a CA_{1b} neuron (3-7 nA, 200 msec) with subthreshold stimulations of the stratum radiatum (30-100 μA, 0.1-0.5 msec; stimulus strength adjusted to 50-60% of orthodromic threshold) resulted in subsequent long-lasting increases in amplitudes of intracellular EPSP evoked by stimulation of the stratum radiatum (intracellular EPSPs as a % of control 15 min after simultaneous pre- and postsynaptic activations: 146.6 ± 8.3; values are Mean ± S.E.M.; n = 6; $p < 0.05$; ANOVA with Duncans' multiple comparisons tests; more quantitative data in Figure 11-11). This post-pairing LTP of

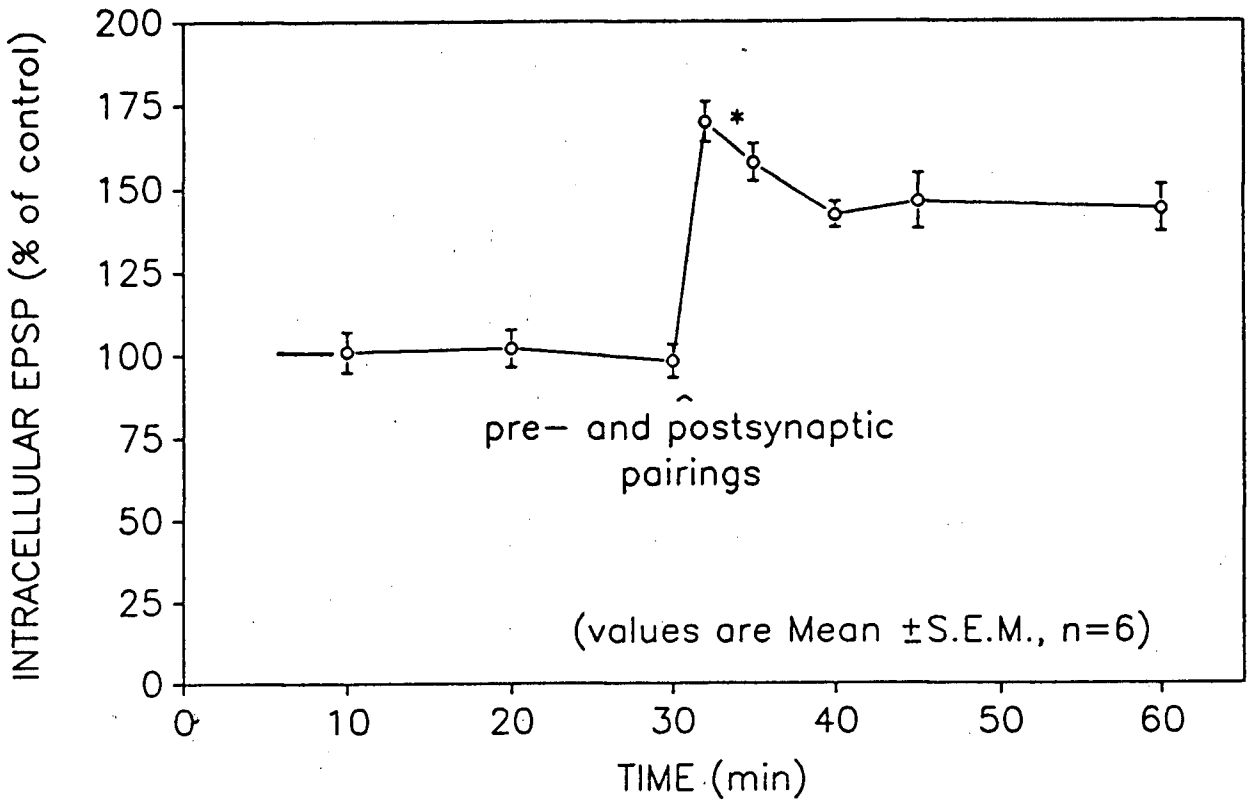

Figure 11-11. Illustration of long-term potentiation induced by simultaneous pairings of conditioning depolarizing current injections into CA_{1b} neurons and stimulation of the stratum radiatum in guinea pig hippocampus in vitro. The graph shows the amplitudes of intracellular EPSP recorded in CA_{1b} neurons evoked by stimulation of the stratum radiatum. Consecutive 10-15 pairings of conditioning intracellular current injections into CA_{1b} neurons with activations of the stratum radiatum at the beginning of each intracellular depolarization, subsequently induced short-term potentiations (*) that decayed within 3-6 min to reveal the underlying long-term potentiation of the intracellular EPSPs (i.e evoked by single stimulations at 0.2 Hz of the stratum radiatum). The potentiated intracellular EPSPs were maintained throughout the 30 min observation period. Picrotoxin (50 μM) was present in the medium.

evoked responses to stimulation of the stratum radiatum was present for periods beyond 30 minutes (Figure 11-11).

11.5 Asynchronous release of transmitter and LTP

During 2-4 min applications of high Ba^{++} medium, short bursts of minEPSPs (minIPSPs and IPSPs were blocked with 50 μM picrotoxin) were observed following single stimulations of the stratum radiatum or after single conditioning depolarizing current injections (0.01-0.2 Hz in both cases) into CA_{1b} neurons (n = 26 neurons; see Table 11-2 for quantitative data). However, the frequencies of evoked minEPSPs in Ba^{++} were increased by at least 50% following the pairings of conditioning depolarizing current injections into CA_{1b} neurons with concurrent stimulation of the stratum radiatum ($p < 0.05$; n = 26 neurons; one-tailed Student's t-test; see quantitative data in Table 11-2). [NB: Stimulation of the stratum radiatum was initiated at the beginning of the direct intracellular depolarization.] These transient bursts of minEPSPs were greatly exaggerated with increasing pairings of conditioning depolarizing current injections into CA_{1b} neurons and stimulations of the stratum radiatum, such that it was not possible to quantify them accurately.

Increases in evoked minEPSPs frequencies were also observed during LTP produced by tetanic stimulations. If hippocampal slices (with the CA_2-CA_3 cell body layer removed) were exposed to high Ba^{++} medium for 2 min, stimulation of stratum radiatum at 0.02-0.2 Hz resulted in a burst of evoked minEPSPs that followed the synchronous EPSP or the synaptically activated action potential (24 of 31 neurons). Slices were then re-exposed to control medium for 15 min and the stratum radiatum was tetanized (400 Hz, 200 pulses; stimulation strength adjusted to evoke subthreshold EPSP only) to induce LTP (18 of the above 24 neurons). LTP was detected as long-last-

Table 11-2. Changes in frequencies of evoked minEPSPs in CA_{1b} neurons during simultaneous pairings of conditioning depolarizing current injections into CA_{1b} neurons and stimulation of the stratum radiatum in guinea pig hippocampus in vitro

	minEPSP amplitudes in mV			
	0.25 - 0.5	0.5 - 1.0	1.0 - 1.5	1.5 - 2.0
Unpaired	11 ± 2	7 ± 2	1 ± 1	---
Paired	17 ± 3*	11 ± 3*	4 ± 2*	1 ± 1

Values are Mean ± S.E.M. per 5 sec (n = 26 neurons). Astericks indicate significant differences as determined by one-way ANOVA with Duncans' multiple comparisons tests. In these experiments, 50 μM picrotoxin was present in the medium. [NB: Numbers rounded up to the nearest integer other than 0.]

ing post-tetanus increases of previously subthreshold EPSPs (intracellular EPSPs as a % of control 15 min after simultaneous pre- and postsynaptic activations: 161.0 ± 10.8; values are Mean ± S.E.M.; $n = 18$; $p < 0.05$; one-tailed paired Student's t-test), sometimes sufficient to reach threshold and elicit orthodromic action potentials following stimulation of the stratum radiatum. When slices were re-exposed to high Ba^{++} medium 15 min after the induction of LTP, the number of minEPSPs following stimulation of the stratum radiatum was at least doubled (evoked minEPSP frequencies, before LTP: 6 ± 3 per 5 sec, after LTP: 22 ± 7 per 5 sec; values are Mean ± S.E.M; $n = 18$ neurons; $p < 0.05$, two-tailed paired Students' t-test; Figure 11-12). The presynaptic volleys during the second Ba^{++} applications were not different from those during the first applications, indicating that the increases in minEPSPs were not due to the activation of more presynaptic axons. Furthermore, if slices were exposed to high Ba^{++} medium twice with a 30 min interval without the LTP-inducing tetanus, the frequencies of minEPSPs were not significantly increased during the second application (evoked minEPSP frequencies; during first Ba^{++} application: 7 ± 2; during second Ba^{++} application: 6 ± 2 per sec; values are Mean ± S.E.M.; $n = 6$ neurons; $p > 0.05$, two-tailed paired Students' t-test). The input resistances determined during the last min of Ba^{2+} applications increased by at least 75% relative to controls, and the EPSPs and/or action potentials "widened" as illustrated in Figure 11-13. But input resistances checked in Ba^{2+} at fixed intervals following the EPSP evoked with stimulation of the stratum radiatum remained unaltered before and after LTP development (R_n in MΩ determined at 100 msec from start of stimulation of the stratum radiatum in Ba^{++}, before LTP: 56.3 ± 2.9; 15 min after induction of LTP: 58.2 ± 3.6; $n = 6$; $p > 0.05$; two-tailed paired Students' t-test).

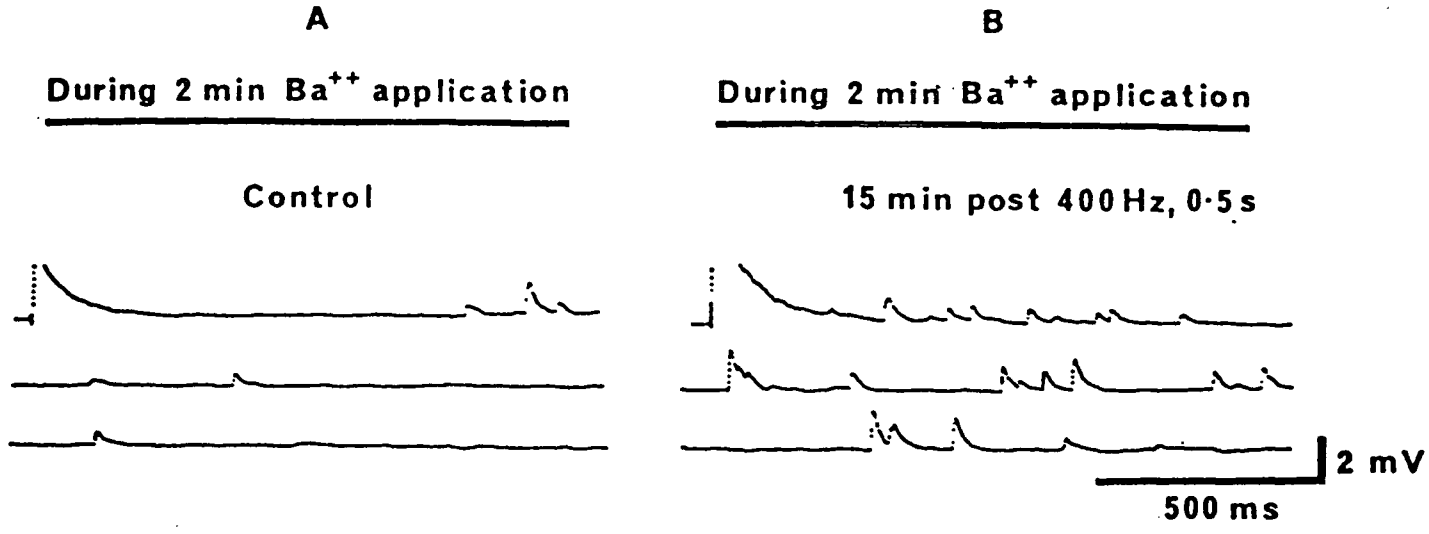

Figure 11-12. Comparisons of the frequency of minEPSPs in CA_{1b} neurons following stimulation of the stratum radiatum before, and after development of long-term potentiation in guinea pig hippocampal slices incubated in Ba^{++}. During a 2 min Ba^{++} application the synaptically activated action potential (truncated) was followed by a burst of minEPSPs (A). [NB: In these experiments spontaneous minEPSPs were usually not present in the control medium or in Ba^{++} medium in the absence of stimulation of the stratum radiatum.] After 15 min of washing out Ba^{++} the stratum radiatum was tetanized (400 Hz, 200 pulses; stimulation strength adjusted to evoke a subthreshold EPSP) to induce LTP. Then 15 min after the induction of LTP (i.e. detected as a long-lasting post-tetanic increase in amplitude of a previously subthreshold EPSP), the slice was re-exposed to a second 2 min Ba^{++} application (i.e. an interval of 30 min between Ba^{++} applications). The number of minEPSPs immediately following the synaptically activated action potential in Ba^{++} was more than doubled during LTP (B) (i.e. same stimulation parameters as in (A). The traces in (A) and (B) were recorded on a strip-chart recorder during the last 30 s of Ba^{++} application. In (A) and in (B) the sweeps were taken in immediate succession. Picrotoxin (50 μM) was present in the medium.

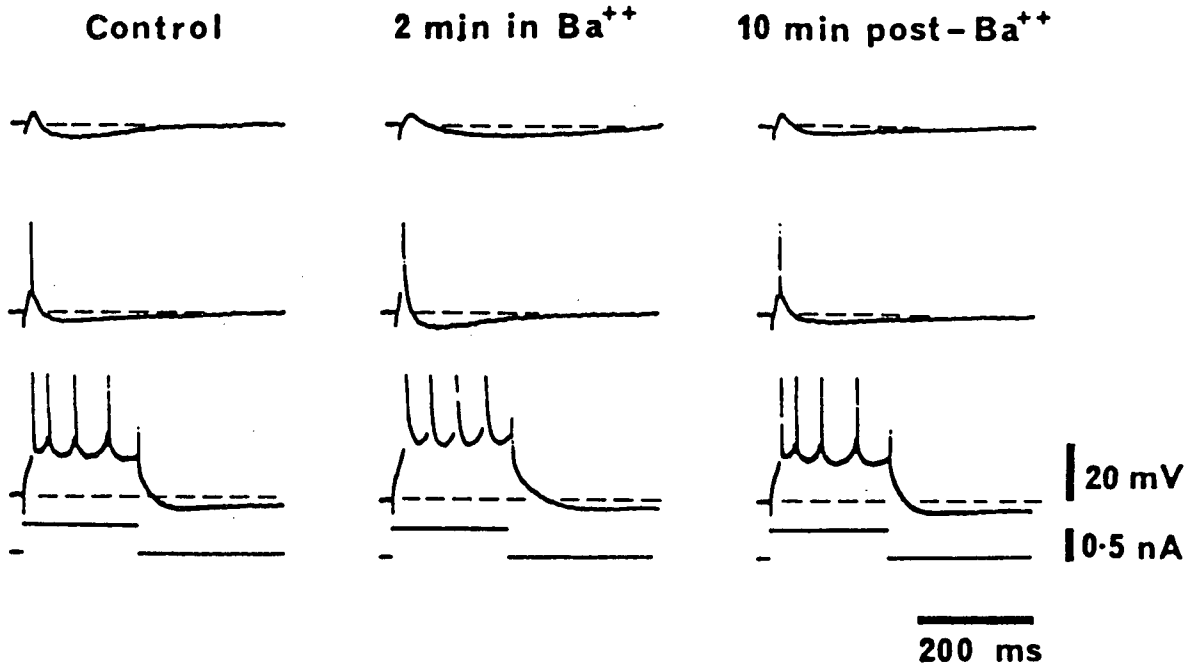

Figure 11-13. Representative changes in evoked intracellular responses in a CA_{1b} neuron in guinea pig hippocampal slices incubated for 2 min in Ba^{++}. The intracellular EPSP (first row) and synaptically driven orthodromic action potential (second row) were evoked following subthreshold and suprathreshold stimulation of the stratum radiatum, respectively. In addition, action potentials in this neuron were also elicited with intracellular depolarizing current pulses (third row; current traces shown in fourth row). The undershoots associated with responses in rows 2 and 3 probably were a mixture of both IPSPs and afterhyperpolarizations (AHP_{++}) [NB: Picrotoxin was not added to the media.] During a 2 min Ba^{++} application, the durations of the EPSP, the synaptically activated action potential, the action potential due to current injections, and all their associated undershoots (IPSPs and/or AHPs) were increased (middle columns in rows 1-3). Furthermore, during Ba^{++} application, note the increase in input resistance which is reflected as an increase in the magnitude of the membrane depolarizing response to intracellular current injection (middle trace versus initial or last traces of row 3). The observed changes during Ba^{++} application were presumably due to Ba^{++} mediated blockade of some K^{+} effluxes from presynaptic and postsynaptic regions. However, the changes in evoked responses during Ba^{++} were reversible as early as 10 min post-application (last column in row 1-3).

11.6 Endogenous substances and synaptic potentiation

Applications of samples collected during tetanic stimulations of the guinea pig hippocampus in vivo (denoted as "THS") for 2 min onto hippocampal slices during stimulations of stratum radiatum at 0.2 Hz subsequently produced synaptic LTP (population spike in CA_{1b} area as a % of control, 15 min after exposure: 130.5 ± 4.8, values are Mean ± S.E.M.; n = 8 slices, $p < 0.05$, one-way ANOVA with Duncans' multiple comparisons tests; Table 11-3). The potentiated synaptic responses had reduced onset latencies (decreases of 10-30 % relative to controls), and LTP was present beyond 60 min (quantitative data in Table 11-3; Figure 11-14). In contrast, applications of HTHS (i.e. heated THS; see methods in chapter 10) (n = 6 slices) or of samples collected in the absence of tetanic stimulations of the guinea pig hippocampus in vivo (denoted as "UHS") had insignificant effects on the population spikes in CA_{1b} area evoked by stimulation of the stratum radiatum (one-way ANOVA, quantitative data in Table 11-3).

Similarly, applications of samples collected during tetanic stimulations of the rabbit neocortex in vivo (denoted as "TNS") caused LTP of the population spike in the CA_{1b} area induced by stimulation of the stratum radiatum (population spike expressed in mV 15 min after exposure: 2.64 ± 0.22, values are Mean ± S.E.M., n = 16, $p > 0.05$, ANOVA with Duncan's multiple comparison tests, quantitative data in Figure 11-15), with associated reductions in onset latencies (onset latencies of population spike as a % of control 15 after induction of LTP: 86.3 ± 4.8; values are Mean ± S.E.M., n = 16, $p < 0.05$; one-tailed unpaired Student's t-test). Neocortical samples collected in the absence of tetanic stimulation (UNS; n = 10 slices) and those TNS fractions that were pre-heated and cooled (HTNS; n = 10 slices) did not produce significant potentiations (quantita-

Table 11-3. Effects of samples collected from guinea pig hippocampus in vivo on CA_{1b} population spike in guinea pig hippocampus in vitro

		Control	Post-application			
		30	10	15	30	60 min
THS:	Mean	100.6	136.6*	130.5*	138.7*	138.0*
	S.E.M.	1.5	3.3	4.8	3.2	3.1
	n	8	8	8	8	8
UHS:	Mean	100.0	97.0	102.0	99.3	103.0
	S.E.M.	2.7	3.3	3.2	1.5	2.0
	n	6	6	6	6	6
HTHS:	Mean	99.5	102.0	98.0	101.3	98.0
	S.E.M.	2.8	1.7	1.8	2.3	2.7
	n	6	6	6	6	6

Reported values are amplitudes of population spikes expressed as of control. Astericks indicate significant differences (relative to control responses) as determined by one-way ANOVA with Duncans' multiple comparisons tests. Abbreviations: THS, tetanized neocortical sample; UHS, untetanized neocortical sample; and HTHS, heated-tetanized neocortical sample.

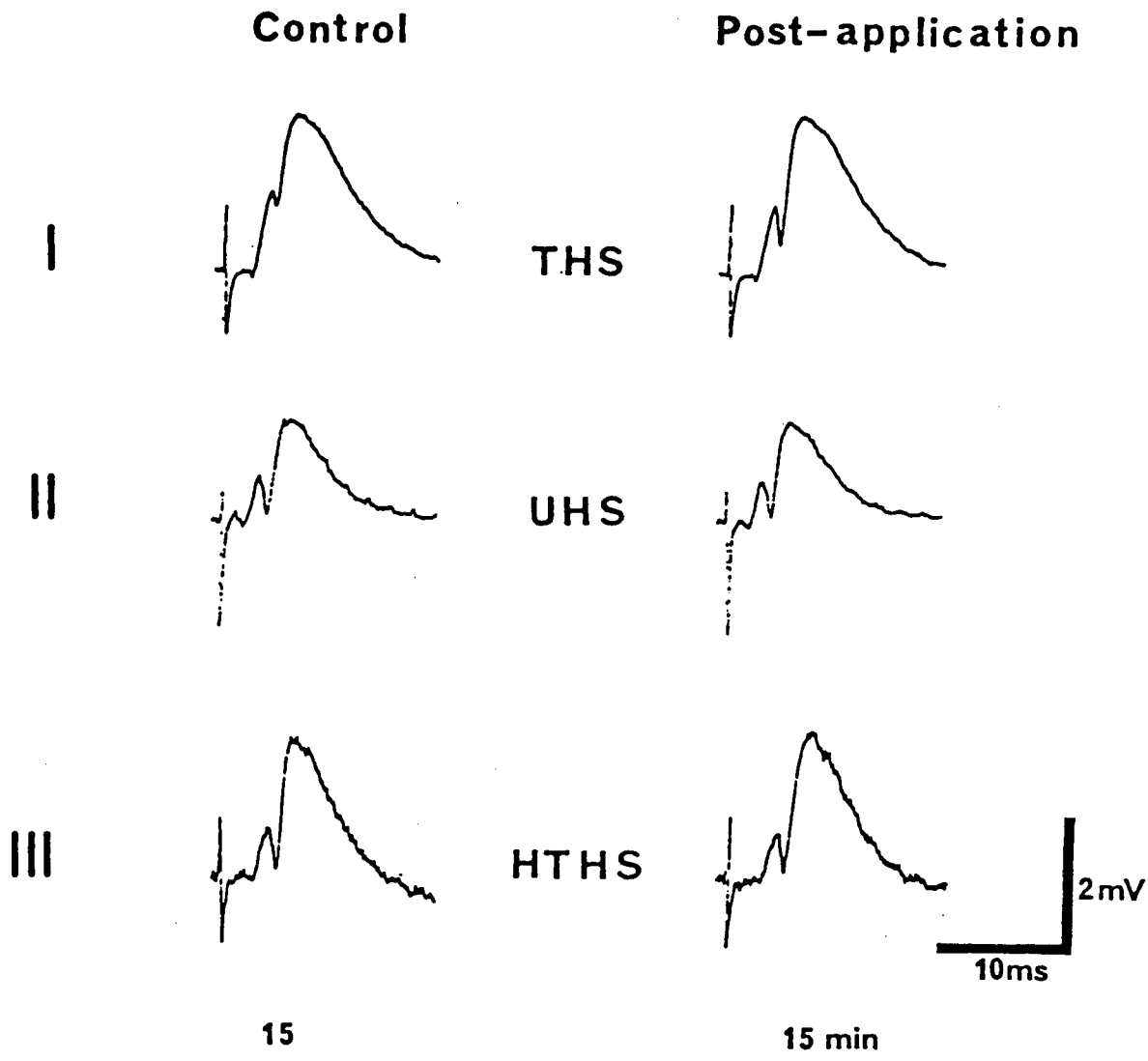

Figure 11-14. Illustration of long-term potentiation in CA_{1b} area of the guinea pig hippocampus in vitro induced with brief applications of samples collected during tetanic stimulations of the guinea pig hippocampus in vivo. The effects of samples collected from guinea pig hippocampus in vivo on population spike in CA_{1b} area evoked by stimulation of the stratum radiatum in representative experiments. Each sample was applied in a different slice. Application of 2ml for 2 min of THS (I) but not UHS (II) or HTHS (III) caused LTP (post-application records taken 15 min after return to control medium).

tive data in Figure 11-15). Presynaptic volleys were not altered by either TNS or THS. In addition, TNS applications without concomitant stimulations of the stratum radiatum caused insignificant changes in evoked synaptic responses (population spikes expressed in mV: controls, 1.03 ± 0.20; and 15 min after application of TNS without stimulation of the stratum radiatum: 0.99 ± 0.17; values are Mean ± S.E.M., n = 6; $p > 0.05$, two-tailed paired Student's t-test). When slices were exposed to TNS in the last 2-3 min during saccharin (10 mM, applied for 10 min) LTP of the population spike in the CA_{1b} area was not induced (population spikes expressed in mV: controls, 1.31 ± 0.14; and 15 min after application of saccharin/TNS: 1.22 ± 0.16; values are Mean ± S.E.M., n = 6 slices; $p > 0.05$, one-way ANOVA, Figure 11-16). When TNS was subsequently applied without concurrent applications of saccharin, LTP of the CA_{1b} population spike was observed (population spikes expressed in mV: controls, 1.34 ± 0.30; and 15 min after exposure to TNS: 2.53 ± 0.35; values are Mean ± S.E.M., n = 6 slices, $p < 0.05$, ANOVA with Duncan's multiple comparisons tests). In separate controls, 2 min applications of glutamate (100 μM) caused post-application depressions of population spikes in CA_{1b} area by 30-70% that lasted for 10-35 min (n = 5 slices). If glutamate samples were pre-heated and cooled (as in the case of HTHS or HTNS) before being applied on hippocampal slices the actions of glutamate were not altered (population spikes expressed as % of controls 15 min after: exogenous glutamate, 73.6 ± 10; and following heated then cooled exogenous glutamate, 71 ± 8.2; n = 5 slices; values are Mean ± S.E.M.; $p > 0.05$; two-tailed Student's t-test), suggesting that the LTP inducing substances in TNS could not have been endogenous glutamate. In other controls, atropine (100 μM; n = 4) or dihydro-β-erythroidine (100 μM; n = 4) did not block LTP when TNS was applied in the last 2-3 min of 10 min

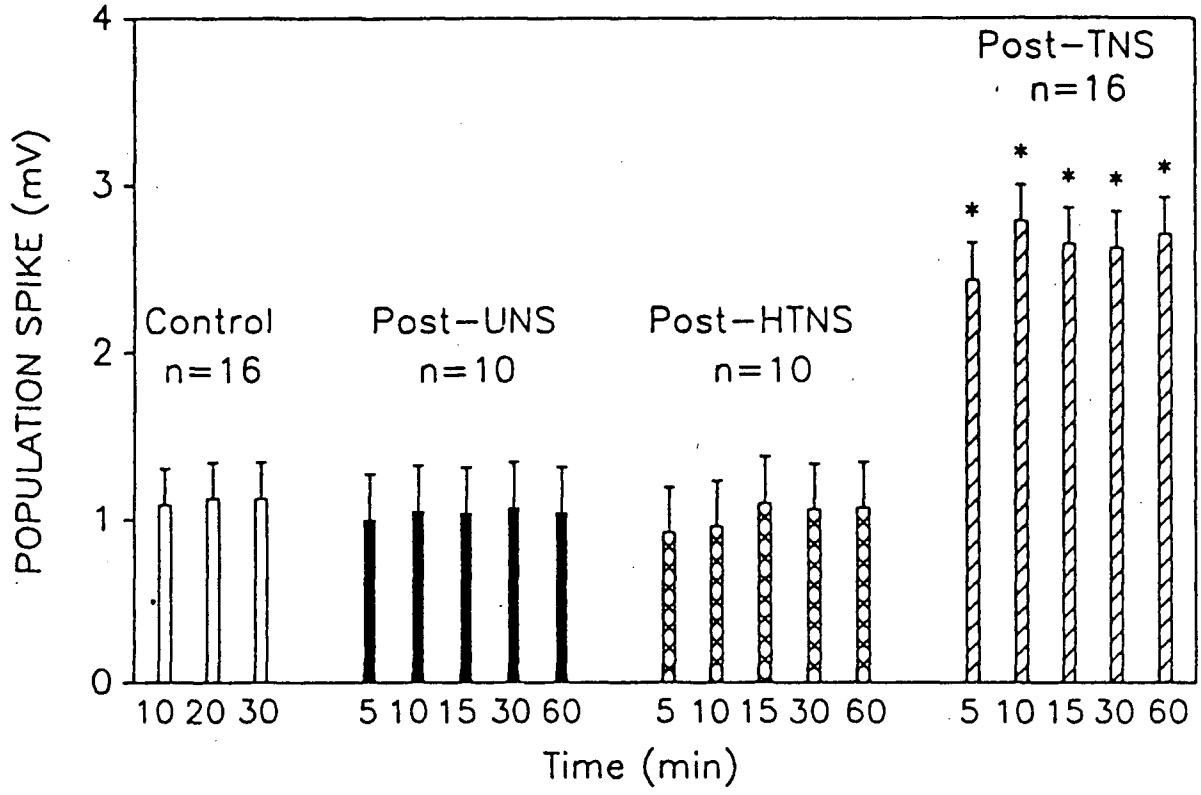

Figure 11-15. Effects of samples collected from rabbit neocortex in vivo on CA_{1b} population spike in guinea pig hippocampus in vitro. The effects of samples collected from rabbit neocortex in vivo on the population spike in CA_{1b} area evoked by stimulation of the stratum in representative slices are shown. The graph shows Mean ± S.E.M. of the population spike amplitudes (in mV). Note that TNS but not UNS or HTNS caused LTP (asterisks indicate significant differences at $p < 0.05$; one-way ANOVA with Duncan's multiple comparisons test).

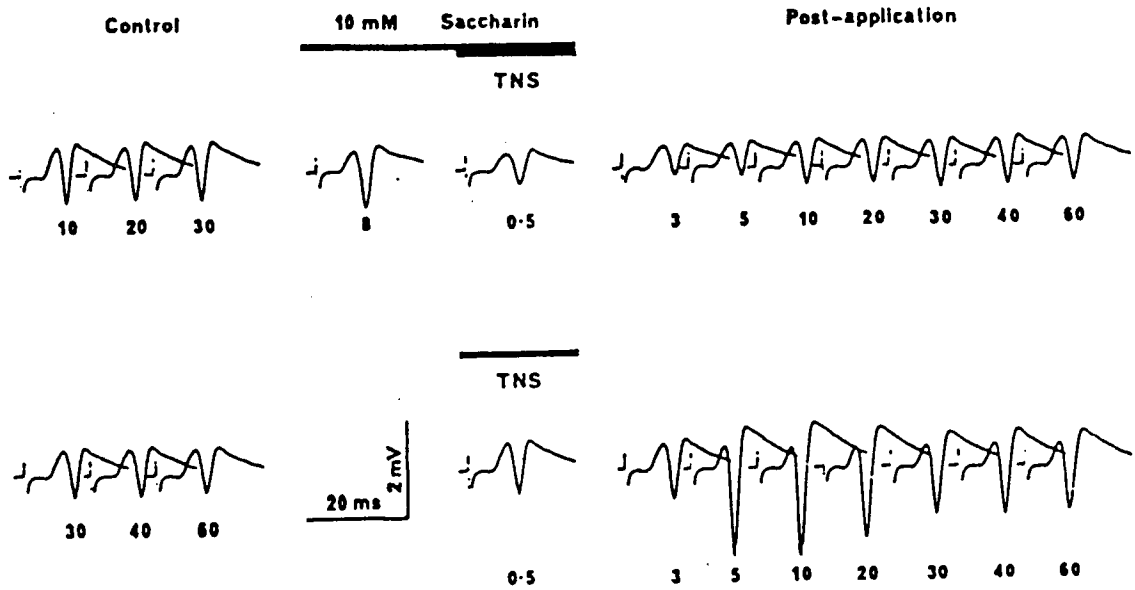

Figure 12-16. Failure to induce long-term potentiation in guinea pig hippocampus in vitro when samples collected during tetanic stimulation of the rabbit neocortex in vivo are applied in the presence of saccharin. The population spike in CA_{1b} area were evoked with stimulation of the stratum radiatum. The top row shows evoked population spikes obtained in control medium, followed by population spikes evoked during a 10 min application of 10 mM saccharin and TNS (i.e. 2 ml of TNS was applied during the last 2 min of saccharin applications), and population spikes recorded after these drug applications. The last three population spikes in the first row (i.e. at 30, 40 and 60 min responses) are shown again at the beginning of the second row. These responses are followed by subsequent population spikes obtained during application of TNS without saccharin, and population spike obtained after the TNS application. Each population spike is an average of 8 consecutive records (0.2 Hz). The time (in min) at which the averaging of the 8 records was initiated are shown underneath each response. Note that TNS applied alone, but not during saccharin, caused LTP.

perfusions of atropine or dihydro-β-erythroidine (population spikes expressed as % of control 30 min after exposure of TNS in the presence of (1) atropine: 203.5 ± 35.0; or (2) dihydro-β-erythroidine; 226 ± 46.3; values are Mean ± S.E.M., n = 4 in each case; $p < 0.05$, one-tailed paired Students' t-test). These results suggested that endogenous acetylcholine that might have been present in TNS, was not responsible for the potentiating effects of THS or TNS. However, TNS contained heat-sensitive substances, probably macromolecules, that were involved in potentiating synaptic transmissions.

Interestingly, 10 mM saccharin applied for 10 min blocked the development of tetanus induced LTP, if the high frequency trains (400 Hz, 200 pulses) were delivered to the stratum radiatum during the last min of saccharin applications (population spikes expressed in mV: controls, 1.11 ± 0.33; and 30 min after tetanic stimulations in saccharin: 1.13 ± 0.47; Figure 11-17). But a subsequent tetanus given in the absence of saccharin produced LTP in the same slices (population spike in mV, 30 min post-tetanus: 2.84 ± 0.44; values are Mean ± S.E.M.; n = 9 slices; $p < 0.05$; ANOVA with Duncan's multiple comparisons test; Figure 11-17).

11.7 Effects of rabbit neocortical samples on cultured PC-12 cells

11.7.1 PC-12 cell growth and neurite induction. The objective of these studies was to examine whether the collected rabbit neocortical samples (see methods in chapter 10) contained neurite-inducing factors. For quantification, PC-12 cells were considered to have developed neurites if they presented with one or more extensions that were longer than the diameter of the cell bodies. On day 2 after plating PC-12 cells in various stages of growth were in small evenly dispersed clusters that were anchored to the floor of each culture dish. None of these cultures showed any

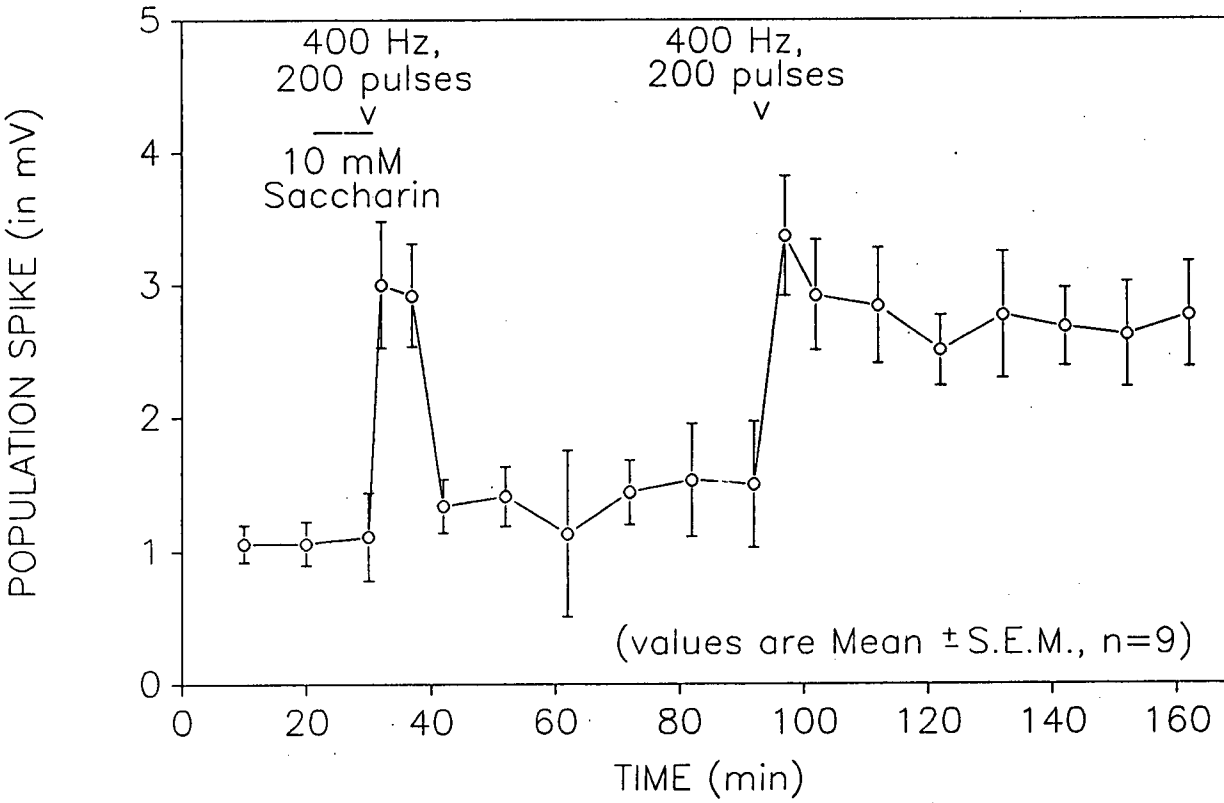

Figure 11-17. The blockade of tetanus-induced long-term potentiation by saccharin in the guinea pig hippocampus in vitro. The graph shows the population spike in CA_{1b} area evoked by stimulation of the stratum radiatum. Tetanic stimulation of the stratum radiatum during a 10 min application of 10 mM saccharin did not cause LTP. However, the same tetanus produced LTP when delivered to the stratum radiatum 60 min after application of saccharin.

significant neurite growth. When PC-12 cell cultures were subsequently incubated in different feeding media, the following results were obtained. PC-12 cell cultures incubated with TNS (n = 6) showed neurite growth on day 3, and these neurites continued to grow throughout the eight-day observation period. However, PC-12 cells did not develop any significant neurites when incubated in feeding media containing (1) TNS with saccharin (10 mM; n = 6), (2) HTNS (n = 6), or (3) UNS (n = 6). Similarly, PC-12 cells that were incubated in plain growth medium throughout these experiments also exhibited insignificant neurite extensions. Some PC-12 cells incubated in growth media containing UNS or HTNS presented with small protrusions (a fraction of the PC-12 cell diameter in length) that seemed to have failed to develop into neurites. Figure 11-18 illustrates the results obtained with PC-12 cell cultures. These results suggested that substances with NGF-like activities were present in the samples collected during tetanic stimulations of the rabbit neocortex.

11.8 Effects of exogenous NGF in the hippocampus

The results in section 11.7 above raised the prospects that samples collected during tetanic stimulation of the rabbit neocortex *in vivo* contained NGF-like substances. Since these neocortical samples could induce LTP when applied in the hippocampus (see results in section 11.6) it was wondered whether similar effects could be mimicked by application of exogenous NGF. When exogenous NGF (2.5 µg/ml, from *Vipera lebetina*) was applied for 10 min during stimulation of the stratum radiatum (0.2 Hz), the population spike in the CA_{1b} area evoked by stimulation of the stratum radiatum was not potentiated during the subsequent one hour of recording (population spike expressed in mV: controls, 1.36 ± 0.2; and 30 min after exposure to NGF with stimulation, 1.34 ± 0.3; values are Means ± S.E.M., n = 9 slices; $p > 0.05$, two-tailed paired Student's t-test). Similarly, if the stimula-

Figure 11-18. Effects of samples collected from rabbit neocortex in vivo on neurite growths in PC-12 cell cultures. PC-12 cells were incubated with 1.5 ml of growth medium containing 0.75 ml each of UNS (A), TNS (B), HTNS (C) and TNS with 10 mM saccharin. PC-12 cells incubated in the presence of TNS (i.e. (B)) and not the other samples developed extensive neurites, thereby indicating the presence of NGF-like substances in TNS. [NB: PC-12 cells were considered to have developed neurites if they presented with one or more extensions that were longer than the diameter of the cell bodies.] The micrographs were taken on the fourth day of incubation. The bar represents 50 μM; n = 6 culture plates for each sample.

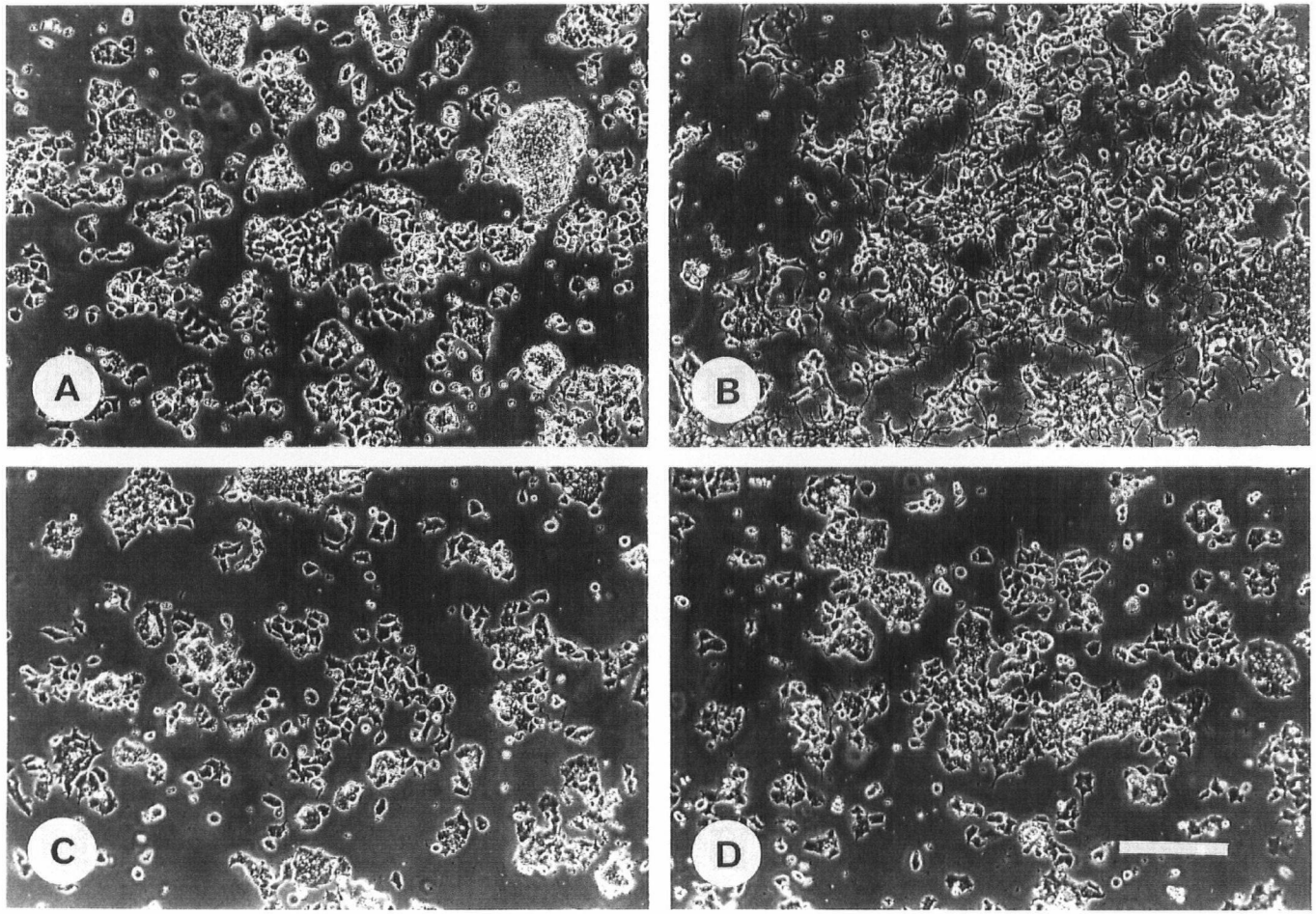

Figure 11-18

tion of the stratum radiatum was interrupted during the application of the peptide, there was no potentiation in any of the slices (population spike expressed in mV: controls, 1.38 ± 0.5; and 30 min after exposure to NGF without stimulation, 1.29 ± 0.45; values are Mean ± S.E.M., n = 4 slices; $p > 0.05$, two-tailed paired Student's t-test).

However, a tetanic stimulation of stratum radiatum which elicited post-tetanic potentiation of the weak CA_{1b} field EPSP (i.e. "weak input") induced LTP if the tetanus was given in the presence of exogenous NGF as illustrated in Figure 11-19 (field EPSP expressed in mV: controls, 0.37 ± 0.02; and 30 min after exposure to NGF and tetanus, 0.59 ± 0.01; values are Means ± S.E.M., n = 6 slices; $p < 0.05$, one-tailed paired Student's t-test). The above potentiating actions of exogenous NGF were blocked if the peptides were given in the last 10 min of 10 mM saccharin (saccharin was applied for a total of 12 min) (field EPSP expressed in mV: control, 0.45 ± 0.02; and 30 min post-tetanus to weak input during saccharin/NGF: 0.42 ± 0.0.05; values are Mean ± S.E.M., n = 6 slices; $p > 0.05$, two-tailed unpaired student's t-test). That exogenous NGF facilitated the development of LTP following stimulation of a weak input was fascinating since it is known in the literature that a weak input can develop LTP when tetanized in conjunction with a strong input (Levy and Steward, 1979). Perhaps tetanic stimulation of a strong input results in the release of chemical signals that subsequently interact with a co-activated weak input. In this regard, it is conceivable that exogenous NGF in the present studies exerted effects similar to those produced by a strong input that is concurrently tetanised with a weak input.

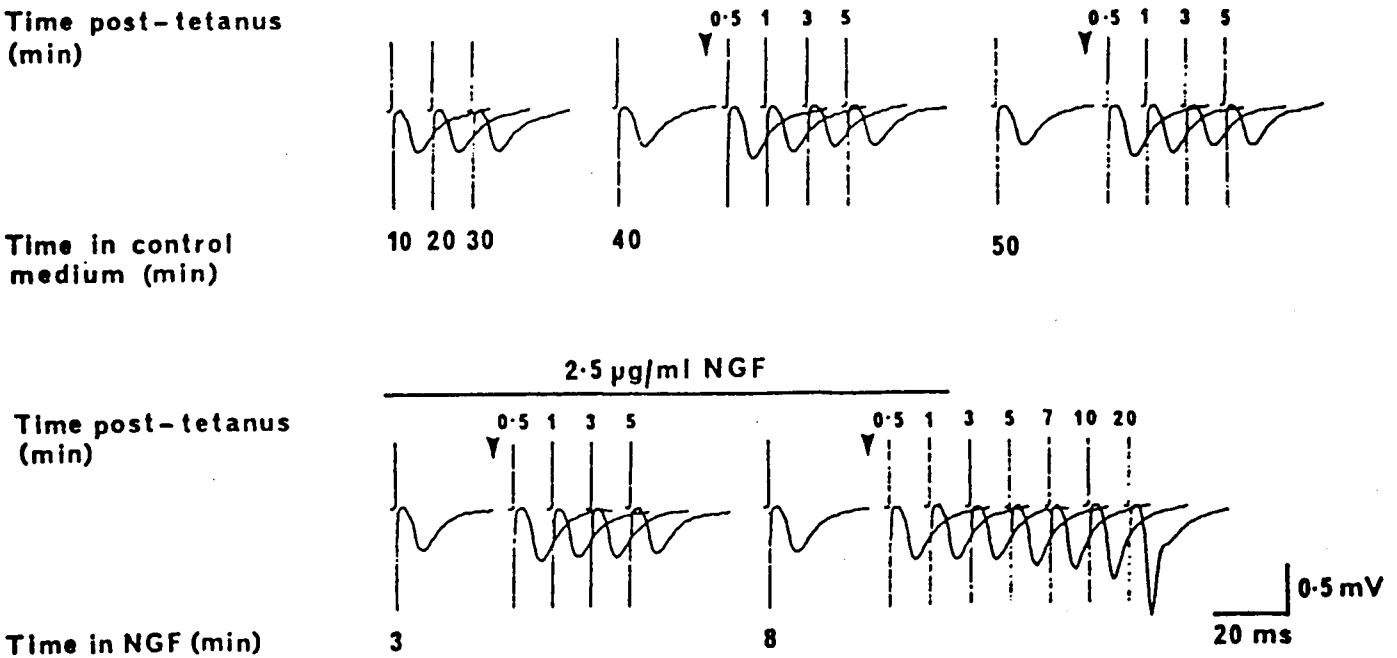

Figure 11-19. Effects of NGF (Vipera lebetina) on weak EPSP recorded in the CA_{1b} dendritic region in guinea pig hippocampus in vitro. The stimulus strengths used to stimulate the stratum radiatum in these experiments were those that evoked a weak field EPSP (i.e., ~0.4-0.5 mV) in the dendritic regions as illustrated. The arrow denotes a tetanic stimulation of the stratum radiatum (50 Hz, 10 pulses). The numbers on top of the records give the post-tetanic times (in min) when responses were recorded. Tetanic stimulation of the stratum radiatum induced only a short-term potentiation in control medium (top row). However, the same tetaus delivered during a 10 min application of exogenous NGF (2.5 µg/ml applied for 10 min) produced LTP (bottom row). Each record represents an average of 8 consecutive sweeps.

11.9 Mechanisms of action of saccharin: Extracellular studies

11.9.1 Saccharin and LTP: Dose-relationships. The following series of experiments were done to determine whether saccharin possessed non-specific electrophysiological effects that could account for its antagonism of LTP development in the hippocampus. Figure 11-20 illustrates the dependency of LTP blockade on saccharin concentrations perfused during tetanic stimulation of the stratum radiatum. Saccharin concentrations of 5 mM (applied for 10 min), failed to block LTP development when the tetanic stimulation was delivered during the last min of drug application (population spike expressed as % of control 15 min after tetanus to the stratum radiatum during saccharin: 201 ± 40; values are Mean ± S.E.M.; $n = 6$; $p < 0.05$, one-way ANOVA with Duncans' multiple comparisons tests). A second tetanic stimulation given after 30 min of washing with control medium was not significantly different from LTP obtained during applications of saccharin (quantitative data in Figure 11-20). These results indicated that the initial tetanic stimulations applied during 5 mM saccharin had induced near maximal LTP and, therefore, could not be potentiated any further with a second tetanus given during wash (cf. Bliss and Lømo, 1973; Bliss and Gardner-Medwin, 1973). In similar experiments, tetanic stimulations to the stratum radiatum during 7.5 mM saccharin applications resulted in relatively reduced magnitudes of LTP (population spike expressed as % of control 15 min after tetanus to the stratum radiatum during saccharin: 126 ± 11; values are Mean ± S.E.M.; $n = 6$; $p < 0.05$, one-way ANOVA with Duncans' multiple comparisons tests; Figure 11-20). Subsequent tetanic stimulations of the stratum radiatum in control medium (i.e. 30 min post-drug) resulted in a significantly greater LTP in all cases (quantitative data in Figure 11-20). Tetanic stimulations of the stratum radiatum during 10 mM saccharin application consistently

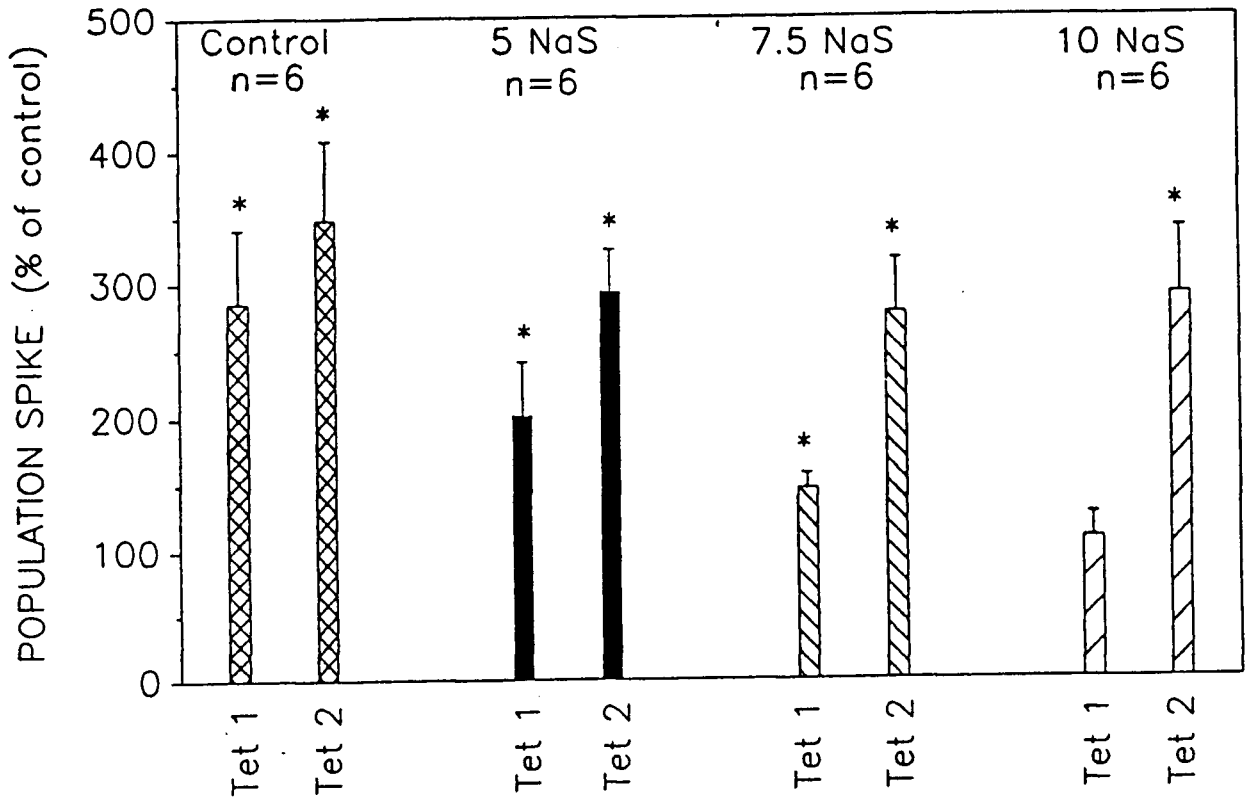

Figure 11-20. Effects of different concentrations of saccharin on LTP production in the CA_{1b} area of the guinea pig hippocampus in vitro. The graph shows the population spike in CA_{1b} area evoked by stimulation of the stratum radiatum expressed as a of control. Each slice was exposed to a single concentration of saccharin that was applied for 10 min, respectively. In saccharin, the first tetanic stimulation (Tet 1) was applied to the stratum radiatum during the 9th min of the drug application. This was followed by the second tetanus (Tet 2) that was applied 30 min after each saccharin application. In slices not exposed to saccharin (0 NaS), there was a 30 min interval between the first tetanus (Tet 1) and the second tetanus (Tet 2). Note that 10 mM saccharin blocked LTP development following tetanic stimulation of the stratum radiatum However, the same tetanus produced LTP when delivered to the stratum radiatum 30 min after 10 mM saccharin. [NB: 400 Hz, 200 pulses used in all experiments; asterisks indicate significant differences relative to control responses; $p < 0.05$; ANOVA with Duncan's multiple comparisons test.]

blocked LTP induction (population spike expressed as % of control 15 min after tetanus to the stratum radiatum during saccharin: 118 ± 18; values are Mean ± S.E.M.; n = 6; $p > 0.05$, one-way ANOVA), and yet tetanic stimulations of the stratum radiatum after 30 min wash elicited LTP in the same experiments (population spike expressed as % of control 15 min after tetanus to the stratum radiatum during wash: 290 ± 50; values are Mean ± S.E.M.; n = 6; $p < 0.05$, one-way ANOVA with Duncans' multiple comparisons tests; quantitative data in Figure 11-20). By comparison, LTP of relatively greater magnitude could be obtained with tetanic stimulation of the stratum radiatum in slices not exposed to saccharin (population spike expressed as % of control 15 min after tetanus in control medium: 290 ± 50; values are Mean ± S.E.M.; n = 6; $p < 0.05$, one-way ANOVA with Duncans' multiple comparisons tests; Figure 11-20). Taken together, the above results illustrated that the minimum concentrations of saccharin that could consistently block LTP induction were between 7.5 and 10 mM.

11.9.2 Saccharin and post-tetanic potentiation. In reduced Ca^{++} medium (i.e. 2 mM Ca^{++} in control medium replaced with 0.5 mM Ca^{++} and 1.5 mM Mg^{++}; see table 9-1 in chapter 9), tetanic stimulation of the stratum radiatum (at 10 - 15 min intervals) repeatedly induced post-tetanic potentiation (PTP) of the population spike lasting for 3 - 6 min. Interestingly, the same tetanic stimulations delivered in the last min of saccharin perfusions (10 mM saccharin applied for 10 min) induced PTP that was not significantly different from that obtained in control medium (population spike expressed as % of control 3 min after tetanus in: (1) control medium, 190 ± 10; and (2) saccharin 204 ± 23; n = 6 slices; values are Mean ± S.E.M.; $p < 0.05$, two-tailed paired Student's t-test; Table 11-4).

Post-tetanic potentiation in the hippocampus, like its counterpart in the peripheral nervous system, is thought to be mediated by presynaptic mechanisms (McNaughton, 1980). It has been suggested that PTP in the peripheral nervous system (where it was first described) is due to transient hyperpolarizations in presynaptic terminals following tetanic stimulations that cause increases in amplitudes and durations of action potentials invading the presynaptic terminals (cf. Eccles and Krnjević, 1959). These changes presumably result in the enhancement of transmitter released during synaptic transmission. Presuming that similar mechanisms underlie PTP in the hippocampus, then results of the present studies indicate that 10 mM saccharin did not interfere with the release of transmitter during synaptic transmission in the hippocampus.

11.9.3 Saccharin and paired-pulse facilitation. The method of paired-pulse facilitation was used to examine further possible effects of saccharin on presynaptic functions. Paired-pulse facilitation is thought to be due to increases in released transmitter induced by the second pulse in the stimulation pair (McNaughton, 1980). Presumably, "residual" effects on transmitter release associated with the first pulse add up with those of the second pulse in the stimulation pair. In this regard, changes in the characteristics of the evoked responses reflect presynaptic conditions and, this feature was utilized in the present studies. Paired-pulse stimulation of the stratum radiatum at a fixed inter-pulse interval of 30 msec evoked a set of population spike denoted as "P_1" and "P_2", respectively, as illustrated in Figure 11-21. The second response, P_2, was consistently larger than the first response, P_1 (ratio of P_2:P_1 was 3.31 ± 1.25; n = 6 experiments; values are Mean ± S.E.M.). Interestingly, during 10 mM

Table 11-4. Effects of saccharin on post-tetanic potentiation of the population spike in the CA_{1b} area induced by stimulation of the stratum radiatum in the guinea pig hippocampus in vitro.

Time after tetanus (min)	Control medium	10 mM Saccahrin
3	1.90 ± 0.18	2.04 ± 0.23
5	1.50 ± 0.20	1.84 ± 0.21

Values are Mean ± S.E.M. in mV (n = 5 experiments). The stimulation strength was adjusted to evoke pre-tetanus population spikes with amplitudes of 1 mV. Tetanic stimulation of the stratum radiatum (400 Hz, 200 pulses) during saccharin (10 mM saccharin applied for 10 min; tetanus given at 9 min of drug application) induced PTP that was not significantly different from PTP obtained in control medium ($p > 0.05$; two-tailed Student's t-test). [NB: Medium used contained reduced Ca^{++} levels (see text).]

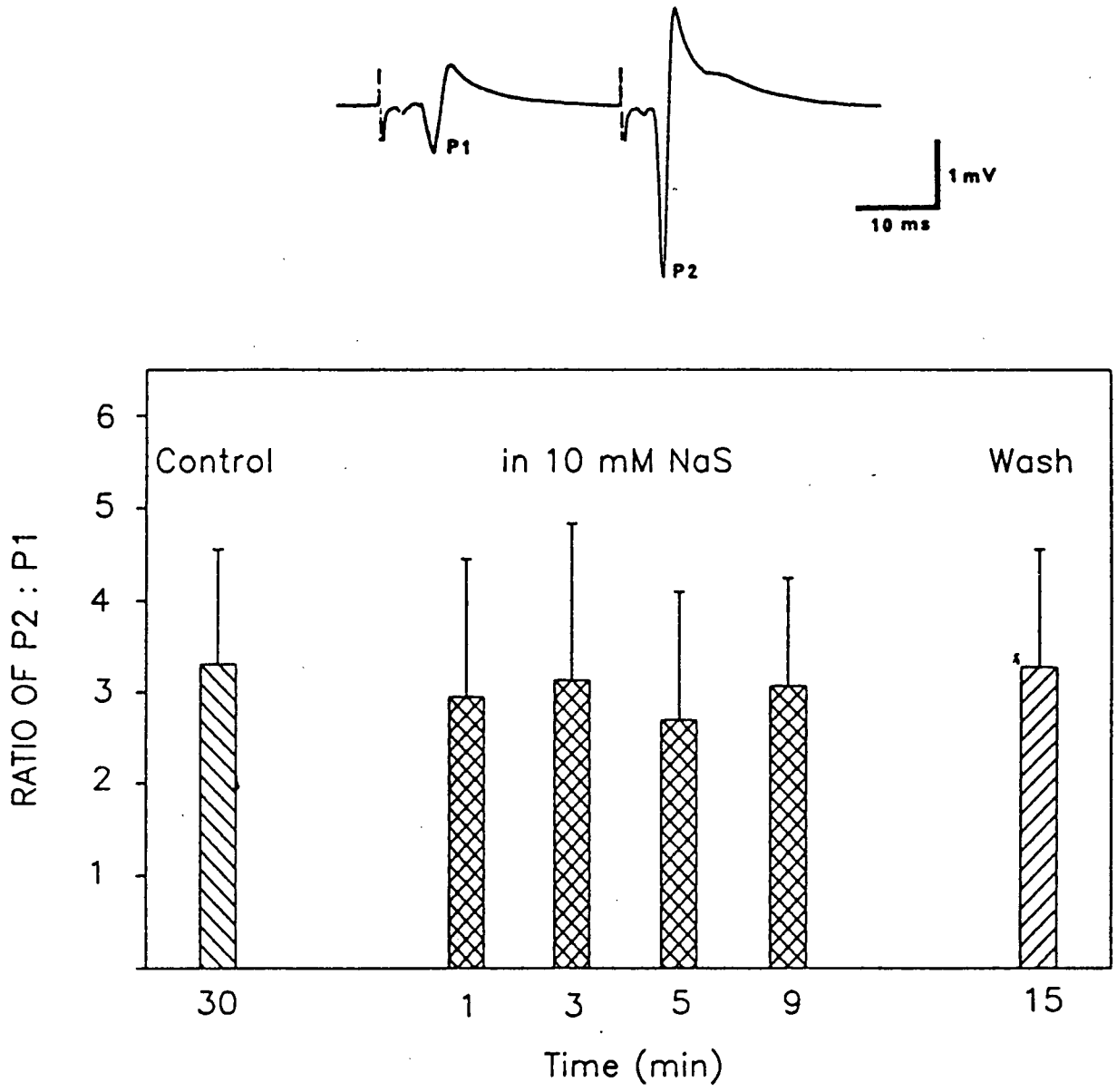

Figure 11-21. Demonstration of lack of effects of saccharin on paired-pulse facilitation in the CA_{1b} area of the guinea pig hippocampus in vitro. Twin pulse stimulation of the stratum radiatum evoked a set of two population spikes (P1 and P2) in the CA_{1b} area as illustrated. The interval between the two pulses in the stimulation pair was 30 msec and, this interval was found to be adequate in causing facilitation of the second response (P2) relative to the first response (P1). Plotted are the calculated ratio of P2 : P1 for each set of evoked responses, respectively. The ratio for sets of responses obtained during 10 mM saccharin that was applied for 10 min were not significantly different from those obtained in control medium before or after application of saccharin.

saccharin (applied for 10 min) the ratio of P_2 to P_1 was not significantly different from that obtained in control medium (ratio of P_2:P_1: in control medium, 3.31 ± 1.25; and in saccharin at 9 min of application, 3.07 ± 1.18; values are Mean ± S.E.M.; n = 6 slices; $p > 0.05$; two-tailed paired Student's t-test; Figure 12-21). These results further indicated that 10 mM saccharin applied for 10 min did not significantly alter transmitter release during synaptic transmission.

11.9.4 Saccharin and dendritic negative wave during tetanus. At this stage in the studies it was becoming evident that 10 mM saccharin had insignificant effects on synaptic responses in CA_{1b} area produced by low frequency stimulation of the stratum radiatum. However, there still remained the possibility that saccharin might interfere with the development of extracellular fields that occur during tetanus (Gustafsson and Wigström, 1988, for review). It is thought that activation of non-NMDA receptors mediate low frequency excitatory synaptic responses in areas such as the CA_1 in the hippocampus (see chapter 6). Under these conditions, NMDA receptor channels are apparently blocked by Mg^{++} and, therefore, do not contribute significantly towards the generation of excitatory synaptic responses. However, tetanic stimulations induce adequate dendritic depolarizations that are sufficient to remove the Mg^{++} block of NMDA receptor channels making it possible for cations (e.g. Ca^{++}) to flow through these open channels. A component of the dendritic negative wave that is abolished by D-2-amino-5-phosphonovalerate (presumed to be a competitive antagonist at the NMDA receptor) is thought to be the electrophysiological correlate of NMDA receptor activation during tetanus. Hence the effects of 10 mM saccharin on the development of the dendritic negative wave during tetanus was examined.

When the slices were exposed to 10 mM saccharin for 10 min, the extracellular negative wave in the apical dendritic area of CA_{1b} neurons, generated during a tetanic stimulation of stratum radiatum, was still present as illustrated in Figure 11-22. The field EPSP, however, was not potentiated subsequent to this tetanus (field EPSP expressed in mV: control, 0.98 ± 0.4; and 15 min after tetanic stimulation in saccharin, 1.05 ± 0.25; values are Mean ± S.E.M.; n = 5 slices; $p > 0.05$, one-way ANOVA; Figure 11-22). A second tetanus given in the absence of saccharin also resulted in a negative field that was not different from that observed during saccharin applications (Figure 11-22). However, the population EPSP was potentiated (field EPSP expressed in mV: control, 0.98 ± 0.4, i.e. same controls as above; and 15 min after tetanic stimulation in control medium, 1.55 ± 0.63; values are Mean ± S.E.M.; n = 5 slices; $p < 0.05$, one-way ANOVA with Duncans' multiple comparisons test; Figure 11-22). This finding showing that saccharin did not affect the development of the dendritic negative wave during tetanus was important for the following reason. NMDA receptors are thought to be involved in the induction of LTP (Bliss and Lynch, 1988; Collingridge, 1985; Gustafsson and Wigström, 1988, for reviews). It, therefore, appeared that 10 mM saccharin was not blocking LTP by interfering with the negative wave in the dendritic region thought to be generated by the activation of NMDA receptors during tetanic stimulations.

11.10 Mechanisms of action of saccharin: Intracellular studies

11.10.1 Effects of saccharin on spontaneous and evoked responses. Applications of 10 mM saccharin for 10 min did not induce any significant changes in resting membrane potentials or input resistances of CA_{1b} neurons tested (RMP in mV, controls: -61 ± 1.9; during the last min of saccharin application: -58.5 ± 2.0; and R_n in MΩ determined from I-V

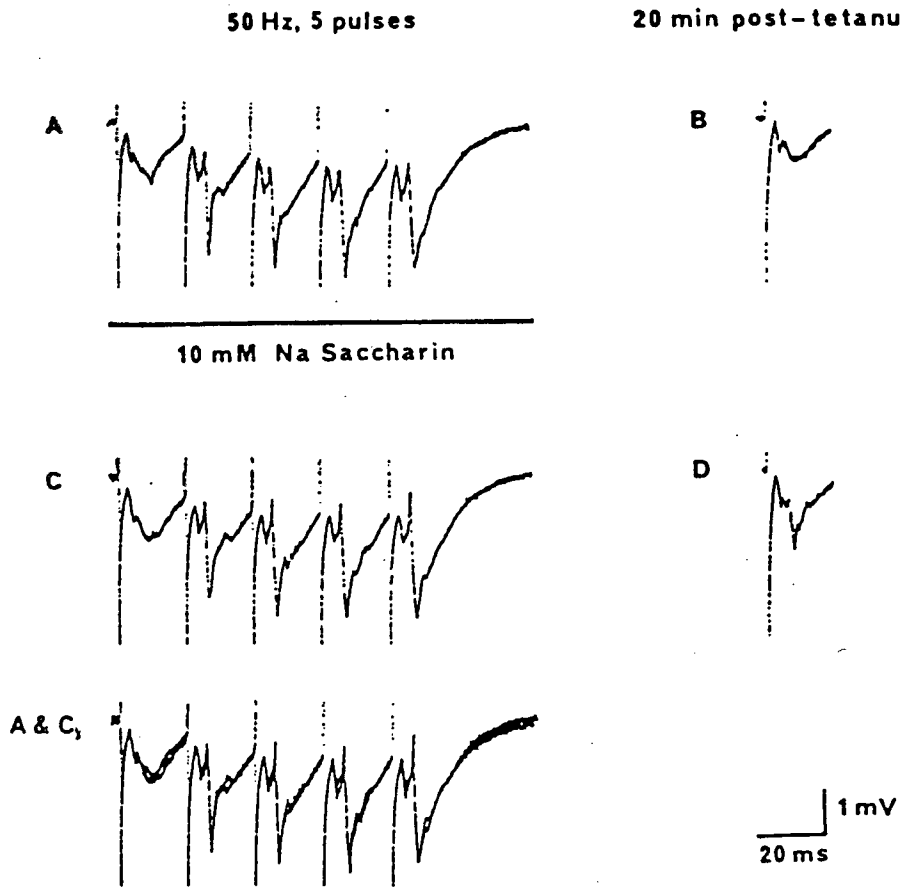

Figure 11-22. Effects of saccharin on the extracellular negative wave in CA_{1b} apical dendrites induced during tetanic stimulations of the stratum radiatum in the guinea pig hippocampus in vitro. The field EPSP recorded in the CA_{1b} dendritic region was evoked by stimulation of the stratum radiatum. In these experiments, 50 µM picrotoxin was added to the physiological medium. Five stimulation pulses, given at 50 Hz, revealed a dendritic negative wave that was superimposed with field EPSPs (A; record taken at 8 min during a 10 min application of 10 mM saccharin). In (B) is shown a dendritic field EPSP evoked by low frequency stimulation (0.2 Hz) of the stratum radiatum in control medium, 20 min after the tetanus of (A). Subsequently, a similar tetanus (50 Hz, 5 pulses), applied in control medium, also induced a dendritic negative wave that was superimposed with dendritic field EPSPs (C). A dendritic field EPSP evoked by a low frequency stimulation (0.2 Hz) of the stratum radiatum was found to be potentiated (D), when checked 20 min after the tetanus of (C). In (E), note that superimposition of traces (A) and (C) gave a perfect fit thereby showing that the responses induced by the tetanus during saccharin applications was of the same magnitude as similar responses obtained in control medium.

relationships, controls: 31.5 ± 1.54; during the last min of saccharin application: 31.9 ± 1.64; values are Mean ± S.E.M.; n = 10 neurons; $p > 0.05$; two-tailed unpaired Student's t-test; Figure 11-23). The frequency of spontaneous miniature postsynaptic potentials (i.e. mixed minEPSPs and minIPSPs, since picrotoxin not added to media) were not affected by the drug (number of spontaneous small potentials, control: 12 ± 2, and during last 2 min of saccharin application: 10 ± 1 per sec in both cases; values are Mean ± S.E.M; n = 10 neurons; $p > 0.05$; two-tailed unpaired Student's t-test). Typical intracellular responses in CA_{1b} neurons obtained in control medium and during application of 10 mM saccharin are illustrated in Figure 11-23. Saccharin applications induced insignificant changes in the magnitudes of EPSPs, IPSPs, action potentials or AHPs (Figure 11-23). The above results supported the notion that 10 mM saccharin was not exerting non-specific effects on electrical properties of CA_{1b} neurons that could account for the drugs' antagonism of LTP production.

11.10.2 Saccharin effects on presynaptic terminal excitability. Presynaptic terminal excitability testing provides an indirect method for assessing some of the electrical properties of presynaptic regions. Wall (1958) suggested that hyperpolarizations of presynaptic terminals, for example, resulted in a decrease in presynaptic terminal excitability. These methods can also be used to identify possible mechanisms mediating increases or decreases in presynaptic terminal excitability (e.g. Cooke and Quastel, 1973; Saint, Quastel and Chirwa, 1986). Hence, the methods of presynaptic excitability testing were used to assess possible effects of saccharin on electrical properties of the presynaptic regions in the CA_{1b} area of the hippocampus. Stimulus rheobasic values extrapolated from strength-duration curves in each experiment, were typically around 3 msec (n = 5 neurons).

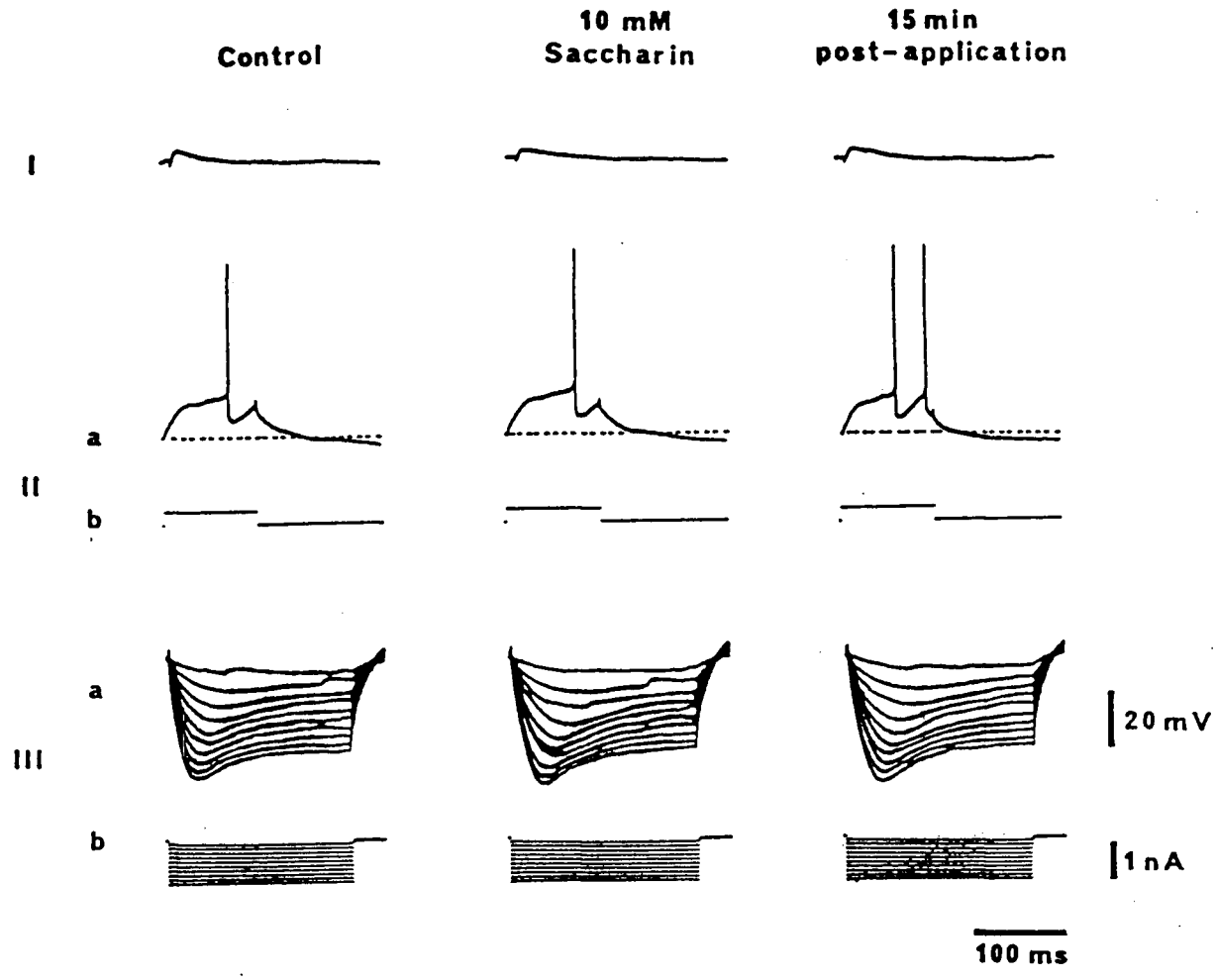

Figure 11-23. Examination of the electrophysiological effects of saccharin in the guinea pig hippocampus in vivo. The intracellular EPSP (I) and synaptically activated action potential (II) were evoked following subthreshold and suprathreshold stimulation of the stratum radiatum, respectively. The action potentials in this neuron were also elicited with intracellular depolarizing current pulses (IIIa, current traces shown in IIIb). The undershoots associated with responses in (II) and (IIIa) probably were a mixture of both IPSPs and afterhyperpolarizations (AHP). In addition, input resistances were checked by recording membrane potential shifts (IVa) evoked with graded hyperpolarizing current injections (IVb). Saccharin (10 mM applied for 10 min, shown records taken during the 6-9th min interval of drug application) exerted insignificant effects on evoked responses as illustrated in (I-IV, middle responses). Furthermore, 10 mM saccharin did not cause changes in input resistance, as can be seen from the nearly identical membrane shifts, both in saccharin (IVa, middle responses) and in control media (IVa, first and last responses), induced by the same graded hyperpolarizing current injections (IVb).

Activations of the Schaffer collaterals at rheobasic thresholds evoked antidromic action potentials in CA_3 neurons. These all-or-none responses had constant onset latencies of 5-6 msec, and they could be evoked in Ca^{++}-free medium. Applications of 10 mM saccharin (for 10 min) did not change the rheobasic thresholds (controls in μA: 11.3 ± 0.4, in saccharin: 10.9 ± 0.2; values are Mean ± S.E.M.; n = 5; p >0.05; two-tailed paired Student's t-test). These results suggested that 10 mM saccharin had insignificant effects on the electrical properties of Schaffer collaterals terminals as determined by the methods described above.

11.10.3 Saccharin and NMDLA responses. The following experiments examined the effects of saccharin on depolarizations induced by NMDLA, the acidic amino acid that is thought to be a selective agonist for NMDA receptors. Applications of NMDLA (25-100 μM) for 1 and/or 1.5 min were associated with long-lasting "desensitizations" of the responses (cf. Fagni, Baudry and Lynch, 1983). These desensitizations were seen as diminutions in the amplitudes of the drug-induced depolarizations during applications. If each cell was re-exposed to the same NMDLA concentration within 5 min after the first application, the resulting depolarizations (to the second NMDLA application) were decreased by as much as 15-40%. However, repeated applications of the same NMDLA concentration elicited similar depolarizations if the drug was given at intervals of at least 10 min. In view of the above results, repeated NMDLA applications were given at intervals of at least 10 min. It was found that NMDLA (25 or 50 μM; applied for 1 min produced depolarizations of 6 to 33 mV in CA_{1b} neurons (intracellular depolarizations in CA_{1b} neurons in mV: 25 μM NMDLA, 9 ± 1; and 50 μM NMDLA, 23.5 ± 5; values are Mean ± S.E.M.; n = 5 neurons in each case) and, these

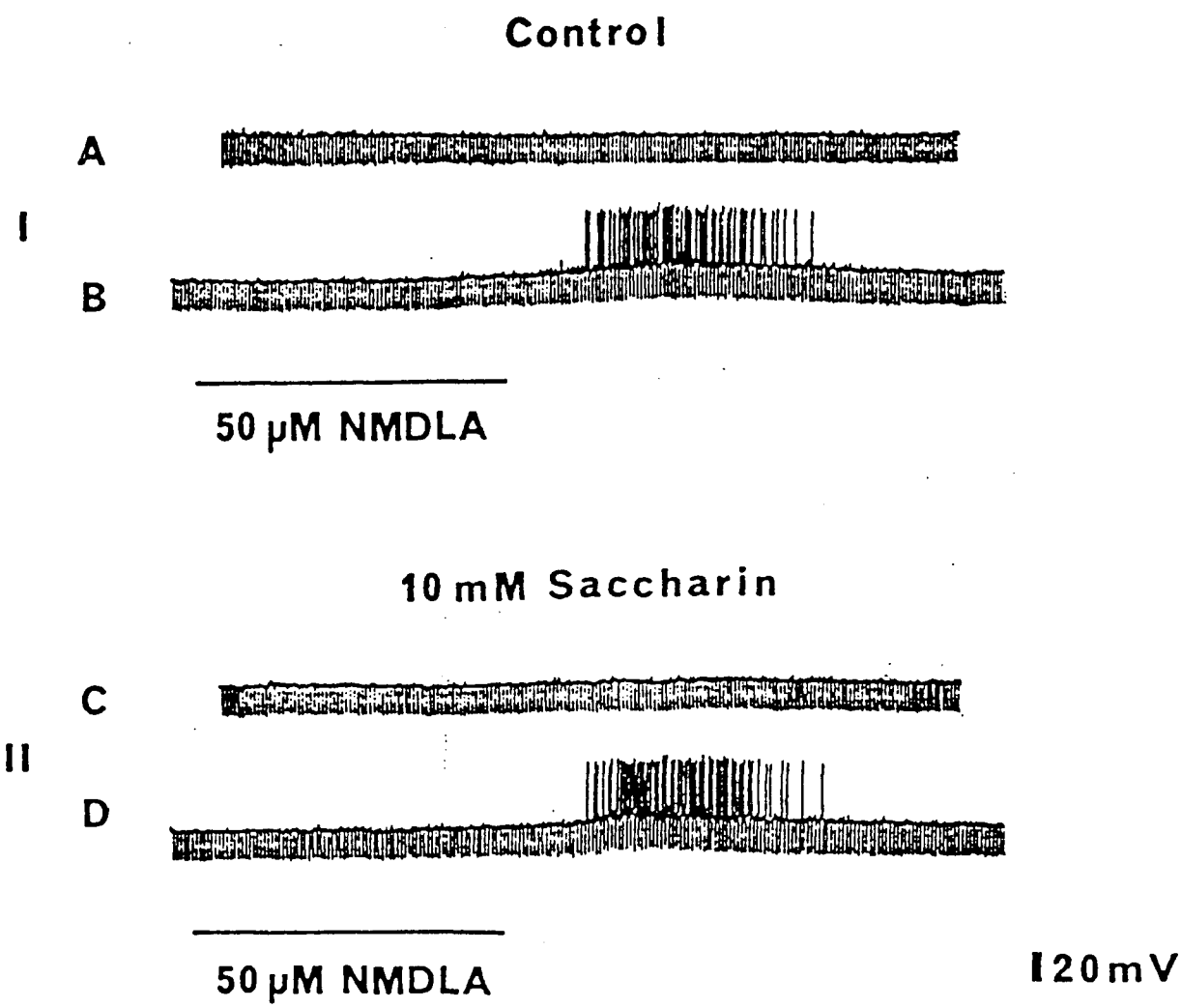

Figure 11-24. Effects of saccharin on the intracellular depolarizations in CA_{1b} neurons induced by NMDLA applications in the guinea pig hippocampus in vitro. The top row shows intracellular recordings of the cell membrane potential and input resistances (as measured with 1 nA hyperpolarizing pulses of 100 msec duration given at 1 Hz) in control medium (A) and during 1 min bath application of 50 µM NMDLA (B). The bottom row shows similar responses in the same cell (i.e. cell membrane potential and input resistances) during a 10 min application of 10 mM saccharin (C) and when 50 µM NMDLA is applied for 1 min towards the end of saccharin application.

intracellular depolarizations were not decreased even if the amino acid was applied in the presence of 10 mM saccharin (i.e. intracellular depolarizations in mV: 25 μM NMDLA/10 mM saccharin, 8.5 ± 1; and 50 μM NMDLA/10 mM saccharin, 24 ± 3; values are Mean ± S.E.M.; n = 5 neurons; $p > 0.05$; two-tailed paired Student's t-test). Furthermore the changes in R_n during NMDLA applications were not altered in the presence of saccharin as illustrated in Figure 11-24.

Taken together, the results from studies with saccharin indicated that this agent blocked LTP development through mechanisms different from either non-specific alterations in CA_{1b} neurons electrical properties or NMDA receptor activation. It seems logical to think that saccharin antagonized the induction of LTP at a step beyond NMDA receptor activation. In this regard, saccharin may turn out to be a useful substance in elucidating mechanisms involved in the production of LTP, subsequent to the postulated NMDA receptor involvement step.

12. DISCUSSION

12.1 General

Most of the electrophysiological studies were conducted in the CA_{1b} area since homosynaptic LTP is well characterized in this region. The CA_{1b} region itself is comprised of cell distributions that are taken to be representative of the whole CA_1 subfields (Lorente De Nó, 1934). Furthermore, the Schaffer collaterals from CA_3 area constituted a readily accessible input to the CA_{1b} neurons. Moreover, the CA_{1b} area was readily discernable in the transverse hippocampal slice, and this facilitated accurate positioning of electrodes. According to Masukawa, Bernado and Prince (1982), CA_{1b} pyramidal neurons exhibit little or no spontaneous bursting activities, unlike pyramidal neurons of the other cornu ammonis subfields. In the present study, this feature was particularly desirable in experiments examining minEPSPs since bursting activities would have interfered with the recording of small potentials.

Samples collected from the neocortex were used since several reports have described the development of LTP in this brain structure. In the neocortex, LTP can be induced with tetanic stimulations of inputs (Artola and Singer, 1987; Komatsu, et al., 1988; Lee, 1983) or pairing of conditioning postsynaptic intracellular depolarizations with activation of presynaptic afferents (Bindman, Meyer and Pockett, 1987). Furthermore, the induction of LTP in the neocortex can be blocked by D-2-amino-5-phosphonovalerate and, this suggests that NMDA receptors are involved in the production of LTP in this structure. The above features of LTP in the neocortex are strikingly similar to those observed in the hippocampus (cf. Bliss and Lynch, 1988). In terms of the studies presented in this thesis, the larger surface

of the rabbit neocortex and its position in the brain was conducive for collection of samples in reasonably adequate quantities.

In general, recorded intracellular and extracellular potentials in the hippocampus exhibited the same features, as have been described for similar responses in the literature (Andersen, Bliss and Skrede, 1971; Andersen, Eccles and Løyning, 1963 and 1964; Johnston and Brown, 1984; Kandel and Spencer, 1961; Kandel, Spencer and Brinley, 1961; Spencer and Kandel, 1961; Schwartzkroin, 1987). Furthermore, small discrete potentials were readily detected in CA_{1b} neurons and minIPSPs, but not minEPSPs, could be blocked with picrotoxin (an antagonist at the $GABA_A$ receptors). Several other investigators have also reported the occurrence of small discrete excitatory potentials in the CA_1 region (Malenka, Ayoub and Nicoll, 1987; Turner, 1988). Presuming that minEPSPs in the hippocampus are analogous to miniature end-plate potentials (MEPP) at the neuro-muscular junction (NMJ), then minEPSPs in CA_{1b} neurons reflected spontaneous quantal (or vesicular) release of transmitters (cf. del Castillo and Katz, 1952). That vesicular release of transmitters occurs in the hippocampus is supported by recent morphometric studies that have identified vesicles in presynaptic boutons in the stratum radiatum, where excitatory synaptic contacts are numerous (Agoston and Kuhnt, 1986; Applegate, Kerr and Landfield, 1987). It was not clear why minEPSPs in CA_{1b} cells had a mixed size distribution (e.g. Figure 11-2). Since these minEPSPs recorded in the soma were coming from synapses with different spatial distributions on dendrites, this could account for the variability in minEPSPs amplitudes. It is also conceivable that minEPSP amplitudes reflected activation of different receptor subtypes (i.e. NMDA, Quisqualate/ Kainate receptors). These issues will need to be resolved in future experiments. Moreover, the size of these minEPSPs is

around 1 mV and, therefore, accurate analysis of size distribution is difficult. In view of this, changes in rise times or amplitudes of spontaneous minEPSPs could not be used as indices for changes occurring at a single synapse (see also section 12.7). This, and the low and extremely variable frequencies of spontaneous minEPSPs among CA_{1b} neurons (e.g. Table 11-1) precluded the use of classic quantal analytical methods in the CA_{1b} area (cf. del Castillo and Katz, 1954). Instead, it was found appropriate to use Ba^{++}, a potent agonist for the asynchronous release of transmitter during stimulation of afferents (Chirwa, 1985; Quastel, et al., 1988; Silinsky, 1978 and 1985; Zengel and Magleby, 1980), to facilitate the occurrence of evoked minEPSPs. This provided a potential method for assessing changes in the release of transmitter, thereby indicating presynaptic mechanisms (cf. Silinsky, 1978).

12.2 LTP in CA_{1b}

Long-term potentiation (LTP) in the CA_{1b} area was readily induced by (1) tetanic stimulations of the stratum radiatum, (2) simultaneous pairings of conditioning postsynaptic depolarizations in a CA_{1b} neuron and activation of the stratum radiatum, or (3) brief applications of samples collected during tetanic stimulation of the guinea pig hippocampus in vivo and rabbit neocortex in vivo. The synaptic potentiations that were observed in the present studies exhibited features as described in the literature, namely, decreased onset latencies, unaltered presynaptic volleys, unchanged RMP or R_n as recorded in the soma (Alger and Teyler, 1976; Andersen, et al., 1980c; Bliss, and Gardner-Medwin, 1973; Bliss and Lømo, 1973; Schwartzkroin and Wester, 1975; Swanson, Teyler and Thompson, 1983).

12.3 K^+ efflux and LTP

It was interesting to find that LTP could still be elicited even though

K^+ channels were blocked by Cs^+ leaking from recording micropipettes. That K^+ currents were diminished by Cs^+ was clearly reflected in the membrane depolarizations that developed in CA_{1b} neurons, and subsequent inactivations of the Na^+ spike generating mechanisms (Brown and Johnston, 1983; Johnston, Hablitz and Wilson, 1980). At first the above results were puzzling since Haas and Rose (1984) reported that intracellular Cs^+ inhibited LTP induction in four CA_1 neurons tested. This suggested that transient accumulations of extracellular K^+ that occur during depolarizations (e.g. Benninger, Kadis and Prince, 1980; Chirwa, 1985), were necessary for LTP development. On reviewing the literature, however, recent studies of other investigators appeared to be inconsistent with the findings of Haas and Rose. For example, when Barrionuevo, et al. (1986) demonstrated that LTP was associated with increased excitatory currents at the mossy fiber-CA_3 synapses, these investigators used recording electrodes containing Cs^+ in some of their experiments (e.g. Figure 6, page 545 of Barrionuevo, et al., 1986). In addition, it has now been demonstrated that agents that diminish K^+ currents during depolarizations facilitate the induction of LTP (e.g. Chirwa, 1985; Lee, Anywl and Kowan, 1986). Moreover, adequate conjunctive depolarizations of presynaptic and postsynaptic regions are thought to be necessary for LTP production (Bliss and Lynch, 1988; Goh, 1986; Gustafsson and Wigström, 1988; Kelso, Ganong and Brown, 1986; Malinow and Miller, 1986; May, Goh and Sastry, 1987; Sastry, Goh and Auyeung, 1986). At the single cell level, therefore, intracellular depolarizations resulting from blockade of K^+ currents could predictably enhance LTP production. But it is feasible that K^+ released from nearby neurons or glia during a tetanic stimulation of afferents could be involved by assisting release of substances that depend on depolarizations and these

substances might further trigger events leading to LTP (see section 12.4).

12.4 Feedback interactions

Reports in the literature indicate that postsynaptic mechanisms mediate LTP production and that presynaptic mechanisms are involved in its maintenance. However, it is not known how the induction of LTP in postsynaptic regions subsequently "triggers" presynaptic mechanisms that are thought to be involved in the maintenance of LTP. In this regard, it can be speculated that feedback interactions occur between postsynaptic and presynaptic regions during LTP production. If this is valid, then it seems unlikely that K^+ effluxes subsequent to subsynaptic membrane depolarizations would act as feedback signals for the following reasons. In a recent review, Smith (1988) suggested that raised extracellular K^+, particularly if large, would be self-limiting in synaptic potentiations since increased excitability would progress to inactivations of Na^+ spikes in afferents (see also Somjen, 1979). Even if increases in extracellular K^+ could facilitate recruitment of inactive synapses, the inductive phase of LTP at these synapses would still have to occur in postsynaptic regions, in keeping with the current ideas implicating postsynaptic mechanisms in the induction of LTP (Bliss and Lynch, 1988; Collingridge and Bliss, 1987; Gustafsson and Wigström, 1988; Teyler and DiScenna, 1987; for reviews). It follows, therefore, that once LTP is induced postsynaptically in these recruited synapses, a "linking" mechanism with the presynaptic regions should subsequently occur to facilitate the development of the postulated presynaptic changes involved in the maintenance of LTP (cf. Bliss and Lynch, 1988).

Alternatively, let us suppose that increased extracellular K^+ depolarizes nearby cells, which might include glia cells (cf. Sastry, Goh, May and Chirwa, 1988). If these cells mediate the changes associated with

LTP development, it still has to be wondered how these cells, in turn, "communicate" with activated synapses to cause increases in synaptic transmission efficacy. In view of the above, it seems logical to think that if LTP production involves feedback interactions, then these are likely to be mediated by voltage-sensitive signals whose major effect is not necessarily to depolarize neuronal elements per se. Rather, it can be hypothesized that such feedback signals, if present, would act as "primary" messengers that initiate secondary cellular processes leading to long-lasting synaptic facilitations. Hence, it was exciting to find that samples collected during tetanic stimulations of the guinea pig hippocampus in vivo or rabbit neocortex in vivo induced LTP when applied in the guinea pig hippocampus in vitro (see section 12.7). It became conceivable that these substances could be likely candidates that mediate the postulated feedback interactions. These ideas are further developed in later sections.

12.5 minEPSPs and depolarizations in Ba^{++}

The experimental evidence for possible feedback interactions discussed in section 12.4 above are apparent in the following results. Ba^{++} was used successfully in facilitating the occurence of evoked minEPSPs in CA_{1b} neurons following stimulation of the stratum radiatum, and this probably reflected the asynchronous release of transmitters (Quastel, et al., 1988; Silinsky, 1978). It is exciting to note that transient but significant increases in frequencies of evoked minEPSPs occur immediately following simultaneous pairings of postsynaptic depolarizations with activated presynaptic afferents in the CA_{1b} area. Presuming that evoked minEPSPs in Ba^{++}-containing medium partly reflect presynaptic functions in the hippocampus, then the simplest interpretation of these results would be that postsynaptic depolarizations were modulating presynaptic activities,

resulting in the facilitation of evoked minEPSPs. This conclusion is based on the following knowledge: At the neuromuscular junction (NMJ), Silinsky (1978) observed an elevation of miniature end-plate frequencies (MEPP) to 5-20 times the control level, if the motor nerve was stimulated at 1 Hz for a brief period (< 1 min). However, Silinsky (1978) found that during tetanic stimulations at frequencies > 1 Hz, there were massive increases in MEPP frequencies which were associated with a steady depolarization of the postsynaptic membranes. In view of the above, it seems likely that in guinea pig hippocampal slices incubated in Ba^{++}, evoked minEPSPs reflect the asynchronous release of transmitter following stimulation of afferents. Therefore, increases in the frequencies of evoked minEPSPs during conjunctive depolarizations of activated presynaptic afferents with intracellular depolarizations of CA_{1b} neurons may be correlated with increases in transmitter release. If this conclusion is valid, then the above results demonstrate that postsynaptic depolarizations modulate the functions of activated inputs in the hippocampus.

Indeed, it is possible that evoked minEPSPs in the presence of Ba^{++} could reflect hitherto unknown events that are unrelated to quantal release of transmitter (see section 12.6). This possibility should be examined in future studies before firm conclusions can be made. However, it is interesting to note that Sastry, Goh and Auyeung (1986) previously found that simultaneous pairings of activated afferents and injections of depolarizing currents into CA_1 neurons induced short-term potentiations (STP) that lasted for 3-5 min and/or LTP. This STP, as well as LTP whenever present, was associated with corresponding decreases in antidromic excitability of Schaffer collaterals (Sastry, Goh and Auyeung, 1986). Sastry, and co-workers (1986) noted the striking similarity between STP produced by

concurrent pairings of activated afferents with injection of depolarizing currents into a CA_1 neuron in the hippocampus and post-tetanic potentiation (PTP) that has been described in the peripheral nervous sytem (Feng, 1937; Guttmann, Horton and Wilber, 1937; Hughes, 1958) and is thought to be mediated by presynaptic mechanisms such as transient post-tetanic hyperpolarizations of presynaptic terminals (Eccles and Krnjević, 1959; Lloyd, 1949; Wall and Johnson, 1958). In this regard, Sastry, Goh and Auyeung (1986) suggested that STP in the hippocampus, like PTP in the peripheral nervous system, could also be mediated by presynaptic mechanisms. However, these authors also suggested that STP might be due to other unknown postsynaptic mechanisms in the hippocampus. But it is intriguing to note that STP and LTP induced by the simultaneous pairing of activated afferents with injection of depolarizing currents into a CA_1 neuron was associated with decreases in Schaffer collaterals terminal excitability (Sastry, Goh and Auyeung, 1986) since this supports the possible involvement of presynaptic regions in the above processes (cf. Wall, 1958).

The results in the present thesis showing increases in bursts of minEPSPs during conjuctive depolarizations of presynaptic inputs and postsynaptic neurons provides further evidence for thinking that postsynaptic depolarizations can modulate presynaptic functions in the hippocampus. How these interactions are achieved and/or their role in LTP production will need to be investigated in future experiments (see section 12.8). However, it would be interesting to examine whether prevention of the speculated feedback interactions might also block the induction of STP and/or LTP.

12.6 minEPSPs and LTP

Another major outcome from the studies with evoked minEPSPs is the demonstration of significant increases in evoked minEPSPs in CA_{1b} neurons

during LTP induced by tetanic stimulations of the stratum radiatum in slices incubated in Ba^{++}. There was no evidence to suggest that the increases in evoked minEPSP frequencies during LTP were caused by changes in presynaptic volleys and altered input resistances or resting membrane potentials, as recorded in the soma. As previously discussed in section 12.5 above, the simplest interpretation for increases in frequencies of evoked minEPSPs is that they reflect increases in transmitter release during LTP (cf. Silinsky, 1978 and 1985). This is further discussed below.

Sustained increases in the release of synaptic transmitter is frequently cited as being the presynaptic change that is involved in the maintenance of LTP (Bliss and Lynch, 1988 for review). These conclusions are based on biochemical studies that have shown significant correlations between increased release of glutamate, the putative excitatory transmitter in the hippocampus, and presence of LTP (Bliss and Lynch, 1988; Bliss, et al., 1986; Dolphin, Errington and Bliss, 1982; Skrede and Malthe-Sørenssen, 1981). However, the full impact of these biochemical results in relation to maintenance of LTP will only be realised once it is confirmed that glutamate is in fact the endogenous excitatory synaptic transmitter in the hippocampus. For example, it may very well be that indirect measurements of glutamate release during LTP reflects increases in metabolic turnover of this excitatory amino acid that is unrelated to transmitter release per se. Clearly, it is necessary to complement the biochemical studies on glutamate release with electrophysiological methods that might demonstrate directly increases in transmitter release during synaptic transmission. The studies on evoked minEPSPs represent one such method for directly assessing possible changes in transmitter release, before and after LTP development.

Indeed, it is tempting to conclude that the observed increase in evoked minEPSPs seen during LTP was due to an increase in transmitter release. Again, this conclusion follows from what is known about the behaviour of MEPPs at the NMJ, where presynaptic mechanisms control the frequencies of MEPPs and their amplitudes are directly related to the conditions of the end-plates (Fatt and Katz, 1952; Katz, 1962). These direct comparisons between activities at NMJ and intrasomatic recordings in the hippocampus, however, need to be made with caution for the following reasons. At the NMJ, the recording of MEPPs is at the end-plate where these discrete signals are generated (Fatt and Katz, 1952). In contrast, in the present studies intrasomatic electrodes were used to pick up minEPSPs that were generated at dendritic sites. These dendrites branch extensively, and they are inundated with a multitude of synapses. Each one of these synapses could give minEPSPs. Moreover, it is feasible that the recruitment of inactive synapses or latent excitatory pathways (cf. Chirwa, Goh and Sastry, 1988; Miles and Wong, 1987) could add to the number of observed minEPSPs during LTP rather than just an increase in the release process per se. It is likely that the increase in the number of minEPSP could also be due to the formation of new synapses. These possibilities will need to be tested in future experiments.

Alternatively, changes in dendritic membrane properties may occur during LTP that could facilitate the propagation of small discrete signals generated at distal synapses that previously failed to reach the soma. However, the studies of Barrionuevo, et al., (1986) indicated that the ability of the EPSP to propagate to the soma does not change during LTP and that any change that contributed to the increase in the intracellular EPSP in the CA_3 neurons during LTP was due to an increase in the synaptic

current. But they could not distinguish between presynaptic and subsynaptic contributions to the enhancement in the synaptic current (Barrionuevo, et al., 1986). A similar situation may exist in CA_{1b} neurons as well. In this regard, the possibility that the increase in the EPSP is due to an increase in the number of subsynaptic receptors appears unlikely. Studies have shown that LTP is not necessarily associated with an increase in subsynaptic receptors (Goh, 1986; Goh, Ho-Asjoe and Sastry, 1986; Lynch, Errington and Bliss, 1985; Sastry and Goh, 1984).

Interestingly, the application of phorbol esters in the hippocampus in vitro potentiated synaptic responses that were similar to LTP, and this potentiation was associated with increased minEPSP frequency (Malenka, Ayoub and Nicoll, 1987; Malenka, Madison and Nicoll, 1986). Quantal analysis of synaptic transmission between mossy fibers and CA_3 neurons in the hippocampus in vitro indicated that potentiation by phorbol esters was accompanied by increases in quantal content (Yamamoto, Higashima and Sawada, 1987 and 1988). In view of the above, it is tentatively concluded that the increase in the frequencies of evoked minEPSPs observed in the present studies support the idea that there is an increase in transmitter released during LTP.

12.7 Quantal transmission in hippocampus

Perhaps it will be useful to discuss some aspects of quantal transmission in the hippocampus that complicate the interpretation of these events. Both inhibitory and excitatory quantal events, under both current- and voltage-clamp conditions, have been resolved in the CA_3 region (Brown, et al., 1988; Johnston and Brown, 1984). A similar situation is thought to exist in the CA_1 region (e.g. Johnston and Brown, 1984, for review). As has been described in earlier sections (12.1, 12.5 and 12.6), these quantal

events are taken to reflect all-or-none discharges of transmitter contents of presynaptic vesicles. At the present time, however, unequivocal quantitative extraction of classical quantal parameters, as has been done at the vertebrate neuromuscular junction (cf, del Castillo and Katz, 1954; McLachlan, 1978) is confounded by factors such as the following.

Many reports have indicated that the dendritic membranes of hippocampal neurons have active properties (Andersen and Lømo, 1966; Andersen, Storm and Wheal, 1987: Cragg and Hamlyn, 1955; Fujita and Sakata, 1962; Miyakawa and Kato, 1986; Schwartzkroin and Slawsky, 1977; Spencer and Kandel, 1961; Wong, Prince and Bausbaum, 1979). The extent to which these active dendritic properties can modify quantal amplitude (q) is not known. Moreover, it is not clear how the expansive spatial distribution of synapses on dendrites (Lorente De Nó, 1934) affects the stochastic distribution of quantal content (m). In this regard, "success" or "failure" to observe a quantal event with intrasomatic recordings may be a function of the location of individual synapses on dendrites, rather than changes in the number of quanta (n) capable of responding or the average probability (p) that they respond. Therefore, postsynaptic mechanisms could contribute towards changes in the distributive patterns of "m". Another troubling feature of quantal studies in the hippocampus pertains to the fact that the role of spine structures in synaptic mechanisms is not known. It is feasible that spine necks, for example, are sites for the all-or-none transfer of transient subsynaptic currents. Assuming that this situation exists in the hippocampus, then spine structures would be expected to directly influence the stochastic behavior of "m" under different conditions of synaptic transmission. Taken together, the above factors raise the real prospect that the relationship,

$$m = np$$

may not be directly applicable in the hippocampus, unless modifications are incorporated in this equation that take into account factors such as synapse location, spine neck and active dendritic properties.

12.8 Endogenous substances and LTP

Perhaps the most important finding of the present studies is the demonstration for the first time that brief applications of samples collected during tetanic stimulations of the guinea pig hippocampus in vivo or the rabbit neocortex in vivo, induce LTP of the population spike in CA_{1b} area evoked by stimulation of the stratum radiatum when applied onto guinea pig hippocampal slices. As previously indicated, the LTP induced by these samples presented with reduced onset latencies of evoked responses and, these synaptic potentiations were not associated with changes in the size of presynaptic volleys. These features are strikingly similar to those seen with LTP induced by tetanic stimulations of afferents in the hippocampus (cf. Bliss and Gardner-Medwin, 1973; Bliss and Lømo, 1973; Bliss and Lynch, 1988). This suggests that the synaptic potentiation induced by application of the above samples has properties that are similar to tetanus-induced LTP.

An intriguing finding in these studies is the observation that the above samples only induced LTP if applied during low frequency activation of the stratum radiatum. In section 12.12 it will be speculated as to how synaptic activations may have been necessary in order to induce LTP with these samples. Because only samples collected during tetanic stimulations of the guinea pig hippocampus in vivo or rabbit neocortex in vivo induced LTP, this raises the prospects that endogenous substances in these samples could mediate feedback interactions during depolarizations, as postulated in section 12.4 above. In terms of possible identities of the endogenous substance(s) responsible for inducing LTP, it is fascinating to note the

following. Neither atropine nor dihydro-β-erythroidine, which are both cholinergic antagonists (e.g. Gilman et al., 1985), blocked the induction of LTP by the above samples. These results indicate that the observed synaptic potentiations were not mediated by endogenous acetylcholine that could be present in the collected samples. Prior heating abolishes the effects of the above samples. The activity is therefore unlikely to be due to endogenous glutamate since prior heating of exogenous glutamate does not abolish the effects of this excitatory amino acid when applied in the hippocampus in vitro. In contrast, it is known that heat denatures proteins and this results in loss of biological activities of these substances (e.g. Lehninger, 1982). In view of this, it seems likely that the potentiating endogenous substances in the collected samples could be macromolecules, possibly proteins.

On reviewing the literature it was interesting to note that protein synthesis inhibitors (Stanton and Sarvey, 1984) and monoclonal antibodies to proteins (Lewis and Teyler, 1986; Stanton, Sarvey and Moskal, 1987) block the induction of LTP with tetanic stimulations of afferents in the hippocampus. Endogenous peptides ranging from 14 to 86 kD are known to be released into the extracellular fluid during LTP in the hippocampus (Duffy, Teyler and Shashoua, 1981; Hess, Hofstein and Shashoua, 1984). It is logical to postulate that, in the present studies, similar proteins were released into the samples collected during tetanic stimulations of guinea pig hippocampus in vivo and rabbit neocortex in vivo and these endogenous proteins may be responsible for the observed LTP. In this regard, a report has appeared in which LTP was induced through brief applications of mast cell degranulating peptides (MCD; see Cherubini, et al., 1987). Prior treatment with trypsin inactivated the potentiating effects of MCD and

Cherubini et al., suggested that MCD may have been mimicking the actions of an endogenous peptide. Clearly, the above reports give credence to the idea that proteins could be involved in the production of LTP in the hippocampus. Future experiments should examine whether prior treatment of the above samples with trypsin would abolish their potentiating actions.

12.9 Endogenous substances and neurite growth

The possible identity for some of the endogenous substances in the samples discussed in section 12.8 above is illustrated in the following results. The growth of neurites in PC-12 cells requires nerve growth factor (NGF) or related substances (Greene and Tischler, 1976). PC-12 cell cultures incubated in growth media containing samples collected during tetanic stimulations of the rabbit neocortex in vivo, presented with extensive neurite growths. These results indicat that NGF-like substances were present in the above neocortical samples and were probably responsible for initiating neurite growth in PC-12 cell cultures. In this regard, it is intriguing to note that the molecular weights of NGF and related compounds range between 14 to 90 kD (Berg, 1984; Wagner, 1986). Coincidentally, these values happen to be similar to the molecular weights of proteins that are known to be released during the induction of LTP with tetanic stimulations in the hippocampus (cf. Hess, Hofstein and Shashoua, 1984). It is possible that there may be similarities in biological activities between NGF and proteins that are released during tetanic stimulations. In fact, this idea was one of the reasons for testing the effects of exogenous NGF (from Vipera lebetina) on synaptic transmission in the hippocampus.

At first, it was disappointing to note that application of exogenous NGF during low frequency stimulation of the stratum radiatum did not induce LTP in the hippocampus. This contradicted the notion that NGF-like

substances could be involved in LTP development! But then it was discovered that exogenous NGF could consistently induce LTP if applied in association with tetanus of a weak input. One possible interpretation of these findings is that exogenous NGF substituted for the endogenous substances that would have otherwise been released by an associative strong tetanus to a strong input (cf. Barrionuevo and Brown, 1983; Levy and Steward, 1979). The reasons for the failure to produce LTP with applications of exogenous NGF alone without tetanus are unclear. Perhaps this reflects species-specific differences in the intrinsic activities or potencies of the applied exogenous NGF. It is also possible that tetanic stimulation releases not only NGF-like substances but other "co-factors", as well, that would not be present in a "purified" sample such as commercially available NGF.

The possible involvement of NGF-like substances in LTP holds promise in view of the following reports in the literature. LTP development is associated with synaptic morphological changes (Desmond and Levy, 1981; 1983; 1986; Fifkova and van Harreveld, 1977; Lee, et al., 1980; Skrede and Malthe-Sørenssen, 1981; Voronin, 1983; Lynch and Baudry, 1984). The mechanisms that mediate these synaptic differentiations are not fully understood. It may be that these post-tetanic morphological changes are regulated by growth related substances, which could be the same substances released during tetanic stimulation (Duffy, Teyler and Shashoua, 1981; Hess, Hofstein and Shashoua, 1984). Future experiments should further examine these possibilities.

12.10 LTP and neurite growth

It is clear that samples collected during tetanic stimulations contained endogenous substances that induced LTP in the hippocampus and caused neurite growth in PC-12 cell cultures. Whether the same substances

in the samples mediated all the above changes was not determined directly. However, other experiments in this thesis provid indirect evidence for the involvement of the same substance(s) in both LTP and neurite growth. This evidence will now be discussed.

Both LTP development and neurite expression exhibit parallel changes to the same treatments that alter the effects of the above samples. For instance, prior heating abolishes the activities of the collected samples in causing synaptic potentiating or neurite growth. Saccharin, a substance that is known to antagonise the NGF-dependent neurite growth (Ishii, 1982), inhibits both the effects of the above samples in inducing neurite growths in PC-12 cell cultures or synaptic potentiations in the hippocampus. Interestingly, saccharin (in concentrations that did not significantly alter synaptic transmission in the hippocampus) antagonises the induction of LTP with tetanic stimulations as well as LTP produced by tetanus of a weak input in the presence of exogenous NGF.

The actions of saccharin were not due to non-specific alterations in the electrical properties of CA_{1b} neurons or NMDA receptor activation, in view of the following results. The dose-response curve to saccharin is spread over two logarithmic scale units, and this is a characteristic feature of drugs exhibiting selective pharmacologic actions (Bowman and Rand, 1980). More importantly, the concentrations of saccharin (10 mM) used do not exert significant effects on evoked synaptic responses in the hippocampus. This is clearly illustrated in the results showing lack of effects of saccharin on paired-pulse facilitations or post-tetanic potentiations. Paired-pulse facilitation is well characterised in the hippocampus (Hess, Kuhnt and Voronin, 1987; MacNaughton, 1980) and it is thought to be due to increases in transmitter that is released with the second pulse in the

stimulation pair (e.g. Hess, Kuhnt and Voronin, 1987). Presumably residual presynaptic depolarizations (or their associated effects, i.e. Ca^{++} influx into terminals) caused by the first pulse add up with depolarizations of the second pulse, thereby augmenting the effects of this latter pulse in the stimulation pair (cf. del Castillo and Katz, 1954). Similarly, PTP is thought to be mediated by presynaptic mechanisms which are responsible for transient increases in transmitter release following tetanic stimulations (McNaughton, 1980). Both paired-pulse facilitation and PTP were unaffected by saccharin. Moreover, the occurrences of minEPSPs and minIPSPs during saccharin applications were the same as in control medium. In addition, the thresholds for antidromic activations of Schaffer collaterals were not altered by saccharin. All these results point to the fact that saccharin in doses used in these studies lacked electrophysiological effects that can account for its blockade of the induction of LTP.

Moreover, it is particularly important to note that saccharin did not alter NMDLA-induced depolarization of the CA_{1b} neurons. Furthermore, saccharin did not antagonise the development of the dendritic negative field that occurs during tetanic stimulations, and is thought to reflect activations of NMDA receptors (Wigström and Gustafsson, 1984; 1986). The above findings were particularly important since tetanus-induced LTP in the CA_1 area is thought to be mediated via the activations of NMDA receptors (Collingridge, Kehl and MacLennan, 1983). These results suggest that saccharin does not antagonize the development of depolarizations that are thought to be necessary for LTP induction (Gustafsson and Wigström, 1988). Taken together, the results with saccharin indicate that the blockade of LTP induction by this agent is probably occurring at a step beyond the activation of NMDA receptors.

12.11 Possible mechanisms of actions of saccharin

A prominent feature of the abundant literature on saccharin is the absence of studies that have examined the electrophysiological effects of this drug. The studies on saccharin reported in this thesis, therefore, provide some of this information. However, the literature on saccharin describes some mechanisms that could help to account for the effects of saccharin on LTP production or neurite growth. These mechanisms will be briefly reviewed here (see chapter 8).

Firstly, saccharin antagonises NGF binding (Ishii, 1982). If it is tentatively accepted that the endogenous substances in the samples collected during tetanic stimulations were close relatives of NGF, then saccharin could have been antagonising the binding of these substances to NGF receptors in the experiments reported in this thesis. However, a major argument against the above possibility is that the binding of NGF and related substances is known to be in pM ranges. Yet the blockade of LTP by saccharin requires drug concentrations greater than 7.5 mM. It is difficult to explain the need for mM concentrations of saccharin in "selectively" antagonising the binding of substances with activities in pM ranges! A plausible explanation for requiring a relatively high saccharin concentration is that the agent acts at intracellular site(s) and is poorly transported into the cell. Hence, to achieve significant intracellular drug levels it is necessary to have mM concentrations of saccharin in the extracellular space. Once internalized, however, saccharin may then antagonise some of the NGF-dependent reactions within the cells.

Secondly, saccharin inhibits the activities of specific phosphorylating enzymes (Best and Brown, 1987; Brown and Best, 1986; Linke and Kohn, 1984; Lygre, 1974; 1976). In this regard, it is interesting to note that

NGF-dependent phosphorylating enzymes have been described in the literature (Halegoua and Patrick, 1980). It is conceivable that some of the enzymes inhibited by saccharin included NGF-dependent phosphorylating enzymes. Perhaps high saccharin concentrations are necessary to diminish the enzyme activities significantly (cf. Best and Brown, 1987). Whatever, the mechanisms of saccharin actions, these will need to be investigated in future experiments.

12.12 Implications in LTP

The studies in this thesis show for the first time that substances that are released during tetanic stimulations are capable of inducing LTP when applied in the hippocampus and neurite growth in PC-12 cell cultures. The exact mechanisms that trigger the release of these substances or how they, in turn, exert changes that lead to LTP or neurite growth in PC-12 cells remain to be determined. However, the present results begin to provide some insights into certain intriguing features pertaining to the phenomena of LTP. Firstly, it is known that tetanic stimulations that induce LTP also cause the release of proteins (Duffy, Teyler and Shashoua, 1981), but the effects of these released proteins on synaptic transmission are not known. Secondly, LTP development is associated with morphological changes to synaptic structures (e.g. Fifkova, 1986). The factors that initiate these morphological changes or how they relate to LTP are not clear. Thirdly, since the induction of LTP is thought to be mediated by postsynaptic mechanisms, it is not known how this postsynaptic change is subsequently "relayed" to presynaptic regions in order to incorporate presynaptic mechanisms which are implicated in the maintenance of LTP (e.g. Bliss and Lynch, 1988).

The studies in this thesis raise the following prospects. Since

tetanic stimulations cause the release of NGF-like substances capable of inducing neurite growth in PC-12 cell cultures, it is conceivable that these same substances mediate the synaptic re-structuring that occur during LTP. It might be that depolarizations (through NMDA receptor activations) induced by tetanic stimulations trigger the release of growth related substances that act as "feedback" messengers that interact with presynaptic and/or subsynaptic membranes and orchestrate changes that produce LTP. In this regard, the proteins that are released during tetanic stimulations (Duffy, Teyler and Shashoua, 1981) could be close relatives of NGF. In a global sense, if the release of NGF-like substances by intense neuronal activity is a common feature of various areas in the nervous system, it becomes important to determine if these substances are ubiquitously involved in improving the quality of synaptic transmission and, therefore, the quality of brain functions in general. In terms of LTP, a speculative case can be made for the involvement of NGF-like substances in its production.

NGF receptors are present on presynaptic terminals and the peptide is known to be taken into the terminals (Hendry et al., 1974; Springer and Loy, 1985). Hence released peptides from postsynaptic regions could interact with NGF receptors on active (but not quiescent) presynaptic terminals (and/or subsynaptic dendritic membranes) and thereby initiate changes leading to LTP development (i.e. changes in dendritic morphology, synaptic rearrangement, enhancement of transmitter release). Presynaptic activity could facilitate the interaction of NGF-like substances with receptors that trigger the internalization of the peptide(s). Therefore, the interaction of these substances with their receptors or the events that follow the peptide(s)-receptor interaction may be voltage dependent. In this regard, it is interesting to note that electrical activity in neurons shapes the

patterns of synaptic connections and that neurite growth on cells in culture is modified by the polarity of the electrical field (Frank, 1987; McCaig, 1987; 1988).

In summary, results in the present study raise the possibility that substances released by tetanic stimulations contain NGF-like proteins that may be involved in synaptic potentiation. These agents could act as one of the messengers in the chain of events leading to LTP production. The possibility that NGF-like substances are universally involved in LTP is, therefore, worth investigating.

13. CONCLUSION

The major findings of the studies in this thesis can be summarized as follows:

1. In hippocampal slices incubated in Ba^{++}, evoked minEPSPs following stimulation of an input probably reflect the asynchronous release of synaptic transmitter.

2. During depolarizations, postsynaptic modulation of presynaptic functions occur in the hippocampus and, this is illustrated in the observed transient increases in frequencies of evoked minEPSPs immediately after pairing of conditioning intracellular depolarizations of a CA_{1b} neuron and activation of the stratum radiatum.

3. The increase in evoked minEPSPs during synaptic potentiation are consistent with previous evidence that LTP may partly be due to an apparent increase in released synaptic transmitter.

4. The induction of LTP with application of samples collected during tetanic stimulations indicates that these samples contained endogenous substances, possibly proteins, that may be involved in the production of synaptic potentiations.

5. The induction of neurites in PC-12 cell cultures incubated in the presence of samples collected during tetanic stimulations of the neocortex illustrates that these samples also contained NGF-like substances.

6. The finding that LTP can be produced by tetanic stimulation of a weak input in conjunction with application of exogenous NGF further supports the notion that close relatives of NGF may be involved in LTP development.

7. Saccharin (a substance known to antagonise NGF binding to its receptors and to inhibit NGF-dependent neurite growth) blocks both the induction of

LTP in the hippocampus and neurite growth in PC-12 cell cultures induced by samples collected during tetanic stimulations of the rabbit neocortex; this supports the idea that common mechanisms may be involved in LTP production and neurite growth.

8. Saccharin also blocks both the induction of LTP by tetanic stimulation of (a) a strong input, or (2) a weak input in conjunction with application of exogenous NGF; this further suggests that NGF-like substances may be involved in LTP development.

9. The concentrations of saccharin used in these studies had insignificant effects on CA_{1b} cell electrical properties, synaptic transmission or NMDA receptor activation; this suggests that saccharin may be blocking induction of LTP at a step beyond NMDA receptor activation.

10. Taken together, the studies in this thesis indicate that intense depolarizations cause the release of diffusable substances, probably growth related proteins, that subsequently interact with activated presynaptic afferents and/or subsynaptic dendritic elements resulting in LTP.

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